# 5

# Physical Chemistry of Milk Fat Globules

**T. Huppertz and A.L. Kelly**

## 5.1. Introduction

The presence of fat globules in milk was first reported by Van Leeuwenhoek in 1674, after 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, or contribute to, 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 can influence enzymatic processes, such as lipolysis. 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.

In this Chapter we will describe important aspects of the physical and colloidal chemistry of milk fat globules, 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 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 $>15\,\mu m$ in diameter; bovine milk typically contains $>10^{10}$ fat

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globules per mL. The composition of the fat in the milk fat globules has been discussed in detail in Chapters 1 and 2. Fat globules in milk are naturally emulsified by a complex layer of surface material, the milk fat globule membrane (MFGM), which accounts for 2–6% of the mass of the fat globules (Keenan and Mather, 2002; Chapter 4). The composition of the MFGM (Table 5.1) is closer to that of a cell membrane, from which it largely derives, 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 fraction, including alkaline phosphatase and Xanthine oxidoreductase, which make up a significant portion of the membrane protein, as well as monoglycerides and free fatty acids. For a more detailed description of the secretion of milk fat globules and the 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 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 prepared 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). However, in more recent years, methods such as dynamic light scattering (Robin and Paquin, 1991; Dalgleish and Hallett, 1995), low-angle laser light scattering (Muir *et al.*, 1991; Michalski *et al.*, 2001a), Coulter counting (Hillbrick *et al.*, 1998), ultrasound (Miles *et al.*, 1990) and electroacoutics (Wade *et al.*, 1996; Wade and Beattie, 1997), have been applied to obtain accurate and reproducible results. Many of the measurement techniques mentioned generate complex primary data,

**Table 5.1.** Estimated average composition of milk fat globule membrane (adapted from Walstra *et al.*, 1999)

| Component | mg/100 g fat globules | mg/m$^2$ 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 | +[a] | + | ? |
| Water | + | + | ? |
| Carotenoids and vitamin A | 0.04 | $2 \times 10^{-4}$ | – |
| Iron | 0.3 | $1.5 \times 10^{-3}$ | + |
| Copper | 0.01 | $5 \times 10^{-5}$ | – |
| *Total* | >2570 | >12.8 | 100 |

[a] + indicates component is present but concentration has not been determined precisely.

which must be processed using specific algorithms or programmes to yield useful data for milk fat globule size.

Avoidance of interference of other milk constituents with measurements is also of importance; for example, dissociation of casein micelles by calcium-chelating agents, such as trisodium citrate or ethylenediamine tetraacetic acid (EDTA), may used to avoid interference of the micelles in particle size measurement, while clusters of fat globules can be disrupted by adding a low level of sodium dodecyl sulphate (SDS).

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, $\Delta d$), 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 fraction, 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 multiplication of 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 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 ($S_n$) is equal to:

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

where $N_i$ is the number of 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:

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

where $\phi$ is the volume fraction of milk fat and $d_{3,2}$ is volume surface-weighted mean diameter. A typical mean value for $A$ is $\sim 2.2\,m^2/g$ fat in unhomogenized bovine milk (range 1.9–2.5 $m^2/g$ fat; Walstra, 1969b).

Average milk fat globule size decreases with advancing stage of 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).

**Table 5.2.** Parameters describing the size distribution of milk fat globules 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[a] |
| Volume mean diameter: $d_v = d_{3,0} = (S_3/S_0)^{1/3}$ | 1.5–2.1 | 1.8[a] |
| Volume surface-weighted mean diameter: $d_{vs} = d_{3,2} = S_3/S_2$ | 2.5–4.6 | 3.34[a] |
| Volume moment-weighted mean diameter $d_{vm} = d_{4,3} = S_4/S_3$ | | 3.53[b] |

[a] Walstra (1969a).
[b] From Huppertz *et al.* (2003).

**Table 5.3.** Fat globule size (volume surface-weighted mean diameter, $d_{vs}$) 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) |
| Goat | 4.89 | Mehaia (1995) |
| | 2.76 | Attaie and Richter (2000) |
| Camel | 4.40 | Farah and Rüegg (1991) |
| | 4.40 | Mehaia (1995) |
| Human milk colostrum | 1.74 | Rüegg and Blanc (1981) |
| Human milk transitional | 1.84 | Rüegg and Blanc (1981) |
| Human milk mature | 4.10 | Rüegg and Blanc (1981) |
| Ewe | 5.00 | Gervilla *et al.* (2001) |
| | 4.95 | Mehaia (1995) |

As illustrated in Table 5.3, considerable interspecies differences in milk fat globule size have been reported. Compared to bovine milk, $d_{3,2}$ is lower for fat globules in caprine (Mehaia, 1995; Attaie and Richter, 2000) and ovine milk (Mehaia, 1995), but is similar in mature human (Rüegg and Blanc, 1981) and camel (Farah and Rüegg, 1991) milk.

