Chapter 14
Role of Differentiated-Size Milk Fat Globules on the Physical Functionality of Dairy-Fat Structured Products

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1 Introduction

Bovine milk fat is one of the most important lipids in the human diet and originally exists as globules dispersed in the milk plasma. The typical size of milk fat globules (MFG) ranges from 0.1 to 15 μm with an average diameter of 4 μm (Walstra, 1995) as illustrated in Fig. 14.1a. The MFG size can be classified into three size fractions, i.e. small (<1 μm), intermediate (1–8 μm) and large (>8 μm) MFG sizes (Michalski, Briard, & Michel, 2001; Walstra & Oortwijn, 1969) with their volume-based percentages of 5%, 80% and 1–2%, respectively. The small size fraction accounts for 80% of the MFG size distribution on the basis of number of globules. Formation of various MFG sizes is governed by processes of assembly, growth and secretion of fat globules in the milk-secreting cells of the mammary gland of mammals (Timmen & Patton, 1988). Generally, MFG origins from the endoplasmic reticulum membranes where tiny intracellular lipid droplets (<0.5 μm) having a triacylglycerol (TAG) core enveloped by a single layer of proteins and polar lipids are generated. These lipid micro-droplets fuse to form bigger droplets, regarded as cytoplasmic lipid droplets, whose droplet-droplet fusion is regulated by specific calcium and protein complexes and fusion-promoting agents, e.g. gangliosides (Valivullah, Bevan, Peat, & Keenan, 1988). The intermediate size MFGs are progressively coated by the plasma membrane when being transported to the apical plasma membrane, resulting in the final tri-layer structure of intact milk fat globule membrane

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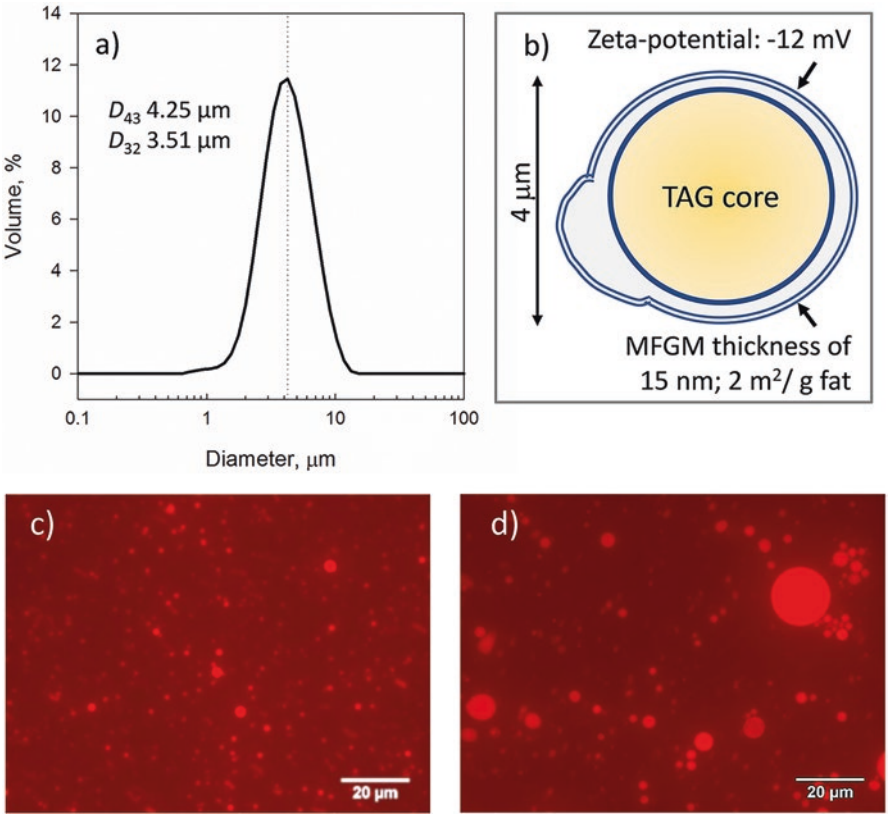


Fig. 14.1 Illustrations of MFG size distribution measured at 25 °C by using laser light scattering technique (**a**) and structure of bovine MFG with TAG core being enveloped by intact MFGM (**b**). Fluorescence microscopic images of small (**c**) and large (**d**) MFG size fractions obtained by two-stage centrifugal separation (scale bar: 20 µm)

(MFGM) (Fig. 14.1b) (Heid & Keenan, 2005). Mean values of specific surface area and zeta-potential of MFG are about 2.2 $m^2\ g^{-1}$ fat and −13.5 mV, respectively (Huppertz & Kelly, 2006; Michalski, Michel, Sainmont, & Briard, 2002). The structure, composition and biochemical functions of MFGM are also MFG-size dependent as discussed elsewhere (Lopez, 2011). It is postulated that the large MFG size is due to post-secretion fusion between large and smaller globules (Timmen & Patton, 1988). Hence, the wide diversity of MFG size in secreted milk is a result of extensive growth of MFG size when being transported from the origins to the secretion sites. Measurement of MFG size can be done using numerous techniques such as microscopy (Ong, Dagastine, Kentish, & Gras, 2010; Precht, 1988; Truong, Morgan, Bansal, Palmer, & Bhandari, 2015), Coulter counting (Cornell & Pallansch, 1966; Walstra & Oortwijn, 1969), laser diffraction, static and dynamic light scattering (McCrae & Lepoetre, 1996; Michalski et al., 2001; Robin & Paquin, 1991), spectroscopy, ultrasound (Miles, Shore, & Langley, 1990), scanning flow cytometry

(Konokhova et al., 2014) and electroacoustics (Wade & Beattie, 1997). Among these measurement methods, laser light scattering techniques is widely adopted to analyse MFG size as well as size distribution. Common expression of mean diameters of MFG size includes number mean (d_n, $D_{1,0}$), volume mean (d_v, $D_{3,0}$), volume surface-weighted mean (d_{vs}, $D_{3,2}$), and volume moment-weighted mean (d_{vm}, $D_{4,3}$) (Fig. 14.1a).

Apart from its physiological role in delivering energy and nutrition to the suckling calf, the wide diversity of size is of industrial interest since each size class may have additional functions. In fact, MFG sizes can be varied among breeds, seasons and lactation stages (Carroll et al., 2006; Mesilati-Stahy & Argov-Argaman, 2014; Wiking, Stagsted, Lennart, & Nielsen, 2004). MFG size distribution can also be altered through milking times and feeding strategy (Avramis, Wang, McBride, Wright, & Hill, 2003; Couvreur, Hurtaud, Marnet, Faverdin, & Peyraud, 2007; Wiking, Nielsen, Bavius, Edvardsson, & Svennersten-Sjaunja, 2006). The effect of breeds and feeding strategy on MFG size has been discussed in **C** of this book. Manipulation of MFG size in post-farm can be achieved by using conventional dairy processing methods such as gravity separation, homogenisation, microfiltration and centrifugation (Dhungana, Truong, Palmer, Bansal, & Bhandari, 2017; Ma & Barbano, 2000; Michalski et al., 2006; Panchal, Truong, Prakash, Bansal, & Bhandari, 2017). It has been reported that differentiated-size MFG possess different chemical composition and physical properties (Lopez et al., 2011; Michalski, Ollivon, Briard, Leconte, & Lopez, 2004). These discrepancies suggest potential industrial strategy of manipulating MFG size in structuring of fat-structured products and developing improved functionalities for dairy fat-containing products such as cream, butter, whipped cream, cheese, yoghurt etc.

The current chapter discusses the importance of MFG size in processing of dairy-fat structured products with the view of potential applications to the production of innovative dairy ingredients and products. It will provide a comprehensive overview of size-dependent variations in physical and chemical properties as well as methodologies to alter the size of both native and emulsified MFGs. Recent studies on utilisation of size-differentiated MFG in dairy-fat structured products will also be highlighted.

