1 Milk Lipids – Composition, Origin and Properties

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1.1 Introduction

Milk is the fluid secreted by the female of all mammalian species, primarily to meet the complete nutritional requirements for the neonate, such as energy, essential amino acids and fatty acids, vitamins, minerals and water. Milk is an effective and balanced source of lipids, proteins (caseins and whey proteins), carbohydrates (mainly lactose), minerals (e.g. calcium and phosphate), enzymes, vitamins and trace elements.

The primary role of the lipids in milk is to provide a source of energy to the neonate. From a practical viewpoint, milk lipids derive a high level of importance from the distinctive nutritional, textural and sensory properties they confer on a wide variety of dairy products such as liquid milk, cheese, ice cream, butter and yoghurt. Milk lipids were long regarded as the most economically valuable constituent of milk and, as a result, the milk price paid to farmers was for many years determined primarily by the concentration of lipids in the milk; only more recently has the concentration of milk proteins attained an equal, or even higher, weighting factor in the determination of milk price. In this chapter, the aim is to provide an overview of the composition, origin and properties of milk lipids. The focus is primarily on the lipids of cow's milk, but comparisons with milk from other major dairy species are made where appropriate.

1.2 Composition of milk lipids

Lipids are esters of fatty acids and related compounds that are soluble in apolar organic solvents and (nearly) insoluble in water and may be divided into three groups:

- Neutral lipids (tri-, di- and monoacylglycerols)
- Polar lipids (phospholipids and glycolipids)
- Miscellaneous lipids (sterols, carotenoids and vitamins)

The term 'lipids' is often readily interchanged with 'fat', but this is incorrect since the latter represents only one subgroup of neutral lipids, the triacylglycerols; hence, the term lipids will be used throughout this chapter. Cow's milk contains ~45 g lipids L^{-1} on average, but this can range from 30 to 60 g L^{-1}, depending on the breed, diet, stage of lactation and health of the cow. There are very large inter-species differences in lipid content between mammals, and the concentration can reach >500 g L^{-1} milk for some species

Table 1.1 Concentration of lipids in the milk of different species.

Species	Lipid content (g L^{-1})	Species	Lipid content (g L^{-1})
Cow	33–47	Marmoset	77
Buffalo	47	Rabbit	183
Sheep	40–99	Guinea pig	39
Goat	41–45	Mink	71
Musk ox	109	Snowshoe hare	134
Dall sheep	32–206	Chinchilla	117
Moose	39–105	Rat	103
Antelope	93	Red kangaroo	9–119
Elephant	85–190	Dolphin	62–330
Human	38	Manatee	55–215
Horse	19	Pygmy sperm whale	153
Monkey	22–85	Harp seal	502–532
Lemur	8–33	Bear	108–331
Pig	68		

Data compiled from Christie (1995).

(Table 1.1). In cow's milk, >98% of lipids are triacylglycerols, but diacylglycerols and monoacylglycerols, free fatty acids, phospholipids, sterols, carotenoids, fat-soluble vitamins and flavour compounds are also found (Table 1.2). In this section, an overview of the various classes of lipids found in milk is provided. More extensive reviews on this subject were compiled by Christie (1995), Jensen (2002), Fox & Kelly (2006) and MacGibbon & Taylor (2006).

Table 1.2 Primary classes of lipids in cow's milk.

Lipid class	% of total
Triacylglycerols	98.3
Diacylglycerols	0.3
Monoacylglycerols	0.03
Free fatty acids	0.1
Phospholipids	0.8
Sterols	0.3
Carotenoids	Trace
Fat-soluble vitamins	Trace
Flavour compounds	Trace

Data compiled from Walstra *et al*. (1999).

1.2.1 *Fatty acids*

A fatty acid is a carboxylic (organic) acid, often with a long aliphatic tail. The fatty acid composition of lipids is particularly important in determining their physical, chemical and nutritional properties. The cow's milk lipids are among the most complex naturally occurring groups of lipids, because of the large number of fatty acids they contain. The fatty acids arise from two sources, synthesis *de novo* in the mammary gland and plasma lipids originating from the feed, as discussed further in Section 1.3. Approximately 400 fatty acids have been identified in cow's milk fat to date, an extensive review of which is provided by Jensen (2002). Primary distinguishing variables among fatty acids are as follows:

- Chain length – An overview of the major fatty acids in cow's milk lipids is given in Table 1.3, from which it is clear that palmitic, oleic, stearic and myristic acids are the principal fatty acids in cow's milk lipids. Short- and medium-chain fatty acids occur in lower amounts, at least when expressed on a weight basis; these have the interesting characteristic that, unlike long-chain acids, they are absorbed into the blood stream in non-esterified form and are metabolised rapidly (Noble, 1978). Furthermore, the chain length influences the melting characteristics of lipids (Section 1.6).
- Degree of saturation – Saturated fatty acids contain no double bonds along the chain; the term *saturated* refers to hydrogen, in that all carbon atoms, apart from the carboxylic acid group, contain as many hydrogen atoms as possible. Saturated fatty acids contain an alkane chain of only single-bonded carbon atoms (–C–C–), whereas

Table 1.3 Major fatty acids in bovine milk lipids.

Number of carbon atoms	Number of double bonds	Shorthand designation	Systematic name	Trivial name	Average range (g 100 g^{-1})
4	0	$C_{4:0}$	Butanoic acid	Butyric acid	2–5
6	0	$C_{6:0}$	Hexanoic acid	Caproic acid	1–5
8	0	$C_{8:0}$	Octanoic acid	Caprylic acid	1–3
10	0	$C_{10:0}$	Decanoic acid	Capric acid	2–4
12	0	$C_{12:0}$	Dodecanoic acid	Lauric acid	2–5
14	0	$C_{14:0}$	Tetradecanoic acid	Myristic acid	8–14
15	0	$C_{15:0}$	Pentadecanoic acid	–	1–2
16	0	$C_{16:0}$	Hexadecanoic acid	Palmitic acid	22–35
16	1	$C_{16:1}$	9-Hexadecanoic acid	Palmitoleic acid	1–3
17	0	$C_{17:0}$	Heptadecanoic acid	Margaric acid	0.5–1.5
18	0	$C_{18:0}$	Octadecanoic acid	Stearic acid	9–14
18	1	$C_{18:1}$	9-Octadecanoic acid	Oleic acid	20–30
18	2	$C_{18:2}$	9,12-Octadecadienoic acid	Linoleic acid	1–3
18	3	$C_{18:3}$	9,12,15-Octadecatrienoic acid	Linolenic acid	0.5–2

Data compiled from Jensen (2002).

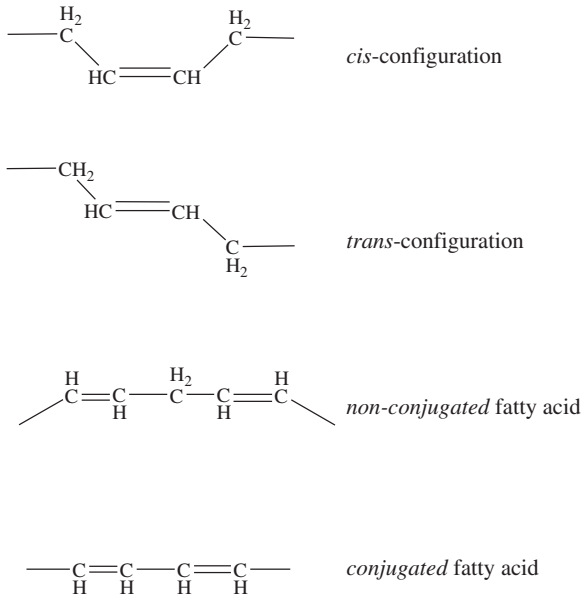


Fig. 1.1 Examples of *cis* and *trans* configuration in unsaturated fatty acids and *non-conjugated* and *conjugated* polyunsaturated fatty acids.

unsaturated fatty acids contain at least one alkene group of double-bonded carbon atoms (–C=C–).

