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Effect of Milk Fat Globule Size on the Physical Functionality of Dairy Products

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Abstract

Bovine milk lipids occur naturally in the form of globules, comprising a triacylglycerol (TAG) core enveloped by a trilayer-structured membrane. Milk fat globules (MFGs) have a widely varied size distribution spanning from 0.1 to 10 μm with an average diameter of 4 μm. Milk fat is a major determinant of the microstructural, rheological and sensorial properties of many fat-containing dairy products such as milk, cream, yogurt, ice cream, cheese, butter and milk chocolate. This book has highlighted the importance of both native and emulsified MFG size as a pivotal processing and functionality parameter in many fat-structured dairy products.

An overview is provided of current knowledge on herd management strategies (breeding, dietary supplement and lactation) and fractionation techniques (gravity separation, centrifugation and microfiltration) to vary native MFG size. The effects of mechanical shear processing (homogenisation, microfluidisation and ultrasonication) to reduce emulsified MFG size are also reviewed. Different size fat globules exhibit differences in TAG composition, physical stability, viscosity, crystallisation properties, optical characteristics and electric conductivity. The influence of fat globule size and structure on the processability and functional properties of dairy fats and fat-containing dairy products is also discussed.

Contents

1 Introduction 1
References 2

2 An Overview of Milk Fat Globules 5
2.1 Secretion Pathway to Create Different Milk Fat Globule Sizes: Small, Intermediate and Large 5
2.2 Primary Components of Milk Lipids 7
References 8

3 Techniques to Measure Milk Fat Globule size 11
3.1 Microscopy 11
3.2 Electrical Sensing Zone Methods (Coulter Counting) 12
3.3 Light Scattering Techniques 12
3.4 Scanning Flow Cytometry 13
References 13

4 Methodologies to Vary Milk Fat Globule Size 15
4.1 Herd Management 15
4.1.1 Breeding 15
4.1.2 Dietary Supplementation 16
4.1.3 Lactation Stage and Milking 18
4.2 Size Fractionation Without Shear 21
4.2.1 Gravity Separation 21
4.2.2 Ultrasonic-Assisted Separation 21
4.2.3 Centrifugation 23
4.2.4 Microfiltration 23
4.3 Mechanical Shear to Reduce Fat Globule Size 23
4.3.1 Homogenisation/High Pressure Homogenisation 24
4.3.2 Microfluidisation 25
4.3.3 Ultrasonication 26
4.4 Effect of Homogenisation on Microstructure of Milk Fat Globules 26
References 27

5 Size-Dependent Variations in Fatty Acid Composition and Lipid Content of Native Milk Fat Globules 31
5.1 Total Fatty Acids 31
5.2 Individual Fatty Acids 31
5.2.1 Short-Chain FAs 31
5.2.2 Medium-Chain FAs 32
5.2.3 Long-Chain FAs 32
5.2.4 Unsaturated FAs 32
5.3 Conjugated Linoleic Acids (CLA) 32
5.4 Polar Lipids 33
References 34

6 Effect of Milk Fat Globule Size on Physical Properties of Milk 35
6.1 Physical Stability 35
6.2 Viscosity 37
6.3 Crystallisation and structural properties 37
6.4 Optical Properties 42
6.5 Electrical Conductivity 43
References 43

7 Effect of Milk Fat Globule Size on Functionalities and Sensory Qualities of Dairy Products 47
7.1 Milk 47
7.1.1 Heat Stability 47
7.1.2 Foaming 49
7.1.3 Gelation 49
7.1.4 Sensory Properties 51
7.2 Cheese 53
7.2.1 Cheese Made from Native Milk Fat Globules 54
7.2.2 Cheese Made from Homogenised Milk Fat Globules 56
7.3 Yoghurt 59
7.3.1 Water Retention and Associated Gel Strength and Syneresis 59
7.3.2 Texture and Firmness 60
7.4 Aerated Dairy Systems 60
7.4.1 Ice-Cream 60
7.4.2 Whipped Cream and Whippable Dairy Emulsions 61
7.5 Butter 62
7.5.1 Churning 62
7.5.2 Firmness, Texture and Mouth-Feel 63
References 64

8 Conclusions 69

Chapter 1
Introduction

Bovine milk lipids exist naturally within colloidal suspensions of emulsified globules, each globule comprising a triacylglycerol (TAG) core enveloped by a tri-layer globule membrane (Lopez et al. 2011). In the cow, the control of size, composition, structure and secretion of native milk fat globules (MFG) is carried out by the cellular regulatory system of the mammary gland (Heid and Keenan 2005; Mather and Keenan 1998). The major physiological role of MFG is in delivering nutrition (particularly energy) and bioactive molecules to the suckling calf. Interestingly, these packages of energy have a wide diversity of size, ranging from 0.1 to 15 μm with a mean diameter of 4 μm (Walstra 1995; Michalski et al. 2001). From a physiological perspective, it is not known whether each size class has additional functions beyond the general role of energy delivery. In fact, there is a variation of the MFG size and size distribution within a single cow in a herd, among breeds and between seasons. They are also changed at various lactation stages and can be modified through feeding and milking times (Logan et al. 2014; Wiking et al. 2004, 2006; Carroll et al. 2006; Hurtaud et al. 2010).

Although different native MFG size distributions can be obtained through breed selection and herd management, a practical limitation of this approach is that it does not yield very discrete size classes and requires complex supply chain management. From an industrial perspective, post-farm strategies for size fractionation and manipulation of MFG may be more feasible. Although still subject to the usual cost-benefit analysis, this might be achieved more readily, for example, through adaptation of conventional dairy processing technologies such as gravity separation, centrifugation, microfiltration or homogenisation.

Milk fat is one of the main ingredients and a key factor in determining the physical functionality, flavour and nutritional profile of many fat-containing dairy products such as cheese, ice cream, yoghurt, butter, etc. In the microstructure of dairy products, milk fat is typically present in the form of native globules (unhomogenised

T. Truong et al., *Effect of Milk Fat Globule Size on the Physical Functionality of Dairy Products*, SpringerBriefs in Food, Health, and Nutrition,
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milk, cream), complex emulsions (homogenised milk, cream), membrane-disrupted free fat in a gel matrix (cheese, yoghurt), agglomerated fat in aerated systems (ice cream, whipped cream) or a continuous, free fat phase (butter, ghee, milk chocolate). Although a few previous studies have reported that different fat globule size fractions can show differences in chemical composition and physical properties (Michalski et al. 2004; Lopez et al. 2011), very little research in this area has been published. On theoretical grounds, it would seem reasonable to expect some such differences to occur, given, for example, the differences in net surface area between two different size fractions of MFG. It is of interest to understand whether it may be feasible to exploit some of these apparent differences on an industrial scale, potentially to be able to use dairy herd management or physical processing of the milk to develop fat-structured, dairy-based foods and ingredients with improved physical functionality, sensory properties and nutritional value.

This book aims to provide an overview of the factors that can influence MFG size and properties, particularly within the context of a potential industrial strategy to produce new types of milk and dairy products and ingredients that are differentiated on the basis of MFG size distribution. The book will focus on fat globule fundamental, size-related physical and chemical properties of both native and emulsified MFG, as well as recent studies on potential applications of size-differentiated MFG in fat-structured dairy products. Compositional differences in milk fat globule membranes (MFGM) and their association with nutritional properties and digestibility are out of scope of this book; these have been highlighted recently by Lopez (2011) and Martini et al. (2013).

References

Carroll SM, DePeters EJ, Taylor SJ, Rosenberg M, Perez-Monti H, Capps V. Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. Anim Feed Sci Technol. 2006;131(3–4):451–73. doi:10.1016/j.anifeedsci.2006.06.019.