Milk fat globule size can be influenced by several treatments applied to milk. Homogenization, as discussed in Section 5.12, is a mechanical treatment classically applied by milk processors to reduce fat globule size and prevent creaming during the 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 volume surface-weighted mean diameter $d_{vs}$, 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.

The composition (i.e., fatty acid composition of the triacylglycerols) of fat globules also varies with globule size. Timmen and Patton (1988) found less $C_{4:0} - C_{10:0}$ and $C_{18:0}$ and more $C_{18:1}$ acids 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 the opposite effect is observed in spring milk; in winter, the levels of $C_{14:0}$ and $C_{16:0}$ acids decrease 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 $C_{18:0}$ acids in small fat globules compared to large globules (Briard *et al*., 2003). The higher levels of $C_{18:1}$ and $C_{18:2}$ acids in small than in 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 larger proportion of $C_{18:1}$ and $C_{18:2}$ acids 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 the average fat globule size in milk and the concentration of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$ and $C_{18:1}$ 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 (Chapter 7; Mulder and Walstra, 1974; Walstra *et al*., 1995). 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 behavior. The crystallize status affects many properties of the milk fat globules (e.g., their susceptibility to partial coalescence and their resistance to disruption). Some reasons why crystallization of fat in globules may differ from that in bulk milk fat as proposed by Mulder and Walstra (1974) are:

- 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.
- Not all fat globules may 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 super-cooling 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, deeper super-cooling was required for crystallization of milk fat inside the globules.
- The surface layer of the fat globule may act as a catalytic impurity (e.g., when it contains mono-glycerides or di-glycerides 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 by other microscopy techniques. Noda and Yamamoto (1994) reported that it is thermodynamically more favorable 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.
- 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.
- The dispersed state has a considerable effect on fat crystal polymorphism. Lopez *et al.* (2000, 2001c) observed that crystallization in milk fat globules is more disordered than in bulk fat. On slow cooling, milk fat crystallizes in the $\alpha$ form in cream (Lopez *et al.*, 2001a), whereas in anhydrous milk fat, it crystallizes first in the $\beta'$ form and then in the $\alpha$ form (Lopez *et al.*, 2001b). Rapid cooling of cream or anhydrous milk fat from 60 to 4°C leads to the formation of $\alpha$ crystalline structures, which transformed into $\beta'$ structures

rapidly in anhydrous milk fat but 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 as the size of the globules decreased.

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 fat within the droplets 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 the crystallization behavior of globular milk fat may offer opportunities to overcome the necessity for super-cooling to obtain a particular level of crystalline fat.

## 5.5. Colloidal Interactions

Colloidal interactions form the basis 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 emulsions 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 distance, $h$ (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 or 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 system 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 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. The approach of two identically charged surfaces leads to an increase in the counter-ion concentration between the surfaces, which generates a repulsive force, as a result of increased osmotic pressure (Dickinson and Stainsby, 1988; Bergenståhl and Claesson, 1990, 1997). The surface charge, as estimated by the zeta-potential, is $\sim -13$ to $-14$ mV for unhomogenized milk fat globules (Jack and Dahle, 1937; Payens, 1963, 1964; Michalski *et al.*, 2001b) and $\sim -20$ mV after homogenization (Wade and Beattie, 1997; Michalski *et al.*, 2001b). Dalgleish (1984) reported slightly lower values for the zeta-potential (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 thus 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 unfavorable reduction in entropy associated

with confining the chains between surfaces (Tadros and Vincent, 1983; Isrealachvili, 1992; Walstra, 1996, 2003). In the case of milk fat globules, steric repulsion is provided by glycoproteins in 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).

## 5.6. Physical Instability of Emulsions

The stability of an emulsion denotes its ability to resist changes in its properties over time (i.e., 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, if it does occur. All food emulsions are thermodynamically unstable and thus will break down if left long enough.