- Configuration of double bonds – The carbon atoms in the chain on either side of the double bond can occur in a *cis* or *trans* configuration (Figure 1.1). In the *cis* configuration, which is most common in nature (>95% of unsaturated fatty acids), the two carbons are on the same side of the double bond. Owing to the rigidity of the double bond, the *cis* isomer causes the chain to bend. In the *trans* configuration, the carbon atoms on either side of the double bond are orientated to the opposite sides of the bond (Figure 1.1) and do not cause the chain to bend much; as a result, their shape is similar to the more linear saturated fatty acids.
- Conjugation of double bonds – For polyunsaturated fatty acids, the term *non-conjugated* indicates that two double bonds in the fatty acid carbon chain are separated by a methylene group (–CH_2–), whereas, in a *conjugated* fatty acid, the double bonds are separated by only one single bond (Figure 1.1); most of the naturally occurring fatty acids are non-conjugated. Particularly in the last decade, conjugated linoleic acid (CLA) has gained major interest in human nutrition, as discussed in Chapter 2 and by Bauman & Lock (2006).

1.2.2 *Triacylglycerols*

A triacylglycerol (also known as a *triglyceride* or *triacylglyceride*) is a glyceride in which glycerol is esterified to three fatty acids; likewise, in a mono- or diacylglycerol, the glycerol is esterified to one or two fatty acids, respectively. The composition of triacylglycerols is

Table 1.4 Total carbon number of triacylglycerols in cow's milk lipids.

Total fatty acid carbon number	% of total
C26	0.1–1.0
C28	0.3–1.3
C30	0.7–1.5
C32	1.8–4.0
C34	4–8
C36	9–14
C38	10–15
C40	9–13
C42	6–7
C44	5–7.5
C46	5–7
C48	7–11
C50	8–12
C52	7–11
C54	1–5

Data compiled from Jensen (2002).

defined in terms of the kinds and amounts of fatty acids present and can be expressed as the total carbon number, that is, the sum of the number of carbon atoms in the three fatty acids. The proportions of triacylglycerols according to total carbon number in cow's milk are given in Table 1.4. Stereospecific analysis has enabled the distribution of the fatty acids on the *sn*-1, *sn*-2 and *sn*-3 positions of glycerol to be determined (Figure 1.2) and shown that the distribution of fatty acids in the triacylglycerols of milk is highly specific (Table 1.5). The short-chain fatty acids, butyric and caproic acids, are esterified almost exclusively at the *sn*-3 position, while the medium-chain acids, lauric and myristic acids are esterified preferentially at the *sn*-2 position. Palmitic acid is esterified preferentially at the *sn*-1 and *sn*-2 positions,

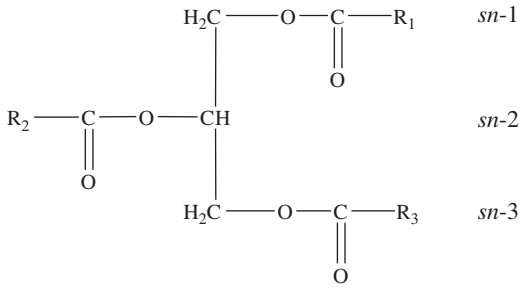


Fig. 1.2 Schematic diagram of a triacylglycerol showing the stereospecific *sn*-1, *sn*-2 and *sn*-3 positions.

Table 1.5 Positional distribution of the major fatty acids in the bovine triacylglycerols.

Fatty acid	Fatty acid composition (mol%)		
	sn-1	*sn*-2	*sn*-3
4:0	–	0.4	30.6
6:0	–	0.7	13.8
8:0	0.3	3.5	4.2
10:0	1.4	8.1	7.5
12:0	3.5	9.5	4.5
14:0	13.1	25.6	6.9
16:0	43.8	38.9	9.3
18:0	17.6	4.6	6.0
18:1	19.7	8.4	17.1

Data compiled from MacGibbon & Taylor (2006).

stearic acid at the *sn*-1 position and oleic acid at the *sn*-1 and *sn*-3 positions (MacGibbon & Taylor, 2006).

1.2.3 *Mono- and diacylglycerols and free fatty acids*

Immediately after milking, milk contains only small amounts of di- and monoacylglycols and free fatty acids, but these levels can increase considerably during storage, due to enzymatic hydrolysis of the ester bonds in triacylglycerols. Enzymatic hydrolysis of triacylglycerols is referred to as lipolysis, and may arise from the action of the indigenous milk enzyme lipoprotein lipase or of bacterial lipases. The diacylglycerols in freshly drawn milk are unlikely to be the products of lipolysis since they are mostly non-esterified at the *sn*-3 position, whereas lipases preferentially attack the *sn*-1 and *sn*-3 position and should thus result in a mixture of diacylglycerols with non-esterified *sn*-1 and *sn*-3 positions. More likely, the diacylglyerols in freshly drawn milk are intermediates in the biosynthesis of triacylglycerols, since the *sn*-3 position is the last to be esterified. The profile of free fatty acids in freshly drawn milk also differs from that of the fatty acids esterified in the triacylglycerols, making it unlikely that free fatty acids are the products of lipolysis. Diacylglycerols have physical properties similar to those of triacylglycerols, but monoacylglycerols, particularly those containing a long-chain fatty acid, are amphiphilic and are thus surface active (Taylor & MacGibbon, 2002; Walstra *et al.,* 2006).

1.2.4 *Phospholipids*

Phospholipids are a class of lipids formed from four components: a backbone (glycerol or sphingosine), fatty acids, a negatively charged phosphate group and a nitrogen-containing compound or sugar. Phospholipids with a glycerol backbone are known as *glycerophospholipids* and have a fatty acid at the *sn*-1 and *sn*-2 positions and a phosphate and a polar head group

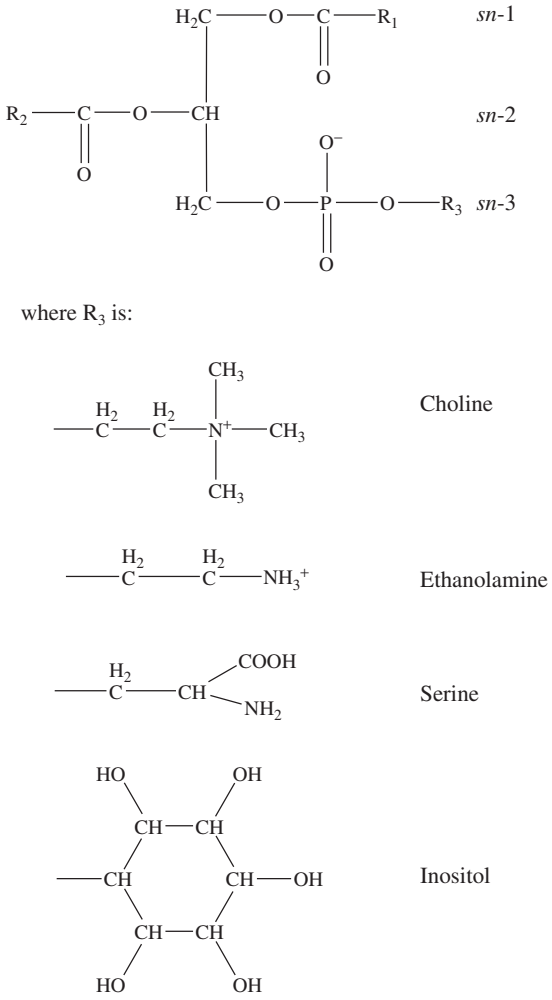


Fig. 1.3 Schematic projection of a glycerophospholipid and its possible polar groups.

(choline, ethanolamide, serine or inositol) at the *sn*-3 position (MacGibbon & Taylor, 2006); a general projection of a glycerophospholipid is given in Figure 1.3. Phosphatidylcholine is commonly referred to as *lecithin*. The major fatty acids in glycerophospholipids are palmitic, stearic, oleic and linoleic acids, with very few shorter chain fatty acids (Bitman & Wood, 1990). Sphingolipids consist of a long-chain amino alcohol, sphingosine, to which a fatty acid is attached through an amide linkage to yield a ceramide. Linkage of phosphorylcholine group to the terminal alcohol group of a ceramide yields sphingomyelin, a sphingophospholipid (Figure 1.4); glycosphingolipids have one or more hexose units (e.g. glucose) attached to the terminal alcohol group of a ceramide (MacGibbon & Taylor, 2002).