2 Variations in Chemical Properties of Differentiated-Size Milk Fat Globules

Bovine milk fat is highly enriched in TAGs, having the average amount of TAGs more than 98% on weight basis. Other primary components accounting for the remaining 2% include monoacylglycerols, diacylglycerols, free fatty acids, and phospholipids (MacGibbon & Taylor, 2006). The bovine milk fat has also been regarded as one of the most chemically complex natural fats existed with numerous types of fatty acids (>400) and TAG species (>200) (Gresti, Bugaut, Maniongui, & Bezard, 1993). As listed in Table 14.1, complex fatty acids such as short-chain

Table 14.1 The amount (%w/w) of principal fatty acids in milk fat (MF) and compositional difference between small and large MFG fractions

Fatty acid	Fatty acid common name	Amount (%w/w)			
		MF[a]	MF-TAGs[b]	Small MFG	Large MFG
$C_{4:0}$	Butyric	2–5	3.6		
$C_{6:0}$	Caproic	1–5	2.4		
$C_{8:0}$	Caprylic	1–3	1.2		
$C_{10:0}$	Capric	2–4	2.9		
$C_{12:0}$	Lauric	2–5	3.5	+4.1%	
$C_{14:0}$	Myristic	8–14	11.2	+5%	
$C_{14:1}$	Myristoleic	0.8	2.0		
$C_{15:0}$	Pentadecanoic	1–2	1.4		
$C_{16:0}$	Palmitic	22–35	29.4	+3.3%	
$C_{16:1}$	Palmitoleic	1–3	3.0	+20.2% (Fauquant et al., 2005; Michalski et al., 2005)	
$C_{17:0}$	Margaric	0.5–1.5	0.8		
$C_{18:0}$	Stearic	9–14	10.6		Enriched (Wiking et al., 2004)
$C_{18:1}$ *cis*	Oleic	20–30	24.2	TAG core: ↑ (Fauquant et al., 2005; Lopez et al., 2011)	↓ (Martini et al., 2006; Timmen & Patton, 1988)
$C_{18:1}$ *trans*		3.9			
$C_{18:2}$	Linoleic	1–3	3.0	TAG core: ↑ (Fauquant et al., 2005; Lopez et al., 2011)	
$C_{18:3}$	Linolenic	0.5–2	0.7[c]		
Conjugated linoleic acids (CLA)					
$C_{18:2}$,cis-9 trans-11	Rumenic acid	75–90 (Bauman, Corl, & Peterson, 2003)		87%; MFG 2.9 μm) (Michalski et al., 2005) ↑; MFG 1.6 μm (Lopez et al., 2011)	82–85% (MFG 4.9–5.7 μm)

[a]Data combined from Kaylegian and Lindsay (1995) and MacGibbon and Taylor (2006)
[b]Data compiled from Wright and Marangoni (2002)
[c]Includes $C_{20:0}$

(C_4–C_8), medium-chain (C_{10}–C_{12}), and long-chain fatty acids (C_{14}–C_{18}) are found in milk lipids with the long-chain fatty acids being abundant (81.9%). The most abundant long-chain fatty acids in milk fat are myristic (8–14%), palmitic (22–35%), stearic (9-14%), and oleic (20–30%) acid (Table 14.1).

Variations in chemical composition of differentiated MFG size fractions are of nutritional interests as they can be used towards the development of nutrient-fortified dairy products. Few attempts have been made to examine the compositional differences across the size range of bovine MFG. Given the differences from sources of milk fat and sampling/analytical methods, a clear tendency in compositional variations between small and large MFGs has not been established. The difficulty is also partly due to overlapping of MFG size ranges being fractionated. Table 14.1 also represents the size-dependent variations in fatty acid composition between small and large MFG size fractions in selected studies.

Regarding individual fatty acids, two separate studies performed by Briard, Leconte, Michel, and Michalski (2003) (small MFG: 1.0–3.3 μm; large MFG: 5.9–7.3 μm) and Lopez et al. (2011) (small MFG: 1.6 μm; large MFG: 6.6 μm) reported that the amount of short-chain fatty acids tended to be unchanged between the small and large MFG fractions. Various investigations on MFG-size dependent changes of saturated, medium-chain fatty acids in secreted cow milk showed that their proportions increased with bigger MFGs (Martini, Cecchi, & Scolozzi, 2006; Mesilati-Stahy, Mida, & Argov-Argaman, 2011; Wiking et al., 2004). Contrast to this finding on varying MFG size by herd management strategy, small and large MFG sizes fractionated by microfiltration technique exhibited a different trend. Fauquant, Briard, Leconte, and Michalski (2005) reported that higher concentrations of lauric, myristic and palmitic acids were found in the small size fraction (2.3–3.7 μm) concerning its large size counterpart (5.2–8.0 μm). Similar tendency, e.g. increasing medium-chain fatty acids in small MFG size fractions, were also reported by Lopez et al. (2011) and Michalski, Briard, and Juaneda (2005). With respect to long-chain fatty acids, the same trend was found across number of studies (Briard et al., 2003; Briard-Bion, Juaneda, Richoux, Guichard, & Lopez, 2008; Fauquant et al., 2005; Michalski et al., 2005; Timmen & Patton, 1988; Wiking et al., 2004) that large MFG size fractions had significantly higher proportion of stearic acid.

Regarding unsaturated fatty acids, the reported differences in the relative amount of palmitoleic (C16:1), oleic (C18:1) and linoleic (C18:2) acids between small and large MFG were contradictory. The small MFG fraction had greater proportion of palmitoleic (Fauquant et al., 2005, Michalski et al., 2005 but less amount of oleic and linoleic acids (Fauquant et al., 2005; Lopez et al., 2011; Wiking et al., 2004). However, other authors (Martini et al., 2006; Timmen & Patton, 1988) reported that the amount of oleic acid was greater in the small MFG fraction. Wiking et al. (2004) also found that palmitoleic acid was enriched with large MFG fraction. Bovine milk fat also contains conjugated linoleic acids (CLA), which have been known to have positive effects on human health such as anti-obesity, anti-carcinogenicity, and anti-diabesity (Belury, 2002). Rumenic acid (C18:2, *cis*-9 *trans*-11), which is a primary component of CLA, was found to be enriched in small MFG fraction (2.9 μm) as compared to its large MFG counterpart (4.9–5.7 μm). Its concentrations in both fractions were 87% and 82–85%, respectively (Michalski et al., 2005). Lopez et al. (2011) also reported similar observation. On the other hand, a few CLA isomers (*trans*-8, *cis*-10, *trans*-11, and *trans*-13) are more concentrated in the larger MFG fraction (Michalski et al., 2005).

3 Dependence of Physical Properties on Milk Fat Globule Sizes

As previously described, milk fat globules have a wide size range (0.1–15 μm) in which, technically, the smallest size class (i.e. 0.1 μm) is about 100 times smaller than its largest counterpart. Considering the small and large MFG fractions at the same bulk volume, the small MFG fraction will have not only a higher total number of globules but also a larger ratio of surface area to volume. Furthermore, there is an increase in curvature with decreasing globule/droplet size. As a result, one can expect no*table* discrepancies in physical properties between the small and large MFG fractions.

