Physical Chemistry of Milk Fat Globules

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5.1 Introduction

The presence of fat globules in milk was first reported by Van Leeuwenhoek in 1674, based on microscopic analysis of milk placed in a fine capillary tube; since then, the physical and colloidal properties of milk fat globules and their size distribution have been the subject of considerable study. These properties of milk fat globules are responsible for some of the properties and phenomena observed in liquid dairy products, e.g. the colour and creaming of milk, and are integral to the manufacture and characteristics of many dairy products, e.g. butter and ice cream. Furthermore, the properties of milk fat globules are also influenced by enzymatic processes, such as lipolysis, with implications for processes such as the ripening of cream and the flavour of some cheese varieties. Finally, milk fat globules can be affected greatly by processes applied to the milk, particularly homogenization, which has significant implications not only for the properties of milk fat globules but also of casein micelles in milk.

This chapter describes important aspects of the physical and colloidal chemistry of milk fat globules, and in particular recent research in the area, which underpins many of the phenomena described in other chapters of this book. The relevant aspects of processes that affect the stability of fat globules, including storage, homogenization and heating, and the resulting interactions with other milk constituents, including caseins and whey proteins, will be reviewed also.

5.2 The Nature and Size Distribution of Milk Fat Globules

Fat in milk is present predominantly in spherical droplets ranging from <0.2 to >15 μm in diameter; bovine milk typically contains $>10^{10}$ globules per mL. The composition of the fat in the milk fat in globules has been discussed in detail in Chapters 1 and 2. Fat globules are dispersed in the continuous phase of milk plasma, which contains casein micelles, serum proteins, sugars and minerals, and can be considered both a colloidal suspension and an oil-in-water emulsion. Fat globules in milk are naturally emulsified by a complex layer of surface material, the milk fat

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Table 5.1. Estimated average composition of milk fat globule membranes

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

Adapted from Walstra *et al.* (1999).

globule membrane (MFGM), which accounts for 2–6% of the mass of the fat globules (Keenan and Mather 2002) and maintains the integrity of the lipid droplets and helps to protect them from destabilization (Walstra *et al.* 1999). The composition of the MFGM (Table 5.1) is closer to that of a cell membrane, from which it is largely derived, than to either milk fat or milk serum. Widely differing compositions of the MFGM, particularly in terms of triglyceride profile, have been reported. Several enzymes are found in the MFGM, including alkaline phosphatase and xanthine oxidoreductase, which make up a significant portion of the membrane protein content, as well as monoglycerides and free fatty acids. For a more detailed description of the secretion of milk fat globules and the exact structure of the MFGM, the reader is referred to Chapter 4.

One of the most important properties of fat globules in milk is their size, both in terms of mean (average) size, but also the range or distribution of sizes and the effects of processes and treatments thereon. A review of the early studies on milk fat globule size was published by Campbell (1932). The size distribution of milk fat globules may vary greatly with the analytical method used, giving a certain degree of unreliability to results obtained using some older methods (Walstra *et al.* 1969).

Various microscopic techniques such as optical microscopy (Tatar *et al.* 2015), fluorescence microscopy (Martini *et al.* 2006; Evers *et al.* 2008), confocal laser scanning microscopy (Ong *et al.* 2010), transmission electron microscopy (Goff *et al.* 1987), scanning electron microscopy (Bermúdez-Aguirre *et al.* 2008) and holographic video microscopy (Cheong *et al.* 2009) can be used to measure milk fat globule size, although these techniques have drawbacks including processing time required to get sufficient data for determination of size distribution (Truong *et al.* 2016). The advantage of microscopic techniques, however, is that they allow direct visualization of fat globule shape, distribution and microstructure of milk fat globules, MFGM and fat crystals.

Methods such as dynamic light-scattering (Robin and Paquin 1991; Dalgleish and Hallett 1995), small-angle laser light scattering (Muir *et al.* 1991; Michalski *et al.* 2001a), Coulter counting (Hillbrick *et al.* 1998), ultrasound (Miles *et al.* 1990) and electroacoustics (Wade *et al.* 1996; Wade and Beattie 1997) provide more accurate and reproducible results. Laser light scattering methods, where particles scatter light from one or two laser beams with an angular pattern directly related to their size, are now favoured for measuring fat globule size. Many of these measurement techniques generate complex primary data, which must be processed using specific algorithms or programmes to yield useful data for milk fat globule size.

Control of interference by other milk constituents with measurements is key to measurement of milk fat globule sizes; for example, dissociation of casein micelles by calcium-chelating agents, such as trisodium citrate or ethylenediamine tetra-acetic acid (EDTA), may be used to avoid interference by the micelles in particle size measurement, while clusters of fat globules can be disrupted by adding a low level of anionic sodium dodecyl sulphate (SDS) or the non-ionic surfactant Tween 20 can be used in combination with EDTA (McCrae and Lepoetre 1996). SDS and Tween 20 act by removing absorbed materials from the oil–water interface and disrupt the agglomerates of fat globules (De Feijter *et al.* 1987; Thiebaud *et al.* 2003) and maintaining the globules in a dispersed form, enabling size measurement of individual globules.

Table 5.2. Parameters describing the milk fat globule size distribution in unhomogenized bovine milk

	Range (μm)	Average (μm)
Number mean diameter: $d_n = d_{1,0} = S_1/S_0$	0.67–1.0	0.81
Volume mean diameter: $d_v = d_{3,0} = (S_3/S_0)^{1/3}$	1.5–2.1	1.8
Volume surface-weighted mean diameter: $d_{vs} = d_{3,2} = S_3/S_2$	2.5–4.6	3.34
Volume moment-weighted mean diameter $d_{vm} = d_{4,3} = S_4/S_3$		3.53[1]

Values from Walstra (1969a).

Table 5.3. Fat globule size in milk from various species

Species	D_{vs} (μm)	Reference
Cow	3.9	Walstra (1969a)
	4.0	Rüegg and Blanc (1981)
	~3.5	Van Boekel and Folkerts (1991)
	5.32	Mehaia (1995)
	3.51	Attaie and Richter (2000)
Reindeer	3.95	Uniacke-Lowe and Fox (2011)
Goat	4.89	Mehaia (1995)
	2.76	Attaie and Richter (2000)
	3.20	El-Zeini (2006)
	2.22	Tatar *et al.* (2015)
Camel	4.40	Farah and Rüegg (1991)
	4.40	Mehaia (1995)
	2.99	El-Zeini (2006)
Human milk colostrum	1.74	Rüegg and Blanc (1981)
	8.9	Michalski *et al.* (2005)
	3.51	Zou *et al.* (2012)
Human milk transitional	1.84	Rüegg and Blanc (1981)
	2.8	Michalski *et al.* (2005)
	3.14	Zou *et al.* (2012)
Human milk mature	4.10	Rüegg and Blanc (1981)
	4.00	Michalski *et al.* (2005)
	3.25	Zou *et al.* (2012)
Mare	1.05	Uniacke-Lowe (2011)
	1.30	Devle *et al.* (2012)
Donkey	1.92	Martini *et al.* (2014)
Ewe	4.95	Mehaia (1995)
	5.00	Gervilla *et al.* (2001)
	3.76	El-Zeini (2006)
Yak	6.09	Luo *et al.* (2018)
Buffalo	8.7	El-Zeini (2006)
	5.0	Ménard *et al.* (2010) and Ahmad *et al.* (2013)

A plot of the number distribution, i.e. the number of globules per unit volume, N, in a certain size class, divided by the width of the size class, Δd, as a function of size, d, shows three sub-distributions (Walstra 1969a, 1995): a subclass of 'small particles', comprising ~80% of the number of particles but only ~3% of the mass of fat, the main fractions, comprising ~95% of fat, and a subclass of large globules, comprising ~2% of the fat. Besides the number distribution, distributions of mass, volume or surface area can also be calculated by multiplying the number frequency by mass, volume or surface area, respectively, for each size class. Plotting volume frequency versus particle diameter is the most common method of presentation of globule size data (Walstra 2003).

Several different parameters can be used to express the mean size of the milk fat globules. These parameters are derived from the so-called moments of the size distribution function; the n^{th} moment of the distribution function is equal to:

$$S_n = d_i^n N_i$$

where N_i is the number particles present and d_i is the particle diameter in size class i. These moments have no physical meaning but are particularly useful as auxiliary parameters in the calculation of characteristic numbers of size distribution. Some common parameters characterizing mean globule size are given in Table 5.2, as are means and ranges of such values for bovine milk. The specific surface area of the fat globules, A, can be derived from the volume surface-weighted mean diameter:

$$A = 6\varphi / d_{3,2}$$

where φ is the volume fraction of milk fat. A typical mean value for A is ~2.2 $m^2\ g^{-1}$ fat in unhomogenized bovine milk (range 1.9–2.5 $m^2\ g^{-1}$ fat; Walstra 1969b).

As illustrated in Table 5.3, considerable interspecies differences in milk fat globule size have been reported. The smallest fat globules are found in equine milk (Uniacke-Lowe 2011) and the largest are found in buffalo milk (El-Zeini 2006). Compared to bovine milk (4.0 μm, Walstra 1969a

and Rüegg and Blanc 1981), $d_{3,2}$ values are lower in caprine (3.2 μm, El-Zeini 2006; 2.76 μm, Attaie and Richter 2000; 2.22 μm Tatar *et al.* 2015), equine milk (1.05 μm, Uniacke-Lowe 2011; 1.3 μm, Devle *et al.* 2012) and asinine milk (1.92 μm, Martini *et al.* 2014) but are higher in ovine milk (4.95 μm, Mehaia 1995 and Gervilla *et al.* 2001), camel milk (4.4 μm, Mehaia 1995 and Farah and Rüegg 1991), yak milk (6.09 μm, Luo *et al.* 2018) and buffalo milk (8.7 μm, El-Zeini 2006). In contrast, $d_{3,2}$ values for mature human milk (4.0 μm, Rüegg and Blanc 1981 and Michalski *et al.* 2005) and reindeer milk (3.95 μm, Uniacke-Lowe and Fox 2011) are similar to those for bovine milk. The mean diameter of human milk fat globule is largest in colostrum, followed by mature milk and smallest in transitional milk (Rüegg and Blanc 1981; Michalski *et al.* 2005; Zou *et al.* 2012).

Milk fat globule size greatly impacts on the formation of milk fat clusters, as in creaming and cold agglutination (see Sections 5.7 and 5.8, respectively, below), which in turn affects the physical stability of milk and dairy products. In general, the smaller the fat globule, the more stable the milk is (Truong *et al.* 2016).

In bovine milk, the average milk fat globule size decreases with advancing lactation (Walstra 1969a) and is positively correlated with the fat content of the milk (Wiking *et al.* 2003) and daily fat yield (Wiking *et al.* 2004). For other species, a positive correlation between fat content and fat globule size has also been reported (El-Zeini 2006), with some exceptions. Equine milk has only 1.4% fat and small fat globules of ~1 μm (Uniacke-Lowe 2011). Similarly, asinine milk is low in fat (1.5%, Malacarne *et al.* 2002) with fat globules of ~1.9 μm (Martini *et al.* 2014). On the other hand, buffalo milk has ~7.5% fat (Ménard *et al.* 2010), and this can be as high as 15% fat depending on lactation stage (Abd El-Salam and El-Shibiny 2011; Ahmad 2013), with fat globules of up to 8.7 μm in diameter (El-Zeini 2006). Reindeer milk has 21–22% fat at peak lactation (Gjøstein *et al.* 2004; Luick *et al.* 1974); but in this case, the fat globules are similar in size to those of bovine milk (Table 5.3). The reason for the formation of larger fat globules during the synthesis of milk fat is important and may be related to limitations in the production of MFGM when fat globules are enveloped during their secretion from the epithelial cells of the mammary gland (Wiking *et al.* 2004). Thus, the level of available MFGM may be a limiting factor in the formation of small fat globules in some high-fat content milks such as buffalo milk.