The proportion of phospholipids in milk lipids is typically 0.9 g 100 g^{-1} milk lipids, but this proportion is considerably higher in skimmed milk (25 g 100 g^{-1}) and buttermilk (22 g 100 g^{-1}); in contrast, the proportion of phospholipids in 40 g 100 g^{-1} fat cream is lower

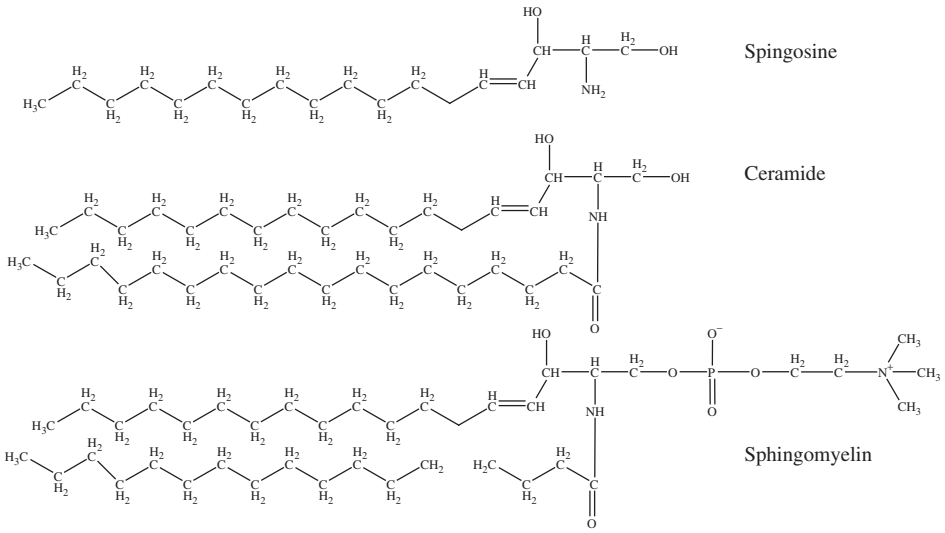


Fig. 1.4 Schematic projection of sphingosine, a ceramide and sphingomyelin.

(∼0.5 g 100 g^{-1} milk lipids) than in whole milk (Mulder & Walstra, 1974). Phosphatidylcholine, phosphatidylethanolamine and sphingomyelin are the principal phospholipids in cow's milk, each representing ∼25–35% of the total phospholipids. Phospholipids are important in dairy products because they are good emulsifiers. Hence, it is not surprising that the majority of phospholipids (60–65%) are present in the membrane surrounding the milk lipid globules (MLGs), whereas the remainder are found in the milk plasma, primarily in soluble fragments of the lipid globule membrane (Huang & Kuksis, 1967). The role of phospholipids in the milk lipid globule membrane (MLGM) is discussed in Section 1.4. Some phospholipids have a number of health-beneficial functions; sphingomyelin shows a strong anti-tumour activity, influences the metabolism of cholesterol and exhibits an anti-infective activity; glycerol phospholipids protect against mucosal damage (Parodi, 2004, 2006).

1.2.5 *Minor constituents*

Sterols or steroic alcohols

These constituents are minor components, accounting for only ∼0.3% of total milk lipids; cholesterol accounts for >95% of total sterols in milk (Walstra *et al*., 1999). Since cholesterol is found primarily in the MLGM, the proportion of cholesterol in milk lipids is, as for phospholipids, considerably higher in skimmed milk and buttermilk than in whole milk and cream (Russel & Gray, 1979). The cholesterol content of various dairy products is summarised in Table 1.6. The association of dietary cholesterol with coronary heart disease has led to consumer preference for food products, including dairy products, containing a low amount of cholesterol. This has led to considerable research into the development of methods for the removal of cholesterol from dairy products; for an overview of biological, chemical and physical processes used for this purpose see Sieber & Eyer (2002).

Table 1.6 Cholesterol content of dairy products.

Dairy product	Lipid content (g 100 g^{-1} product)	Cholesterol content (mg 100 g^{-1} product)
Skimmed milk	0.3	2
Whole milk	3.3	14
Medium cream	25.0	88
Skimmed milk powder	0.8	20
Cream cheese	34.9	110
Ice cream	11.0	44
Cheese		
Cheddar	33.1	105
Brie	27.7	100
Swiss	27.5	92
Butter	81.1	219

Data compiled from Jensen (2002).

Carotenoids occur only in trace amounts (μg g^{-1} lipids) in cow's milk lipids; β-carotene accounts for >95% of total carotenoids in cow's milk. The level of β-carotene is highly variable, depending both on the concentration of carotene in the feed, as well as on the breed of the cow. β-Carotene is responsible for the yellow colour of milk fat (Walstra *et al*., 2006).

The lipid-soluble vitamins (i.e. vitamins A, D, E and K) are also found in the lipid fraction of milk; milk is a significant source of vitamin A, but the concentrations of vitamins D, E and K are too low to make significant contributions to the consumers' vitamin requirements.

Finally, milk lipids contain a large number of flavour compounds, especially lactones, fatty acids, aldehydes and methyl ketones, which contribute to the overall organoleptic properties of milk. The concentrations of flavour compounds are influenced primarily by the feed of the cow. For a description of the flavour compounds in milk lipids, the reader is referred to Schieberle *et al*. (1993).

1.3 Origin of milk lipids

1.3.1 *Biosynthesis and origin of the fatty acids in milk lipids*

The fatty acids of milk arise from two sources: (a) synthesis *de novo* in the mammary gland and (b) uptake from the circulating blood. The composition of the fatty acids derived from the two sources differs markedly. The fatty acids produced *de novo* are all those with a carbon chain of 4–14 atoms and a proportion of the C_{16} fatty acids, whereas the remainder of the C_{16} fatty acids and almost all of the C_{18} acids arise from the blood. In this section, a brief overview of the two sources of fatty acids for bovine lipids are given; for more extensive reviews, the reader is referred to Hawke & Taylor (1995), Barber *et al*. (1997) and Palmquist (2006).

1.3.2 *De novo synthesis of fatty acids*

Ruminants use acetate (C_2) and β-hydroxybutyrate (C_4) that is synthesised by bacteria in the rumen as the carbon sources for fatty acid synthesis; acetate contributes ~92% of the total carbon in milk fatty acids. Monogastric animals use glucose as the principal source of carbon source for fatty acid synthesis (Palmquist, 2006). In ruminants, the first step of fatty acid synthesis is the conversion of acetate, derived from the blood, to acetyl coenzyme-A by the cytosolic enzyme acetyl coenzyme-A synthase, according to Figure 1.5.

$$acetate + coenzyme\ A + ATP \rightarrow acetyl\text{-}coenzyme\ A + ADP.$$

Acetyl-coenzyme A is converted to malonyl coenzyme A (Figure 1.5):

$$acetyl\text{-}coenzyme\ A + HCO_3^- + ATP \rightarrow malonyl\text{-}coenzyme\ A + ADP + P_i$$

The synthesis of fatty acids subsequently occurs under the influence of the multi-enzyme system 'fatty acid synthase', according to Figure 1.5.

$$acetyl\text{-}coenzyme\ A + n\ malonyl\text{-}coenzyme\ A + 2n\ NADPH + 2nH^+ \rightarrow$$
$$fatty\ acid + nCO_2 + (n{+}1)\ coenzyme\ A + 2n\ NADP^+ + (n-1)\ H_2O$$

where the number of carbon atoms in the fatty acid is $(2n + 2)$. Activation of β-hydroxybutyrate leads to the formation of β-hydroxybutyl-coenzyme A, which contributes equally to acetyl-coenzyme A as the primer for fatty acid formation by fatty acid synthase, but does not contribute to chain elongation. The synthesis of fatty acids by fatty acid synthase is

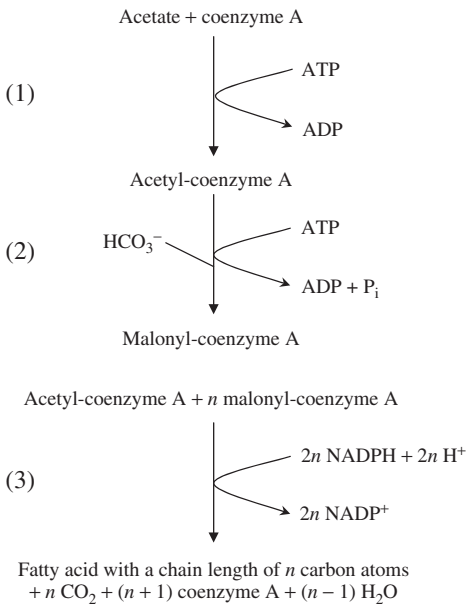


Fig. 1.5 Schematic representation of the synthesis of a fatty acid.

described in detail by Smith *et al*. (2003). Cycling through the fatty acid synthase system, with the addition of C_2 units from malonyl-coenzyme A, continues until the nascent fatty acid reaches a chain length of 6 to 16 carbon atoms, when a thioesterase specific for that chain length releases the fatty acid and terminates the cycle (Palmquist, 2006).