Physical Stability Bovine milk has been regarded as an oil-in-water emulsion or a colloidal suspension since its microstructure composes of milk fat globules dispersed in the milk plasma, which contains serum proteins, casein micelles, sugars and minerals (Walstra, Geurts, Noomen, Jellama, & Van Boekel, 1999). Thus, milk fat globules are readily subjected to physical instabilities such as droplet aggregation, creaming, flocculation and partial coalescence, causing alteration in their structural organisation and spatial distribution. Milk is homogenised to reduce the MFG size below 1 μm to enhance the physical stability for shelf-life extension of drinking milk. Typical size of homogenized milk fat globules is about 0.4 μm, which renders adequate physical stability to homogenized milk against creaming phenomenon. Recent work performed on recombined and standardised commercial cream (23–28% w/w fat) covering three MFG size ranges of 0.13, 0.6 and 3.9 μm showed that the sub-micron- (0.6 μm) and nano-sized (0.13 μm) droplets were relatively stable after 1 month of storage at 4 °C (Hussain, Truong, Bansal, & Bhandari, 2017). Since small MFG size is less prone to partial coalescence and creaming, the small MFGs are undesirable for butter making. This is due to the large MFG size that facilitates partial coalescence, improving the efficiency of the churning process (Walstra, Wouters, & Geurts, 2005). The MFG size also affects cold agglutination in raw milk. When raw milk is subjected to cooling, agglutinin causes a precipitation of cryoglobulins in raw milk onto the MFG. The cold agglutination induces aggregation of MFGs, causing formation of large floccules and a subsequent creaming layer. Thus, raw milk contains small MFGs will be more stable against cold agglutination because it needs more agglutinin covering greater surface area with smaller MFG size (Walstra et al., 2005).

Viscosity In general, there is a slight increase in viscosity of milk and dairy emulsions with smaller MFG size regardless of fat content. This is due to smaller droplet size and/or narrow size distribution causing greater colloidal repulsion and monodispersed close packing (Pal, 1996). As such inter-droplet resistance increases, leading to a corresponding increase in the bulk viscosity (Long, Zhao, Zhao, Yang, & Liu, 2012). Kietczewska, Kruk, Czerniewicz, Warminska, and Haponiuk (2003) reported that reduction in MFG size from 2.7 to 1.0 μm in 3.3% fat milk resulted in

higher viscosities (1.8–1.96 mPa s). Regarding dairy emulsion systems, the viscosities of both low- (10%) and high- (36%) fat containing emulsions having various MFG size ranges also increased with smaller emulsion droplet sizes (Lopez, 2005; Truong, Bansal, & Bhandari, 2014). Measurement of apparent viscosities in recombined dairy cream (23% fat) also showed that the apparent viscosities increased from 0.026 to 3.14 Pa s when the droplet size was reduced from micron- (3.9 μm) to sub-micron size range (0.24–0.59 μm) (Hussain et al., 2017).

Crystallisation Properties Crystallisation behaviour and crystalline structure of milk fat in milk, natural/recombined cream and milk fat emulsions are dependent on MFG size (Bugeat et al., 2011; Lopez et al., 2002; Michalski, Ollivon, et al., 2004; Truong et al., 2015; Truong, Bansal, Sharma, Palmer, & Bhandari, 2014). Regarding native MFG, it was reported that the small MFG size fraction (0.93 μm) obtained by microfiltration exhibited delayed crystallisation as compared to its larger MFG size fraction (7.15 μm). The longitudinal packing of milk fat crystals (e.g. double chain length) tended to be more in the large MFG size fraction (Michalski, Ollivon, et al., 2004). In this work, it is also pointed out the difference in crystallisation properties of differentiated-size native MFGs within the size range of 0.93–7.14 μm may be governed by thermal history and cooling rate rather than the direct influence of MFG size (Michalski, Ollivon, et al., 2004). The dependence of crystallisation properties on MFG size is more evident in milk fat emulsion systems where anhydrous or fractionated milk fat is fabricated in the form of dispersed droplets surrounding by aqueous phase containing dairy-based emulsifiers (whey proteins and/or caseins). In these milk fat emulsion systems, crystallisation temperature, solid fat content and melting enthalpy were found to be lower with smaller droplet size (Bugeat et al., 2011; Lopez et al., 2002; Truong, Bansal, Sharma, et al., 2014). These differences are partly attributed to lack of impurities to catalyse numerous amount of smaller droplet size, resulting in limiting the rate of crystallisation. Confinement of milk fat into tiny droplet boundary at nano-size scale seemed to alter the crystalline structure of milk fat. For instance, triple chain length structure of crystalline milk fat was absent in milk fat nanoemulsions (~200 nm) enriched in unsaturated fatty acids as compared to that of micron-sized emulsions contained the same composition (Bugeat et al., 2011; Truong et al., 2015). More information on the effects of MFG size on crystallisation of milk fat can be found in **C** of this book.

Structural Properties of Milk Fat Crystals When milk fat is crystallised into solid-state, MFG size had an impact on arrangement (crystalline structure) and shape (crystal morphology) of milk fat crystals. Depending on the microscopic techniques used and the MFG size range investigated, features of milk fat crystals have been described differently. Classification of milk fat crystals can be based on birefringence of the crystals under polarised light microscopy (Walstra, 1967). As illustrated in Fig. 14.2a, four main types of crystals were visualised in cream, namely O (no birefringence), N (needle-type), L (layer-type) and M (mixed type, e.g. combination of L and N types). Goff (1997) reported that MFG contained needle-type fat crystals within the interior part (Fig. 14.2b). Lopez et al. (2002) used this classification to

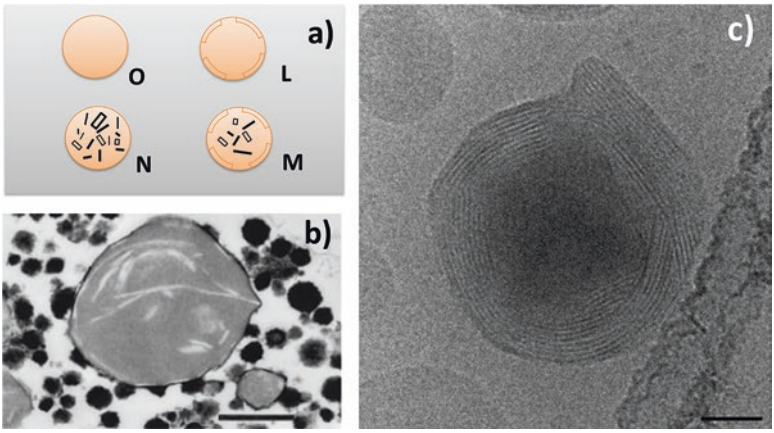


Fig. 14.2 Four main types of milk fat crystals (O, L, M and N) as observed under polarised light microscope (redrawn from Walstra (1967)) (**a**); visualisation of crystal morphologies in MFG as captured by electron microscopic techniques showing needle-type crystals within interior part of MFG (adapted from Goff, 1997); (**b**) and straight orientation of lamellar TAG layers in nano-sized dairy emulsion droplets (adapted from Truong et al., 2015). Scale bars in (**b**) and (**c**) represents 0.5 μm and 50 nm, respectively

describe the size dependence of structural properties in native milk fat globules. Accordingly, the largest MFG exhibited N-type whilst fat crystals were tiny in the smallest MFG fraction which can be deemed as type O. Nevertheless, under slow cooling regime (0.5 °C min^{-1}), the largest and smallest MFG was found to contain type M and spherulite-shaped crystals, respectively (Lopez et al., 2002). Observation of milk fat globules upon crystallisation using freeze-fracturing and electronic microscopy revealed the location of crystal shell as well as the presence of concentric layers of 5 mm in thickness surrounding the globules (Precht, 1988). Usage of high-resolution electron microscopy permits observation of milk fat emulsions in nano-sized range. As shown in Fig. 14.2c, arrangement of TAG lamellar layers at the outer part were of a straight orientation when the milk fat nanoemulsion (200 nm) was cooled at a very slow cooling rate (0.1 °C min^{-1}) (Truong et al., 2015). Truong et al. (2015) also reported the impact of droplet size in milk fat emulsions on morphologies of milk fat crystals. Owing to the physical confinement of nano-sized droplets, it is likely that typical crystals could not bend along the extreme curvature of such tiny droplets. Therefore, straight orientation of TAG lamellar layers is favourable, leading to protrude of fat crystals at the outer part (Truong et al., 2015).