Heid HW, Keenan TW. Intracellular origin and secretion of milk fat globules. Eur J Cell Biol. 2005;84(2–3):245–58. doi:10.1016/j.ejcb.2004.12.002.

Hurtaud C, Faucon F, Couvreur S, Peyraud JL. Linear relationship between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid composition and butter properties. J Dairy Sci. 2010;93(4):1429–43. doi:10.3168/jds.2009-2839.

Logan A, Auldist M, Greenwood J, Day L. Natural variation of bovine milk fat globule size within a herd. J Dairy Sci. 2014;97(7):4072–82. doi:10.3168/jds.2014-8010.

Lopez C. Milk fat globules enveloped by their biological membrane: unique colloidal assemblies with a specific composition and structure. Curr Opin Colloid Interface Sci. 2011;16(5):391–404. doi:10.1016/j.cocis.2011.05.007.

Lopez C, Briard-Bion V, Menard O, Beaucher E, Rousseau F, Fauquant J, Leconte N, Robert B. Fat globules selected from whole milk according to their size: different compositions and structure of the biomembrane, revealing sphingomyelin-rich domains. Food Chem. 2011;125(2):355–68. doi:10.1016/j.foodchem.2010.09.005.

Martini M, Salari F, Altomonte I. The macrostructure of milk lipids: the fat globules. Crit Rev Food Sci Nutr. 2013. doi:10.1080/10408398.2012.758626.

Mather IH, Keenan TW. Origin and secretion of milk lipids. J Mammary Gland Biol Neoplasia. 1998;3(3):259–73. doi:10.1023/A:1018711410270.

Michalski MC, Briard V, Michel F. Optical parameters of milk fat globules for laser light scattering measurements. Lait. 2001;81(6):787–96.

Michalski MC, Ollivon M, Briard V, Leconte N, Lopez C. Native fat globules of different sizes selected from raw milk: thermal and structural behavior. Chem Phys Lipids. 2004;132(2):247–61. doi:10.1016/j.chemphyslip.2004.08.007.

Walstra P. Physical chemistry of milk fat globules. In: Fox PF, editor. Advanced dairy chemistry, vol. 2: lipids. London: Chapman & Hall; 1995. p. 131–78.

Wiking L, Stagsted J, Lennart B, Nielsen JH. Milk fat globule size is affected by fat production in dairy cows. Int Dairy J. 2004;14(10):909–13. doi:10.1016/j.idairyj.2004.03.005.

Wiking L, Nielsen JH, Bavius AK, Edvardsson A, Svennersten-Sjaunja K. Impact of milking frequencies on the level of free fatty acids in milk, fat globule size, and fatty acid composition. J Dairy Sci. 2006;89(3):1004–9.

Chapter 2
An Overview of Milk Fat Globules

2.1 Secretion Pathway to Create Different Milk Fat Globule Sizes: Small, Intermediate and Large

Assembly, growth and secretion of MFG takes place in the milk-secreting cells of the mammary gland of mammals. In the original state, tiny intracellular lipid droplets (<0.5 μm) are formed at the endoplasmic reticulum membranes, which is the site of origin of TAGs. These discrete small droplets have a TAG core coated by a single layer of polar lipids and proteins. They migrate from the endoplasmic reticulum to the cytosol, fuse together and form bigger droplets (Heid and Keenan 2005; Deeney et al. 1985). The formation of these cytoplasmic lipid droplets by droplet-droplet fusion is assumed to be governed by calcium and protein complexes originating from the cytosol and fusion-promoting agents, gangliosides (Valivullah et al. 1988). However, the coalescence of cytoplasmic lipid droplets to form larger droplets is not facilitated. It is assumed that the regulation of droplet size might be associated with the difference in composition of surface coat between the micro-lipid and cytoplasmic lipid droplets (Deeney et al. 1985). The lipid droplets are then transported to the apical plasma membrane in which they are discharged from the epithelial cell and secreted. At this point the lipid droplets are progressively coated by the plasma membrane to form the outer bilayer milk fat globule membrane (MFGM), rendering the final trilayer structure of intact MFGM upon secretion (Heid and Keenan 2005) (Fig. 2.1). With its dense protein coat (10–50 nm thick) and complex molecular organization, the MFGM is considered to be a true biological membrane (Keenan and Mather 2006) (Fig. 2.1). The MFGM is enriched in polar lipids and also possesses size-related biochemical and structural differences (Lopez 2011).

The substantial growth of MFG size during the transit time from the origin to the secretion sites is evidenced by the polydispersity of its size in secreted milk as shown in Fig. 2.2 (Scow et al. 1980; Michalski et al. 2001a). Size measurement of native full fat milk using laser light scattering after dissociating casein micelles

T. Truong et al., *Effect of Milk Fat Globule Size on the Physical Functionality of Dairy Products*, SpringerBriefs in Food, Health, and Nutrition,
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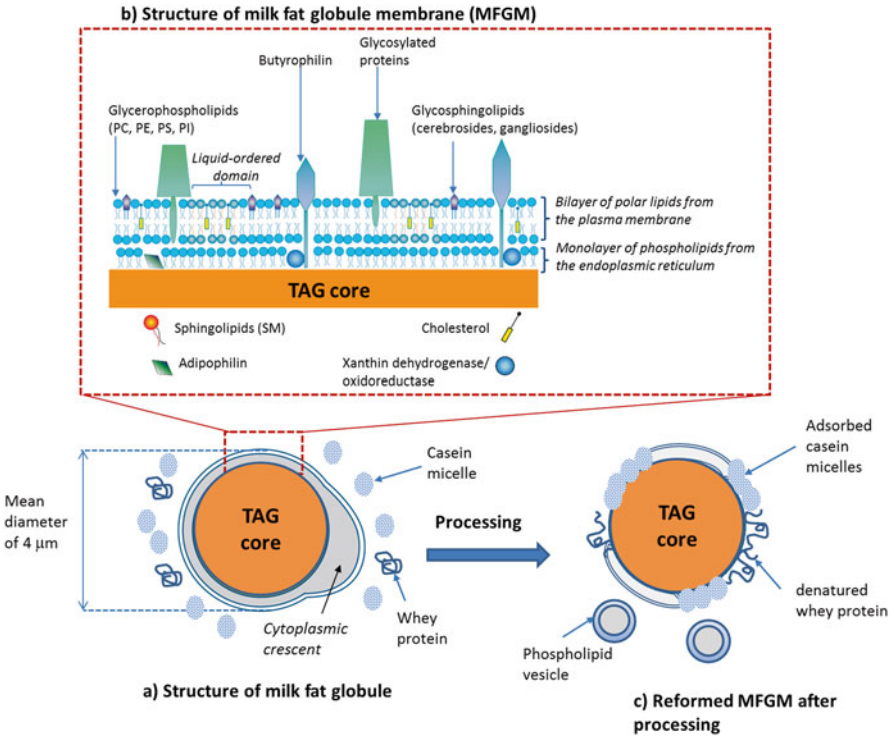