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 considered in this chapter; for more information, the reader is referred to Chapters 11 and 12. Physical instability results in an alteration in the spatial distribution or structural organization of the globules (i.e., the dispersed phase of the emulsion). A number of important mechanisms responsible for the physical instability of emulsions, as depicted in Figure 5.1, 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*). Creaming of milk will be discussed in more detail in Section 5.7.

*Droplet aggregation* is said to occur when droplets stay together for a time much longer than they would in the absence of colloidal interactions, (i.e., than can be accounted for by collisions due to Brownian motion) (Walstra, 2003). Mechanisms responsible for the physical instability of droplets through aggregation are flocculation, coalescence or partial coalescence.

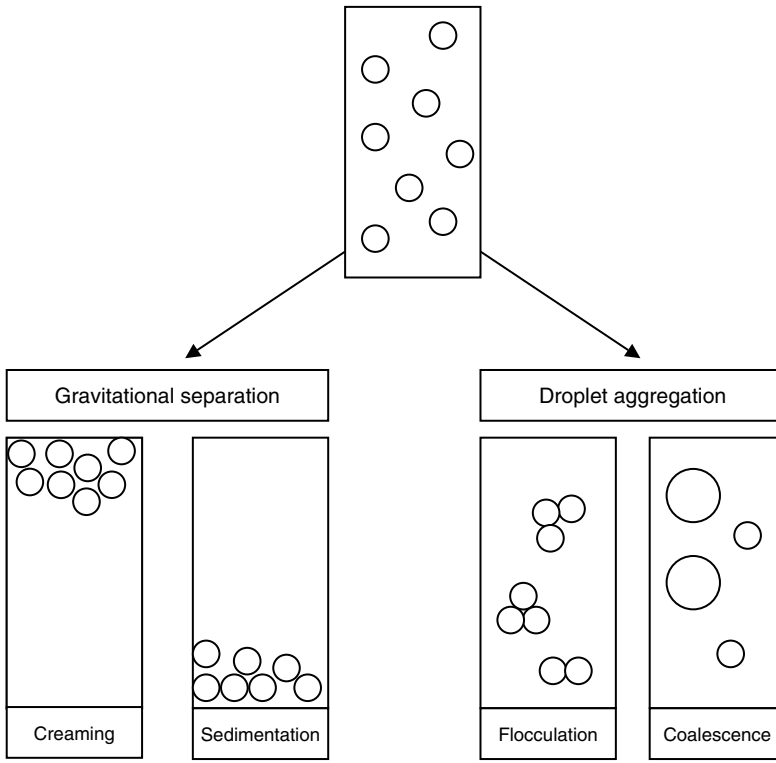


**Figure 5.1.** Schematic overview of types of instability of emulsions.

- *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 collisions; 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.
- *Coalescence* is the process in 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 for 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.

## 5.7. Separation of Milk

Because milk fat has a lower density than milk plasma, it tends to rise under the influence of a gravitational or centrifugal force. For perfect spheres, the rate of rise, $v$, is given by Stokes' Law:

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

where $a$ is the acceleration due to gravitational or centrifugal force, $\rho_p$ is the mass density of the plasma, $\rho_f$ is the mass density of the fat, $d$ is the diameter of the fat globule and $\eta_p$ is the viscosity of the plasma. For gravity creaming, $a = g \approx 9.8\,\mathrm{m/s^2}$. For creaming under centrifugal force, $a = R^2\omega$ where $R$ is the effective centrifugal radius and $\omega$ 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:

- Globules must be perfect and homogeneous spheres;
- Other particles in the plasma must be considerably smaller than the fat globules;
- Brownian motion must be small compared to the rate of rise;
- Counter-flow of liquid due to globule movement must be negligible;
- Mutual interaction between globules must be absent.

Troy and Sharp (1928) found that, in milk highly diluted with milk plasma, the 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):

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

This parameter shows a linear relationship with 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 accelerated significantly 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 5.2. The objective of centrifugal separation is to achieve the lowest possible fat content in the skimmed milk, while removing the fat as a greatly (~tenfold) concentrated 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 an elevated temperature, as the factor $(\rho_p - \rho_f)/\eta_p$ increases more then 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., $< 1\,\mu m$), which are the most difficult to separate, and the level of non-globular fat.