Optical Properties Dispersed particles in milk, particularly fat globules and casein micelles, influences the colour and opacity of milk. Since milk fat has a wide distribution of globule size, it tends to have stronger light scattering than that of casein micelles (Walstra et al., 2005). Thus, estimation of milk fat globule size has been undertaken using spectroturbidimetry in early days (Ashworth, 1951; Goulden,

1958), static light scattering (Michalski et al., 2001), dynamic light scattering (Truong, Bansal, & Bhandari, 2014) and visible and near-infrared (Vis/NIR) spectroscopy (Aernoutsa et al., 2015) in recent days. The latest technique revealed that there is a reduction of the visible and near-infrared bulk scattering coefficient and scattering anisotropy factor with decreasing milk fat globule size obtained by ultrasonic homogenization of raw milk (Aernoutsa et al., 2015). This implies that milk fat globule size influences the optical properties of milk. Since smaller fat globules cause an increase in light scattering, the appearance of milk appears to be whiter with smaller MFGs (Fox & McSweeney, 1998). For example, it is reported that luminosity of sonicated milk (MFG size <1 μm; $L*$: 92.37) is remarkably greater that of raw milk ($L*$: 87.820 (Fox & McSweeney, 1998).

Electrical Conductivity Electrical conductivity of milk varies between 4.0 and 5.5 mS cm^{-1} at ambient temperature. Major components of milk such as casein micelles, milk fat globules, lactose and salts contribute to the electrical conductivity in which contribution from soluble salts is the greatest. Lactose and proteins have an indirect influence on the electrical conductivity by their impact on the viscosity of milk. Fat itself has poor conductivity and the presence of milk fat globules also immobilises the charge-carrying ions; thus, it can be deduced that the electrical resistance of milk will be higher with increasing milk fat content. Few attempts have been made to measure electrical conductivity of milk having smaller fat globule size (Banach, Żywica, & Kiełczewska, 2008; Mabrook & Petty, 2003). It was reported that smaller MFG size caused a slight increase in conductance (5.05 ± 0.03 mS versus 4.85 ± 0.03 mS) in commercial full-fat milk (Mabrook & Petty, 2003). Regarding homogenised milk, larger droplet size within 1.5–5 μm did not exhibit any significant difference in conductance properties. Nevertheless, when milk was homogenised at higher pressure (20 MPa) to obtain smaller MFG size (1.07 μm), the impedance remarkably decreased. The difference in conductivity is attribu*table* to the effect of homogenisation on disintegration of casein micelles rather than any direct influence of MFG size. The shearing force exerted during homogenisation process may cause dissociation and solubilisation of the colloidal calcium phosphate from the micelles to a certain extent, resulting in the imbalance of mineral salts in milk serum. As such, the electrical conductivity is altered.

4 Main Approaches to Manipulate MFG Size

There have been many attempts to manipulate MFG size distribution since different MFG size fractions might have potential benefits in nutritional properties, processability and physical functionality of dairy fat-based products and ingredients. In general, methodologies to vary MFG size can be categorised into three main strategies, e.g. herd management, fractionation on MFG size basis and shear processing, as summarised below. The MFG size reported in this book chapter is based on volume-weighted mean diameter, unless otherwise specified.

4.1 Herd Management

Strategies of herd management involve in selection of different breed of cows, modification of cow feed inputs and milking practice such as milking frequency and milking at various stages of lactation.

It has been known that variation in MFG size naturally exists between individual cows (Couvreur et al., 2007; Logan, Auldist, Greenwood, & Day, 2014) with the span in MFG size can be up to 1 μm (Mulder & Walstra, 1974). The discrepancy in mean MFG size among a single herd of Holstein-Friesian cows ($n = 78$) was noted to be as wide as 2.5–5.7 μm (Logan, Auldist, et al., 2014). There is also a correlation between breed of cows and MFG size. For example, measurement of the average diameter of MFG produced by Italian Friesians, German Friesians and Jersey cows using florescent microscopy showed that their MFG sizes were different (5.3, 4.93 and 4.97 μm, respectively) (Martini, Cecchi, Scolozzi, Leotta, & Verita, 2003). Similar observation was reported by Banks, Clapperton, Muir, and Girdler (1986) and Carroll et al. (2006). That is, MFG size in Jersey milks tended to have a greater number of large fat globules (>5 μm) and wider size distribution that those of Friesians milks.

Alteration of MFG size can also be achieved by modifying dietary supplementation for cow diets. Previous studies on influence of lipid dietary supplements on bovine milk composition found that cows on diets enriched in unsaturated lipids secreted smaller fat globules (fish oil: 1.84 versus 2.31 μm; linseed oil: 4.56 versus 4.73 μm, whole soybean: 4.07 versus 4.18 μm; and fresh grass: 3.65 versus 3.94 μm) with narrower size distribution in their milks (Avramis et al., 2003; Couvreur et al., 2007; Hurtaud, Faucon, Couvreur, & Peyraud, 2010; Lopez et al., 2008). In contrast, MFG size seemed to increase with the addition of saturated fatty acids into the dietary supplements (Wiking, Bjorck, & Nielsen, 2003). The modification of cow diets in this way is thought to be associated with synthesis and secretion of milk fat (Wiking et al., 2003, 2004). Given that MFGM material is available, the addition of a greater amount of dietary fat into feeding inputs induces higher lipid content, resulting in the formation of larger MFG. Nevertheless, it was found that enzyme activity of γ-glutamyl transpeptidase, which is an indicator of production of membrane material, decreased with resultant large MFG size obtained from high lipid diets. This implies that the supply of polar lipids, the main component of membrane material, is limited in secretory cells, in this case, causing the preferential production of greater MFG size (Wiking et al., 2004).

It appears that MFG size changes along lactation stage (Mesilati-Stahy & Argov-Argaman, 2014; Wiking et al., 2004) in response to alteration of energy balance, which is positive towards late lactation. This results in greater availability of MFGM material that can sufficiently cover greater proportion of micro-lipid droplets whereby a larger amount of small fat globules can be formed (Martini, Altomonte, Pesi, Tozzi, & Salari, 2013; Wiking et al., 2006). Walstra et al. (2005) also found that there is a decrease in MFG size (from 4.4 to 2.9 μm) with advancing lactation stage. Few studies reported the influence of milking times and frequency on MFG

size. The increase in milking times facilitates the growth of MFG size with possible shift from medium- to large-sized fat globules (Wiking et al., 2006). Fat globules also tended to grow larger (4.28 ± 0.06 to 4.39 ± 0.07 μm) with increased daily milking frequency from two to four (Wiking et al., 2006). On the other hand, milking system and milk interval did not have a significant influence on MFG size (Abeni, Degano, Calza, Giangiacomo, & Pirlo, 2005).

Taken together, the herd management strategies can alter the native MFG size and size distribution to a range of 3.0–5.3 μm. Given that discrete size fractions are hardly yielded whereas complex supply chain management is required, this approach limits practical application from an industry perspective. Thus, manipulation of MFG size by adaptation of conventional dairy processing technologies may be more feasible. The post-farm strategies such as gravity separation, centrifugation, microfiltration and homogenisation are discussed as followings.