1.3.3 *Uptake of fatty acids from the blood*

Lipids taken up by the mammary gland from the blood can originate from the digestive tract or from mobilised body-fat reserves. To overcome their insolubility in aqueous media, dietary triacylglycerols are transported in the form of lipoproteins or, more specifically, a subclass of lipoproteins, the very-low density lipoproteins. In the mammary gland, fatty acids are de-esterified from triacylglycerols by lipoprotein lipase, a process described by Barber *et al*. (1997). Fatty acids released from body-fat reserves by the action of a hormone-sensitive lipase are also taken up by the mammary gland, although their contribution is thought to be limited. As mentioned previously, almost all C_{18} fatty acids are taken up from the blood, but $C_{16:0}$ is derived from the blood and by *de novo* synthesis. When the level of dietary lipid intake is low, almost all $C_{16:0}$ is synthesised *de novo*, but the proportion of $C_{16:0}$ synthesised *de novo* can decrease by $>70\%$ when the uptake from the blood increases (Palmquist, 2006).

1.3.4 *Desaturation of fatty acids*

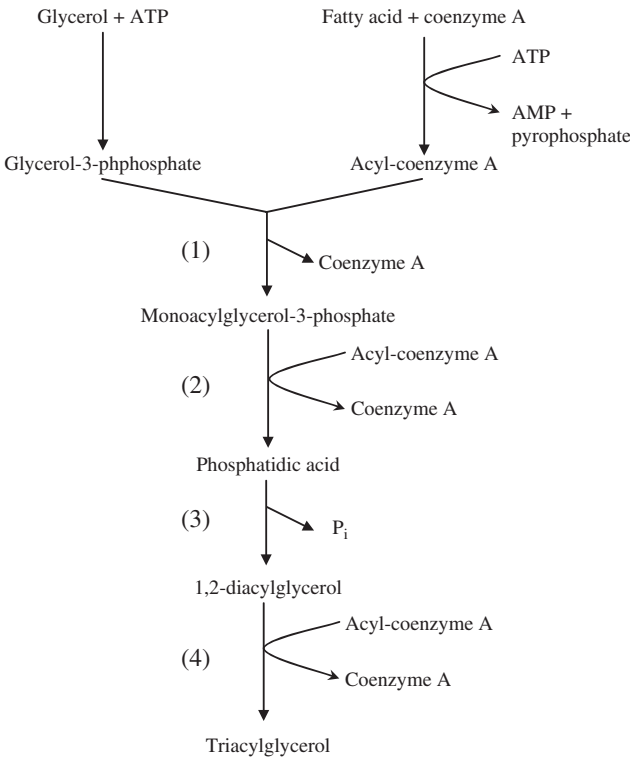
From Section 1.3.1, it is clear that only saturated fatty acids are synthesised *de novo* in the mammary gland; furthermore, due to the very low redox potential in the rumen, extensive hydrogenation of unsaturated fatty acids in the diet occurs. Hence, the initial concentration of unsaturated fatty acids in the mammary gland is low and an extensive desaturation must occur to achieve the levels of unsaturated fatty acids found commonly in bovine milk. The rate-limiting enzyme in this desaturation process is stearoyl-coenzyme A desaturase, the activity of which is particularly high in the lactating mammary gland, but low in non-lactating mammary gland tissue. Stearyl-coenzyme A is located primarily in the endoplasmic reticulum (ER) and its principal substrates are stearoyl-coenzyme A and palmitoyl-coenzyme A (Palmquist, 2006).

1.3.5 *Synthesis of triacylglycerols*

The glycerol-3-phosphate pathway is the primary route for triacylglycerol synthesis in the mammary gland and is presented in Figure 1.6. In this multistep enzyme-catalysed pathway, the *sn*-1, *sn*-2 and *sn*-3 positions of glycerol are esterified consecutively. The resulting triacylglycerols associate into spherical droplets, which become covered with a membrane layer, as described in Section 1.5.

1.4 Factors affecting the composition of milk lipids

When considering the composition and origin of milk lipids, it is important to consider that the composition of milk lipids is not a static, but a dynamic, phenomenon, which is influenced



Steps (1), (2), (3) and (4) are catalysed by the following enzymes:
- Acyl-coenzyme A:sn-glycerol-3-phosphate acyl-transferase
- Acyl-coenzyme A:1-acyl-sn-glycerol-3 phosphate acyl transferase
- Phosphatide phosphate
- Acyl-coenzyme A:1,2-diacylglycerol acyl-tranferase

Fig. 1.6 Schematic representation of the synthesis of triacylglycerols in the mammary gland.

by both physiological and nutritional factors. Physiological factors that influence milk lipid composition include other species of mammals, as well different breeds of a species. Milk lipid composition is also affected by the stage of lactation. Colostrum is rich in C_{12}, C_{14} and C_{16} fatty acids, but the relative proportions of C_4–C_{10} and C_{18} acids increase rapidly afterwards and the relative proportions of fatty acids generally stabilise 1 week postpartum. During the subsequent lactation, the relative proportions of fatty acids synthesised *de novo* increases, whereas the proportion of dietary fatty acids decreases concomitantly (Palmquist *et al.,* 1993; Palmquist, 2006).

The fatty acid profile of milk lipids is also influenced strongly by dietary factors, which have been reviewed extensively (Grummer, 1991; Palmquist *et al.,* 1993; Palmquist, 2006), and only a few points are mentioned here:

- Low-fat diets greatly reduce the proportion and yield of C_{18} acids in milk lipids, whereas increasing the level of C_{18} acids in a low-fat diet linearly increases the level of C_{18} acids in milk.

- The incorporation of high levels of unsaturated fatty acids in the diet has little effect on the degree of unsaturation of milk lipids, due to extensive biohydrogenation in the rumen.
- The concentrations of C_{16} and C_{18} acids in milk lipids can be increased by increasing the dietary uptake of these fatty acids.

1.5 Intracellular origin of milk lipid globules and the milk lipid globule membrane

1.5.1 *Secretion of milk lipid globules*

The secretion of MLG is represented schematically in Figure 1.7. The precursors for the MLG originate in the ER, as the so-called micro lipid droplets (MLD), which have a diameter <0.5 μm and consist of a triacylglycerol-rich core surrounded by a coat consisting of proteins and polar lipids (Keenan & Mather, 2006). Since the size distribution of MLG is considerably broader than that of the MLD, considerable growth must occur after micro lipid droplet formation. Whether droplet growth is a controlled or random process is unknown. Although there are several potential mechanisms through which the droplet growth can occur, fusion of droplets appears to be the only mechanism for which there is both biochemical and morphological evidence (Keenan & Mather, 2006). Such fusion is restricted to the small droplets, which can fuse with each other or with larger droplets; apparently, large

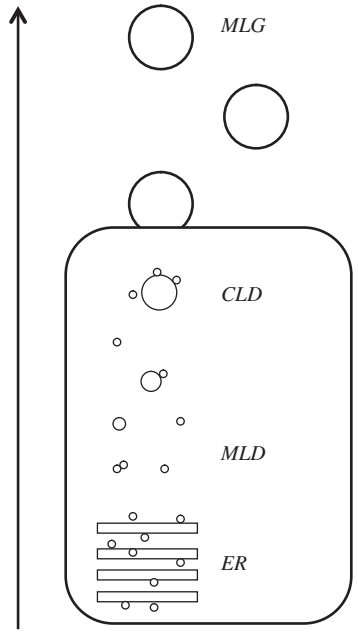


Fig. 1.7 Schematic representation of the secretion of milk lipid globules. ER, endoplasmic reticulum; MLD, micro lipid droplet; CLD, cytoplasmic lipid droplet; MLG, milk lipid globule. The upward arrow indicates the direction in which the secretion process progresses.

droplets (>2 μm) cannot fuse. The larger droplets resulting from the fusion of MLD are termed *cytoplasmic lipid droplets*. The micro and cytoplasmic lipid droplets move through the cell to its apical region. Upon arrival at the apical membrane, the droplets come into contact with a dense proteinaceous coat on the inner face of the plasma membrane and are gradually surrounded by plasma membrane and subsequently released into the lumen as MLG (Figure 1.7). The composition of the membrane surrounding the MLG is described in the following section.