According to the Laplace equation, large globules have lower stability against rupture and a lower resistance to deformation and coalescence under mechanical pressure than the small globules (Walstra *et al.* 2006). Therefore, the larger fat globules in buffalo milk indicate that the globules are more prone to disruption during churning (Hammad 1993). Similarly, yak milk is reported to be suitable for making butter and ghee products due to its large fat globules (Luo *et al.* 2018). The Laplace pressure of buffalo milk is reported to be 0.8–1.6 kPa, compared to 1.1–2.2 kPa for bovine milk (Ménard *et al.* 2010).

Milk fat globule size can also be influenced by several treatments applied to milk. Homogenization, as discussed in Section 5.12, is a mechanical treatment which has long been applied by milk processors to reduce fat globule size and prevent creaming during storage of liquid milk. Van Boekel and Folkerts (1991) reported that batch heating or indirect ultra-high temperature (UHT) heating of milk at 90–150 °C did not influence d_{vs}, whereas direct UHT heating reduced d_{vs} progressively with increasing temperature. Treatment of milk or cream at a high hydrostatic pressure (up to 600 MPa) has little effect on milk fat globule size (Dumay *et al.* 1996; Gervilla *et al.* 2001; Huppertz *et al.* 2003).

5.3 Differences in the Composition of Milk Fat Globules

Walstra and Borggreve (1966) reported that in milk from a single milking of a single cow, considerable differences in refractive index existed between milk fat globules of similar diameter, indicating differences in the composition of the globules. Furthermore, the observation by Walstra (1967) that the fat in a small proportion of the fat globules in milk melts at a temperature considerably higher than the average melting

point (37 °C) also indicates compositional differences between fat globules.

It is now accepted that the composition (i.e. fatty acid composition of the triacylglycerides) of fat globules also varies with globule size. Timmen and Patton (1988) found less $C_{4:0}$ – $C_{10:0}$ and $C_{18:0}$ fatty acids and more $C_{18:1}$ in smaller than in larger fat globules. The fatty acid composition of globules also differs with season; the $C_{18:1}$ and $C_{18:2}$ acid content of milk obtained in winter increases with fat globule size, but a reverse effect is observed in spring milk; in winter, the content of $C_{14:0}$ and $C_{16:0}$ fatty acids decreased with fat globule size (Briard *et al.* 2003). In both spring and winter, there was significantly more $C_{14:0}$, $C_{16:1}$ and less $C_{18:0}$ fatty acids in small fat globules compared to large globules (Briard *et al.* 2003). Higher levels of $C_{18:1}$ and $C_{18:2}$ in small compared to large globules in spring milk can be explained partially by the fact that the fat globule membrane, which represents a larger proportion of the mass of smaller globules, contains a higher proportion of these fatty acids than bulk fat (Jensen and Nielsen 1996); however, the higher proportion of $C_{18:1}$ and $C_{18:2}$ in the membrane alone cannot fully explain compositional differences between globules of different size; thus, it may be assumed that their level in the fat core is also higher (Briard *et al.* 2003). Wiking *et al.* (2004) reported a positive correlation between average milk fat globule size in milk and the concentration of C_{16}, $C_{16:1}$, C_{18} and $C_{18:1}$ fatty acids.

5.4 Fat Crystals in Globules

Crystallization of fats (triglycerides) is a complex phenomenon, especially for milk fat, due to its very broad fatty acid composition (see Chapter 1). Principles of crystallization of milk fat have been reviewed extensively elsewhere (Mulder and Walstra 1974; Walstra *et al.* 1995; Chapters 7 and 8). Whether a quantity of milk fat is present as a continuous mass (e.g. anhydrous milk fat or butter oil) or in numerous small globules (e.g. as in milk or cream) has a considerable influence on its crystallization behaviour. The crystallization state affects many properties of the milk fat globules, e.g. their susceptibility to partial coalescence and their resistance against disruption. Some reasons why crystallization of fat in globules may differ from that in bulk milk fat are (Mulder and Walstra 1974):

1. Heat dissipation in bulk fat is considerably slower than in milk or cream; this is related to the lower thermal conductivity of bulk fat and, in particular, the fact that bulk fat cannot be agitated efficiently.
2. Not all fat globules contain the catalytic impurities required to start heterogeneous nucleation, so that nuclei would have to form spontaneously in those globules. Söderberg *et al.* (1989) observed that deeper supercooling was necessary to induce crystallization in milk fat globules than in bulk fat, whereas Lopez *et al.* (2002a) observed that, with decreasing globule size in cream, a deeper super-cooling was required for crystallization of milk fat inside the globules.
3. The surface layer of the fat globule may act as a catalytic impurity, e.g. when it contains mono- or diglycerides with long-chain fatty acid residues; however, there is still some uncertainty as to whether this process actually occurs (see Walstra 1995). Although concentric layers of apparently crystalline fat have been observed in electron micrographs of freeze-etched or freeze-fractured milk or cream samples (Buchheim 1970; Henson *et al.* 1971), these observations could not be confirmed using other microscopy techniques. Noda and Yamamoto (1994) reported that it is thermodynamically favourable for fat crystals to be located at the oil/water interface, rather than in the interior of the droplet, which may explain the presence of fat crystals at the membrane.
4. The composition of bulk fat is uniform, but differences from globule to globule are known to occur (see Section 5.3); consequently, considerable differences may occur in the final melting point of the fat between different globules. Shi *et al.* (2001) proposed that a higher ratio of higher-melting triglycerides to lower-melting ones in bulk milk fat was associated with faster crystallization rates.

The dispersed state has a considerable effect on fat crystal polymorphism. Lopez *et al.* (2000, 2001c) observed that fat crystallization in milk fat globules is more disordered than in bulk fat. On slow cooling, milk fat crystallizes in the α form in cream (Lopez *et al.* 2001a), whereas, in anhydrous milk fat, it crystallizes in the β′ form and then in the α form (Lopez *et al.* 2001b). Rapid cooling of cream or anhydrous milk fat from 60 to 4 °C led to the formation of α crystalline structures which transformed into β′ structures rapidly in anhydrous milk fat, and more slowly in cream. On prolonged storage, these crystal structures evolve further, leading to the co-existence of α, β′ and β structures (Lopez *et al.* 2002b). Furthermore, Lopez *et al.* (2002a) observed a greater disorder and smaller size of the fat crystals in milk fat globules with reduced fat globule size.

Crystallization of milk fat in globules is also influenced by exposure to high hydrostatic pressure. High pressure (HP) treatment at 100–500 MPa at 23 °C induces crystallization of milk fat within the globules, and crystallization proceeds further during storage at 23 °C (Buchheim and Abou El-Nour 1992; Buchheim *et al.* 1996). Acceleration of crystallization of milk fat by HP treatment is due to a shift of the solid/liquid transition temperature towards a higher value (Frede and Buchheim 2000). HP-induced crystallization of milk fat was strongly delayed by a reduction in fat globule size (Buchheim *et al.* 1996). These HP-induced changes in crystallization behaviour of globular milk fat may offer opportunities to overcome the necessity for super-cooling to obtain particular levels of crystalline fat.

5.5 Colloidal Interactions

Colloidal interactions form the basis of several of many of the properties of emulsions, as well as the changes observed in emulsions over time; such interactions govern whether droplets remain as separate entities or aggregate. In this section, a brief overview of the predominant colloidal interactions of importance for the stability of emulsions of milk fat globules is given.

The interactions between two emulsion droplets can be described in terms of the interaction energy, or inter-droplet pair potential $w(h)$, which is the energy required to bring two emulsion droplets from an infinite distance apart to a surface-to-surface separation difference, h (McClements 1999):

$$w(h) = w_{\text{attractive}}(h) + w_{\text{repulsive}}(h)$$

If attractive forces dominate at all separations, $w(h)$ is always positive, and the interaction energy, i.e. the free energy needed to bring two droplets from an infinite distance closer together, will be negative, and the droplets will tend to aggregate. Conversely, if repulsive forces dominate at all separations, and the positive interaction energy is several times larger than the average kinetic energy involved in the encounter of two particles by Brownian motion, droplets tend to remain as individual entities. In many cases, however, $w(h)$ is neither positive nor negative over the entire distance h.

The classical DLVO (Derjaguin-Landau-Verwey-Overbeek) theory (Derjaguin and Landau 1941; Verwey and Overbeek 1948) states that the stability of a colloidal suspension essentially depends on two independent interactions between colloidal particles: van der Waals attractions and electrostatic repulsion:

$$w(h) = w_{\text{van der Waals}}(h) + w_{\text{electrostatic}}(h)$$

Van der Waals forces are attractive forces which act between all molecules; they arise from the attraction between electrostatically or orientationally polarized molecules. Their strength decreases with droplet separation, increases with droplet size and depends on the physical properties of the droplets and the surrounding medium and on the thickness and composition of the absorbed emulsifier layer (Bergenståhl and Claesson 1997; Friberg 1997; McClements 1999).

Electrostatic interactions occur between molecules that contain a permanent electrical charge, such as ions and polar molecules. The approach of two identically charged surfaces leads to

increases in the counter-ion concentration between the surfaces, which generate a repulsive force as a result of increased osmotic pressure (Dickinson and Stainsby 1988; Bergenståhl and Claesson 1990, 1997).

The surface charge of milk fat globules cannot be measured directly, but the zeta potential (ζ), which approximates the potential at a certain distance from the particle surface, can be measured electrokinetically (Tuinier and De Kruif 2002). The ζ of milk fat globules can be defined as the potential at the shear layer, indicating the degree of globule surface coverage by plasma proteins (Michalski *et al.* 2002). The surface charge, as estimated by ζ, is ~ −13 to −14 mV for unhomogenized bovine milk fat globules (Jack and Dahle 1937; Payens 1963, 1964; Michalski *et al.* 2001b) and ~ −20 mV after homogenization (Wade and Beattie 1997; Michalski *et al.* 2001b). Dalgleish (1984) reported slightly lower values for ζ, i.e. −10 mV for unhomogenized and − 13 to −17 mV for homogenized milk fat globules. The overlap of electric double layers will cause a local increase in potential, implying that work must be performed to bring particles closer together.

Thus, according to the DLVO theory, aggregation of milk fat globules should occur if the van der Waals attraction is larger than the electrostatic repulsion. However, calculation of these forces for milk and application of the data to the DLVO theory results in a negative interaction energy at all distances (Walstra 1995), so that immediate aggregation of milk fat globules should be observed. Aggregation of fat globules, however, does not occur, even when electrostatic interactions are minimal. Thus, there must be a second repulsive force acting, i.e. steric repulsion; the DLVO theory may then be extended to:

$$w(h) = w_{\text{van der Waals}}(h) + w_{\text{electrostatic}}(h) + w_{\text{steric}}(h)$$

Repulsive steric forces are encountered when the outer segments of two polymer-covered surfaces begin to overlap. These interactions usually lead to a repulsive force due to the unfavourable reduction in entropy associated with confining the chains between surfaces (Tadros and Vincent 1983; Israelachvili 1992; Walstra 1996). In the case of milk fat globules, steric repulsion is provided by glycoproteins of the milk fat globule membrane, which have highly hydrophilic moieties protruding from the globule surface. Hydrolysis of these glycoproteins by papain causes aggregation of milk fat globules (Shimizu *et al.* 1980).

From a colloidal stability point of view, milk fat globules with high ζ potential are electrically stabilized, while globules with low ζ potentials tend to coagulate or flocculate (Zou *et al.* 2012). Human milk fat globules have a lower ζ potential (−7.8 mV, Michalski *et al.* 2001b; −7.6 mV, Lopez and Menard 2011 or −7.25 mV, Zou *et al.* 2012;) than the ζ potential of milk fat globules from other mammals such as equine (−10.3 mV, Uniacke-Lowe 2011), bovine (−13.5 mV, Michalski *et al.* 2001b), yak (−11.9 mV, Luo *et al.* 2018), buffalo (−11.0 mV, Ménard *et al.* 2010), and reindeer (−12.5 mV, Uniacke-Lowe and Fox 2011) milk fat globules. The ζ potentials for human colostrum, transitional and mature milk fat globules are −5.60, −6.72 and − 7.25 mV, respectively, indicating an increasing trend as lactation progresses (Zou *et al.* 2012).