4.2 Fractionation of MFG on Size Basis

Gravity Separation The separation of MFG size fractions on gravity basis resembles the natural creaming process of milk. Owing to the difference in density of serum and fat phases in milk, lower density material (i.e. fat) rises over higher counterpart (i.e. serum/water) according to Stokes' law. This phenomenon also depends on the size of the milk fat globules as well as relative difference in composition between the small and large size fractions of MFG (Ma & Barbano, 2000). Since large MFG contain less mass of MFGM than the volume of fat, their density is lower than that of smaller MFG. Thus, large MFG tended to be risen over smaller MFG. For example, largest MFG size fraction (3.6 μm) was found on the top layer of aged (2–48 h at 4 or 15 °C) milk (3.75%w/w fat content) contained in a vertical column container. This was followed by smaller size fractions of 2.8, 2.3 and 1.2 μm toward to the bottom (Ma & Barbano, 2000). Using similar gravity separation method on standardised cream having 10% w/w fat, it was shown that the gradient of MFG size spans from 2.8 to 4.8 μm (Eden, Dejmek, Lofgren, Paulsson, & Glantz, 2016). The rising speed of MFG is also a function of temperature and aging time. The MFG size (4.5 ± 0.06 μm) remained the same when whole milk was kept at 5 (4.4 ± 0.03 μm) and 40 °C (4.3 ± 0.07 μm). It became larger (4.62 ± 0.05 μm) at the top layer as well as creamed faster when the temperature was at 25 °C. A study performed on gravity-based fractionation of MFG shows that extended aging time (48 h at 4 °C) facilitates greater fractionation efficiency with achievable small MFG size fraction of 1.2 μm (Ma & Barbano, 2000). The gravity separation method can also be done in a two-stage procedure in which the control milk (3.58 μm) was fractionated at 4 °C for 6 h to yield semi-skim milk in the first stage. The second step of separation of semi-skim milk obtained skim and cream portions. MFG size of the latter fraction (3.45 μm) was slightly different to that of control milk (O'Mahony, Auty, & McSweeney, 2005).

Ultrasonic-Assisted Separation Ultrasonication technique utilises primary acoustic radiation force to induce physical destabilisation of milk fat globules whereby the creaming process is facilitated due to formation of floccules and clusters of MFG (Juliano et al., 2011; Leong et al., 2014). There are numerous factors controlling the separation efficacy of MFG using ultrasonication such as physical properties of the MFG (size, solid/liquid proportion of fat) and ultrasonic operating parameters (frequency of ultrasound, processing time, mode of operation, specific energy density input etc.) (Juliano et al., 2011; Leong et al., 2014). A previous study performed on recombined emulsion (3.5% fat) showed that smaller fat droplets (2.7 μm) were more resistant to creaming in comparison to larger droplets in raw milk (4.9 μm) and coarser emulsion (9.3 μm) upon sonication (400 kHz or 1.6 MHz) for 5 min at 35 °C. Based on the discrepancy in MFG size in top and bottom fractions obtained in sonicated natural whole milk (4.3–4.5 μm), it was found that the milk pre-cooled to 5 °C had the lowest differentiation (4.39 and 4.44 μm). The separation efficacy improved with broadest discrepancy in MFG size pre-heated at 25 °C (4.0–4.9 μm) (Leong, Juliano, et al., 2014). Possible explanations for this observation could be due to alteration of immunoglobulins in sonicated milks and/or associated change in the solid/liquid ratio of the milk fat, impacting the formation of floccules of MFGs. Use of higher frequency ultrasound results in greater separation efficacy of MFG. Sonication of natural whole milk at 1 MHz appears to yield greater differentiation in MFG size fractions at the top (4.9 μm) and bottom (4.0 μm) than those obtained with 600 kHz sonication (4.7 and 4.4 μm, respectively) (Leong, Juliano, et al., 2014). An attempt has also been made to further improve ultrasonic separation efficacy with multi-stage ultrasonic fractionation. The smallest achievable size was about 3.38 μm, which was 0.9 μm different to the MFG size of the original milk (4.28 μm) (Leong et al., 2016). The ultrasound-assisted separation of MFG is fully covered in Chap. 18.

Centrifugation Centrifugation is a well-established method to concentrate MFG in commercial manufacture of various dairy-fat based products such as skim milk, cream and butter. Similar to the naturally occurring gravitation separation method, the centrifugation method is also based on a density basis but with 6500-fold faster sedimentation velocity (TetraPak, 2009). As compared to the gravity separation method, a larger differentiation in small (2.5–3.0 μm) and large (5.0 μm) MFG size fractions can be achieved with mild centrifugation at 150 × *g* (Logan et al., 2014). A two-step centrifugal method has been developed aimed at fractionating more discrete MFG size fractions (Timmen & Patton, 1988). Timmen and Patton (1988) reported that skim and cream portions obtained from the two-step centrifugal method enriched in small MFG (1.02–1.77 μm) and large MFG (2.76–3.33 μm). There are few attempts to modify the cream separators to obtain more discrete MFG size fractions. Eden et al. (2016) modified the geometry of the bowl disc and used only four discs to centrifuge standardised milk (4% w/w fat) at 1200 × *g*, obtaining the skim and cream portions. The fractionated cream was further concentrated using an unmodified cream separator at 5300 × *g*. The processing temperature of both fractionation and concentration stages was 55 °C. The MFG size of the resultant creams (35.8–44.1% w/w fat) was varied about 0.5 μm from the original milk (4.3 μm) in the range of 4.1–4.9 ± 0.1 μm. Dhungana et al. (2017) reported that

larger differentiation (2.9 μm) in MFG size fractions (1.35–4.28 μm) of fractionated creams (33–65% w/w fat) can be achieved when using a novel two-stage centrifugal fractionation method. This method utilised a commercial cream separator as a continuous centrifuge in the first stage by removing all the cones from separating disc. Normal set up of commercial cream separator (no removal of the cones) was employed for the second stage to concentrate the skim and cream fractions obtained in the previous step. Figure 14.1c and d represent microscopic images of small and large MFG fractions in native dairy creams obtained by this two-stage centrifugal fractionation method (unpublished data).

Microfiltration Microfiltration is a membrane processing technique that can fractionate MFG size effectively (Michalski et al., 2006). Utilisation of different membrane pore sizes (2–12 μm) and operating parameters of membrane processing (permeate flux, tangential shear stress, volume reduction factor etc.), one can obtain discrete MFG size classes as small as 0.9–3.3 μm in permeate and much larger MFG size in retentate (5–7.5 μm) from the original milk of 4.2 μm (Michalski et al., 2006). The microfiltration technique was found to maintain the integrity of fat globules without damage of MFG owing to shear or cavitation as reflected by unchanged zeta-potential values of the resultant MFG size fractions (Michalski, Michel, et al., 2002). However, this process is susceptible to membrane fouling (Michalski et al., 2006).

Up to date, among available methods for fractionating native MFG on size basis without shear, centrifugation method seems to be commercially viable since it has the potential to be applicable and effective in terms of cost-efficiency due to use of conventional processing equipment, high throughput and size differentiation. It is thought that with further modification, the two-stage centrifugal separation method could provide an efficient mean of producing differentiate-sized creams for improved dairy-fat based products.

4.3 Downsize of Milk Fat Globules with Mechanical Shear

Reduction of MFG size based on mechanical shear processing is a common practice in industrial processing of milks, aiming at improving their physical stability. Depending on homogenisation conditions, the fat globule size can be reduced to the sub-micron range. Owing to rupture forces of cavitation, shear and high turbulence, the MFGs are divided into finer droplets in accompany with disruption of milk fat globule membrane into fragments. Since the globule surface area increases with reduction of MFG size, stabilisation of newly generated droplets in homogenised milks is governed by dairy proteins (whey protein and adsorbed caseins) and the MFGM fragments (Michalski, Cariou, Michel, & Garnier, 2002). As such, the shear-processing can lead to alteration of physicochemical properties of homogenised milks regarding their native counterparts. This is due to differences in the fat globule size as well as properties of emulsified layers in the reformed MFGs. Beyond that, use of high pressure homogenisation can cause dissociation of casein micelles into casein micellar fragments and modification of whey proteins (Lee, Lefèvre, Subirade, & Paquin, 2009). Thus, the reduced MFG size, the emulsified MFG

properties and the modified structures might have either positive or negative impacts on functionalities of dairy products made from homogenised milks.