Fig. 2.1 Schematic illustrations of bovine MFG (**a**) and its tri-layer membrane (**b**) (not to scale) (Redrawn with modifications from Lopez et al. 2011; Lopez et al. 2008; Waninge et al. 2004). Heating, mixing and homogenisation of milk can disrupt the intact MFGM resulting in adsorption of casein micelles and denatured whey protein and their incorporation into the membrane of the emulsified globule. Processing can also cause the release of small amounts of membrane material into the milk, which may then form discrete phospholipid vesicles (**c**)

showed that size distribution of bovine MFG spans from 0.03 to 11 µm with the main peak at 4 µm (Lopez 2005). The number of globules per mL of milk is about 1.5×10^{10} (Walstra 1995; Michalski et al. 2001a). The wide variation in size of native bovine MFG can be categorised into three size fractions, namely small (<1 µm), intermediate (1–8 µm), and large globule sizes (>8 µm) (Walstra 1969; Michalski et al. 2001a). In fact, 80 % of the fat globule number constitutes the small globule size. However, the intermediate globule size has the highest volume-based percentage of approximately 80 %, followed by the small and large globule sizes (5 and 1–2 %, respectively). The range of specific surface area of fat globules is 1.9–2.5 m^2 g fat^{-1} with a mean value is about 2.2 m^2 g fat^{-1} (Huppertz and Kelly 2006). Milk fat globules are negatively charged with a zeta-potential value of about −13.5 mV for unhomogenized or natural MFG (Michalski et al. 2001b). According to Timmen and Patton (1988), there are three possible pathways to regulate the formation of various globule sizes. The small size fraction could be a result of direct

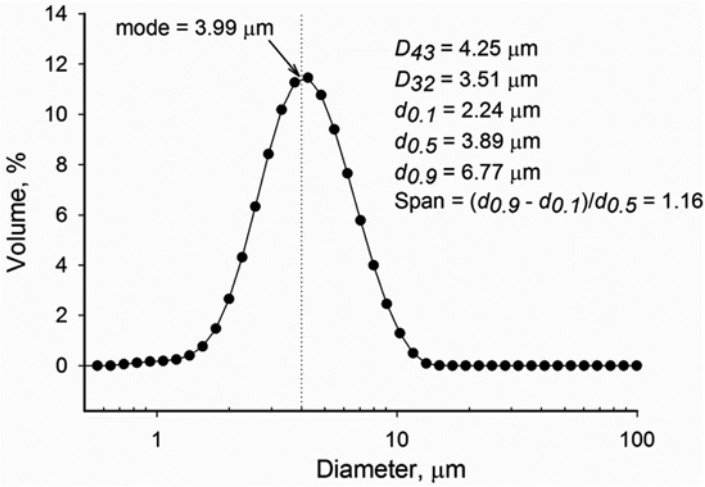

Fig. 2.2 A typical size distribution of raw bovine MFG measured by laser light scattering at 25 °C using the general model (distribution of spherical particles is unknown). Common expressions of mean diameters of MFG size: mode, volume-weighted mean D_{43}; surface-weighted or Sauter mean D_{32}; $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ are the globule diameters that are equal or larger than those of 10, 50 and 90 %, respectively, of the volume distribution. Span is the size distribution of MFG

secretion and/or limited fusion of lipid micro-droplets. The intermediate globule sizes are formed by the fusion of micro-droplets as described above. The large globule size might result from post-secretion fusion of the large globules with the smaller ones. However, the exact mechanism of regulation of differently-sized milk fat globules is still unclear.

2.2 Primary Components of Milk Lipids

The primary component of milk lipids are TAGs (more than 98 %) with the remainder including monoacylglycerols, diacylglycerols, free fatty acids, phospholipids, and traces of sterols, carotenoids, fat-soluble vitamins, and flavour compounds (MacGibbon and Taylor 2006). At least 400 different fatty acids and 200 different TAG species have been detected in milk lipids (Gresti et al. 1993). The fatty acids in milk lipids can be differentiated according to variables in chain length, degree of saturation, configuration and conjugation of double bonds (Walstra et al. 1999). It has been found that bovine milk lipids are made up of complex fatty acids including short-chain (C_4-C_8), medium-chain (C_{10}-C_{12}), and long-chain (C_{14}-C_{18}) fatty acids at 8.3, 6.6, and 81.9 %, respectively (Jensen 2002). The difference in chain length of fatty acids renders the unique characteristics of milk lipids and fractionated milk fats. For example, the short-, medium-, long-chain fatty acids are in order of increasing melting point.

Table 2.1 The principal fatty acids found in milk fat (MF) and anhydrous milk fat (AMF)

Fatty acid	Fatty acid common name	Amount (%w/w)			
		MF[a]	MF[b]	AMF[c]	MF-TAGs[c]
$C_{4:0}$	Butyric	2–5	3.9	4.0	3.6
$C_{6:0}$	Caproic	1–5	2.5	2.7	2.4
$C_{8:0}$	Caprylic	1–3	1.5	1.3	1.2
$C_{10:0}$	Capric	2–4	3.2	3.0	2.9
$C_{12:0}$	Lauric	2–5	3.6	3.6	3.5
$C_{14:0}$	Myristic	8–14	11.1	11.0	11.2
$C_{14:1}$	Myristoleic	–	0.8	1.8	2.0
$C_{15:0}$	Pentadecanoic	1–2	1.2	1.3	1.4
$C_{16:0}$	Palmitic	22–35	27.9	29.4	29.4
$C_{16:1}$	Palmitoleic	1–3	1.5	2.9	3.0
$C_{17:0}$	Margaric	0.5–1.5	–	0.8	0.8
$C_{18:0}$	Stearic	9–14	12.2	10.7	10.6
$C_{18:1}$ *cis*	Oleic	20–30	17.2	23.9	24.2
$C_{18:1}$ *trans*			3.9		
$C_{18:2}$	Linoleic	1–3	1.4	3.0	3.0
$C_{18:3}$	Linolenic	0.5–2	1.0	0.8[d]	0.7[d]

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

The TAG -rich core of MFG consists of varying chain length fatty acids. The most abundant fatty acids are myristic, palmitic, stearic and oleic acid (11, 30, 12 and 23 %, respectively) as shown in Table 2.1. The TAG-rich core of the native MFG is emulsified by the MFGM, which is in the form of a trilayer structure (Fig. 2.1). The MFGM accounts for 2 % of the total mass of milk lipids (Walstra et al. 1999), enveloping the MFG with proteins, phospholipids and plasma membrane. The primary components of MFGM are lipids (33 %—mainly TAGs and glycerophospholipids), glycoproteins (20–60 %—rich in butyrophilin, a transmembrane protein), and milk enzymes (Keenan and Mather 2006). The MFGM accounts for about 60 % of the total amount of phospholipids in milk. Significant quantities of cholesterol, phosphatidylcholine, sphingomyelin, glycolipids have also been detected in MFGM (Keenan and Mather 2006).

References

Deeney JT, Valivullah HM, Dapper CH, Dylewski DP, Keenan TW. Microlipid droplets in milk secreting mammary epithelial-cells – evidence that they originate from endoplasmic-reticulum and are precursors of milk lipid globules. Eur J Cell Biol. 1985;38(1):16–26.

Gresti J, Bugaut M, Maniongui C, Bezard J. Composition of molecular-species of triacylglycerols in bovine-milk fat. J Dairy Sci. 1993;76(7):1850–69.

Heid HW, Keenan TW. Intracellular origin and secretion of milk fat globules. Eur J Cell Biol. 2005;84(2–3):245–58. doi:10.1016/j.ejcb.2004.12.002.

Huppertz T, Kelly AL. Physical chemistry of milk fat globules. In: Fox PF, McSweeney PLH, editors. Advanced dairy chemistry volume 2: lipids, vol. 2. 3rd ed. New York: Springer; 2006.

Jensen RG. The composition of bovine milk lipids: January 1995 to December 2000. J Dairy Sci. 2002;85(2):295–350.

Kaylegian KE, Lindsay RC. Handbook of milkfat fractionation technology and applications. Champaign: AOCS; 1995.

Keenan TW, Mather IH. Intracellular origin of milk fat globules. In: Fox PF, McSweeney PL, editors. Advanced dairy chemistry 2: lipids, vol. 2. 3rd ed. New York: Springer; 2006.