The differences in ζ potential of human milk fat globules at different stages of lactation or from different species are largely due to differences in the composition of polar lipids and proteins in the MFGM, and minerals present in the aqueous environment. However, the low ζ potential of human milk fat globules may be of special benefit to the digestion and metabolism of human milk fat by the infant (Zou *et al.* 2012). The fat globules of reindeer milk, which are similar in size to those in bovine milk (Table 5.3), form an exceptionally stable emulsion and show little or no tendency to flocculate compared to those of bovine milk, which is attributed to the higher zeta potential of reindeer fat globules, reflecting significant differences in the electrical character of the surface layers of the fat globules of reindeer and bovine milk (Aikio *et al.* 2002).

5.6 Separation of Milk

Because milk fat globules have a lower density than milk plasma, they tend to rise under the influence of a gravitational force. For perfect spheres, the rate of rise, v, is given by Stokes' Law:

$$v = a\left(\rho_p - \rho_f\right)d^2 / 18\eta_p$$

where a is the acceleration due to the gravitational force, ρ_p is the mass density of the plasma, ρ_f is the mass density of the fat, d is the diameter of the fat globule and η_p is the viscosity of the plasma. For gravity creaming, $a = g \approx 9.8$ m s^{-2}. For creaming in a centrifugal field, $a = R^2\ \omega$, where R is the effective centrifugal radius, and ω is the angular velocity (= $2\pi n/60$, where n is the number of revolutions per minute).

To predict v correctly, several prerequisites must be met (Mulder and Walstra 1974; Walstra and Oortwijn 1975; Walstra 1995), most notably: (1) globules must be perfect and homogeneous spheres; (2) other particles in the plasma must be considerably smaller than the fat globules; (3) Brownian motion must be small compared to the rate of rise; (4) counter-flow of liquid due to globule movement must be negligible; and (5) mutual interaction between globules must be absent. Troy and Sharp (1928) found that in milk highly diluted with milk plasma, the rate of rise of individual milk fat globules, as well as roughly spherical clusters of milk fat globules, correlated well with Stokes' law. However, Walstra and Oortwijn (1975) observed that the rate of rise of fat globules in undiluted milk systems under the influence of gravity was lower than predicted by Stokes' law, in particular for milk of high fat content or containing small fat globules.

The creaming rate (defined as the proportion of the fat arriving in the cream layer per unit time) is proportional to the creaming parameter, H (Walstra and Oortwijn 1975):

$$H = S_5 / S_3 = \ N_i d_i^5 \ / \ \ N_i d_i^3$$

This parameter shows a linear relation to the creaming rate if the effect of aggregation of the globules is excluded (Rüegg and Blanc 1981); it can be seen that larger globules, in particular, affect H, and thus the creaming rate.

The presence of clusters of fat globules affects creaming considerably. Such clusters will rise faster than the individual globules because of their larger size. Clusters may be formed due to cold agglutination (see Section 5.8) or due to inefficient homogenization (i.e. formation of homogenization clusters, see Section 5.13). Also, small clusters of fat globules may be formed during sterilization of heat-evaporated milk at the onset of heat-induced coagulation (Schmidt *et al.* 1971).

The separation of milk can be significantly accelerated by application of a centrifugal force, which is the principle of separation (skimming) of milk in industrial practice; the design of a separator is depicted in Figure 5.1. The objective of centrifugal separation is to achieve the lowest possible fat content in the skimmed milk while removing the fat greatly (~tenfold) concentrated in a cream phase. With the exception of high-fat products, Stokes' law can be applied rather accurately to the rate of rise of milk fat globules in a centrifugal field. Centrifugal separation is more efficient at elevated temperatures, as the factor $(\rho_p - \rho_f)/\eta_p$ increases more than tenfold in a linear fashion over the temperature range 0–80 °C (Mulder and Walstra 1974). The fat content of the skimmed milk depends on the proportion of the fat in very small globules (e.g. <1 μm), which are the most difficult to separate, and the level of non-globular fat.

5.7 Physical Instability of Emulsions

The stability of an emulsion denotes its ability to resist changes in its properties over time, i.e. a higher emulsion stability implies slower change in emulsion properties. When considering the stability of an emulsion, it is of major importance to distinguish between thermodynamic stability and kinetic stability. Thermodynamics predict whether or not a process will occur, whereas kinetics predict the rate of the process,

Figure 5.1. Principle of operation of a centrifugal milk separator. (Reproduced with permission from *Dairy Processing Handbook*, Tetra Pak Processing Systems AB, Lund, Sweden, 1995).

if it does occur. All food emulsions are thermodynamically unstable and will break down if left long enough; thus, it is kinetic stability which is responsible for the occurrence of the different types of instability that are observed in food emulsions.

Instability of an emulsion may be physical or chemical in nature. Chemical instability, which results in an alteration in the chemical structure of the lipid molecules due to oxidation or hydrolysis (McClements 1999), will not be covered in this chapter; for more information, the reader is referred to Chapter 11. Physical instability results in an alteration in the spatial distribution or structural organization of the globules (i.e. the dispersed phase of the emulsion). The key mechanisms responsible for the physical instability of emulsions, as depicted in Figure 5.2, can be divided into two categories: gravitational separation and droplet aggregation.

Gravitational separation involves the movement of emulsion droplets due to the fact that they differ in density from the surrounding liquid. If the droplets have a lower density than the surrounding medium, they tend to move upwards, a process referred to as *creaming*. Conversely, droplets or particles that have a density higher than the surrounding medium tend to move downwards under the influence of a gravitational force, i.e. *sedimentation*.

Droplet aggregation is said to occur when droplets stay together for a time much longer than they would in the absence of colloidal interactions (Walstra 2003), i.e. than can be accounted

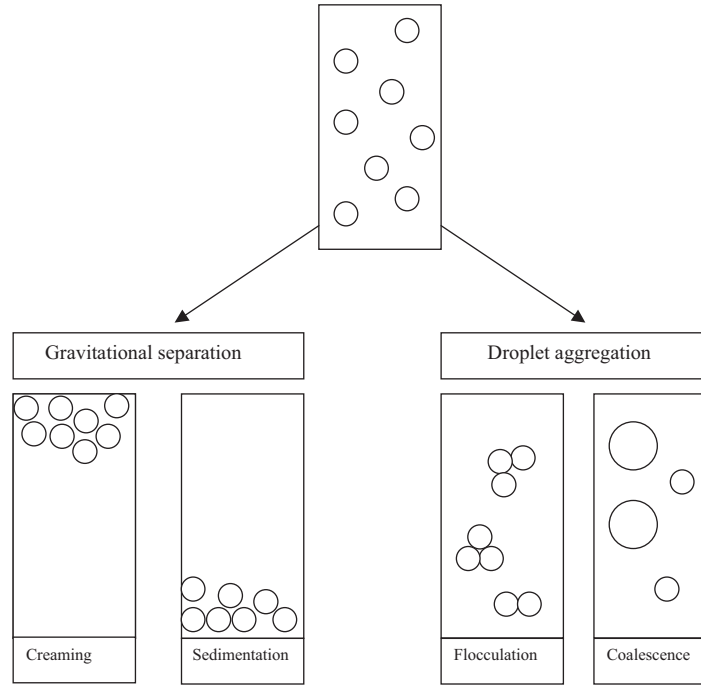

Figure 5.2. Schematic overview of types of instability of emulsions.

for by collisions due to Brownian motion. Mechanisms responsible for the physical instability of droplets through aggregation are flocculation, coalescence or partial coalescence.

- *Flocculation* of droplets is defined as the aggregation of droplets to give three-dimensional floccules, wherein the droplets remain as individual entities (Tadros and Vincent 1983). Flocculation can be distinguished from coagulation by the fact that the former denotes weak, reversible interactions, whereas the latter denotes strong, and often irreversible, interactions (Walstra 2003). Flocculation occurs as a result of collision; the extent of flocculation is determined by both the total number of droplet collisions per unit time per unit emulsion volume and the likelihood that an encounter between droplets will lead to aggregation. The most effective way to control the rate and extent of flocculation is by regulating the colloidal interactions between the droplets.
- *Depletion flocculation* of droplets can occur in samples containing different-sized colloids, wherein it is energetically favourable for the droplets to be surrounded by particles of the same size. This phenomenon is known to exist in systems containing both emulsion droplets and polymers and is known to occur for mixtures of milk and casein-stabilized emulsions (Ten Grotenhuis *et al.* 2003). Depletion flocculation becomes particularly important at small interparticle distances, i.e. when volume fractions of particles are high (e.g. >0.3).
- *Coalescence* is the process by which two or more fat globules merge to form one larger spherical fat globule through the rupture of the liquid film between emulsion droplets. It is the principal mechanism by which an emulsion moves towards its thermodynamically stable state, through a decrease in free energy as a result of the decrease in contact area between the oil and water phases (Tadros and Vincent 1983; McClements 1999; Walstra 1996, 2003). Coalescence of milk fat globules will be discussed in more detail in Section 5.9.
- *Partial coalescence* involves the formation of anisometrically shaped conglomerates of droplets due to the fact that true coalescence is prevented, e.g. because the globules contain a network of crystalline fat (Walstra 1996, 2003;

McClements 1999). The ultimate driving force behind partial coalescence is a decrease in interfacial free energy, although other processes are also involved (Walstra 2003). Partial coalescence of milk fat globules will be discussed in more detail in Section 5.9.

The primary form of destabilization relevant to bovine milk is creaming. The creaming of bovine milk has been the subject of research since, at least, the work of Babcock (1889). The relevant literature has been reviewed by Hammer (1916), Dunkley and Sommer (1944), Mulder and Walstra (1974), Euber and Brunner (1984), Brunner (1978), Walstra and Jenness (1984) and Keenan *et al.* (1988). However, the creaming of only a few other species has been studied, and then only superficially.

5.8 Analytical Methods for Evaluating Creaming of Milk

From an analytical perspective, the simplest method of following gravitational separation and creaming in milk is visual observation. One of the earliest methods of measuring the volume of cream produced by a milk sample consisted of standing bottles of identical size and shape beside each other and comparing the depth of the cream layer or measuring the distance from the top of the bottle to the line dividing the cream from the milk. Harding *et al.* (1922) developed a relatively precise method which is still in use today. Milk samples are poured into round-bottomed test tubes, of precise dimensions, to a specific height. The tubes are cooled in ice water and held at 4 °C for ~20 h, and the depth of the resulting cream layer is measured in mm, where each mm of cream represents a certain proportion of cream by volume (Harding *et al.* 1922).

Creaming can alternatively be defined as the volume of cream produced from a specified volume of milk in a glass tube, of various dimensions, at a stated temperature after certain time intervals, usually up to 24 h. The rate of creaming can be derived from the data obtained from this type of experiment if a sufficient number of sampling times is used. The creaming index can be calculated from the ratio of cream height over the total height of the emulsion upon standing (see Hammer 1916; Dunkley and Sommer 1944; Keynon *et al.* 1966; Euber and Brunner 1984).