The three common sizing methods to reduce fat globule size in dairy processing are homogenisation, microfluidisation and ultrasonication. Among them, the homogenisation of milk is a conventional practice in dairy industry in which pressure differences (caused by homogenisation valve and collision with the impact ring) result in rupture forces (i.e. shear, cavitation and high turbulence). Generally, milk is pre-heated to 50–60 °C and subjected to the first- and second-stage valves at 10–30 MPa and 3–5 MPa, respectively. The former aims to break up the fat globules, whereas the latter prevents formation of agglomerates (Walstra et al., 1999). Homogenisation of milks at high pressure in the range of 50–350 MPa has also been investigated. With microfluidisation technique, the fat globule size is reduced by intensive disruption forces generated in an interaction chamber where two streams of product (e.g. milk, cream) collide with each other, causing breaking up of fat globules. It is reported that microfluidisation technique has peak shear rate as high as 10^8–10^9 s^{-1} (Mason, Wilking, Meleson, Chang, & Graves, 2006). In ultrasonication technique, homogenisation of milk using ultrasonic waves generated by acoustic power (typically 20 kHz frequency) creates intense mechanical vibrations. This generates cavitation forces whereby milk fat globules can be disrupted.

Table 14.2 presents selected operating conditions of downsizing of milk fat globules using the mechanical shear. Conventional homogenisation of milks at 18–20 MPa can reduce the MFG size about tenfold with average MFG size (Hayes & Kelly, 2003; Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003). However, MFG size seems not to be significantly further reduced with high pressure homogenisation. As shown in Table 14.2, comparable MFG size range (0.4–0.9 μm) was obtained when milks were homogenised at 200 MPa as against conventional homogenisation (0.7–0.8 μm). It is reported that usage of higher pressure up to 300 MPa can reduce the MFG size as small as 0.15–0.16 μm and 0.30–0.43 μm in milk (Serra, Trujillo, Quevedo, Guamis, & Ferragut, 2007) and cream (Rodarte, Zamora, Trujillo, & Juan, 2018), respectively. The ineffectiveness of high pressure homogenisation in further reduction of MFG size may be related to shortage of the available emulsifiers to stabilise the newly generated surface area resulting from numerous amount of smaller fat globules. Thus, the addition of extraneous emulsifiers may facilitate greater reduction of MFG size. A recent study performed on dairy cream (38% fat) showed that nano-sized range of MFG (0.13–0.14 μm) can be achieved with the addition of 4–5% sodium caseinate into cream prior to microfluidisation (Panchal et al., 2017). When pressure is applied at a moderate level, microfluidisation method appears to be more effective than conventional homogenisation in reduction of MFG size. A previous study showed that microfluidisation and homogenisation of milk at the same pressure (40 MPa) resulted in different MFG size range, i.e. 280 and 400 nm, respectively (Dalgleish, Tosh, & West, 1996). Major factors influence the efficiency of microfluidisation in reduction of MFG size include microfluidising pressure and fat content of the sample (Hardham, Imison, & French, 2000; Olson, White, & Richter, 2004). Downsizing of MFG using ultrasonication is a function of ultrasonic power and treatment time (Table 14.2). In general, application of higher ultrasonic power with prolonged treatment duration caused greater reduction of MFG size in both milk and cream (Bermudez-Aguirre, Mawson,

Table 14.2 Selected studies on downsizing of milk fat globules using mechanical shear via conventional/high pressure homogenisation, microfluidisation and ultrasonication

Sample	Operating conditions	MFG size obtained	References
Conventional and high pressure homogenisation (HPH)			
Milk	18 MPa	D_{43} 0.7 μm	Hayes & Kelly, 2003)
Milk	1–2 stages: 50–200 MPa	D_{43} 0.62–3.20 μm	
Pre-warmed milk prior to 2-stage HPH		D_{43} 0.4–0.5 μm	
Milk (4, 14, and 24 °C)	200 MPa	D_{43} 0.90, 0.65 and 0.37 μm, respectively	Thiebaud et al. (2003)
Milk	Conventional	D_{43} 0.88 μm	Hayes, Fox, and Kelly (2005)
	Pasteurized and HPH	D_{43} 0.48–0.86 μm	
Milk (40 °C)	200 MPa	0.15 μm	Serra et al. (2007)
Milk (30 °C)	300 MPa	0.16 μm	
Cream (20% fat)	300 MPa; single stage	D_{43} 0.30–0.43 μm	Rodarte et al. (2018)
Microfluidisation			
Milk	40 MPa	280 nm	Dalgleish et al. (1996)
Milk	50 and 100 MPa	460 and 304 nm	Olson et al. (2004)
	150 and 200 MPa	361 and 383 nm	
Milk (42 and 54 °C)	75, 125 and 170 MPa	0.39–0.50 μm	Bucci, Van Hekken, Tunick, Renye, and Tomasula (2018)
Cream (38% fat) with extraneous protein added)	62 MPa	D_{32} 0.13 μm	Panchal et al. (2017)
Ultrasonication			
Milk	20–40 W for 1–10 min	2–5 μm	Wu et al. (2000), Ertugay et al. (2004), Bermudez-Aguirre et al. (2008)
Milk	400–450 W	0.5–0.7 μm	
Milk (70–75 °C)		0.57–0.95 μm	Villamiel and de Jong (2000)
Milk	31 W for 30 min; 50 °C	1.49 μm	Chandrapala et al. (2016)
Cream (42% fat)	50 W for 10 min; 10 and 50 °C	3.35 and 1.63 μm, respectively	

& Barbosa-Canovas, 2008; Chandrapala et al., 2016; Ertugay, Sengul, & Sengul, 2004; Villamiel & de Jong, 2000; Wu, Hulbert, & Mount, 2000).

There is an effect of temperature on size reduction of MFG in milks and creams regardless of homogenisation techniques employed (Chandrapala et al., 2016; Serra et al., 2007; Thiebaud et al., 2003). As summarised in Table 14.2, smaller MFG size was obtained with a higher inlet temperature of milks and cream. Thiebaud et al. (2003) reported that MFG size of milk being homogenised at 4, 14 and 24 °C with 200 MPa was in the range of 0.9, 0.65 and 0.37 μm, respectively. Ultrasonication of cream at 50 °C yielded smaller MFG size (1.63 μm) as compared to that of lower temperature (10 °C; 3.35 μm) (Chandrapala et al., 2016).

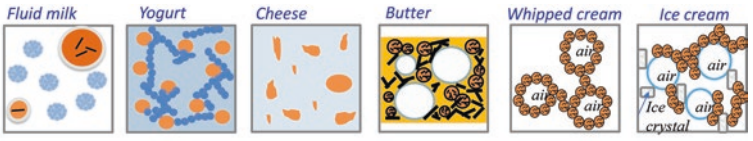
5 Influence of MFG Size on Properties and Functionalities of Dairy-Fat Structured Products

Milk fat, casein micelles and whey proteins are major components of various dairy products (Fig. 14.3) in which the fundamental characteristics of each component will affect the product properties. As previously discussed, there is a dependence of chemical composition and physical characteristics on MFG size. Thus, variation of MFG size in dairy products can be expected to influence texture, flavour, sensory perception and physical functionalities. Baes on previous findings in the dairy-related field, a summary of possible influence of differentiated-size MFG on fundamental characteristics (e.g. interfacial properties, crystallisation properties, physical stability and optical properties) and resultant alterations on physical functionalities of milk, yoghurt, cheese, butter, whipped cream and ice cream is presented in Fig. 14.3.

5.1 Milk and Dairy Cream

Variation in MFG size was found to influence various physical properties and functionalities of fluid milk such as thermal stability, gelation, foaming and sensory properties.