## 5.8. Cold Agglutination

When bovine milk is stored in the cold under quiescent conditions, a cream layer will form due to the rise of milk fat globules. However, the rate of rise of the milk fat globules is considerably faster than can be accounted for by Stokes' law for individual globules (Troy and Sharp, 1928). This is due to the fact that milk fat globules tend to rise in large clusters, which rise at a considerably higher rate than individual globules. Merthens (1933b) reported that addition of colostrum to milk enhanced creaming

**Figure. 5.2.** Principle of operation of a centrifugal milk separator. Milk enters at the bottom of the unit and separates into heavier shim (dark grey) and lighter cream (pale grey) fractions which are recovered at the top of the separator (Reproduced with permission from *Dairy Processing Handbook*, Tetra Pak Processing Systems AB, Lund, Sweden, 1995).

considerably, suggesting that one or more agents enriched in colostrum promoted creaming. Detailed studies, including an extensive survey of older work in this area, were reviewed by Dunkley and Sommer (1944). 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 reconstituted 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 (''Samuelson effect''). Homogenization at a pressure as low as 1 MPa, or even mild shearing, impairs the agglutinating tendency of skimmed milk (Walstra, 1980). The globulin 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 globulin 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).

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 raw 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-induced or HP-induced increases in creaming of milk has not yet been described.

Heating milk at a temperature $>62°C$ (Orla-Jensen *et al.*, 1929; Rowland, 1937), or treating it at a pressure $\geq 400$ 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 interactions of caseins or whey proteins with the MFGM may also prevent cold agglutination (Van Boekel and Walstra, 1995). Addition of colostral globulin to heated milk restores its creaming capacity (Keynon and Jenness, 1958).

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), ovine (Fahmi *et al.*, 1956) buffalo (Fahmi, 1951; Abo-Elnaga, 1966; Wahba *et al.*, 1977; Ismail *et al.*, 1972), camel (Farah and Rüegg, 1991) or carabao (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.

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 milk cream and skimmed milk from bovine and camel milk, respectively (Farah and Rüegg, 1991). The poor creaming properties of buffalo milk were attributed to its poor clustering ability (Abo-Elnaga *et al.*, 1966). 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 cows' cream and skimmed carabao milk, whereas a mixture of agglutinin-rich bovine skimmed milk and carabaos' cream creamed extensively. Further experiments showed that carabao 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.

## 5.9. 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 membrane of the droplets. Coalescence moves an emulsion towards a thermodynamically stable state, because it reduces 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 developed. 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 membrane to rupture. Coalescence may be induced by collisions or by prolonged contact between the emulsion droplets. Collision-induced coalescence can be 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). The probability of film rupture is greater if the interfacial tension, $\gamma$, 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.

Indirect UHT treatment can cause aggregation of fat globules, due to partial heat 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 on cooling.

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 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., whipped cream or ice cream). Walstra *et al*. (1999) reported that the following factors affect the rate of partial coalescence in milk:

- *Application of a velocity gradient* or 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).
- An increased *fat content* increases the rate of clumping.
- The *proportion of solid fat* is crucial. Partial coalescence can not 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).
- *Fat globule size* also influences the extent of partial coalescence. Larger globules are less stable against partial coalescence, due to the fact they have larger fat crystals and the probability of a crystal sticking out far enough is thus higher.
- 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 has not been kept at a sufficiently low temperature, referred to as "bitty cream" or "broken cream." Bacterial phospholipases can hydrolyse

up to 60% of the phospholipids in 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.

## 5.10. 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 an undefined 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 value, a high calcium content or a low level of caseinate emulsifier, as well as by temperature fluctuations during storage.

Recent studies in 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 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 triacylglycerols 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 are necessary to establish if this mechanism also applies to dairy cream.

## 5.11. 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 transfer of Xanthine oxidoreductase 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 induces 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 pressure differences in the frozen products developing due to the different expansion coefficients of ice and fat (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 oxidoreductase, 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 if the order of these steps is reversed (Sharma and Dalgleish, 1994). The association of denatured whey proteins, 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 to their displacement by denatured whey proteins during heating (Houlihan *et al.*, 1992), although the exact mechanism is thus far unknown (Lee and Sherbon, 2002). The effects of heat treatment and homogenization on fat globules are compared schematically in Figure 5.3. 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 of milk can also reduce the triacylglycerol content of the MFGM (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 MFGM phospholipids content 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 membranes 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, as discussed in Chapters 10 and 13.