Farah and Rüegg (1991) adapted the methods above and added 2 drops of nigrosine solution per 100 mL milk in a creaming assay on camel milk, carried out in graduated measuring cylinders, which allowed clear optical distinction between the aqueous (blue) and fat (white) phases. The relatively simple method of Speroni and Bertoni (1984) to measure natural creaming in milk is frequently used prior to the production of Gruyère, Parmigiano-Reggiano and Grana Padano cheeses when milk is allowed to cream naturally before cheese production (e.g. Abeni *et al.* 2005). Milk (14 mL) is placed in plastic tubes for 3 h in flowing water at 15 °C. Fat on the surface is removed by aspiration, and then 2 mL of milk is aspirated from the bottom of the tube using a syringe and analysed for milk fat content. Natural creaming is calculated as:

$$\%\text{natural creaming} = \left(\text{MF1} - \text{MF2}\right) / \text{MF1} \times 100$$

where MF1 = milk fat content before natural creaming and MF2 = milk fat content after natural creaming.

In the last decade, dispersion stability analysers such as the LUMiSizer (LUM, Berlin, Germany) and the Turbiscan (Formulaction, Toulouse, France) have been used to assess and quantify creaming in milk samples. Both instruments use a near-infrared (NIR) light source to detect instabilities such as creaming or sedimentation in samples by scanning the entire length of a sample over a defined time period, at a defined temperature, which can typically be varied (in a range from 4 to 60 °C); however, the principles of how they operate differ, with the LUMiSizer accelerating physical instability by application of centrifugal force (200 to 4000 rpm), whereas the Turbiscan studies separation under gravity.

The LUMiSizer can also perform high-resolution particle size analysis in the range of 10 nm to 300 μm (depending on physical proper-

ties), while particle sizes of 1 mm to 10 nm can be measured by the Turbiscan. The migration rate of particles is dependent on Stokes Law, and hydrodynamic diameter can thus be calculated from the migration rate.

The LUMiSizer uses artificial conditions to monitor sample stability, especially for forms of instability such as creaming where fat globules are in weak equilibrium within a liquid phase, and can predict broad stability under accelerated conditions, where instability is not dependent on longer time-scale changes in a sample. The Turbiscan, on the other hand, does not use external stress and evaluates stability as the sample is in a natural state. Increasing temperature rather than using an external force is regarded by some researchers as being preferable for acceleration of destabilization processes while maintaining realistic testing conditions.

5.9 Cold Agglutination

When bovine milk is stored in the cold under quiescent conditions, a cream layer will form rapidly due to the rise of milk fat globules under the influence of gravity, and the rate of separation is faster and the mobility of large fat globules faster at 4 °C than at higher temperatures (Ma and Barbano 2000). The rate of rise of milk fat globules during creaming is considerably faster than can be accounted for by Stokes' law for individual globules (Troy and Sharp 1928). A cream layer may be evident in bovine milk within 20 min of milking. However, the appearance of a cream layer, if formed as a result of the rise of individual fat globules of ~4 μm diameter, according to Stokes' equation, would take approximately 50 h (Fox *et al.* 2015). This is due to the fact that milk fat globules tend to rise in large clusters, which rise at a considerably faster rate than individual globules. Merthens (1933b) reported that addition of colostrum to milk enhanced creaming considerably, suggesting that one or more agents enriched in colostrum promoted creaming. The clustering of milk fat globules during cold storage markedly resembles the agglutination of bacteria or red blood cells, due to the action of the immunoglobulin IgM, in terms of dependence on pH, concentration and valency of cations. Hence, the clustering of milk fat globules in the cold is referred to as cold agglutination.

In terms of understanding the mechanism for cold agglutination of milk fat globules, two of the most important phenomena are the 'Merthens effect' and the 'Samuelson effect'. Merthens (1933a) observed that milk recombined from homogenized skim milk and unhomogenized cream has poor creaming ability ('Merthens effect'). It was proposed initially that this is due to denaturation of the agglutinin on homogenization, but Koops *et al.* (1966) showed that this was not the case. Samuelson *et al.* (1954) showed that two components are required for cold agglutination: a homogenization-labile component and a heat-labile component ('Samuelsson effect'). Homogenization at a pressure as low as 1 MPa, or even mild shearing, impairs the agglutinating tendency of skimmed milk (Walstra 1980). The euglobulin fraction of milk, implicated by many early investigators as the agglutinin, associates with the milk fat globules, particularly in the cold, and is not homogenization-labile (Payens 1964). Subsequent studies (Payens 1964, 1968; Payens *et al.* 1965; Gammack and Gupta 1967; Payens and Both 1970; Stadhouders and Hup 1970) identified immunoglobulin M (IgM) as the heat-labile agglutinin in the euglobulin fraction of milk. Gammack and Gupta (1970) showed that lipoprotein particles in the aqueous phase are a prerequisite for the rapid creaming of milk, which supports the earlier observations by Hansson (1949) that creaming of milk is enhanced by the addition of phospholipids.

Euber and Brunner (1984) proposed a mechanism for cold agglutination which involves three components: (1) the milk fat globules, (2) IgM, the heat-labile component, which functions as a cold agglutinin; and (3) the so-called skim milk membrane (SMM), the homogenization-labile component, consisting of lipoprotein particles present in the aqueous phase of milk. Euber and Brunner (1984) suggested that these components interact through specific carbohydrate moieties. IgM can interact with both SMM and the fat

globules, whereas SMM interacts with IgM only. Fat globules can be clustered to a limited extent by IgM alone, but clustering is considerably more extensive in the presence of SMM, which acts as a cross-linking agent. Environmental factors that affect the uptake of IgM by fat globules or SMM include ionic strength, dielectric constant, pH and the temperature of the suspending medium (Euber and Brunner 1984). Based on studies using confocal microscopy and fluorescent labelling of immunoglobulins, Hansen *et al.* (2019) reported that immunoglobulin-driven agglutination of milk fat globules was more extensive at 5 °C than at 15 °C and hardly occurred at 20 or 37 °C. The key mechanism involved appeared to involve the presence of an immunoglobulin receptor on the MFGM, which was not found at warm temperatures.

Cold agglutination is influenced also by processing conditions. Agitation of milk during cold storage impairs creaming, but heating milk to 40–50 °C normally restores the creaming capacity of the milk on cold storage (Merthens 1933a). Heating milk at a higher temperature, up to ~62 °C, improves the creaming capacity, relative to that of fresh milk (Rowland 1937). A similar increase in creaming capacity was observed after high-pressure treatment at 100–250 MPa by Huppertz *et al.* (2003), who showed that clusters of milk fat globules formed on cold storage of milk treated at 200 MPa were larger than those formed in unpressurized milk. However, the exact mechanism for heat- or HP-induced increases in creaming of milk has not yet been described.

Heating milk at >62 °C (Orla-Jensen *et al.* 1929; Rowland 1937), or treating it at a pressure ≥ 400 MPa (Huppertz *et al.* 2003), impairs the rate of creaming of milk fat globules. Huppertz *et al.* (2003) showed that clustering of milk fat globules on cold storage did not occur in milk treated at 600 MPa. Thermal or high-pressure-induced inhibition of cold agglutination is probably the result of denaturation of IgM; heat-induced denaturation of immunoglobulins reduced agglutination in a manner proportional to the level of denaturation (Hansen *et al.* 2019). Heat-induced interactions of caseins or whey proteins with the MFGM may also prevent cold agglutination (Van Boekel and Walstra 1995). Addition of colostral euglobulin to heated milk restores its creaming capacity (Keynon and Jenness 1958).

The mechanism of gravity separation of cream in milk remains an active topic of research, particularly in the context of pre-treatment of milk for some cheese types (such as Parmigianno Reggiano). D'Inecco *et al.* (2018) reported that partial coalescence occurred in milk creamed at a temperature higher than 22 °C, and that warming milk to 37 °C followed by separation at cold temperatures altered the fat globule size distribution, potentially by affecting IgA- and IgM-mediated interactions of fat globules with clostridial spores; adding purified immunoglobulins to raw milk increased their partition into the cream layer. The role of IgM in the separation of spores through their interaction with fat globules was also demonstrated by Geer *et al.* (2014a), while Caplan *et al.* (2013) proposed that gravity separation of milk could result in reduced-fat milk with decreased bacterial and somatic cell counts.

Interestingly, Geer *et al.* (2014b) reported that during gravity separation, somatic cells in milk were critical for the upward movement of fat globules (and also bacterial spores), and that immunoglobulins alone were not sufficient to cause such separation. They proposed that somatic cells may interact with immunoglobulins and that these then aggregate with fat, with the cells providing buoyancy for the upward separation due to entrapped air.

Clustering of milk fat globules in the cold, followed by rapid creaming, is not a universal phenomenon. As described earlier, it occurs in bovine milk, but not, or to a considerably lower extent, in caprine (Jenness and Parkash 1971), buffalo (Fahmi 1951; Abo-Elnaga 1966; Wahba *et al.* 1977; Ismail *et al.* 1972), camel (Farah and Rüegg 1991) and reindeer (Uniacke-Lowe and Fox 2011; Gonzales-Janolino 1968a) milk. This has been related to the fact that clustering of milk fat globules does not occur in the milks from these species. The clustering of fat globules has been monitored in a thin (1 mm) body of milk between glass plates (Dunkley and Sommer

1944; Keynon *et al.* 1966); the clusters can be seen with the naked eye or photographed. Dispersion analysers such as the Turbiscan (as described above) can be used to accurately monitor clustering of fat globules during creaming of milk. Changes in the particle size due to coalescence can be detected on the basis of the back-scattering signal in the middle zone of the sample cell. If the fat globules grow with time due to agglomeration or coalescence, the number of scattering centres decreases in the middle zone of the sample cell, decreasing the back-scattering signal.

Jenness and Parkash (1971) showed that milk reconstituted from caprine cream and bovine skim milk creams rapidly, whereas milk reconstituted from bovine cream and caprine skim milk shows a very low level of creaming. Similar results were observed on reconstituting cream and skimmed milk from bovine and camel milk (Farah and Rüegg 1991). The poor creaming properties of buffalo milk were attributed to its poor clustering ability (Abo-Elnaga *et al.* 1966; Ahmad 2013). Addition of euglobulin, isolated from buffalo colostrum, considerably increased the creaming capacity of buffalo milk (Wahba *et al.* 1977). Gonzales-Janolino (1968b) observed poor creaming of mixtures of cow's cream and caribou' skimmed milk, whereas a mixture of agglutinin-rich bovine-skimmed milk and caribou' cream creamed extensively. Further experiments showed that caribou milk lacks the homogenization-labile component (Gonzales-Janolino 1968b). Thus, it is apparent that cold agglutination of fat globules in milk is highly dependent on the species of origin.

Ongoing research (authors' unpublished data) has confirmed the significant differences found in the creaming behaviours of fresh milk samples from a variety of species. Milk from seven species (porcine, bovine, human, reindeer, buffalo, goat and camel) was assayed in a LUMiSizer at 14 °C at 500 rpm (~ 36 g). The intensity of transmitted NIR light, measured over the entire length of the samples, was recorded every 120 s for 2.5 h (75 profiles recorded). The slope (%/h) of the integral transmission versus time curves was calculated as an indicator of the initial creaming rate for each sample. The higher the slope value, the more creaming that has occurred under these experimental conditions. Slopes calculated were 0.1147 for porcine milk, 4.6551 for bovine milk, 14.0912 for human milk, 0.2107 for reindeer milk, 0.8383 for buffalo milk, 0.2409 for goat milk and 0.8840 for camel milk. Human milk, under these conditions, creamed significantly more than any of the other samples, while goat and reindeer milk did not cream (Figure 5.3). Furthermore, human milk became more translucent as it creamed, due to the small size and low concentration of human casein micelles rendering the contrast in colour between skim milk and cream phases much greater than in milk of other species. Buffalo and camel milk creamed very poorly, as reported earlier. Freezing milk for periods from 1 day to 2 months had little effect on the creaming of the milk of all species studied once the milk was thawed slowly overnight at 4 °C.