Thermal Stability It is known that reduction of MFG size by homogenisation affects heat stability of milk. The homogenisation needs to be carried out prior to the pre-warming or concentration step to maintain the heat stability of milk. The reverse order will cause a reduction of the heat stability. It was demonstrated that heat coagulation time at 120 °C increased with homogenised milk having MFG size



Physical state	Liquid	Gel	Plastic solid	Solid	Foam	Solid foam
Compositional difference → interfacial properties	Heat stability Foamability Foam stability	Syneresis Firmness	Water retention Free oil leakage Syneresis Firmness Cheese yield	Water retention	Foamability Foam stability	
Crystallisation properties			Rheology	Churning of cream	Foaming	
Physical stability	Creaming		Creaming			
Optical properties	Colour		Colour			

Fig. 14.3 Illustrations of role of milk fat in structuring wide variety of dairy products and associated influence of MFG size on physical functionality of the products

of less than 0.4 μm (Whiteley & Muir, 1996). Since no changes in heat stability was in association with MFG size above 0.4 μm, it was suggested that the varied MFG size did not have any direct effect on the heat stability. Instead, alteration of protein conformation upon homogenisation may govern the heat stability of homogenised milk. A recent study performed on heat stability of native creams (Dhungana, Truong, Bansal, & Bhandari, 2019) provides insights into the role of MFG size as well as the connection between heat treatment with complexity of serum phase in the context of MFG size reduction. In this study, five fractions of MFG size (1.45, 2.45, 3.85 and 4.50 μm) were obtained from fractionation of native creams (18 and 36% fat content) using the two-stage centrifugal method (Dhungana et al., 2017). Testing of heat stability of all cream samples at 140 °C showed that the heat stability depended on both the MFG size and the fat content. The smaller MFG size fraction was more heat s*table* with low fat content. In contrast, the larger MFG size fraction having high fat content tended to maintain heat stability better than that of smaller size fraction. It was proposed that apart from MFG size, possible influence of the heat stability may include changes in the composition of serum phase, fat content and MFGM properties.

Gelation Gelation of milk can be induced by rennet addition and/or acidification in which the protein network is built up by fusion and/or linkage of caseins, providing pores (typically 4 and 15 μm in rennetted gels and 1–30 μm in acid gels) for fat globules to be embedded within (Cho, Lucey, & Singh, 1999; Mellema, Heesakkers, van Opheusden, & van Vliet, 2000; Michalski, Cariou, et al., 2002). Depending on the relative size between fat globules and pore size, the fat globules can be fitted into or excluded from the void spaces, resulting in enhancing the gel firmness or weakening the gel integrity, respectively (Logan et al., 2015; Michalski, Cariou, et al., 2002). It was postulated that native MFGs act as inert fillers since their intact MFGM do not interact with the casein network. In homogenised and recombined milks, the surface of MFGM is generally modified whereby adsorption of whey proteins and caseins permits interaction with the gel structure. Thus, the effect of MFG size on gelation of milk not only involves in optimum packing between fat globule and gel pores size but also presence and volume fractions of other interacting particles forming the gel network. For both rennet- and acid-induced gels, Michalski, Cariou, et al. (2002) found that G′ increased with smaller MFG size fraction obtained from microfiltrated milk (2.8 vs. 4.6 μm), homogenised milk (1.6–2 μm) and reconstituted skim milk (1.9–3.9 μm). In another study performed on fresh milk obtained from selection of cow breeding, it was reported that rennet-induced gel contained large MFG (4.6–5.15 μm) tended to firmer than that of small MFG (3.5–3.6 μm) at the similar size of casein micelles (Logan et al., 2015). Regarding acid-induced gel, measurement of yield stress and strain of homogenised skim milk (50–850 bar) showed that the measured rheological values were higher with smaller MFG size (0.2 versus 2.0 μm) (Ji, Lee, & Anema, 2011). In this case, the reinforcement of gel structure might be partly related to changes in protein adsorption behaviour and characteristics of other interacting particles caused by homogenisation process (Ji et al., 2011).

Foamability and Foam Stability Foaming characteristics of whole milk homogenised at different pressures to obtain different particle sizes (0.39, 0.44, 0.76, and 1.50 μm) have been investigated (Borcherding, Hoffmann, Lorenzen, & Schrader, 2008). It was shown that the foaming properties at 50 °C were governed by milk protein fractions contained in the skim milk phase rather than a direct influence of MFG size. However, the influence of fat globules was more evident when foaming was carried out at lower temperature 20–30 °C (Borcherding et al., 2008). At this point, it is not clear whether the physical state of milk fat across a temperature range of 20–50 °C have had any impact on the foaming properties observed in this temperature region. Dependence of foamability and foam stability on MFG size (0.2, 0.6 and 1.2 μm) of various milk fat emulsions (10% anhydrous milk fat or milk fat fractions) has also been studied (Truong, Bansal, & Bhandari, 2014). In general, the extremely small droplets (i.e. 0.2 μm) could not retain in the liquid films, causing thinning of the films and subsequent foam collapsing. Further investigation is needed to elucidate the role of MFG size in foamability and foam stability of milks where associated alterations of protein conformation is minimized.

Sensory Properties Perception of dietary fat has been known to have multimodal stimulus in which there is an involvement of sensory modalities (in-mouth tactile sensations, vision, taste, olfaction) and product-related factors (structure of food matrix, viscosity, aroma, tasting temperature) (Le Calve et al., 2015; Mattes, 2009; Schoumacker et al., 2017). Few attempts have been made to relate the perception of creaminess in milk with different MFG sizes (Goudedranche, Fauquant, & Maubois, 2000; Richardson & Booth, 1993). For instance, Richardson and Booth (1993) reported that the smaller MFG obtained by homogenisation in thickened milk (18.6 Pa s) contributed to greater creaminess. This can be partly attributed to the greater surface area and numerous amount of smaller MFG in combination with adequate thickness, providing overall perception of creaminess in the thickened milk. From milk fat emulsion (4% fat) perspective, it was found that fat aggregates having average size above 5 μm contributed to powdery feeling and textural perception such as thickness and chalkiness (Fibrianto, 2013).

5.2 Yoghurt

As previously discussed, MFGs play an important role in determining the gelation properties of milk. In yoghurt system, depending on their interactions with the protein matrix in either active or inert modes, milk fat globules can promote or disrupt the microstructure of yoghurts, respectively. The influence of MFG on water retention and associated gel strength as well as syneresis of yoghurts have been investigated. It was reported that yoghurt made from thermo-sonicated milk with smaller MFG size (0.4 μm) had two-fold water retention than that of made from the conventional method (Riener, Noci, Cronin, Morgan, & Lyng, 2009). Reduction of MFG size using microfluidisation technique showed that larger gel particles can be formed

with smaller MFG. Nevertheless, associated changes in texture and the amount of water retained in the matrix of yoghurt made from microfluidised milk had only a slight improvement (Ciron, Gee, Kelly, & Auty, 2010). Usage of high pressure homogenisation was able to further reduce MFG size as small as D_{32} 0.12–0.16 μm. Such small MFG was found to have positive effects on gel strength, gel firmness and syneresis of yoghurt as compared with the sample made from skim milk powder using conventional manufacturing method (Serra et al., 2007). In those studies, reduction of MFG size was done using mechanical shear processing that has altered the globule membrane reactivity with inclusions of whey proteins and casein. Thus, it will be interesting to examine the effect of native MFG size on the physical functionality of yoghurt products.

5.3 Cheese

The primary structure of cheese is a protein matrix composed of aggregated and linked casein micelles in which water, salts and MFG are dispersed. MFG can act either as inert fillers or interacting particles within the cheese protein network depending on the integrity of MFG. Generally, native MFGM is non-interactive whereas homogenised MFGM being partly replaced with caseins and/or whey proteins is prone to interacting with the cheese protein network through hydrophobic interactions (Everett & Olson, 2000; Lucey, Johnson, & Horne, 2003). Regarding cheese prepared from native MFG, small MFG fraction obtained by microfiltration was found to improve water binding capacity in Emmental and Camembert cheeses. This enhancement with the cheeses containing small MFG fraction was due to greater surface area to volume ratio and associated changes in MFGM material, leading to the higher moisture content and softer texture in the cheeses (Michalski et al., 2003, 2004). Proteolysis and lipolysis seem to be enhanced with smaller MFG in Camembert, Emmental, and Italian cheese (Jana & Upadhyay, 1992; Michalski et al., 2003, 2007; Michalski, Camier, et al., 2004). This may be partly explained by more specific sites are available for enzyme activity with a greater proportion of MFGM in smaller MFG fraction. A contrasting observation was reported in Emmental cheese and miniature Cheddar cheese that large MFG (5.6 and 4.68 μm, respectively) caused greater lipolysis upon ripening with remarkable increase in free fatty acid (O'Mahony et al., 2005). It was postulated that the differences in liberation of free fat in these cheese matrices may be caused by different crystallisation behaviour of fat within small MFG. Since the larger MFG may contain crystalline structures that were able to disrupt the MFGM (O'Mahony et al., 2005), making it more prone to the enzyme activity.