1.5.2 *The milk lipid globule membrane*

The MLGM, which is also referred to as the milk fat globule membrane (MFGM), has been the subject of considerable research and was reviewed by King (1955), Patton & Keenan (1975), Keenan & Dylewski (1994) and Keenan & Mather (2002, 2006).

As described above, the MLGM originates from the membranes of the milk-secreting cells. The droplets released from the endoplasmatic reticulum are coated primarily by proteins and phospholipids; this surface layer has no discernable bilayer characteristics (Keenan & Dylewski, 1995; Keenan & Mather, 2006). On arrival at the apical region, the droplets interact with the plasma membrane prior to secretion. During this process, the droplets are enveloped with plasma membrane. As a result, the composition of the MLGM, which is given in Table 1.7, closely resembles that of the cell membrane from which it is derived. The current view of the molecular organisation of the MLGM is that it comprises a true bilayer membrane with a thick (10–50 nm) dense proteinaceous coat on the inner surface of the bilayer membrane, plus an innermost layer that existed before secretion of the globules (Keenan & Mather, 2006). From Table 1.7, it is apparent that lipids and proteins, including enzymes, are the principal constituents of the MLGM and these are described in Sections 1.5.3, 1.5.4 and 1.5.5. The MLGM material has attracted considerable attention from both nutritional and technological viewpoints in recent years and was reviewed by Ward *et al*. (2006).

Table 1.7 Gross composition of the milk lipid globule membrane.

Constituent class	Amount
Proteins	25–60 g 100 g^{-1}
Total lipids	0.5–1.1 mg mg^{-1} protein
Neutral lipids	0.25–0.88 mg mg^{-1} protein
Phospholipids	0.13–0.34 mg mg^{-1} protein
Glycosphingolipids	13 μg mg^{-1} protein
Hexoses	108 μg mg^{-1} protein
Hexosamines	66 μg mg^{-1} protein
Sialic acids	20 μg mg^{-1} protein
RNA	20 μg mg^{-1} protein
Glycosaminoglycans	0.1 μg mg^{-1} protein

Data compiled from Keenan & Mather (2002).

1.5.3 *Lipids of the milk lipid globule membrane*

The principal classes of lipids in the MLGM are summarised in Table 1.8, from which it is apparent that triacylglycerols and phospholipids, in a ratio of ∼2:1, are the most abundant lipid classes. The triacylglycerols of the MLGM contain a higher proportion of long-chain fatty acids than the triacylglycerols of the milk lipid globule core. Di- and monoacylglycerols are also present, but it is unclear whether they are true membrane constituents or the products of lipolysis (Keenan & Mather, 2006). Of the total phospholipids in milk, ∼60% are present in the MLGM. The distribution and fatty acid composition of phospholipids in the MLGM are similar to those in skimmed milk, suggesting that they are derived from a common source (Keenan & Dylewski, 1995).

1.5.4 *Proteins of the milk lipid globule membrane*

Proteins account for 25–60% of the mass of the MLGM (Table 1.8); these proteins are extremely diverse and were reviewed by Mather (2000). Electrophoresis on the basis of molecular mass reveals eight major protein bands, which are discussed briefly. Most of these proteins are known under several different names; the nomenclature proposed by Mather (2000) is followed here.

Butyrophilin (*BTN*) is the most abundant protein of the MLGM, comprising ∼40% of total membrane protein in the milk of Holstein cows. BTN is a transmembrane protein with an externally oriented N-terminus; its association with the membrane is probably stabilised by disulphide bonds. The protein is expressed specifically in the lactating mammary gland and is concentrated at the apical cell membrane and on the MLG. BTN is a glycosylated protein with a molecular mass of ∼66 kDa. A comprehensive review of the molecular and cellular biology of BTN is provided by Mather & Jack (1993).

Table 1.8 Lipids of the milk lipid globule membrane.

Lipid class	% of total lipids
Triacylglycerols	62
Diacylglycerols	9
Monoacylglycerols	0–0.5
Unesterified fatty acids	0.6–6.0
Phospholipids	26–31
Sphingomyelin	22[a]
Phosphatidyl choline	36[a]
Phosphatidyl ethanolamine	27[a]
Phosphatidyl inositol	11[a]
Phosphatidyl serine	2[a]

[a]Concentration expressed as a percentage of total phospholipids in milk.
Data compiled from Keenan & Mather (2002).

Xanthine dehydrogenase/xanthine oxidoreductase (XDH/XO), which is an internally disposed constituent of the proteinaceous coat of the MLGM, is the most abundant protein with a known enzyme activity in the MLGM and represents ~20% of membrane-associated protein. XDH/XO is an iron- and molybdenum-containing oxidoreductase, which can exist as a dehydrogenase or an oxidase; the structure and properties of the oxidase form that predominates in milk were reviewed by Harrison (2006). XDH/XO, a homodimer with a monomeric mass of ~147 kDa, plays a key role in the terminal steps of the purine metabolism, catalysing the oxidation of hypoxanthine to xanthine and uric acid. In milk, XDH/XO may play a role by providing hydrogen peroxide for the antimicrobial hydrogen peroxide–lactoperoxidase–thiocyanate system in milk.

Mucin 1 (*MUC-1*) is a heavily glycosylated (~50% carbohydrate) protein that displays allelic polymorphism and has a mass of ~56 kDa. MUC-1 occurs in milk at a concentration of ~40 mg L^{-1} and is incorporated into the lipid globules during the contact of the lipid droplet with the plasma membrane. Its biological function is unclear but, since MUC-1 is a transmembrane protein with an externally oriented N-terminal and appears to be present in filamentous structures extending up to 1 μm from the membrane, it may provide the membrane with steric stabilisation and protect it against physical damage and invasive microorganisms (Mather, 2000).

Periodic acid Schiff 6/7 (PAS 6/7) is an externally disposed, extrinsic MLGM protein; it appears as a doublet with a molecular mass in the range of 48 to 54 kDa on electrophoretic separation on the basis of molecular mass; this rather broad size range is due primarily to post-translational modification, particularly glycosylation. The specific functions of PAS 6/7 in milk lipid globule secretion or lactation are unknown (Mather, 2000).

Periodic acid Schiff III (PAS III) is a rather poorly characterised glycoprotein with a molecular mass of ~100 kDa; it is located on the apical surfaces of the epithelial cells in the mammary gland.

Cluster of differentiation 36 (CD 36) has a molecular mass of ~77 kDa and is heavily glycosylated; carbohydrates, excluding sialic acids, account for ~24% of its mass. It is an integral protein of the MLGM and remains associated with the membrane upon destabilisation of the globules. CD36 is a transmembrane protein with short N- and C-terminal segments internally disposed and a large hairpin loop disposed externally (Mather, 2000).

Adipophilin (*ADPH*) was recognised as a constituent of the MLGM only recently, because its molecular mass (~52 kDa) overlaps with that of PAS 6/7, and it has a relatively low solubility using conventional methods for the preparation of samples for electrophoresis. ADPH is not glycosylated, but may be acetylated with long-chain fatty acids, and may play a role in the secretion of MLG (Mather, 2000).

Fatty acid binding protein (*FABP*) has a molecular mass of ~13 kDa, and is the smallest of the major proteins of the MLGM; FABP is not glycosylated, but may be modified posttranslationally. Within the mammary epithelial cells, FABP may serve as a transporter of fatty acids, but its role in milk lipid globule formation and secretion is unknown.

1.5.5 *Enzymes of the milk lipid globule membrane*

The MLGM is a significant source of indigenous milk enzymes, although, except for XDH/XO, whose concentrations are too low to be considered as major constituents.

Table 1.9 Enzymes found in the milk lipid globule membrane.

Xanthine oxidoreductase	Alkaline phosphatase	β-Galactosidase
Lipoamide dehydrogenase	Acid phosphatase	Plasmin
NADPH oxidase	Phosphatic acid phosphatase	Inorganic pyrophosphatase
NADP oxidase	5′-Nucleotidase	Adenosine triphosphatase
Sulphydryl oxidase	Glucose-6-phosphatase	Nucleotide pyrophosphatase
Catalase	Phosphodiesterase I	Aldolase
γ-Glutamyl transpeptidase	Ribonuclease I	Acetyl-coenzyme-A carboxylase
Galactosyl transferase	UDP-glycosyl hydrolases	
Choline esterase	β-Glucosidase	

A summary of enzymatic activities found in the MLGM is given in Table 1.9. More than half of the enzymatic activities in the MLGM are hydrolases, with oxidoreductases and transferases also representing major proportions. Biological roles of enzymes of the MLGM remain largely unstudied, and studies on their functional significance have focussed largely on effects on the processing characteristics and organoleptic properties of milk and dairy products (Keenan & Mather, 2006).