## 5.12. 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. Shearing, cavitation or turbulence, 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

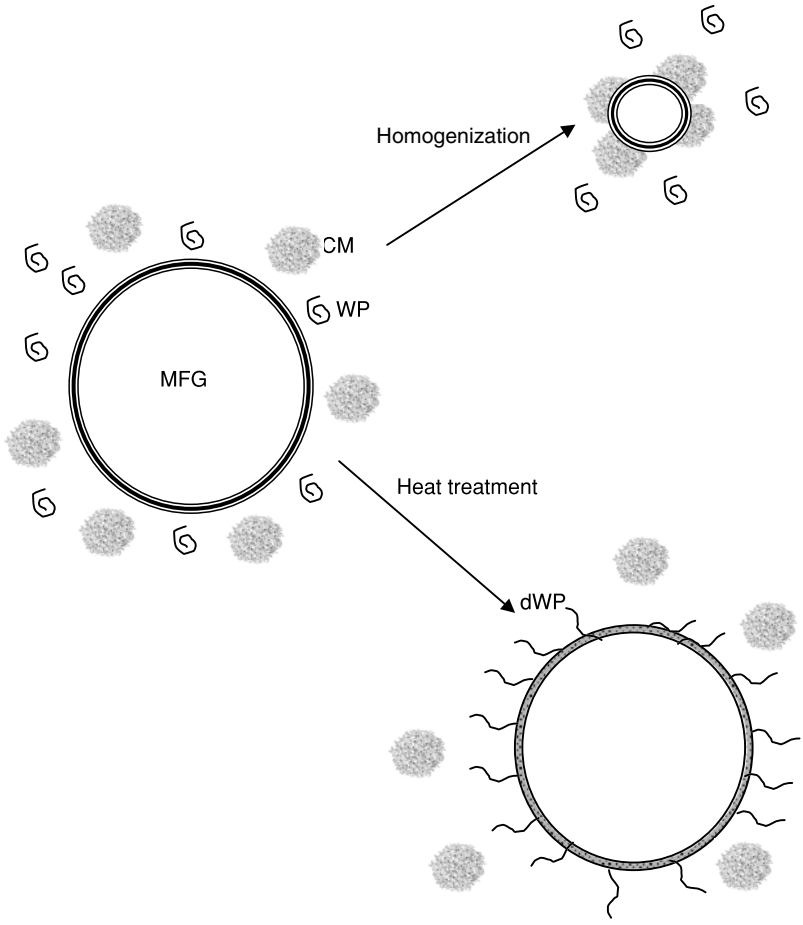


**Figure 5.3.** Schematic illustration of the relative effects of heating and homogenization on fat globules in milk. MFG = milk fat globule, CM = casein micelle, WP = whey protein, dWP = denatured whey protein.

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 (Walstra, 2003):

$$P_L = 2\gamma/R$$

where $\gamma$ is the interfacial tension and R is the radius of the droplet. The value for $\gamma$ 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, $\eta S$ (where $\eta$ 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 ($\sim$40°C) milk (in which the fat is in a liquid state; homogenization is less effective when the fat is partially solid) is passed through a small orifice at a pressure of 10–20 MPa. 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 $< 2\,\mu$m in diameter; Figure 5.4). The principle of operation of a valve homogenizer is shown in Figure 5.5. The extent of the reduction depends on a number of factors, including the geometry of the homogenizer valve 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:

$$\log d_{vs} = k - 0.6 \log p_h$$

where the constant, k, 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 the occurence of pressure fluctuations under turbulent flow conditions (Walstra, 1995). Creaming of fat globules in homogenized milk is considerably slower than 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 thereby decreases the rate of rising, as well as through inactivation of the homogenization-labile component involved in cold agglutination.