5.10 Coalescence and Partial Coalescence

When two or more emulsion droplets come into contact, a thin film of the liquid continuous phase forms between them. Coalescence is the process whereby liquid droplets merge to form a single larger droplet as a result of the rupture of both this liquid film and the interfacial membranes of the droplets. Coalescence moves an emulsion towards a thermodynamically stable state, because it involves a decrease in the contact area between the phases (Tadros and Vincent 1983; Walstra 1996; McClements 1999).

The current state of understanding of coalescence is unsatisfactory because of the number of variables involved and the fact that some fundamental problems have not been resolved fully (Walstra 2003), but some general understanding has been obtained. The susceptibility of droplets to coalescence is determined by the nature of the forces that act on and between the droplets and the resistance of the droplet membranes to rupture. Coalescence may be induced by collisions or by prolonged contact between the emulsion droplets. Collision-induced coalescence can be

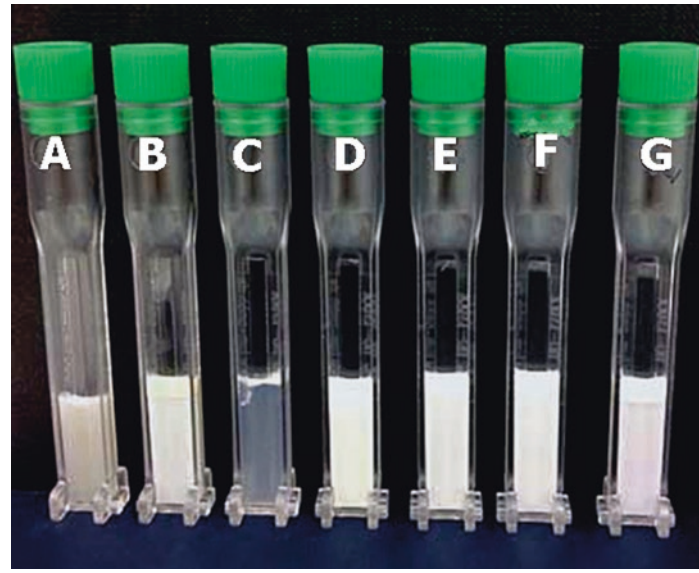

Figure 5.3. Images showing the creaming ability of the milk from seven species after analysis using a LUMiSizer at 14 °C for 2.5 h at 500 rpm. *From left*: A, porcine; B, bovine; C, human; D, reindeer; E, buffalo; F, goat and G, camel milk.

due to movement of the droplets by Brownian motion, gravity or applied mechanical forces. Coalescence induced by prolonged contact occurs spontaneously after the droplets have been in contact for a sufficient period, e.g. in emulsions which contain flocculated droplets or droplets that have accumulated at the top or bottom of the emulsion due to gravitational separation, respectively. The probability of film rupture will be larger if the interfacial tension, γ, is small, and if the colloidal repulsion between the droplets is stronger (Walstra 2003). Furthermore, susceptibility to coalescence increases with droplet size.

Shimizu *et al.* (1980) reported that removal of the polar head of phospholipids in the milk fat globule membrane by phospholipase C results in oiling-off; thus, it appears that the polar head of the phospholipids plays an important role in the stability of milk fat globules against coalescence. It is notable that several species of psychrotrophic bacteria isolated from raw milk can produce phospholipases, which may contribute to spoilage phenomena in milk (Sadiq *et al.* 2016; Vithanage *et al.* 2016).

Indirect UHT treatment can cause aggregation of fat globules due to partial heat-induced coagulation; direct UHT treatment, which involves greater turbulence and flash boiling, does not cause aggregation (Melsen and Walstra 1989). Mulder and Walstra (1974) reported that coalescence of fat globules in cream may occur during treatment in a heat exchanger, but Van Boekel and Folkerts (1991) could not confirm this for direct or indirect UHT treatment of unhomogenized milk. Streuper and Van Hooijdonk (1986) observed coalescence on UHT treatment of milk, but only if back-pressure in the apparatus allowed some boiling of the liquid.

Whereas true coalescence is of limited importance in the case of milk and dairy products, partial coalescence is of far greater importance, in particular in the preparation of products such as whipped cream, butter and ice cream. Partial coalescence occurs when two or more partially crystalline emulsion droplets come into contact. A fat crystal protruding from a globule may pierce the film between close globules, which leads to conjunction of the globules, resulting in the formation of an irregularly shaped aggregate (Van Boekel and Walstra 1981). The aggregate partially retains the shape of the globules from which it was formed, because the fat crystal network within the droplets prevents a complete merger. Partial coalescence differs from true coalescence in that it tends to be much faster and that, due to the formation of irregular aggregates

or clumps, it increases the effective volume fraction of the dispersed phase. Prerequisites for partial coalescence include the presence of a network of fat crystals in the globules (Boode *et al.* 1993) and that the fat crystals are located at the oil–water interface; if fat crystals are totally wetted by either the oil or water phase, they do not affect emulsion stability (Boode and Walstra 1993).

In the case of milk fat globules, partial coalescence can lead to the formation of irregularly shaped granules, e.g. butter clumps, or the formation of a continuous network, e.g. in whipped cream or ice cream. Walstra *et al.* (1999) reported that the following factors affect the rate of partial coalescence in milk:

1. *Application of a velocity gradient* of shear rate increases the rate of collision between fat globules and presses globules closer together, thus enhancing the possibility of a protruding crystal bridging the gap between globules. However, above a certain velocity, the rate of partial coalescence decreases (Boode *et al.* 1993).
2. An increased *fat content* increases the rate of clumping.
3. The *proportion of solid fat* is crucial. Partial coalescence cannot occur if there are no fat crystals, but if there is too much solid fat, there may not be enough liquid fat to hold globules together (Boode *et al.* 1993).
4. *Fat globule size* also influences the extent of partial coalescence. Larger globules are less stable against partial coalescence, due to the fact that they may have larger fat crystals and the probability of a crystal sticking out far enough is thus higher;
5. The *surface layer on the globules* plays an important role. Natural fat globules are reasonably stable, but the presence of a surface layer of protein, e.g. after homogenization or recombination, increases the stability of the globules considerably through colloidal repulsion.

Partial coalescence is probably also involved in a defect in unhomogenized milk that is not kept at a sufficiently low temperature, referred to as ‘bitty cream’ or ‘broken cream’. Bacterial phospholipases can hydrolyse up to 60% of the phospholipids on the milk fat globule membrane (O’Mahony and Shipe 1972), making the globules more susceptible to partial coalescence, which leads to the formation of large particles of cream floating in the milk (Stone 1952a, b; Stone and Rowlands 1952; Labots and Galesloot 1959). In market milk, the bitty cream defect has been largely eliminated by homogenization.

5.11 Rebodying

The term rebodying is often used for the phenomenon whereby cooled cream, when warmed (e.g. to 30 °C) and subsequently re-cooled, becomes more viscous, or even (at a sufficiently high fat content) solid-like (Hoffmann 1999). Rebodying is caused by partial coalescence (Oortwijn and Walstra 1982b) and occurs in unhomogenized or weakly homogenized systems, in which much of the natural MFGM is retained. The extent and nature of the rebodying phenomenon depends on the rate of cooling (which determines whether the number [fast cooling] or size [slow cooling] of the fat crystals increases) and the temperature history of the cream, particularly cycling through higher and lower temperatures. Repeated rebodying can cause fat separation. Warming cream that has undergone rebodying to 30 °C increases fat globule size, probably as a result of full coalescence of globules that are already in the partially coalesced state (Oortwijn and Walstra 1982b).

In UHT-treated whipping cream, rebodying results in clumping of fat or the formation of cream plugs (small lumps of partially solidified fatty material), which cannot be redispersed in the product by gentle shaking. The fat droplets in the cream plug are aggregated and partially coalesced. Streuper and Van Hooijdonk (1986) reported that the firmness of the plug in UHT-treated cream increased with increasing rate of cooling of the cream. The formation of a cream plug in heat-treated unhomogenized cream can be prevented completely by addition of carrageenan, in combination with protein-fat powder (Precht *et al.* 1987). Dickinson *et al.* (1989) introduced the term ‘cohesive cream’ to describe

a concentrated emulsion layer in which the flocculated oil or fat droplets have become compressed into a coherent structure that cannot be redispersed by mild agitation; these authors also reported that the formation of cohesive cream in liqueurs is enhanced at a low pH, a high calcium content or a low level of caseinate emulsifier, as well as by temperature fluctuations during storage.

Studies on model creams have further clarified the possible mechanism for rebodying. For an increase in viscosity to occur on re-cooling, it is necessary that, after the warming step, <10% of the fat remains solid; if all fat is melted, rebodying does not occur (Boode *et al.* 1991; Noda and Yamamoto 1994; Mutoh *et al.* 2001). Sugimoto *et al.* (2001) observed that the increase in viscosity on re-cooling warmed cream is accompanied by a substantial increase in the concentration of protein on the fat globule surface and proposed the following mechanism for an increase in viscosity on re-cooling of warmed cream. At a critical level of solid fat (<10%) in cream, fat crystals approach the oil droplet surface, which causes conformational changes in the proteins absorbed at the oil-droplet surface. A rapid decrease in the fluidity of triacylglycerides on cooling causes further changes in the conformation and charge of the surface proteins, which leads to attraction between serum proteins and those on the surface; this results in an increase in viscosity and solidification of the cream. However, further studies may be necessary to establish if this mechanism also applies to dairy cream.

5.12 Factors That Affect the Surface Layers of Fat Globules in Milk and Cream

The surface layers of the fat globules in milk are affected by various treatments. Effects of homogenization are described in Sections 5.13 and 5.14; however, other treatments such as cooling and heating, as well as environmental conditions, also influence the surface layers of milk fat globules and are described in this section.

Cooling of milk induces the release of up to 15% of phospholipids from the MFGM (Koops and Tarassuk 1959; Baumrucker and Keenan 1973), resulting in an increase in the phospholipid content of the milk plasma (Patton *et al.* 1980). Cooling also causes a shift in xanthine oxidase activity from the fat to the plasma phase and results in the reversible adsorption of the cryoglobulins onto the fat globules (Mulder and Walstra 1974). Furthermore, cooling induced the migration of copper from the milk fat globules to the milk plasma (Mulder and Walstra 1974). Freezing and subsequent thawing cause considerable clumping of milk fat globules, particularly in cream, primarily caused by mechanical pressure differences in the frozen products (Mulder and Walstra 1974).

Heat treatment can also affect the composition of the MFGM. The amount of protein associated with the fat globules increases on heating; the newly bound protein is largely denatured whey protein, particularly β-lactoglobulin (Dalgleish and Banks 1991; Corredig and Dalgleish 1998). Interactions of whey proteins with the MFGM probably occur primarily via sulphydryl-disulphide interchange reactions (Kim and Jimenez-Flores 1995; Lee and Sherbon 2002). Heating can also result in the formation of high molecular weight protein complexes between xanthine oxidase, butyrophilin and denatured whey proteins (Ye *et al.* 2002); the kinetics of such reactions were reported by Ye *et al.* (2004).

If milk is heated prior to homogenization, less whey protein is incorporated into the MFGM than would be the case if the order of these steps is reversed (Sharma and Dalgleish 1994). The association of denatured whey protein, principally β-lactoglobulin, with the MFGM increases its protein content and reduces that of lipids proportionately. Membrane glycoproteins, such as PAS-6 and 7, become less evident on electrophoretic (SDS-PAGE) analysis following heat treatment (Houlihan *et al.* 1992; Iametti *et al.* 1997; Lee and Sherbon 2002), which may be due to mechanical damage to the globules caused by pumping and circulation through the pasteurizer (Iametti *et al.* 1997) or due to their displacement by denatured whey proteins during heating (Houlihan *et al.*

1992), although the exact mechanism is not clear (Lee and Sherbon 2002). The effects of heat treatment and homogenization on fat globules are compared schematically in Figure 5.4.