Lopez C. Focus on the supramolecular structure of milk fat in dairy products. Reprod Nutr Dev. 2005;45(4):497–511. doi:10.1051/Rnd:2005034.

Lopez C. Milk fat globules enveloped by their biological membrane: unique colloidal assemblies with a specific composition and structure. Curr Opin Colloid Interface Sci. 2011;16(5):391–404. doi:10.1016/j.cocis.2011.05.007.

Lopez C, Briard-Bion V, Menard O, Rousseau F, Pradel P, Besle JM. Phospholipid, sphingolipid, and fatty acid compositions of the milk fat globule membrane are modified by diet. J Agric Food Chem. 2008;56(13):5226–36. doi:10.1021/Jf7036104.

Lopez C, Briard-Bion V, Menard O, Beaucher E, Rousseau F, Fauquant J, Leconte N, Robert B. Fat globules selected from whole milk according to their size: different compositions and structure of the biomembrane, revealing sphingomyelin-rich domains. Food Chem. 2011;125(2):355–68. doi:10.1016/j.foodchem.2010.09.005.

MacGibbon AKH, Taylor MW. Composition and structure of bovine milk lipids. In: Fox PF, McSweeney PL, editors. Advanced dairy chemistry 2: lipids, vol. 2. 3rd ed. New York: Springer; 2006.

Michalski MC, Briard V, Michel F. Optical parameters of milk fat globules for laser light scattering measurements. Lait. 2001a;81(6):787–96.

Michalski MC, Michel F, Sainmont D, Briard V. Apparent zeta-potential as a tool to assess mechanical damages to the milk fat globule membrane. Colloids Surf B Biointerfaces. 2001b;23:23–30.

Scow RO, Blanchettemackie EJ, Smith LC. Transport of lipid across capillary endothelium. Fed Proc. 1980;39(9):2610–7.

Timmen H, Patton S. Milk-fat globules - fatty-acid composition, size and in vivo regulation of fat liquidity. Lipids. 1988;23(7):685–9. doi:10.1007/Bf02535669.

Valivullah HM, Bevan DR, Peat A, Keenan TW. Milk lipid globules - control of their size distribution. Proc Natl Acad Sci U S A. 1988;85(23):8775–9. doi:10.1073/pnas.85.23.8775.

Walstra P, Oortwijn H. Estimating Globule-Size Distribution of Oil-in-Water Emulsions by Coulter Counter. J Colloid Interf Sci. 1969;29(3):424–31.

Walstra P. Physical chemistry of milk fat globules. In: Fox PF, editor. Advanced dairy chemistry vol. 2: lipids. London: Chapman & Hall; 1995. p. 131–78.

Walstra P, Geurts TJ, Noomen A, Jellama A, Van Boekel MAJS. Dairy technology: principles of milk properties and processes. New York: Marcel Dekker; 1999.

Waninge R, Kalda E, Paulsson M, Nylander T, Bergenstahl B. Cryo-TEM of isolated milk fat globule membrane structures in cream. Phys Chem Chem Phys. 2004;6(7):1518–23. doi:10.1039/B314613h.

Wright AJ, Marangoni AG. The effect of minor components on milkfat crystallization, microstructure, and rheological properties. In: Marangoni AG, Narine SS, editors. Physical properties of lipids. New York: Marcel Dekker; 2002. p. 589.

Chapter 3
Techniques to Measure Milk Fat Globule size

The first micrograph of MFGs was captured by Van Leeuwenhoek in 1674 using primitive microscopy (Kernohan and Lepherd 1969). Microscopy is a useful technique as it not only provides measurements of individual MFG size but also visualises shape, distribution and microstructure of MFG, MFGM and fat crystals (Truong et al. 2015; Ong et al. 2010; Precht 1988). Along with microscopy numerous techniques such as Coulter counting (Cornell and Pallansc 1966; Walstra and Oortwijn 1969), laser diffraction, static and dynamic light scattering (Michalski et al. 2001; Robin and Paquin 1991; McCrae and Lepoetre 1996), spectroscopy, ultrasound (Miles et al. 1990), scanning flow cytometry (Konokhova et al. 2014) and electroacoustics (Wade and Beattie 1997) have been employed to estimate the size and size distribution of MFG. These techniques yield complex primary data, which need to be processed mathematically to obtain MFG size data (Huppertz and Kelly 2006). Among these techniques, particle size analysis by small angle light scattering is now widely used to measure MFG size and its distribution.

Depending on the technique used, MFG size can be described by number, diameter, volume and/or surface area. Mean diameters of MFG size are commonly expressed as 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}, $D_{4,3}$). Size distribution of MFG is typically presented as a plot of volume frequency versus globule diameter (Fig. 2.2).

3.1 Microscopy

Various microscopic techniques, such as optical microscopy, fluorescence microscopy, confocal laser scanning microscopy (CLSM), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and holographic video microscopy can be used for measurement of MFG size. High resolution (resolution

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limit ~0.4 nm) electron microscopy allows characterisation of the smallest globules, which cannot be captured by light microscopy, which has a much lower resolution limit (~0.2 μm). In term of size measurement, the main drawbacks of microscopy are poor reproducibility, inaccuracy and processing time, as compared to other methods. Holographic video microscopy, which combines an inverted light microscope and collimated laser beam, is a technique that records the interference pattern generated by scattered light of individual MFG and the unscattered portion of the laser beam. The holographic image is then converted to MFG radius and optical properties (i.e. refractive index) using the Lorenz-Mie light scattering theory (Cheong et al. 2009). Using this technique, the radii of MFG were reported to be 0.562, 0.590, 0.643, and 0.693 μm for fat-free, 1 %, 2 %, and whole milk commercial samples, respectively. The averaged refractive index (1.464 at wavelength of 633 nm) was in good agreement with that of obtained by light scattering (1.470 and 1.460 at 466 and 633 nm) (Michalski et al. 2001). However, as with electron microscopy, this technique is limited by relatively small statistical sampling (sample size ~100).

3.2 Electrical Sensing Zone Methods (Coulter Counting)

The number and volume of MFG can also be determined using a Coulter counter (Kernohan and Lepherd 1969; Walstra and Oortwijn 1969), a technique based on the electrical sensing zone principle. When MFGs are suspended in an electrolyte and passed through an orifice of known size, the stream of MFGs is subjected to an electric field using electrodes on both sides of the orifice. The passage of the MFGs through the orifice causes changes in the electrical impedance generating voltage pulses. The amplitude of these voltage pulses is in proportional to the volumes of the MFGs. These pulses are then counted and grouped according to size using a pulse height analyser. The sample has to be diluted enough to ensure that MFGs pass the orifice droplet by droplet. The output is generated in the form of number based particle size distribution. The volume-surface diameter of MFG of mixed breed cows determined by a 30-μm orifice Coulter counter was reported to be 2.6 μm (Kernohan and Lepherd 1969). The Coulter counter was noted to be fairly accurate compared to microscopic and turbidimetric analyses (Walstra and Oortwijn 1969). However, this method does not provide good representation of total size distribution because small MFG such as in homogenised and skim milk cannot be counted (Cornell and Pallansc 1966; Walstra and Oortwijn 1969).