1.6 Physicochemical stability of milk lipid globules

As will be described in more detail in the later chapters of this book, the fact that milk lipids are present primarily in the form of globules has implications for many properties of dairy products. Hence, the physicochemical stability of MLG is of major importance to the dairy industry. An excellent and unrivalled overview of this area was provided by Mulder & Walstra (1974); the more recent reviews of Walstra (1995) and Huppertz & Kelly (2006) update knowledge in this area. This section will provide a summary of the work reviewed in the aforementioned publications.

1.6.1 *Size distribution of milk lipid globules*

As mentioned previously, the lipids in milk are present predominantly in the form of globules, which in cow's milk range from ~0.2 to 15 μm in diameter. Cow's milk contains $>10^{10}$ lipid globules mL^{-1}, of which ~80% have a diameter <1 μm; however, these small globules comprise only ~3% of the total mass of the lipids in milk. The majority (>90%) of milk lipids are in globules with a diameter in the range 1 to 10 μm, with a small proportion of globules having a diameter >10 μm (Walstra, 1969, 1995).

The most common parameters used to express average globule size are derived from the so-called 'moments' of the particle size distribution, which are particularly useful auxiliary parameters. The *n*th moment of a particle size distribution is given by

$$S_n = \sum d_i^n N_i$$

Table 1.10 Common parameters used to express average milk lipid globule size.

Name	Abbreviation	Calculation	Average value for cow's milk (μm)
Number mean diameter	d_n or $d_{1,0}$	S_1/S_0	0.81[a]
Volume mean diameter	d_v or $d_{3,0}$	$(S_3/S_1)^{1/3}$	1.8[a]
Volume surface-weighted mean diameter	d_{vs} or $d_{3,2}$	S_3/S_2	3.3[a]
Volume moment-weighted mean diameter	d_{vm} or $d_{4,3}$	S_4/S_3	3.5[b]

[a]Data compiled from Walstra (1969).
[b]Data compiled from Huppertz *et al*. (2003).

where N_i and d_i are the number and diameter of the particles in size class i (Walstra, 2003). By calculating the various moments of the particle size distribution, usually S_1, S_2, S_3 and S_4, a number of parameters representing average particle size can be derived, as summarised in Table 1.10. The size of MLG can be determined by a variety of methods, including dynamic or static light scattering, Coulter counting, electroacoustics, ultrasonic spectroscopy and light or electron microscopy.

1.6.2 *Colloidal stability of milk lipid globules*

Colloidal interactions form the basis of the stability or instability of the MLG emulsion and will, for instance, govern whether the droplets remain as discrete entities or aggregate. For MLG, colloidal stability is governed by a balance between attractive forces (van der Waals attractions) and repulsive forces (electrostatic and steric repulsions) (Walstra, 1995; Huppertz & Kelly, 2006). Electrostatic repulsion occurs between molecules with a permanent electrical charge. For MLGs, repulsion arises from the net negative charge on the globule membrane. However, even when electrostatic repulsion is minimised, MLGs remain as discrete entities, indicating that considerable stability is derived from steric repulsion. In the case of MFGs, such steric stabilisation is provided by various glycoproteins of the MLGM (Section 1.5.4); hydrolysis of these proteins, for example, by papain, causes aggregation of MFGs (Shimizu *et al*., 1980).

All food emulsions, including those containing MFGs, are physically unstable over time. Such instability may be evident as gravitational separation or droplet aggregation, as outlined in Figure 1.8. Gravity separation of MLGs is a result of a density difference between the globules and the milk plasma and may occur under the influence of a gravitational or centrifugal force. As MLGs have a lower density than milk plasma, the predominant type of gravitational separation of MLGs is creaming, which is described in Section 1.6.3. Sedimentation of MLGs occurs only under extreme circumstances, for example, when the protein load on the globule membrane is sufficiently high to increase the density of the globule above that of the milk plasma. Aggregation of droplets occurs when they stay together for a time longer than can be accounted for in the absence of colloidal interactions. Flocculation implies the aggregation of droplets to give three-dimensional structures, but is rarely observed in MLGs. Coalescence is the process by which two or more droplets merge to form a larger

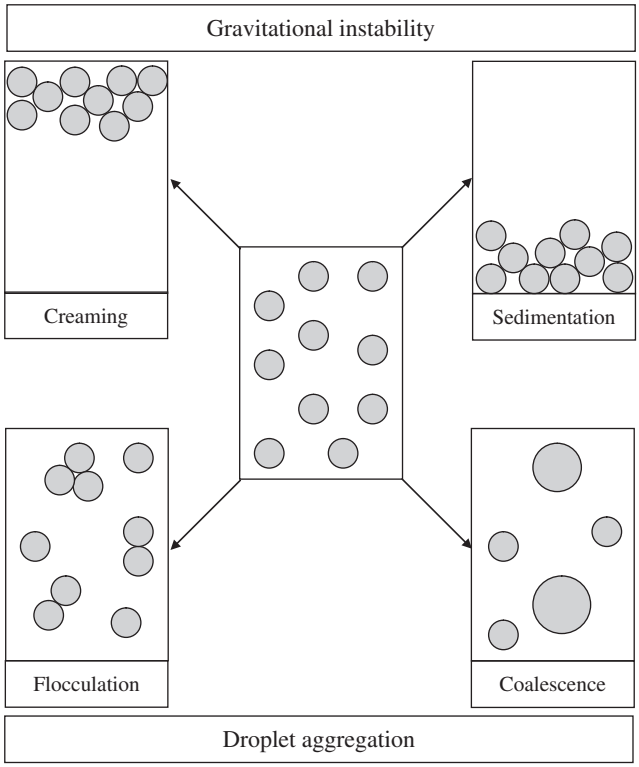


Fig. 1.8 Schematic representation of types of physical instability of emulsions.

droplet; it may be partial when anisometrically shaped conglomerates are formed because true coalescence is prevented. True and partial coalescence are described in Section 1.6.4.

1.6.3 *Creaming of milk lipid globules*

Creaming of MLGs implies the rise under gravitational or centrifugal force due to a difference in density between the globules and the milk plasma. For perfectly spherical globules, the rate of rise of globules, v, is given by Stokes' Law:

$$v = a\frac{(\rho_p - \rho_l) \cdot d^2}{18 \cdot \eta_p}$$

where d is the diameter of the globules, ρ and η are the density and viscosity, respectively, the subscripts p and l refer to the density of the plasma and lipid phase of milk, respectively, and a is the acceleration, which is 9.8 m s^{-2} for gravitational separation. For centrifugal separation, $a = R\omega^2$, where R is the centrifugal radius and ω is the angular velocity, which is equal to $2\pi n/60$, where n is the number of revolutions per minute (rpm). Centrifugal creaming of milk forms the basis of industrial separation of the cream and skimmed milk phases of milk and is described in Chapter 4.

Considerable creaming of cow's milk occurs under quiescent conditions; in fact, when raw, unhomogenised milk is stored at refrigeration temperature, the rate of rise of MFGs is much higher than can be accounted for by Stokes' Law for individual MLGs. This is due to the fact that the globules in such milk rise as large clusters, up to 1 mm in diameter, which are formed as a result of cold agglutination of MLG (Dunkley & Sommer, 1944). Cold agglutination of MLGs requires three components, that is, MLGs, immunoglobulin M (IgM) and lipoproteins present in the milk plasma; the latter fraction is often referred to as the *skimmed milk membrane* (*SMM*). On cold agglutination, IgM interacts with both the globules and SMM, whereas the latter cannot interact with each other (Euber & Brunner, 1984). Heating milk at a temperature $\sim 62^{\circ}$C impairs cold agglutination due to the denaturation of IgM, a heat-labile component, whereas homogenisation disrupts the SMM, a homogenisation-labile component, and thereby impairs cold agglutination (Walstra, 1995; Huppertz & Kelly, 2006). Cold agglutination is almost unique to bovine milk; it does not occur, or if it does, to a limited extent, in sheep, goat, buffalo and camel milk (Huppertz & Kelly, 2006).