Heat treatment can also result in the release of H_2S from the globules and transfer of copper from plasma to globules (Mulder and Walstra 1974). Heating can also reduce the triacylglyceride content of the MFMG (Houlihan *et al.* 1992), but conflicting results have been published on the effect of heat treatment on the phospholipid content of the MFGM. Koops and Tarassuk (1959) and Greenbank and Pallansch (1961) observed a reduction in the phospholipid content of the MFGM on heating, but Houlihan *et al.* (1992) reported that heat treatment did not influence this parameter. In a system comprising milk fat globules in simulated milk ultrafiltrate, warming to 45–50 °C for 10 min resulted in the loss of up to 50% of total protein from the MFGM, perhaps due to the melting of the lipid phase and subsequent rearrangement of the globule surface (Ye *et al.* 2002).

Because concentration of milk by thermal evaporation can also damage the MFGM, and drying can damage the MFGM considerably, milk to be used for evaporation or drying is usually homogenized to strengthen the globule mem-

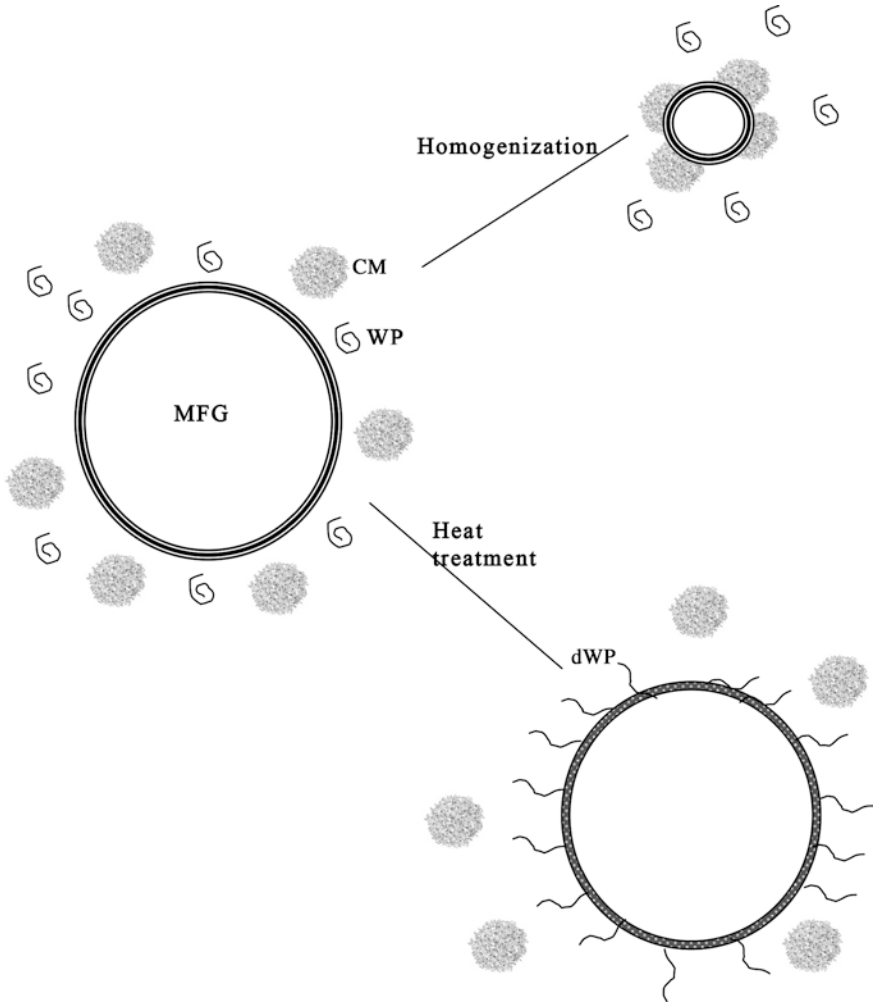

Figure 5.4. Schematic illustration of the relative effects of heating and homogenization on fat globules in milk. *MFG* milk fat globule, *CM* casein micelle, *WP* whey protein, *dWP* denatured whey protein.

branes by binding of caseins. Furthermore, contact with air bubbles can change the MFGM, which has important implications for products such as ice cream and whipping cream (see Chapter 9).

5.13 Disruption of Globules

Fat globules are relatively fragile, particularly when the fat is liquid, and can be disrupted readily by a number of conditions experienced in dairy processing operations. Viscous (shearing) or inertial (cavitation, turbulence) forces, in particular, can damage the MFGM and cause physical rupture and sub-division of the globules. Rupture occurs when droplets are deformed beyond a critical value for longer than a critical time. Resistance to deformation is related to the Laplace pressure and the ratio of the viscosity of the fat to that of the plasma. The Laplace pressure refers to the difference between the pressures at the concave and convex sides of a curved interface of two fluids. For a spherical droplet, the Laplace pressure, P_L, can be expressed as follows (Walstra 2003):

$$P_L = 2\gamma / R$$

where γ is the interfacial tension and R is the radius of the droplet. The value for γ for native milk fat globules is very small, but increases as globules are deformed. To disrupt a droplet, it is necessary to apply an external force which is considerably larger than P_L, and the duration of application must be longer than the time required to deform and disrupt the droplet (McClements 1999).

Globules can be deformed by the shearing action of liquid; if the viscous stress, ηS (where η is the viscosity of milk serum and S is the velocity gradient), equals or exceeds P_L, the globule may be disrupted; this typically requires very high velocity gradients, and, even then, the ratio of the viscosity of the milk fat to plasma protects all but the largest fat globules from shear-induced disruption, in cases of non-turbulent flow (Walstra 1995). Disruption occurs more readily under turbulent flow conditions, depending on the amount of turbulent energy dissipated per unit time and per unit volume of liquid. Such conditions are encountered only very transiently, e.g. in the valve of homogenizers or at the top of a rapidly rotating stirrer blade.

Homogenization is a process designed to reduce the size of the milk fat globules and thus retard separation of fat globules to such an extent that a cream layer does not form in homogenized milk products during their shelf-life. During homogenization, pre-warmed (~ 40 °C) milk (in which the fat is in a liquid state; homogenization is less effective when the fat is partially solid) at a pressure of 10–20 MPa is passed through a small orifice. Shearing, impact and distortion effects combine to stress the fat globules to such an extent that they split into a greater number of smaller globules (usually <2 μm diameter; Figure 5.5). The principle of operation of a homogenizer is shown in Figure 5.6. The extent of the reduction depends on a number of factors, including the valve geometry of the homogenizer used, the number of passes through the valve and, in particular, the homogenization pressure (Walstra 1975). The relationship between d_{vs} and homogenization pressure (p_h) is given by:

$$\log d_{vs} = \text{constant} - 0.6 \log p_h$$

where the constant varies between −2 and − 2.5, depending on the type of homogenizer and other processing conditions (Walstra 1975). The principal mechanism responsible for globule disruption during homogenization is probably pressure fluctuations under turbulent flow conditions (Walstra 1995). Creaming of fat globules in homogenized milk is considerably slower than that of the original globules, due to the reduction in fat globule size and the adsorption of milk proteins onto the fat globules, which increases their density and therefore reduces the rate of creaming, as well as causing inactivation of the homogenization-labile component involved in cold agglutination.

Michalski *et al.* (2002) studied the types of particles in homogenized milk and identified three classes: (1) disrupted globules covered mainly by caseins and some of the original MFGM (the surface layer of fat globules in

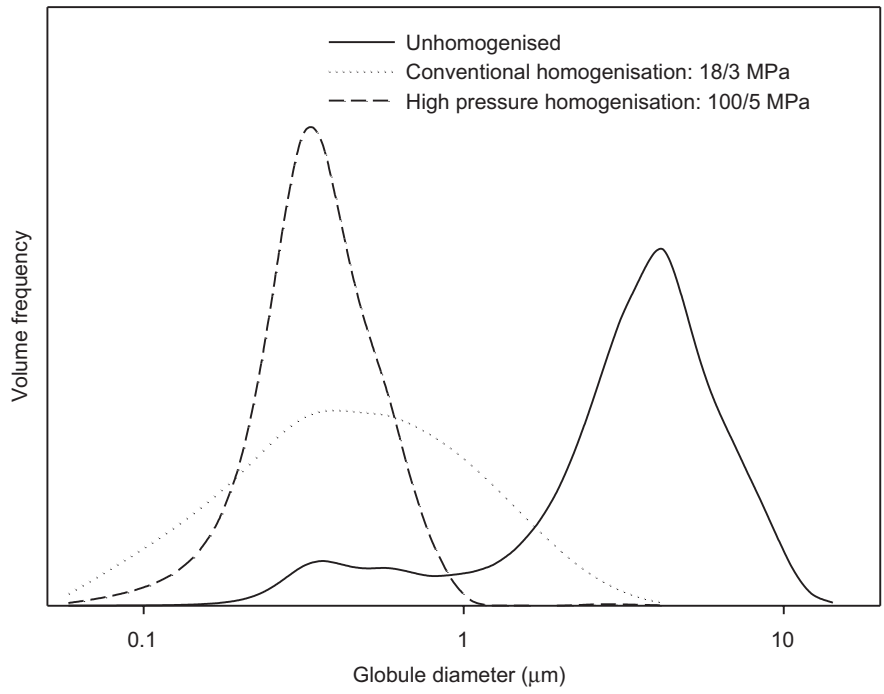

Figure 5.5. Effect of conventional (18/3 MPa) and high pressure (100/5 MPa) homogenization on the volume frequency distribution of fat globules in bovine milk.

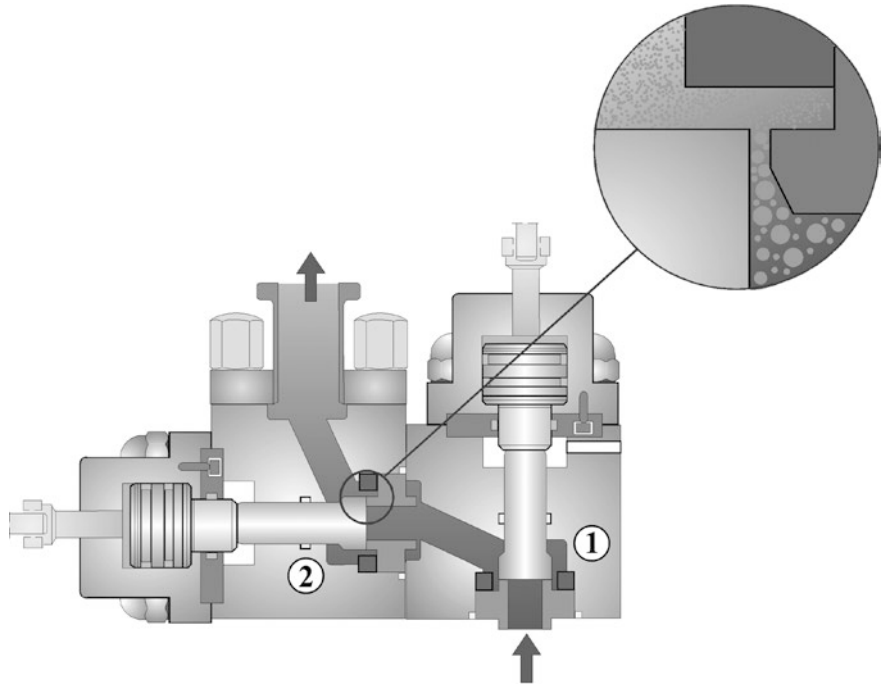

Figure 5.6. Principle of operation of a typical two-stage homogenizer for liquid milk, indicating the first (1) and second stages (2). (Reproduced with permission from *Dairy Processing Handbook*, Tetra Pak Processing Systems AB, Lund, Sweden, 1995).

homogenized milk is discussed in Section 5.13); (2) a population of very small native fat globules of around 100 nm in diameter, which appeared unaffected by homogenization, and (3) small newly formed lipid-protein complexes with a new casein-rich membrane, a type of particle also produced by ultrasonication or pumping. The presence of the population of small fat globules that are apparently unaffected by homogenization may be explained by the fact that their size is smaller than the Kolmogorov scale (Michalski *et al.* 2002), which approximates the minimum size of particles that can be affected at a given homogenization pressure, i.e. 320, 240, 180 or 140 nm at 5, 10, 20 or 40 MPa, respectively (Mulder and Walstra 1974).