3.3 Light Scattering Techniques

Light scattering techniques can be either static or dynamic. Static scattering techniques measure the time-averaged structure of a system. In static light scattering (also known as laser diffraction, (near-) forward light scattering and low-angle laser light scattering), a monochromatic laser light is scattered by the fat globules to be

analysed. Hence, MFG size is measured based on the intensity characteristics of the scattering pattern produced by the fat globules in a sample. The scattering pattern is measured using a series of detectors positioned at various angles. The scattering pattern is dependent on the intensity of scattered light, which in turn is dependent on the size of the MFGs, refraction index and the wavelength of incident light. The signal measured by the detectors is then converted into a MFG size distribution using various theoretical models such as Mie and Fraunhofer theories. Figure 2.2 presents the milk fat globule size and size distribution determined by laser light scattering, showing the span of MFG size from 0.1 to 10 μm. In fact, all colloidal particles in the milk system (MFG, casein micelles etc.) scatter light and thus contribute to the yielded volume distribution counts. To eliminate the interference of casein micelles, (typical size range 0.1–0.3 μm) milk fat samples are commonly diluted (1:1 volume) with alkaline casein-dissolving buffer such as EDTA/NaOH, pH 7.0 to dissociate casein micelles and any aggregates (Michalski et al. 2001).

In dynamic light scattering, MFG size can be examined by correlating variations in light intensity to the Brownian movement of the particles. The yielded hydrodynamic radius of the particles and size distribution are converted from the diffusion speed of the particle. The sample needs to be highly diluted to prevent multiple scattering, which tends to limit the application of this technique to fluid milk and diluted dairy emulsions (Dalgleish and Hallett 1995). The effective size-range for this technique (0.002–6 μm) also allows the particle size of dairy-based nanoemulsions (176–200 nm) to be tracked by dynamic light scattering (Truong 2013).

3.4 Scanning Flow Cytometry

Scanning flow cytometry is a recent advance in MFG size characterisation, which has been reported to provide nanometric precision (Konokhova et al. 2014). This is based on single particle analysis that allows simultaneous determination of diameter and refractive index of individual MFG without recourse to ancillary measurements (Cheong et al. 2009). Scanning flow cytometry minimises the dependence on the refractive index of globules as a priori knowledge in the conversion of diffraction patterns to MFG size values. The MFG size of raw milk and processed milks (2.5 and 3.2 % fat) measured by scanning flow cytometry was reported to be 2.285, 1.115 and 1.133 μm, respectively. The corresponding refractive indices were in the range of 1.474–1.482 (Konokhova et al. 2014).

References

Cheong FC, Xiao K, Grier DG. Technical note: characterizing individual milk fat globules with holographic video microscopy. J Dairy Sci. 2009;92(1):95–9.

Cornell DG, Pallansc MJ. Counting and sizing fat globules electronically. J Dairy Sci. 1966;49(11):1371–5.

Dalgleish DG, Hallett FR. Dynamic light-scattering – applications to food systems. Food Res Int. 1995;28(3):181–93.
Huppertz T, Kelly AL. Physical chemistry of milk fat globules. In: Fox PF, McSweeney PLH, editors. Advanced dairy chemistry volume 2: lipids, vol. 2. 3rd ed. New York: Springer; 2006.
Kernohan EA, Lepherd EE. Size distribution of fat globules in cows milk during milking, measured with a coulter counter. J Dairy Res. 1969;36(2):177–82.
Konokhova AI, Rodionov AA, Gilev KV, Mikhaelis IM, Strokotov DI, Moskalensky AE, Yurkin MA, Chernyshev AV, Maltsev VP. Enhanced characterisation of milk fat globules by their size, shape and refractive index with scanning flow cytometry. Int Dairy J. 2014;39(2):316–23.
McCrae CH, Lepoetre A. Characterization of dairy emulsions by forward lobe laser light scattering - application to milk and cream. Int Dairy J. 1996;6(3):247–56.
Michalski MC, Briard V, Michel F. Optical parameters of milk fat globules for laser light scattering measurements. Lait. 2001;81(6):787–96.
Miles CA, Shore D, Langley KR. Attenuation of ultrasound in milks and creams. Ultrasonics. 1990;28(6):394–400.
Ong L, Dagastine RR, Kentish SE, Gras SL. The effect of milk processing on the microstructure of the milk fat globule and rennet induced gel observed using confocal laser scanning microscopy. J Food Sci. 2010;75(3):E135–45.
Precht D. Fat crystal structure in cream and butter. In: Garti N, Sato K, editors. Crystallization and polymorphism of fats and fatty acids. New York: Marcel Dekker; 1988. p. 305–61.
Robin O, Paquin P. Evaluation of the particle-size of fat globules in a milk model emulsion by photon-correlation spectroscopy. J Dairy Sci. 1991;74(8):2440–7.
Truong T. Physical properties of milk fats in nanoemulsions. The University of Queensland; 2013.
Truong T, Morgan GP, Bansal N, Palmer M, Bhandari B. Crystal structures and morphologies of fractionated milk fat in nanoemulsions. Food Chem. 2015;171:157–67. doi:10.1016/j.foodchem.2014.08.113.
Wade T, Beattie JK. Electroacoustic determination of size and zeta potential of fat globules in milk and cream emulsions. Colloids Surf B. 1997;10(2):73–85.
Walstra P, Oortwijn H. Estimating globule-size distribution of oil-in-water emulsions by coulter counter. J Colloid Interface Sci. 1969;29(3):424–31.

Chapter 4
Methodologies to Vary Milk Fat Globule Size

Apart from the basic role of milk fat in delivering energy in the form of fat globules to the suckling calf, there has been an increasing interest in the significance and potential industrial usefulness of different MFG size fractions. On theoretical grounds, it seems reasonable to suggest that significant variations in MFG size might have implications for the processability, physical functionality and nutritional properties of some fat-based dairy foods and ingredients (Argov et al. 2008). Three main approaches have been used in attempting to manipulate MFG size distribution. These are (1) herd management strategies (selection and breeding, modification of cow diet, milking at different stages of lactation, milking frequency); (2) fractionation (gravity- and density-based sedimentation and microfiltration); and (3) shear processing (high pressure homogenisation, microfluidisation and ultrasonication). The scope of these various strategies is summarised in Fig. 4.1.

4.1 Herd Management

4.1.1 Breeding

Several studies have established a correlation between cow genetics and MFG size. It was observed that Jersey cows tended to produce larger MFG (average diameter > 5.31 μm) than other breeds such as Holstein, Friesians, and Brown Swiss (Carroll et al. 2006; Martini et al. 2003; Banks et al. 1986). This is assumed to be due to the presence of high percentage of large-size population (>6 μm) in Jerseys even though their number of fat droplets are much smaller (3.55×10^9) compared to Italian and German Friesians cows (4.33×10^9 and 4.19×10^9, respectively) (Martini et al. 2003). Using a Coulter particle size counter, Banks et al. (1986) reported that the number of large fat globules (>5 μm) in Jersey cows was 50-fold greater than that of Friesian

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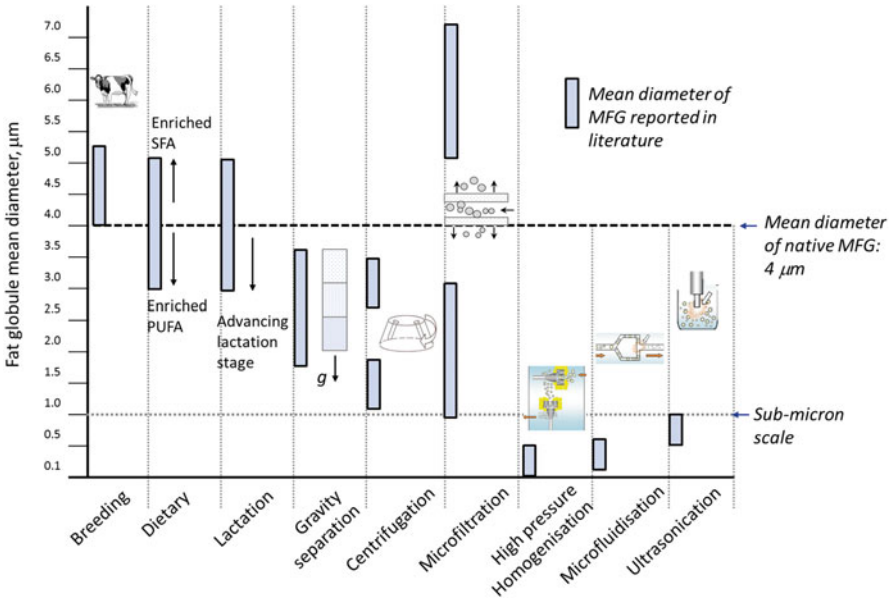