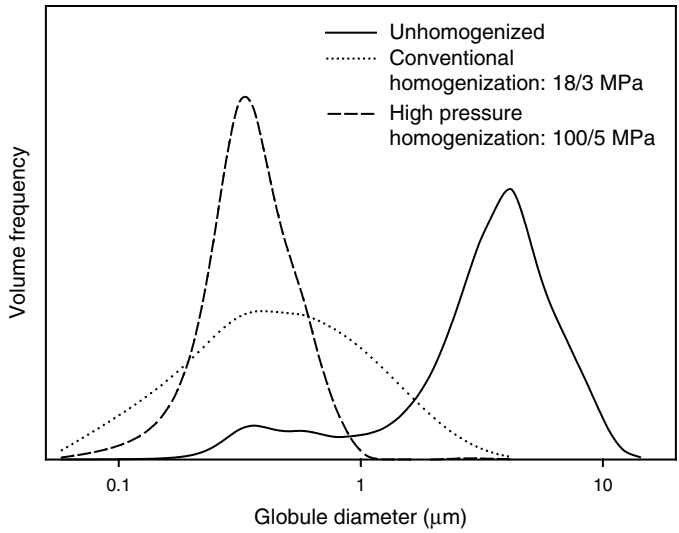


**Figure. 5.4.** Effect of two stage conventional (18/3 MPa) or high pressure (100/5 MPa) homogenization on the volume frequency distribution of fat globules bovine milk.

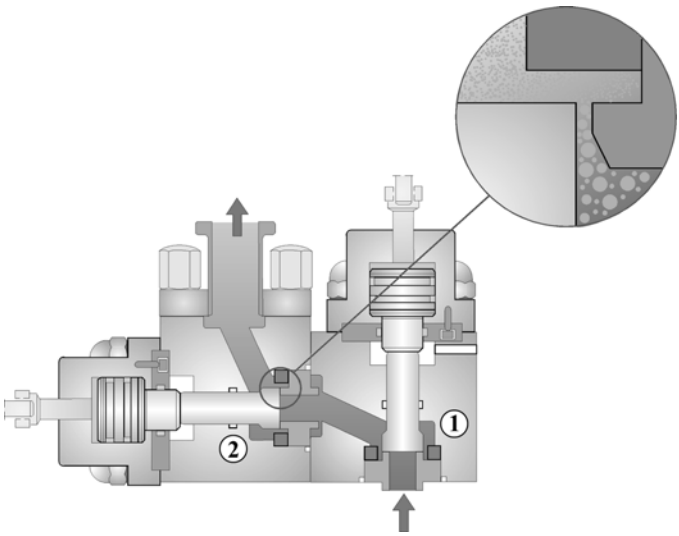


**Figure. 5.5.** Principle of operation of a typical two-stage homogenizer for liquid milk, indicating the first (1) and second stages (2). (Reproduced with permission from *Dairy Processing Handbook*, Tetra Pak Processing Systems AB, Lund, Sweden, 1995).

Michalski *et al.* (2002) studied the types of particles in homogenized milk and identified three classes:

- Disrupted globules covered mainly by caseins and some of the original MFGM material (the surface layer of fat globules in homogenized milk is discussed in Section 5.13).
- A population of very small native fat globules of around 100 nm in diameter that appeared unaffected by homogenization.
- Small newly formed lipid–protein complexes with a new casein-rich membrane, a type of particle also produced by ultrsonication or pumping. The presence of small fat globules that are apparently unaffected by homogenization may be explained by the fact that they are 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 5.4), such as high pressure homogenizers (Hayes and Kelly, 2003a; Thiebaud *et al.*, 2003) and microfluidisers (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. In a microfluidiser, forces are generated by impinging high-velocity fluid jets (Paquin, 1999). 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).

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

## 5.13. 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 proteins also becoming attached if homogenization is combined with heat treatment (Darling and Butcher, 1978; Garcia-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 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 rise 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-coagulation, acid-coagulation 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 yogurt, 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 neighboring 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 (e.g., 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 $>\sim$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 microfluidiser, some fat clustering occurred at 35 MPa, but none 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 flavor defect results from conversion of methionine to methional, catalyzed by riboflavin activated by photo-oxidation; however, the exact mechanism through which homogenization influences this process has thus far not been described. However, homogenized milk is less prone to copper-catalyzed 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 throughout milk following homogenization, and are less likely to propagate lipid oxidation (Tarassuk and Koops, 1960).

## 5.14. Milk Fat Globules in Recombined Milk

Altered fat globule surface layers are of considerable importance in recombined milks. 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 the fat globules in recombined milk contains no original MFGM material; the nature of the new membrane is influenced strongly 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 in the membrane is smaller than that in milk (Oortwijn and Walstra, 1982a; Sharma *et al.*, 1996a,b).