In recent years, novel homogenizing devices that reduce milk fat globule size considerably more than the traditional homogenizers by operating at a higher pressure (100–300 MPa; Figure 5.5), such as high-pressure homogenizers (Hayes and Kelly 2003a; Thiebaud *et al.* 2003) and microfluidizers (McCrae 1994; Strawbridge *et al.* 1995; Hardham *et al.* 2000) have been used. The operating principle of a high-pressure homogenizer is generally similar to that of a conventional two-stage mechanical homogenizer, but it operates at a significantly higher pressure. The forces exerted by high-pressure homogenization, including shear, cavitation, impacts, turbulence and frictional heating, can also kill bacteria, inactivate enzymes, denature whey proteins and alter several properties of milk (Hayes and Kelly 2003a, b; Thiebaud *et al.* 2003; Hayes *et al.* 2005).

In a microfluidizer, forces are generated by impinging high-velocity fluid jets (Paquin 1999). Bucci *et al.* (2018) reported formation of a stable emulsion on microfluidization of milk at pressures of 75–170 MPa, leading to particle sizes substantially lower than those achieved by conventional homogenization. Microfluidization has been found to improve the textural properties of low-fat yoghurt through modification of the acid gel structure (Ciron *et al.* 2011, 2012).

Ultrasonic treatment can also disrupt milk fat globules, probably through cavitation and other shear and shock effects (Villamiel *et al.* 1999). Wu *et al.* (2001) reported that high-amplitude ultrasound homogenization of milk for yoghurt manufacture achieved similar effects as conventional homogenization.

High-pressure jet processing, in which milk is subjected to a high pressure (up to 600 MPa) and then pushed through a very narrow orifice (1–10 μm), subjects fat globules to forces including shear, pressure, turbulence, impingement and cavitation. There have been a number of reports of the impact of such processing on milk proteins, but fewer on whole milk; Tran *et al.* (2018) reported a bimodal particle size distribution in such milk, with the formation of stable casein-fat particles of relatively high density.

Heffernan *et al.* (2011) compared high-pressure homogenization, microfluidization, ultrasound homogenization and homogenization using orifice nozzles and radial diffusion principles for the emulsification of cream liqueurs. Microfluidization produced the smallest globule sizes, while some of the processes evaluated yielded a wide range of particle sizes. For all technologies, processing pressure was inversely correlated with droplet size, and small droplet sizes were generally associated with increased physical storage stability (except in the case of ultrasound homogenization). The applications of a range of homogenization technologies for milk systems were reviewed by Tobin *et al.* (2015).

5.14 Milk Fat Globules in Homogenized Milk and Cream

The decrease in fat globule size on homogenization results in a significant increase in globule surface area. This new surface area is far too large to be covered and stabilized by the original amount of MFGM material; therefore, the surface becomes covered by casein micelles, or fragments thereof, with some whey protein also becoming attached when homogenization is combined with heat treatment (Darling and Butcher 1978; García-Risco *et al.* 2002; Lee and

Sherbon 2002). The protein load per unit surface area increases as homogenization pressure is increased, but homogenization pressure has little effect on the composition of surface protein (Cano-Ruiz and Richter 1997). The membrane in homogenized milk is thicker than that in fresh raw milk, and the proportion of the casein in milk that becomes associated with the fat globules has been estimated to be ~6–8% (Fox 2002). In extreme cases, a high protein load on the globules may lead to gravitational or centrifugal sedimentation instead of upward separation in evaporated milk (Fox *et al.* 1960) or homogenized raw milk (Michalski *et al.* 2002), respectively.

Fat globules in homogenized milk, due to their small size and the presence of a high level of casein on their surface, can, in effect, behave like casein micelles, which has significant implications for the heat-, acid- or rennet-coagulation properties of milk and hence for the properties of resulting products. These effects can be positive in the case of acidified milk products, such as yoghurt, but they are generally undesirable in the case of cheese. Fat-casein complexes may accelerate the kinetics of coagulation, particularly heat coagulation; homogenization reduces the heat stability of whole milk (Sweetsur and Muir 1983).

As discussed in Section 5.9, milk fat globules are naturally susceptible to partial coalescence or clumping. Freshly homogenized fat globules are particularly unstable and tend to coalesce into clusters or clumps, i.e. homogenization clusters; these clusters are formed as a result of the sharing of casein micelles between globules (even a single casein micelle can form a bridge between two neighbouring globules), because the amount of surface-active material is insufficient to cover the newly formed interface (Ogden *et al.* 1976; Darling and Butcher 1978). The formation of homogenization clusters is usually prevented by use of a second, or even third, homogenization stage, usually at a lower pressure than the first stage (Kiesner *et al.* 1997). The tendency to form homogenization clusters is enhanced by a high fat content, small fat globule size and a high surface protein load. Intense heat treatment before homogenization can also increase the tendency of globules to form clusters (Walstra 1995).

The presence of homogenization clusters increases the viscosity of cream (Niar *et al.* 2000), particularly during product storage; high-fat cream can acquire the consistency of a thick paste. This has implications for processes where only the cream is homogenized, followed by recombination with skim milk (i.e. liquid milk processing); if clusters form, products will cream readily. When fat is emulsified directly in skim milk, clusters are formed if the fat concentration exceeds 12% (w/w) (Oortwijn and Walstra 1982b).

At a given homogenization pressure, average fat globule size decreases with increasing fat volume fraction up to a certain level, above which an increase is observed (Phipps 1983), indicating the formation of homogenization clusters. In a conventional valve homogenizer, fat clustering in milk or cream occurred at a pressure > ~20 MPa (McCrae 1994; Noda and Yamamoto 1996; McCrae and Lepoetre 1996), whereas on high-pressure homogenization, milk fat globule size decreased up to 250 MPa, but increased at 300 MPa, due to the formation of homogenization clusters (Thiebaud *et al.* 2003). In a microfluidizer, some fat clustering occurred following treatment at 35 MPa, but none was reported after treatment at 103 MPa (McCrae 1994).

Homogenized milk is very susceptible to hydrolytic rancidity, as the protective function of the MFGM has been compromised (Iametti *et al.* 1997); for this reason, homogenization should be combined with pasteurization to inactivate lipases in milk. Several methods have been developed to evaluate damage to the MFGM (Iametti *et al.* 1997; Evers 2004). Homogenized milk is also more susceptible to the so-called sunlight flavour or light-activated flavour defect (Dunkley *et al.* 1962). This flavour defect results from conversion of methionine to methional catalysed by riboflavin activated by photo-oxidation; however, the exact mechanism through which homogenization influences this process has thus far not been described. Furthermore, homogenized milk is less prone to copper-catalysed lipid oxidation than unhomogenized milk (Tarassuk and Koops

1960; Dunkley *et al.* 1962), which is probably due to the fact that oxidation-susceptible phospholipids are more uniformly distributed following homogenization and are less likely to propagate lipid oxidation (Tarassuk and Koops 1960).

5.15 Milk Fat Globules in Recombined Milk

A class of products in which the altered fat globule surface layers is of considerable importance is that of recombined milk. Typically, in the manufacture of recombined milk, skim milk powder, water and a source of milk fat (e.g. anhydrous milk fat; AMF) are mixed and homogenized to emulsify the fat and yield a stable product. Since AMF contains little or no MFGM material, the membrane surrounding fat globules in recombined milk does not contain any original MFGM material; the nature of the new membrane is highly influenced by adsorption conditions (e.g. composition of the continuous phase, agitation, temperature, heat treatment and fat: protein ratio). Both caseins and whey proteins are present in the membrane of recombined milk (Oortwijn *et al.* 1977), but the proportion of whey proteins on the membrane is lower than that in milk (Walstra and Oortwijn 1982; Sharma *et al.* 1996a, b). In addition to recombined milk, various other products are also prepared by recombining a fat source and milk protein sources, e.g. infant formula products as well as clinical nutrition products.

As the ratio of protein to fat in recombined milk is increased, the surface protein load on the fat globules increases; at higher ratios, little further effect is observed (Sharma *et al.* 1996a). The protein load per unit surface area on the fat globules in recombined milk is influenced markedly by the form of protein present in the liquid phase, i.e. it is markedly higher when casein micelles are present, compared to when only whey protein or sodium caseinate is present (Oortwijn and Walstra 1979; Sharma and Singh 1998, 1999). Furthermore, the surface protein load decreases with increasing temperature during emulsification (Oortwijn and Walstra 1979; Sharma *et al.* 1996a) and with increasing homogenization pressure (Sharma *et al.* 1996a) and is increased by heat treatment of milk prior to emulsification (Oortwijn and Walstra 1979; Sharma *et al.* 1996a). Heat treatment prior to emulsification also increases the level of β-lactoglobulin in the membrane (Sharma *et al.* 1996a).

The casein micelles on the globule surface in recombined milk often appear to be disrupted, which may be either due to homogenization or to the process of adsorption (Sharma *et al.* 1996b). The extent of disruption of micelles in recombined milk is greater than that in freshly homogenized milk (Sharma *et al.* 1996b) and increases with the temperature of homogenization (40–70 °C; Oortwijn and Walstra 1982a). Disruption of casein micelles in recombined milk was not observed after fixation of casein micelles with glutaraldehyde or addition of the surfactant Tween 20 prior to homogenization (Oortwijn *et al.* 1977); the latter effect is probably due to the preferential absorption of Tween 20 compared to casein micelles on the micelle surface. The addition of surfactants before or after recombination also decreases the protein surface load (Oortwijn and Walstra 1979). Destabilization of casein micelles by reducing the colloidal calcium phosphate content reduces the protein load on fat globules in recombined milk and alters the proportions of individual caseins at the globule surface (Sharma *et al.* 1996a). Sharma *et al.* (1996b) reported that it is far more difficult to remove κ-casein than α_s-caseins or β-casein from the fat globule surface in recombined milk and suggested that a portion of the κ-casein is associated directly with the globule surface, which was confirmed by Su and Everett (2003).

Inclusion of certain emulsifiers prevents fat separation in UHT-processed recombined milk, with Tween 20 being most effective; refined monoglyceride actually enhanced creaming slightly, perhaps due to protein displacement from surface layers, thus reducing the effective density of the globules (Mayhill and Newstead 1992). Addition of soy lecithin may reduce the stability of fat globules in recombined cream against coalescence (Melsen and Walstra 1989).

Heating of recombined milk products at 130 °C at pH 6.7 leads to the formation of chains of fat globules and casein particles, linked via the latter. Furthermore, at pH values <6.7, the surface of the casein micelles on the fat globule surface develops appendages on heating, possibly whey protein aggregates, while those in milk of pH >6.7 remained free of whey protein on heating (Singh *et al.* 1996). Addition of AMF (without homogenization) or homogenization (in the absence of AMF) did not influence the heat coagulation time of the skim milk, but the heat stability of milk recombined from AMF and skim milk is considerably lower than that of the skim milk (Sharma and Singh 1999). Furthermore, the heat stability of recombined milk decreased with decreasing fat globule size, which may be linked to a higher surface protein concentration and a lower proportion of κ-casein on smaller fat globules than larger ones (Sharma and Singh 1999). McCrae *et al.* (1994) reported that heat-induced interactions between whey protein and casein adsorbed at the fat globule surface promote the heat coagulation of recombined milk. However, the relatively low heat stability of recombined milk can be increased considerably by addition of soy lecithin, either pre- or post-homogenization (McCrae and Muir 1992).