Fig. 4.1 Schematic illustration of strategies used to vary MFG size and corresponding mean diameter of MFG values reported in literature. Shear-processing downsizes the mean diameter of native MFG from 4 µm to sub-micron size. *SFA* saturated fatty acids, *PUFA* polyunsaturated fatty acids, *g* gravitational force

cows. Wider size distribution (1–12 µm) was also found in Jerseys as compared to Friesians (1–10 µm). Carroll et al. (2006) also pointed out that Jersey milks had the highest value of $d_{0.9}$ parameter in particle size analysis using a Malvern Mastersizer, followed by Holstein and Brown Swiss cows. However, neither $d_{0.5}$ nor $d_{0.1}$ and mean surface-weighted globule size and size distribution were different (Table 4.1).

Naturally variation in MFG size between individual cows also exists (Couvreur et al. 2007; Logan et al. 2014a). The discrepancy in globule size from cow to cow can be up to 1 µm (Mulder and Walstra 1974). A recent study found that there was a wide span of mean globule size (2.5–5.7 µm) among a single herd of 78 Holstein-Friesian cows (Logan et al. 2014a). Hence, the authors suggested that size-based selection of cows might be an alternative route to mechanical fractionation to obtain industrial quantities of MFG size-classified milks.

4.1.2 Dietary Supplementation

It has been reported that cows on diets with a high proportion of saturated fatty acids tend to produce larger MFG above 4 µm (Wiking et al. 2003). In contrast, reduction in globule size (about 0.3–1.0 µm) and narrower size distribution can be obtained by

Table 4.1 Fat globule size of different breed of cows

Breeds	Mean size and range	Size measurement method	References
Jerseys	$D_{3,2}$ 1.65 μm; $d_{0.5}$ 3.25 μm	Malvern Mastersizer	Carroll et al. (2006)
	Span: 1.75		
Holstein	$D_{3,2}$ 1.66 μm; $d_{0.5}$ 3.31 μm		
	Span: 1.77		
Brown Swiss	$D_{3,2}$ 1.59 μm; $d_{0.5}$ 3.00 μm		
	Span 1.67		
Jerseys	D_{vs}: 7.68 μm	Microscopy	Czerniewicz et al. (2006)
Holstein-Friesian	D_{vs}: 6.19 μm		
Jerseys	5.31 μm	Fluorescent microscopy	Martini et al. (2003)
Friesians (Italian)	4.93 μm		
Friesians (German)	4.97 μm		
Holstein	2.76–3.33 μm	Light microscopy	Timmen and Patton (1988)
Holstein + Brown Swiss	D_{32} 3.31 μm; D_{43} 3.88 μm	Malvern Mastersizer	Menard et al. (2010)
	Mode 3.56 μm		
Holstein-Friesians	D_{43} 3.8–3.9 μm	Malvern Mastersizer	Logan et al. (2014a)

adding unsaturated lipids such as fish oil, canola oil, linseed oil, whole soybean and fresh grass into cow diets (Avramis et al. 2003; Couvreur et al. 2007; Lopez et al. 2008) as listed in Table 4.2.

It is apparent that the modification of feed inputs in this way can regulate both the synthesis and secretion of milk fat (Wiking et al. 2004). For bovine milk, it was found that total lipid content is higher with an increase in amount of dietary fat in cow diets (Wiking et al. 2003). For the same globule size, more MFG would be formed in response to a higher lipid content, provided that an increased amount of membrane material was also available. However, feeding trials have suggested that the supply of membrane material (principally polar lipids) to secretory cells can become limiting in these circumstances, leading to the preferential formation of larger MFG (Couvreur et al. 2007; Menard et al. 2010; Wiking et al. 2003, 2004). Consistent with this hypothesis, studies have also shown an association between high lipid diets, increased fat globule size and a decrease in activity of the enzyme γ-glutamyl transpeptidase, an indicator of membrane material production (Wiking et al. 2004). Quantitative chemical analyses have also shown that larger MFG have a smaller (TAG : phospholipid) ratio of triacylglycerol over phospholipids as compared to smaller MFG (Lopez et al. 2011; Mesilati-Stahy et al. 2011).

4.1.3 Lactation Stage and Milking

It is widely accepted that there are differences in quantity and composition of fatty acids and polar lipids as well as MFG sizes depending on lactation stage (Mesilati-Stahy and Argov-Argaman 2014; Wiking et al. 2004). In one example, the mean diameter of MFG decreased from 4.4 to 2.9 μm with advancing lactation stage (Walstra et al. 2005). Reduction in globule size is associated with the changing energy balance along lactation stages. Towards late lactation, energy balance is positive and MFGM material is more available to coat the microlipid droplets, resulting in a greater proportion of smaller fat globules upon secretion (Martini et al. 2013).

In another study, MFG size became slightly larger when milking frequency was increased from two (4.28 ± 0.06 μm) to four (4.39 ± 0.07 μm) daily. A comparison of MFG distribution between the two milking times showed that the small-size population ($d_{0.1}$) was unchanged while large-size population ($d_{0.9}$) grew bigger with increased milking time (Wiking et al. 2006). Since the fat yield and fat content remained the same across the milking times, in this case, the growth of globule size with milking times can be best explained by a shift from medium to large globule size (Wiking et al. 2006). However, in another study (Abeni et al. 2005) neither milking system (automatic milking system, milking parlour) nor milking interval had a marked impact on MFG size range.

Table 4.2 Selected studies on effect of lipid dietary supplements on bovine milk composition and physical properties

Cow breeds and type of feed	Mean size and range	Remarks	References
Holstein cows:	Control: 2.31 µm	Fish meal:	Avramis et al. (2003)
Corn-based diet + protein supplement containing fish meal (60 % herring + 15 % feather + 25 % wheat)	Fish meal: 1.84 µm	– MFG size ↓	
		– Casein micelle size ↓ (FM: 170.8 nm; control: 187.5 nm)	
		– Fat level ↓	
		– α_{s1}-casein, β-casein ↓	
		– Unchanged: protein, lactose, somatic cell counts and remaining milk proteins	
		– Longer churning time in butter	
Holstein and Montbeliarde cows:	Control:	Linseed-enriched:	Lopez et al. (2008)
Maize silage (grassland hay, mixture of cereals, soybean meal) + linseed	D_{43}: 4.73 µm;	+ 18 % phospholipids ↑	
	D_{32}: 3.66 µm;	+ 30 % sphingomyelins ↑	
	Span: 1.29	+ 1.7-fold stearic acid ↑	
	Linseed-enriched:	+ 1.36-fold UFA ↑	
	D_{43}: 4.56 µm;	+ 3.7-fold $C_{18:1}$ trans ↑	
	D_{32}: 3.93 µm		
	Span: 1.07		
Holstein cows:	Corn-silage:	Pasture:	Couvreur et al. (2007)
Pasture (perennial ryegrass and white clover)	D_{43}: 3.94 µm;	– MFG size ↓	
	D_{32}: 3.38 µm.	– Monounsaturated and polyunsaturated FA ↑	
	Pasture:	– Protein content, calcium mineralisation ↓	
	D_{43}: 3.65 µm;		
	D_{32}: 3.15 µm.		