1.6.4 *Coalescence of milk lipid globules*

As mentioned in Section 1.6.2, coalescence is the process whereby lipid globules merge to form a single, larger globule. This process stabilises the emulsion thermodynamically, as it reduces the contact area between the globule membrane and the milk plasma. Although the general process of coalescence is not fully understood, it is clear that coalescence can be induced by collisions and prolonged contact of the lipid globules (Walstra, 2003). Coalescence of MLGs, which can be induced by the enzymatic removal of the polar head of phospholipids in the MLGM, is of limited importance in milk and dairy products (Shimizu *et al*., 1980).

Partial coalescence is of far greater importance for milk and dairy products, particularly in the preparation of ice cream, whipped cream (Chapter 4) and butter (Chapter 5). Partial coalescence occurs when two lipid globules, in which the lipids are partially crystalline, come into contact. The aggregate will partially retain the shape of the individual globules, because the presence of a solid lipid phase prevents complete merger. Partial coalescence is enhanced by applying a velocity gradient, increasing the lipid content, carefully manipulating the concentration of solid lipids and minimising the repulsion provided by the globule membrane (Walstra *et al*., 1999, 2006). Partial coalescence plays a major role in the textural defect 'bitty' or 'broken' cream, which is highlighted by the presence of large cream particles floating on top of milk or cream. Partial coalescence also plays a major role in re-bodying, a phenomenon whereby cooled cream, when warmed to $\sim 30^{\circ}$C and subsequently recooled, becomes extremely viscous or even solid-like. These textural defects are described in more detail in Chapter 4.

1.6.5 *Homogenisation and properties of homogenised milk lipid globules*

As outlined in Section 1.6.3, MLGs cream readily, which is an undesirable feature of liquid milk products. From Stokes' Law, it is apparent that the rate of rise of MLGs can be reduced by a number of measures, of which a reduction in size is the most effective. For this purpose,

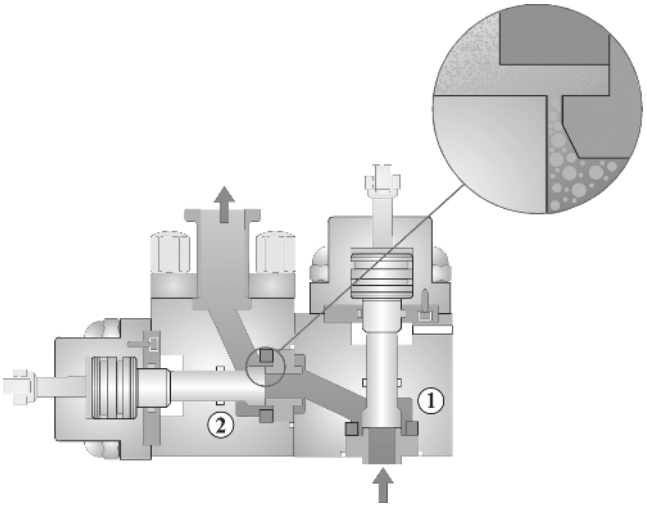


Fig. 1.9 Cross-sectional view of a homogeniser. Reproduced by permission of Tetra Pak, Lund, Sweden.

a process called homogenisation has been commonly applied in the dairy industry for a century. Homogenisation involves forcing milk through a small orifice at high pressure; the principle of operation is shown in Figure 1.9. The effectiveness of a homogeniser in reducing particle size depends on a number of factors, including homogenisation pressure, valve geometry and the number of passes through the homogeniser (Mulder & Walstra, 1974). The relationship between homogenisation pressure (P_h, in MPa) and average particle size is given by

$$\log d_{3,2} = k - 0.6 \cdot \log P_h$$

where k is a constant which generally varies between -2 and -2.5 (Walstra, 1975). Conventionally, a pressure in the range 10 to 20 MPa (100–200 bar or 14 500–29 000 psi) is used. In recent years, several so-called high-pressure homogenisers have been developed, which are capable of reaching pressures up to 400 MPa. The influence of both conventional and high-pressure homogenisation on the size distribution of MLGs is shown in Figure 1.10. Homogenisation is most effective when the milk lipids are in a liquid state, so prewarming the milk to $>40^{\circ}$C prior to homogenisation is required.

During homogenisation, MFGs are deformed and disruption occurs if the force of deformation is larger than the resistance to deformation, which depends on the Laplace pressure in the globule and the difference in viscosity between the plasma and lipid phases. Following disruption of the lipid globules in the homogenising valve, the surface of the lipid globules far exceeds that which the amount of original globule membrane material can cover, so adsorption of casein micelles and fragments thereof, and also, at high temperature, of some whey proteins, on the lipid-milk plasma interface occurs (Huppertz & Kelly, 2006). Freshly homogenised MLGs are susceptible to clustering if the globule surface is not covered sufficiently rapidly and, as a result, share casein micelles on their interface. The formation of homogenisation clusters is prevented by the application of a second homogenisation stage,

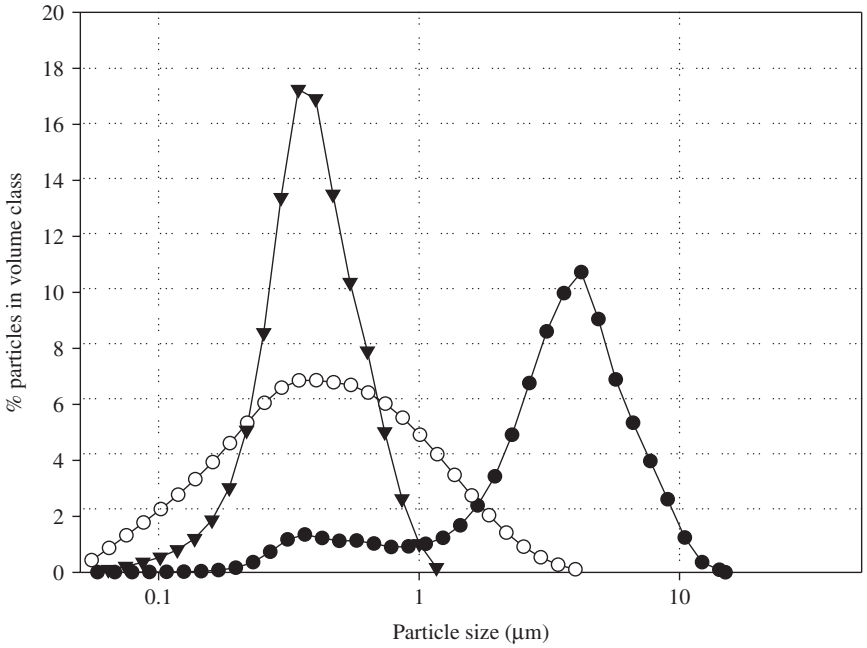


Fig. 1.10 Particle size distribution in unhomogenised milk (●) or in milk homogenised using a conventional homogeniser at 20 MPa (○) or using a high-pressure homogeniser at 200 MPa (▼). Data from T. Huppertz.

at a considerably lower pressure than the first stage (Kiesner *et al*., 1997). Homogenisation clusters are particularly evident in products containing a high level of lipids, for example, homogenised cream, where the casein:lipid ratio is too low to provide sufficient surface coverage following homogenisation.

As a result of the altered composition of the lipid globule membrane, homogenised lipid globules behave differently than natural globules. Due to their coverage by caseins, the homogenised lipid globules behave like casein micelles, which can be beneficial in the case of yoghurt manufacture; however, the rennet-induced coagulation of milk and the stability of milk against heat-induced coagulation are impaired by homogenisation (Walstra, 1995; Huppertz & Kelly, 2006).

1.6.6 *Temperature-induced changes in milk lipid globules*

The MLGM is influenced considerably by temperature. Cooling of milk induces the transfer of phospholipids from the membrane to the milk plasma. Freezing and subsequent thawing of milk, and particularly cream, can result in the clumping of the lipid globules. Heat treatment of milk increases the amount of protein associated with the MLGM; proteins associated with the membrane as a result of heat treatment are mainly whey proteins, which presumably associate with membrane proteins via sulphydryl-disulphide interchange reactions. Heat treatment can also reduce the level of triacylglycerols in the MLGM, but the influence on the phospholipid content is unclear, with both heat-induced increases and decreases being reported (Mulder & Walstra, 1974; Walstra, 1995; Huppertz & Kelly, 2006).