5.16 Free Fat

'Free fat' is a term used in the literature to denote a particular parameter that has been claimed to correlate with the degree of damage to, or stability of, fat globules; various definitions have been given for this ambiguous term (Evers 2004), e.g. 'fat inside damaged globules' (Fink and Kessler 1983), 'fat that is enclosed insufficiently by an undamaged membrane' (Kessler and Fink 1992) or 'fat that has leaked out of globules' (Fink and Kessler 1983). Other authors have defined free fat as a method-dependent parameter, e.g. the proportion of fat extracted by centrifugation at 60 °C (Te Whaiti and Fryer 1975) or solvent-extractable fat (Deeth and Fitz-Gerald 1978).

The question of whether free fat actually occurs in milk or cream is, in fact, controversial, with frequent suggestions that it is an artefact of the method used for its measurement (e.g. that organic solvents can damage the MFGM and extract some fat; Evers *et al.* 2001). Walstra and co-workers (Van Boekel and Walstra 1989, 1995; Van Boekel and Folkerts 1991; Walstra 1995; Walstra *et al.* 1999) suggested that there is more than sufficient protein in milk to cover any uncovered fat very rapidly, e.g. in ~10 ms. The efficiency of methods to quantify the level of free fat was recently reviewed by Evers (2004), who, in agreement with Walstra *et al.* (1995), concluded that these methods have poor repeatability due to their poor robustness, i.e. very precise experimental control is required to obtain repeatable results. In some cases, the extraction method used may damage the fat globules, thereby magnifying, or even generating, the extractable fat level (Evers *et al.* 2001).

While free fat remains a controversial concept in liquid dairy products, it undoubtedly has an important role in whole milk powder (WMP); for certain applications (e.g. chocolate manufacture) high free fat WMP is favoured. Keogh *et al.* (2003) reported that the particle size in chocolate mixes after refining and the viscosity of the molten chocolate decreases as the free fat content of the WMP increased; such changes have significant implications for the mouthfeel and smoothness of chocolate. Schmidmeier *et al.* (2019) found high levels of free fat in a coarse (large particle size) fraction of a fat-filled milk powder which was prone to the formation of white flecks on reconstitution, and linked this to poor emulsion stability of such powder on reconstitution. The topic of free fat in dairy powders was reviewed by Vignolles *et al.* (2007), who concluded that both processing conditions and composition strongly affect free fat in dairy powders.

When considering so-called free fat in dairy powders, care should be taken to distinguish between values based on traditional solvent extraction methods and those obtained by methods such as X-ray photoemission spectroscopy (XPS). The latter focusses only on the actual composition of the surface layer of the particle, and values are typically expressed as % of particle surface covered by fat. For solvent extraction,

however, not only surface fat but also some accessible fat further into the particle is extracted. Values for this method are typically expressed as g extractable fat per 100 g total fat or powder (Vignolles *et al.* 2007).

For more information on the role of fat in milk powder and chocolate, see Chapter 9.

5.17 Influence of Fat Globules on Rheological Properties of Milk and Cream

Rheological properties of emulsions are of importance in food science for various reasons. Some sensory attributes (e.g. creaminess, smoothness, thickness and flowability) of food emulsions are directly related to their rheological properties. Furthermore, the shelf-life of many food emulsions depends on rheological characteristics of the phases; for example, the rate of creaming of milk depends on the viscosity of the milk plasma (McClements 1999). The content of lactose or whey proteins in milk has little influence on the viscosity of milk; fat content has a major influence, although by far the greatest influence is that of the casein content (McCarthy 2003). The influence of milk fat globules on the rheological properties of milk and cream, in particular the viscosity, will be discussed in this section.

If fat globules are present as separate particles, the fat content is ≤40% and the milk fat completely molten, milk and cream behave as Newtonian fluids at intermediate and at high shear rates (Phipps 1969; McCarthy 2003), i.e. its viscosity is not influenced by shear rate ($\tau = \eta \times \gamma$, where τ is the shear stress [*Pa*], η is the viscosity [*Pa s*] and γ is the shear rate [s^{-1}]). For a Newtonian fluid, the Eilers equation (Eilers 1941) is generally obeyed (Walstra 1995):

$$\eta = \eta_0 \left[1 + \frac{1.25\varphi}{1 - \varphi / \varphi_{max}} \right]^2$$

where η is the viscosity of the product, η_0 is the viscosity of the continuous phase, ϕ is the volume fraction of spherical particles and ϕ_{max} is the hypothetical volume fraction when the particles are in the closest possible packing arrangement. Van Vliet and Walstra (1980) showed that, if ϕ is taken as ϕ_{fat} + 0.16 (ϕ for skim milk ≈ 0.16), $\eta_0 = 1.02\,\eta_{water}$ and $\phi_{max} = 0.88$, values calculated from the Eilers equation are in good agreement with the experimental data from Phipps (1969).

For studying the viscosity of particle suspensions, a number of other models (e.g. the Einstein model, the Batchelor model and the Krieger-Dougherty model) have also been proposed and applied over the years (e.g. Walstra 2003; Willenbacher and Georgieva 2013). However, the Einstein model is relevant only for infinite dilutions and assumes a linear relationship between concentration and viscosity. The Batchelor model addresses some issues of the Einstein model by including a factor accounting for particle interactions, but still applies only to rather dilute systems. A more useful model is the Krieger-Dougherty model, which is not only applicable to dilute but also to concentrated particle suspensions; it states that

$$\eta = \eta_{serum} \left(1 - \frac{\varphi}{\varphi_{max}} \right)^{-[\eta]\varphi_{max}}$$

where η and η_{serum} are the viscosity of the sample and of the serum phase of the sample, φ is the volume fraction of particles, φ_{max} is the maximum volume fraction of particles on random packing and [η] is the intrinsic viscosity. For monodisperse spherical particles, φ_{max} = 0.64, and this value increases with polydispersity of the particle size distribution. In addition, for systems with bi- or multi-modal particle size distributions, φ_{max} may become as high as 0.85. For solid hard spheres, [η] = 2.5, but on swelling of particles and on deviations from spherical shape, [η] increases. The aspects are of particular importance when flocculation or aggregation of emulsion droplets occurs, as the fractal geometry of the flocs or aggregates formed essentially determines [η].

At a temperature < 40 °C, milk does not behave as a Newtonion fluid; the deviation from Newtonian flow is larger at a temperature further below 40 °C (Randhahn 1973; Wayne and

Shoemaker 1988; Kristensen *et al.* 1997). Rather, milk viscosity decreases with increasing shear rate at a temperature below 40 °C (Randhahn 1973), which Mulder and Walstra (1974) suggested may be due to the disruption of clusters of coalesced milk fat globules which were formed as a result of cold agglutination.

Rheological properties of milk and cream are influenced by various processes, e.g. heat treatment, cooling or homogenization. McClements (1999) reported that the main factors which determine the rheological properties of emulsions can be divided into five groups:

5.17.1 Dispersed Phase Volume Fraction

With an increase in dispersed-phase volume fraction, the viscosity of an emulsion increases. This increase in viscosity is linear at a low droplet concentration (McClements 1999); the viscosity of an emulsion of milk fat globules in milk plasma increases linearly with fat content up to 30% (Bakshi and Smith 1984; Kyazze and Starov 2004), whereas the viscosity of low-fat milk (≤2.0% fat) increases in a near-linear fashion with fat content (Phillips *et al.* 1995). However, above a certain volume fraction of the dispersed phase, the droplets in emulsions are packed so closely that flow is impaired, giving the emulsion a gel-like character (McClements 1999). For instance, the viscosity of cream increases very significantly with increasing fat content when the fat content is >50% (Prentice 1968; Mulder and Walstra 1974).

5.17.2 Rheology of the Component Phases

The viscosity of an emulsion is directly proportional to the viscosity of the continuous phase; any alteration in the rheological properties of the continuous phase results in a corresponding alteration in the rheology of the whole emulsion (McClements 1999). The rheological properties of the dispersed phase, i.e. the milk fat globules in the case of milk and cream, have only a minor influence on the rheology of the emulsion (Walstra 1996; McClements 1999). This is illustrated well by the influence of temperature on milk viscosity. A decrease in temperature, particularly below ambient temperature, results in an increase in milk viscosity (Randhahn 1973; Prentice 1992; Kristensen *et al.* 1997); even though considerable crystallization of fat occurs in the globules on cooling, changing the rheological properties of the fat, increases in milk viscosity are dominated by changes in the milk serum, primarily increases in hydration of casein micelles (Prentice 1992).

5.17.3 Droplet Size

The viscosity of dilute emulsions is independent of the size of its droplets when long-range attractive and repulsive colloidal interactions between droplets are negligible, and the thickness of the surface layer is small compared to the droplet diameter; however, when long-range colloidal interactions are present and/or the thickness of the surface layer is a significant proportion of particle diameter, particle size has a considerable effect on emulsion viscosity. The viscosity of an emulsion increases with increasing thickness of the surface layer, due to an increase in the effective volume fraction of the dispersed phase (Pal 1996). Homogenization of milk leads to an increase in milk viscosity (Whitnah *et al.* 1956; Randhahn 1973), which Prentice (1992) suggested is due to the adsorption of casein particles on the fat globule surface, thereby increasing the effective volume fraction of the dispersed phase. The formation of homogenization clusters also leads to increases in viscosity of the product, as discussed in Section 5.13.

5.17.4 Colloidal Interactions

Colloidal interactions between emulsion droplets play a primary role in determining emulsion rheology. If attractive interactions predominate over repulsive interactions flocculation can occur, which leads to an increase in the effective volume

fraction of the dispersed phase and thus increases viscosity. As discussed in Sections 5.5 and 5.6, flocculation of natural milk fat globules does not occur. However, clustering of milk fat globules due to cold agglutination increases the effective volume fraction of the milk fat globules, thereby increasing viscosity (Prentice 1992).

5.17.5 Particle Charge Interactions

As discussed earlier (Section 5.5), the charge on an emulsion droplet can influence the rheological properties of the emulsion. First, the charge determines whether droplets tend to aggregate (see Section 5.6). Furthermore, droplet charge can also influence the rheological properties of the emulsion through the primary electroviscous effect (Pal 1996); movement of a charged droplet through a fluid results in distortion of the surrounding cloud of counter-ions, which causes an attraction between the charge on the droplet and the charge associated with the 'cloud' of counter-ions that lags slightly behind it. This attraction opposes the movement of droplets and thus increases the viscosity of the emulsion because more energy is needed to cause droplets to move (Pal 1996; McClements 1999). This mechanism may be involved in the increase in viscosity observed on homogenization of milk (Whitnah *et al.* 1956; Randhahn 1973). The fat globules in homogenized milk have a higher net-negative surface charge than those in unhomogenized milk (Dalgleish 1984; Michalski *et al.* 2001b), which may thus, through the primary electroviscous effect, result in increased milk viscosity, although this has to be confirmed with experimental data.

Overall, it is apparent that although fat globules are not the predominant milk constituent affecting the rheological properties of milk and cream, they still exhibit a considerable influence.

5.18 Conclusions

Whole milk or cream are emulsions of milk fat globules in milk plasma. The physico-chemical properties of the milk fat globules affect many properties of liquid dairy products such as milk and cream, and as such should always be considered when studying the stability of liquid dairy products. The physico-chemical properties of the milk fat globules can also be influenced through a wide variety of common industrial processes, as described in this chapter, and once chosen and controlled carefully, these processes can be efficiently used to give products desired characteristics, e.g. in terms of storage stability or rheological properties. Although much is known concerning physico-chemical properties of the milk fat globules, and instability of dairy emulsions can be controlled well with the current state of knowledge, gathering further information concerning the physical chemistry of milk fat globules, and the underlying fundamental problems, remains crucial. Pursuit of fundamental problems often leads to good results, sometimes in unexpected ways. Thus, it is important to continue the constant enhancement of our understanding of areas such as those described in this chapter.

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