(continued)

Table 4.2 (continued)

Cow breeds and type of feed	Mean size and range	Remarks	References
Holstein cows:	Barn-fed:	Pasture-fed:	Timmen and Patton (1988)
Barn-fed (grass or wheat silage)	Cream: 3.17 µm	–MFG size ↓	
Pasture (corn cob silage)	Skim milk: 1.72 µm		
	Pasture-fed:		
	Cream: 2.81 µm		
	Skim milk: 1.41 µm		
Holstein cows:	Control: 4.18 µm	Linseed-enriched:	Hurtaud et al. (2010)
Control (corn silage 70 %, cereal-based, soybean)	Linseed 1: 4.07 µm	– Monounsaturated and polyunsaturated FA ↑	
Linseed-enriched 1 (corn silage 70 %, cereal-based, extruded linseed 2.1 %)	Linseed 2: 4.49 µm	– *Butter making*: churning time ↓	
Linseed-enriched 2 (corn silage 70 %, cereal-based, extruded linseed 4.3 %)		– Physical properties of butter	
		Fat loss and moisture ↑	
		Fat content and iodine value ↓	
		Firmness ↓, brightness, yellow index ↓, spreadability ↑	

4.2 Size Fractionation Without Shear

4.2.1 Gravity Separation

Gravity separation is a traditional method to separate milk fat relying mainly on the density difference of two phases (water and fat) as in the natural creaming process of milk. However, gravity-based fractionation of MFG is not only subject to Stokes' law (i.e. lower density material rises over higher density material) but also depends on the size of the fat globules (Ma and Barbano 2000). After an aging time of 2–48 h at 4 or 15 °C, a gradient of largest to smallest globule fraction from the top to the bottom of a vertical column allowed the separation of different size fractions of 1.2, 2.3, 2.8 and 3.6 μm. This separation also appeared to be related to compositional differences between large and small fat globules (Ma and Barbano 2000). Smaller fat globules tend to have greater mass of milk fat globule membrane than the volume of fat. Since the membrane proteins have higher density than that of fat, the density of smaller fat globules is higher than the larger ones. Temperature and aging time also influence the rate of sedimentation and fractionation efficiency. Leong et al. (2014b) reported that whole milk creamed fastest at 25 °C. At this temperature after 5 min, the MFG size (4.5 ± 0.06 μm) became larger at the top layer ($D_{4,3}$ 4.62 ± 0.05 μm) whereas the MFG sizes at 5 and 40 °C were unchanged (4.4 ± 0.03 and 4.3 ± 0.07 μm, respectively). The rising speed of the fat globules is faster with increasing temperature (3.6 μm fraction obtained after 2 h at 15 °C) and smaller fat globules can be achieved with longer aging time (1.2 μm fraction after 48 h at 4 °C) (Ma and Barbano 2000). Gravity separation of milk fat globules has also been tested in a two-stage procedure. In this method, the primary stage is undertaken under quiescent conditions at 4 °C for 6 h to obtain semi-skim milk. In the second stage, the semi-skim milk is separated into skim and cream portions. The supernatant (i.e. cream) retained after the second stage had average globule size of D_{43} 3.45 μm, which is slightly different to the control whole milk (D_{43} 3.58 μm) (O'Mahony et al. 2005).

The gravity separation of MFG is advantageous in helping to preserve the intact native MFGM and in small-scale production where there is no access to a centrifugal cream separator (Ma and Barbano 2000). A potential application of this process has been proposed in the traditional manufacturing procedure for Grana Padano and Parmigiano-Reggiano cheeses, in which gravity separation is part of the cheese-making processes (O'Mahony et al. 2005). The main drawback of this simple fractionation technique is its inefficiency in separating discrete fat globule size fractions. For example, in the study of Ma and Barbano (2000), the smallest size globules were still present in the largest size fraction. Also, milk needed to be aged for a relatively long time (48 h) to obtain the smallest size fraction.

4.2.2 Ultrasonic-Assisted Separation

Ultrasonication has also been employed in MFG separation and size fractionation. When ultrasonic standing waves are applied to the milk system, physical destabilisation of MFG is driven by a so-called primary acoustic radiation force, which

initiates formation of floccules and clusters of MFG and can be used to accelerate the creaming process (Juliano et al. 2011; Leong et al. 2014b). The effectiveness of ultrasonic-assisted creaming was reported to be a function of size and physical properties of MFG. The larger the MFG size, the stronger the manipulation by ultrasound and influence of buoyancy. For instance, upon sonication at 400 kHz or 1.6 MHz for 5 min at 35 °C, smaller MFG in fine recombined emulsion (3.5 % fat; $D_{4,3}$ 2.7 μm) are resistant to creaming whereas creaming is enhanced with larger MFGs in coarse recombined emulsion ($D_{4,3}$ 9.3 μm) and raw milk ($D_{4,3}$ 4.9 μm) (Juliano et al. 2011). Interfacial surface characteristics of MFG also played an important role in the coalescence rate. Initiation of flocculation/coalescence using the ultrasonication is more difficult in milk having intact MFGM, which acts as a stabilising barrier, compared to self-assembly of casein micelles and milk proteins on surface of recombined milk emulsions (Leong et al. 2014a). As solid/liquid state of milk fat is temperature dependent, temperature is also an influential factor in ultrasound separation efficacy. Investigation on ultrasonic separation (1 MHz frequency ultrasound for 5 min) of natural whole milk (4.3–4.5 μm) at 5, 25, and 40 °C showed that high discrepancy in MFG sizes between the top (4.9 μm) and bottom (4.0 μm) samples can be obtained with milk preheated at 25 °C, followed by 40 °C (bottom layer: 4.1 μm; top layer: 4.8 μm). The ultrasound separation of natural whole milk is less effective at 5 °C with lower differentiation of MFG sizes in the bottom and top fractions (4.39 and 4.44 μm, respectively) (Leong et al. 2014b). It was postulated that the dependence of ultrasound separation of MFG size on temperature is attributable to agglutination, which drives the flocculation process. If this is the case, the ultrasound separation would be highly effective at 5 °C because agglutinin-driving flocculation decreases with increasing temperature. However, the fat separation efficacy is lowest at 5 °C in the study of Leong et al. (2014b). A possible cause might be due to changes of immunoglobulins upon ultrasonication (Leong et al. 2014b). At this point, it should be mentioned that ultrasonication is capable of inducing crystallisation of bulk fat (Martini et al. 2008; Wagh et al. 2013). While the impact of ultrasonication on crystallisation behaviour of emulsified fat, i.e. MFG, is still unclear; it is possible that ultrasonication may alter the solid/liquid fraction of the fat, in turn, affecting the flocculation and/or coalescence of MFGs.

Beyond the nature of MFG, ultrasonic-assisted creaming process is also influenced by frequency of ultrasound. A corresponding higher acoustic force created by applying high frequency ultrasound resulted in better separation of MFG. It was reported that applying 1 MHz of ultrasound frequency on natural whole milk at 25 °C (4.5 μm) promoted larger change of MFG size (the top: 4.9 μm, the bottom: 4.0 μm) than that of 600 kHz (the top: 4.7 μm, the bottom: 4.4 μm) (Leong et al. 2014b). Furthermore, such high frequency of ultrasound (1 MHz) did not damage the integrity of MFG as confirmed by unchanged zeta-potential values of MFG (Leong et al. 2014a).

Other ultrasonic conditions impacting on separation efficacy of MFG include processing time, specific energy density input, mode of operation (single or dual transducer(s)), geometry of vessel etc. (Leong et al. 2014a, b; Juliano et al. 2011).