1.7 Crystallisation and melting of milk triacylglycerols

The crystallisation behaviour of the triacylglycerols greatly affects the consistency of high-fat dairy products (e.g. butter), as well as the occurrence and rate of partial coalescence of MLGs; hence, knowledge of the crystallisation behaviour of the triacylglycerols of milk is very important for achieving and maintaining the desired product characteristics. Crystallisation of triacylglycerols is a complicated subject and the extremely wide and variable composition of milk triacylglycerols adds further complications. In the following section, a brief description of crystallisation of milk triacylglycerols and some factors affecting it are presented. For more detailed information, the reader is referred to the excellent reviews by Mulder & Walstra (1974), Walstra *et al*. (1995) and Wright & Marangoni (2006).

As milk fat is composed of hundreds of different triacylglycerols, it has a very broad melting range, rather than a discrete melting temperature. Milk fat is not completely solid until it reaches a temperature below -40°C, and must be warmed to $+40^{\circ}$C to ensure complete melting; the solid fat content in the range $0-40^{\circ}$C is of industrial significance and is shown as a function of temperature in Figure 1.11 for a typical milk fat.

For crystallisation of fat to occur, nucleation is required, which occurs when the molecules are 'supercooled', that is, cooled to a temperature below their melting temperature. Supercooling is the thermodynamic driving force that initiates crystallisation. On supercooling, triacylglycerols aggregate continuously into very small clusters, most of which redissolve rapidly. Only when a certain size is reached does the cluster become stable and can it start to act as a nucleus for crystallisation. The minimum size required for cluster stability decreases

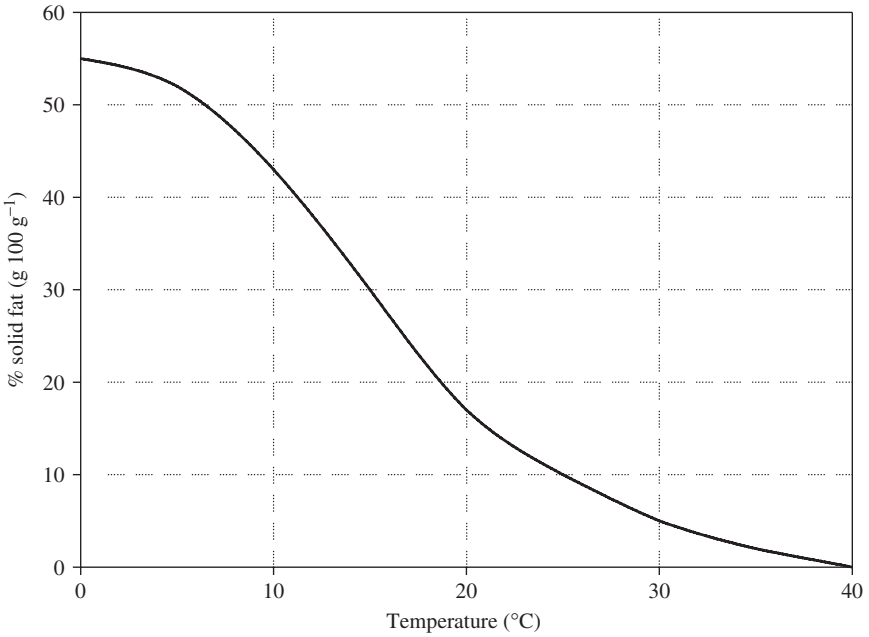


Fig. 1.11 Solid fat content of typical milk fat as a function of temperature. Redrawn using the data of Wright & Marangino (2006).

with temperature. Three types of nucleation can generally occur in fats (Walstra *et al.,* 1995; Wright & Marangoni, 2006):

- *Primary homogeneous nucleation* occurs in the absence of foreign materials and interfaces. This process generally requires very deep supercooling and is very rare in milk fat because sub-zero temperatures are required for this process to occur.
- *Primary heterogeneous nucleation* is much more common for milk fat and is initiated at the surface of catalytic impurities. The degree of primary heterogeneous nucleation decreases with decreasing temperature. For milk fat, micelles formed by monoacylglycerides may act as catalytic impurities.
- *Secondary nucleation* occurs at the interface of fat crystals that have formed during cooling and is also important in milk fat.

Following nucleation, growth of crystals can occur to a degree which depends on the degree of supersaturation, the rate of molecular diffusion to the crystal surface and the time required for triacylglycerols to fit into the growing crystal lattice. Crystals of milk fat can occur in three polymorphic modifications, designated the α, β′ and β forms. The α crystals have the simplest and least densely packed structure and are often formed first on rapid cooling; subsequently, the metastable α crystals may undergo molecular rearrangements to form the more thermodynamically stable β′ and ultimately β forms, as depicted in Figure 1.12; *vice versa* transitions, that is from β to β′ to α do not occur. In milk fat, the majority of fat crystals remain in the β′ form, even after prolonged storage (Wright & Marangoni, 2006).

In a complex mixture of triacylglycerols, like milk fat, impure crystals are formed. Milk fat may also form mixed or compound crystals, which contain two or more molecular species; the probability of these compound crystals being formed is greater when the compounds are more alike. Mixed crystals are formed most readily in the α form, where the packing density is not too dense and there is some conformational freedom to fit different molecules into the crystal lattice. Compositional variation is considerably more restricted in the β′ and, particularly, in the β crystal form (Walstra *et al*., 1995). The presence of mixed crystals has important consequences for the influence of temperature on the solid fat phase (Mulder & Walstra, 1974; Walstra *et al.,* 1995):

- The melting range of the fat is narrower than it would be if the crystals contained only one molecular species.

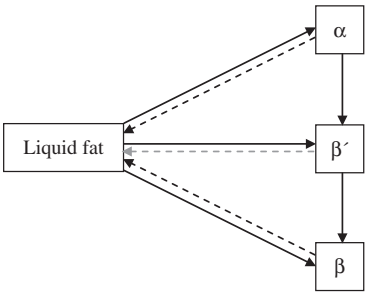


Fig. 1.12 Polymorphic transitions in fats.

- The temperature at which most of the fat melts depends on the temperature at which crystallisation occurred.
- Stepwise or slow cooling produces less crystalline fat than rapid cooling.
- Precooling to a lower temperature followed by bringing the fat to the final temperature results in greater crystallisation than direct cooling to the final temperature.

From the above, it is apparent that whether a quantity of milk fat is present as a continuous mass (e.g. anhydrous milk fat (AMF) or butter oil) or in numerous small globules (e.g. as in milk or cream) has a considerable influence on its crystallisation behaviour. Some reasons why crystallisation of fat in globules may differ from that in bulk milk fat are as follows (Mulder & Walstra, 1974; Huppertz & Kelly, 2006):

- Because of the lower thermal conductivity of bulk fat and the fact that bulk fat cannot be agitated efficiently, heat dissipation in bulk fat is considerably slower than in milk or cream.
- Not all lipid globules may contain the catalytic impurities required to initiate heterogeneous nucleation, so that nuclei would have to form spontaneously in those globules.
- The surface layer of the lipid globule may act as a catalytic impurity, for example, when it contains, mono- or diglycerides with long-chain fatty acid residues.
- The composition of bulk fat is uniform, but there are inter-globular differences in milk lipid composition, resulting in differences in crystallisation behaviour.

1.8 Conclusions

Many desirable organoleptic attributes of dairy products are due to the lipids they contain. As a result, milk lipids have always been valued highly and have been the subject of scientific interest for more than a century. As summarised in this chapter, extensive research has provided considerable insight into the lipids of milk. The >400 different fatty acids in milk, which are derived from the feed as well as *de novo* synthesis, are esterified into tri-, di- and monoacylglycerols and phospholipids and exist in emulsified state, that is, in MLG, which are surrounded by a membrane. The properties and stability of the MLGs are of crucial importance for many dairy products, as described in the later chapters of this book. To optimise the functionality and value of milk lipids, the fundamentals described in this chapter need to be considered carefully and explored further. The great English physicist Michael Faraday (1791–1867) is reported to have said 'Nothing is too wonderful to be true if it be consistent with the laws of nature, and in such things as these, experiment is the best test of such consistency' and while he did not refer to milk lipids, it is undoubtedly true that pursuit of fundamental knowledge in this area can yield surprising and fascinating new insights that will increase further the value of milk lipids.

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