Native MFG size also influences product properties and functionalities in Camembert, Emmental, mini Swiss and fresh cheeses. The cheeses made from smaller MFG appeared to be softer with lower rheological values (Goudedranche et al., 2000; Michalski et al., 2003, 2007; Michalski, Camier, et al., 2004). Improved stretching and elasticity was also found in Emmental cheese containing small

MFG. With regarding to sensory properties, the cheeses made from smaller MFG was perceived as smoother and more elastic texture (Goudedranche et al., 2000; Michalski et al., 2003). St-Gelais, Passey, Hache, and Roy (1997) also reported effect of MFG size on low-fat Cheddar cheese manufactured from low mineral retentate powder in which large MFG fraction improved colour, flavour and texture of the cheese.

Reduction of MFG size via homogenisation approach has been shown to affect enzyme activity (lipolysis, proteolysis), processability (fat loss to whey, free oil release, cheese yield), physicochemical properties (moisture, viscosity, firmness, whiteness, rennet time, syneresis), and functionality (flowability, stretchability) (Green, Marshall, & Glover, 1983; Lemay, Paquin, & Lacroix, 1994; Michalski, Camier, et al., 2004; Rowney, Hickey, Roupas, & Everett, 2003; Rudan, Barbano, Gu, & Kindstedt, 1998; Schenkel, Samudrala, & Hinrichs, 2013). These influences are attributed to MFG size reduction and modification of MFG surface upon homogenisation (Green et al., 1983). For instance, casein may be less available to build up the cheese protein network since it is incorporated into the homogenised MFGM, resulting in weaker structure of the cheese protein matrix (Green et al., 1983).

5.4 Butter

Butter is made from churning of dairy cream whereby a phase inversion of oil-in-water to water-in-oil emulsions can be occurred. Thus, the physical properties of initial dairy cream significantly influence the processability of butter making and physical functionality of resultant butter. It has been demonstrated that churning time of cream is MFG-size dependent. A previous study performed on secreted milks having large (2.3 μm) and small MFGs (1.84 μm), which was obtained by modifying diet of cow with fish meal, showed that the small MFG induced longer churning time (Avramis et al., 2003). When cow diets were modified with extruded linseed, it appears that small MFGs (3.49 μm) had shorter churning time as compared to that of larger ones (4.18 μm). In these studies, the discrepancy in churning time may be related to possible changes in MFG composition resulting from the different cow diets. In another study, Goudedranche et al. (2000) found no difference in churning abilities between small (below 2 μm) and large (above 2 μm) MFGs fraction obtained by microfiltration of native MFG in cream. The churnability of differentiated-sized MFG prepared from recombined cream (38% fat; emulsifying anhydrous milk fat with sodium caseinate) within the range of 0.17–3.50 μm has also been studied (Panchal et al., 2017). Since the recombined cream having smaller size tended to be less prone to destabilisation, churning time was extended with reduction of MFG size. Addition of low molecular surfactant (Tween 80) into the sodium caseinate stabilised cream samples promotes the "competitive destabilisation" at the interface of water and oil, leading to a significant reduction of churning time (Panchal et al., 2017). Apart from churning time, few studies showed that the small MFG had a negative impact on process and product such as higher fat loss

during churning and greater water retention in the resultant butter (Goudedranche et al., 2000; Hurtaud et al., 2010).

Few attempts have been made to prepare butter made from differentiated-size dairy creams. Goudedranche et al. (2000) reported that the butter made from smaller fat globules obtained by microfiltration method tended to be greasy, oily than that of prepared from control butter. In contrast, Avramis et al. (2003) and Hurtaud et al. (2010) found that small MFG improved physical functionality of the resultant butter, i.e. more spreadable, softer texture and better mouthfeel. Since the small MFG was obtained from modifying cow diet with meal enriched in unsaturated fatty acid, it is difficult to interpret whether the associated changes in physical functionality related to MFG size or the compositional differences with varied MFG size. Thus, it would be interesting to further explore the feasibility of manipulating MFG size to improve the physical functionality of butter.

5.5 Whipped Cream and Ice Cream

Whipped cream and ice cream are common dairy aerated products in which partial coalescence of MFG is essential to stabilise air cells embedded in the dairy matrices (Goff, 1997). Formation of foam and foam stability in those products are governed by several factors such as whipping conditions (time, intensity and temperature), ice crystal size, serum viscosity and interactions between fat, emulsifiers/stabilisers at the air-water interface (Goff, 1997). Apart from those influential factors, MFG size has been found to impact on foamability and foam stability of whipped cream and ice cream. It has been demonstrated in native dairy creams having different MFG size (3.8–4.9 μm) obtained by gravity separation and subsequent bowl disk centrifugal separation that an increase in MFG size reduced whipping time by 22 ± 7% with greater overrun. Similar tendency was noted with the homogenised creams having similar MFG size range, indicating that the foamability is dependent on true MFG size (Eden et al., 2016). In this study, no difference in serum drainage (based on MFG size) in whipped cream was found. In another study, it has been reported that dairy foam containing smaller MFG fraction, which was prepared from microfiltration technique, was more s*table* with less foam collapse (12%) as against the foam made from large MFG fraction (29%) and control (25%) whipped cream (Michalski et al., 2006). Regarding ice cream system, influence of MFG size in homogenised cream has been evaluated whereas there is little information on manipulation of native MFG size in ice cream manufacturing. Koxholt, Eisenmann, and Hinrichs (2001) used homogenised cream within size range of 0.44–3.33 μm to study melt-down of ice cream. It was found that foam structures containing smaller fat particles (0.44–0.85 μm) had faster melt-down that their larger counterpart (0.85–3.33 μm). The contrasting observations on the effect of MFG size in whipped cream and dairy cream systems suggest that these different influences may be also attributed from the discrepancy in dairy processing and associated MFG preparation.

6 Conclusion

This book chapter underscores the importance of manipulating MFG size, which spans from 0.1 to 15 μm in naturally bovine MFG, to improve processability and physical functionality of a wide variety of dairy-fat structured products. Beyond the impact of MFG size, there is a compositional difference among MFG size fractions, giving rise to discrepancies in the fundamental properties of MFG. Herd management, physical separation and microfiltration are effective methods to preserve the integrity of MFGM in native size-classified milks. However, further improvement needs to be done to obtain more discrete MFG size fractions with these methods. Mechanical processing is a common practice to reduce the MFG to nano-sized scale (i.e. approximately 0.2 μm). When being subjected to the mechanical shear, the MFGM of emulsified globules is disrupted whereby caseins and whey proteins are incorporated into the reformed MFGM. This alteration makes the emulsified MFG become interacting particles with the protein network whereas non-interacting native MFG acts as inert fillers within the dairy matrices. As such, the inactive and active modes of MFG also contribute to particle interactions within the dairy matrices, apart from the influence of true MFG size. It has been demonstrated that differentiated-sized MFG fractions participate in structuring dairy-fat containing products where the associated differences in composition, structure and crystallisation behaviour of milk fat can be used as a means of enhancing physical functionality and processability of fluid milks, dairy creams, cheeses, yoghurts, butter, whipped cream and ice cream. With availability of advanced techniques for dairy processing and characterisation of dairy products, it is suggested that further research to be undertaken to obtain more discrete MFG size fraction with wider size ranges. The next advance can be manipulation of the complex interactions between differentiated-sized fat globules and other particles in dairy matrices to develop innovative, low-fat, health-promoting dairy products.

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