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 of surface area of the fat globules in recombined milk is influenced markedly by the form of protein present in the continuous phase (i.e., it is markedly higher when casein micelles are present), than 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 due either 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 adsorption of Tween 20 over casein micelles on the micelle surface. Addition of surfactants before or after recombination also reduces the protein surface load (Oortwijn and Walstra, 1979). Destabilization of casein micelles by reducing the colloidal calcium phosphate content reduces the protein load on the fat globules in recombined milk and alters the proportions of individual caseins on the globule surface (Sharma *et al*., 1996a). Sharma *et al*. (1996b) reported that it is far more difficult to remove κ-casein than $\alpha_s$-casein or β-casein from the fat globule surface in recombined milk, suggesting that part 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 21 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 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 a pH $\leq$ 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 remain free of whey proteins 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 on larger ones (Sharma and Singh, 1999). McCrae *et al*. (1994) reported that heat-induced interactions between whey proteins and casein adsorbed at the fat globule surface promote the heat-induced coagulation of recombined milk. 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).

## 5.15. 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 separated 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 (1995), concluded that these methods have poor repeatability due to their poor robustness (i.e., a 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 favored. 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. For more information on the role of fat in milk powder, see Chapter 13

## 5.16. Influence of Fat Globules on Rheological Properties of Milk and Cream

Rheological properties of emulsions are important 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 milk plasma (McClements, 1999). The content of lactose or the whey proteins in milk influence the viscosity of milk only little; 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 $\leq$40% and the milk fat completely molten, milk and cream behave as Newtonian fluids at intermediate and high shear rates (Phipps, 1969; McCarthy, 2003), {i.e., its viscosity is not influenced by shear rate ($\tau = \eta \times \gamma$, where $\tau$ is the shear stress [Pa], $\eta$ is the viscosity [Pa s] and $\gamma$ is the shear rate[1/s])}. For a Newtonian fluid, Eilers' equation (Eilers, 1941) is generally obeyed (Walstra, 1995):

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

where $\eta$ is the viscosity of the product, $\eta_0$ is the viscosity of the continuous phase, $\phi$ is the volume fraction of spherical particles and $\phi_{\max}$ is the hypothetical volume fraction when the particles are in the closest possible packing arrangement. Van Vliet and Walstra (1980) showed that if $\phi$ is taken as

$\phi_{fat} + 0.16$ ($\phi$ for casein micelles, lactose and whey proteins in skim milk $\approx$ 0.16), $\eta_0 = 1.02\eta_{water}$ and $\phi_{max} = 0.88$, values calculated from Eilers equation are in good agreement with experimental data (Phipps, 1969).

At a temperature <40°C, milk does not behave as a Newtonion fluid; the deviation from Newtonian flow becomes larger as the temperature decreases (Randhahn, 1973; Wayne and Shoemaker, 1988; Kristensen *et al.*, 1997). Viscosity of milk decreases with increasing shear rate at a temperature below 40°C (Randhahn, 1973), which Mulder and Walstra (1974) suggested may be due to disruption of clusters of 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 that determine the rheological properties of emulsions can be divided into five groups:

### 5.16.1. Volume Fraction of the Dispersed Phase

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 ($\leq$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 rapidly with increasing fat content when the fat content is >50% (Prentice, 1968; Mulder & Walstra, 1974).

### 5.16.2. 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 change 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 occurs of fat occurs in the globules on cooling, changing the rheological properties of the fat, increases in milk viscosity are almost completely due to

changes in the milk serum, primarily increases in the hydration of casein micelles (Prentice, 1992).

### 5.16.3 Droplet Size

The viscosity of a dilute emulsion 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.

### 5.16.4. Colloidal Interactions

Colloidal interactions between emulsion droplets play a primary role in determining emulsion rheology. If attractions predominate over repulsive forces, flocculation can occur, which leads to an increase in the effective volume fraction of the dispersed phase and thus increases viscosity (McClements, 1999). Clustering of milk fat globules due to cold agglutination increases the effective volume fraction of the milk fat globules, thereby increasing viscosity (Prentice, 1992).

### 5.16.5. Particle Charge

The charge on an emulsion droplet can influence the rheological properties of the emulsion. Firstly, the charge determines whether droplets tend to aggregate (see Section 5.6). Furthermore, droplet charge can also influence the rheological properties of an 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 charged droplet and the cloud of counterions 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, 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.

## 5.17. Conclusions

Whole milk or cream can be regarded as an emulsion 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 physicochemical properties of the milk fat globules can be influenced through a wide variety of 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 knowledge often leads to good results, sometimes in unexpected ways. Thus, it is important to continue to enhance our understanding of areas such as those described in this chapter.

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