4.2.3 Centrifugation

A well-established method for isolation of MFG is centrifugation, which can be used to separate milk fat on density basis. This is used commercially in the manufacture of skim milk, cream and butter. In general, the sedimentation velocity of centrifugal method is 6500-fold faster than that of naturally occurring gravity separation (TetraPak 2009). Applying only mild centrifugation ($150 \times g$) is able to generate a gradient of milk fat from about 5 μm to 2.5–3 μm from top to the bottom layers, respectively (Logan et al. 2014b). Using a two-step centrifugal method at $300 \times g$ for 15 min in the first step to obtain skim milk, which was then further centrifuged at $33{,}000 \times g$ at 4 °C, large fat globules aggregated in the cream layer (2.76–3.33 μm) whilst smaller fat globules (1.02–1.77 μm) were retained in the skim milk (Timmen and Patton 1988).

4.2.4 Microfiltration

Membrane processing based on fat globule size can also be effective in separating different MFG size classes discretely (Lopez et al. 2011; Michalski et al. 2006; Goudedranche et al. 2000; Fauquant et al. 2005). For example, cross-flow microfiltration is able to generate 1 μm larger (retentate; 5–7.5 μm) and smaller (permeate; 0.9–3 μm) size fractions as compared to the raw whole milk (4.2 μm) by utilising various membrane pore sizes (i.e. 2–12 μm) and hydrodynamic conditions (volume reduction factor, permeation flux, tangential shear stress etc.) (Michalski et al. 2006). This membrane process is also beneficial to maintaining the integrity of native MFGM as confirmed by unchanged zeta-potential values of size-differentiated micro-filtered MFG (Michalski et al. 2002b). It was also reported that tangential shear stress at the wall (T_w) as high as 200 Pa did not induce any break-up of MFG due to shear and cavitation. The MFGM was undamaged as zeta-potential values of both permeate and retentate at $T_w = 200$ Pa were similar to the original raw milk (Michalski et al. 2006). Milk aging, as required in gravity separation, is unnecessary in the microfiltration process. Membrane fouling is the main disadvantage of this method (Michalski et al. 2006).

4.3 Mechanical Shear to Reduce Fat Globule Size

Mechanical shear-processing (commonly various types of homogenisation) is widely used in the industrial processing of milk and dairy products to reduce MFG size and enhance emulsion stability. In comparing these sizing methods with other techniques, it is important to note that shear-processing of milk and dairy ingredients commonly results not only disruption of the MFGM but also incorporation of dairy proteins and other surface-active molecules (emulsifiers) into the resultant, reformed and

emulsified MFG. Shear-processing will thus result in both chemical and physical changes to the MFGM that may give rise to differences in emulsified MFG properties beyond those that can be accounted for simply by differences in MFG size.

4.3.1 Homogenisation/High Pressure Homogenisation

Conventional milk homogenisation is essentially a process of mechanical disruption of fat globules into finer ones by employing rupture forces of cavitation, shear and high turbulence. These mechanical forces result from pressure differences in the homogenising valve and collision with the impact ring (Paquin 1999). Two-stage homogenisation of milk is widely practiced in dairy industry. Here, pre-heated milks (50–60 °C) are processed to go through the first stage valve at high pressure (10–30 MPa) to break up the fat globules and the subsequent second stage valve at much lower pressure (3–5 MPa) to separate any agglomerates formed (Walstra et al. 1999). Homogenising raw milk at 20 MPa can result in a tenfold reduction in MFG size in the processed milk raw milk and a significant narrowing of particle size distribution (Lopez 2005). This reduction in size range improves emulsion stability and helps to prevent the aggregation of fat globules upon storage of milk and dairy beverages.

High pressure homogenisation (HPH; 50–350 MPa), which has similar operating principles to conventional homogenisation, seems to have a similar effect on reduction of MFG size. It was found that there was no significant difference in fat globule size reduction between conventional homogenisation (18 MPa; D_{43} 0.7 μm) and either one-stage or two-stage HPH (50–200 MPa; 0.62–3.20 μm). Reduction of MFG size was further extended to a mean size of 0.4–0.5 μm when fat globules were converted to a liquid state by pre-warming the milk prior to two-stage HPH (Hayes and Kelly 2003; Thiebaud et al. 2003). A similar trend was also observed in the combined pasteurisation and HPH (D_{43} 0.48–0.86 μm) as against commercial milk processed by conventional homogenisation (D_{43} 0.88 μm) (Hayes et al. 2005). Serra et al. (2007) found that the smallest achievable fat globule size could be obtained at 200 MPa and inlet temperature of 40 °C (0.15 μm) or 300 MPa and inlet temperature of 30 °C (0.16 μm). Clusters of fat globules occurred at high pressures above 230 MPa causing formation of larger globule size (0.48–4.25 μm) due to aggregation (Serra et al. 2007; Thiebaud et al. 2003).

This apparent inefficiency in attempting to downsize of MFG by HPH might be partly explained by an insufficient amount of emulsifier (i.e. milk proteins) or by the limited effectiveness of the available emulsifier to further reduce the interfacial tension between fat particles and aqueous phase in the system. Upon homogenisation, when droplet size decreases, the surface area increases about 5–10 times. The available amount of native MFGM is insufficient to stabilise the newly generated globules. In homogenised milk, stabilisation of the newly formed milk emulsion is achieved by replacement or reinforcement of the MFGM with milk proteins, mainly caseins in form of casein micellar layer (about 90 nm in thickness) and whey proteins, adsorbed onto the droplet surface (Fig. 2.1) (Mcpherson et al. 1984; Sharma

and Dalgleish 1993; Dalgleish et al. 1996). It has been demonstrated that the adsorbed protein layer is significantly thinner (<10 nm thick) in the case of smaller droplet sizes. Fragmentation of casein micelles may be required to stabilise such a thin layer (Dalgleish et al. 1996). In recombined emulsion systems of corn oil, it was found that at the same amount of emulsifier, an increase in oil concentration resulted in a larger droplet size (Pandolfe 1995). Whilst similar effects have also been observed in dairy systems, it has also been demonstrated that HPH can be very effective in achieving much further reduction in MFG size, provided that sufficient emulsifier is present to stabilise the system. For example, in recombined milk fat emulsion systems stabilised by dairy-based emulsifiers (whey proteins and sodium caseinate at 0.5–3.0 % w/w) it was possible to use high homogenisation pressure (87–123 MPa) to reduce MFG size to a modal mean of about 200 nm, with a narrow size distribution (Truong 2013).

4.3.2 Microfluidisation

Although the rupture forces (shear, turbulence, cavitation) caused by microfluidisation are similar to those associated with homogenisation, interaction with the product and the mechanism of generation of these forces is somewhat different. A microfluidiser consists of an interaction chamber where the product is accelerated by an intensifier pump and divided to two streams passing through two opposite microchannels. Intensive disruption forces are generated firstly by forcing the material through these narrow channels and secondly when these two rapidly flowing streams collide with each other in the interaction chamber, causing droplet break-up (Paquin 1999). The peak shear rate of a microfluidiser is estimated to be around 10^8–10^9 s^{-1} (Mason et al. 2006) and commercial systems can be pressurised up to about 200 MPa (Microfludics 2010).

In processing milk and cream systems, microfluidisation is more effective in reduction of MFG size than HPH at moderate pressure ranges (Mccrae 1994; Hardham et al. 2000; Dalgleish et al. 1996). At the same homogenising pressure (40 MPa), globule size of microfluidiser milks (280 nm) is smaller than that of conventionally homogenized milks (400 nm) (Dalgleish et al. 1996). To achieve a similar D_{32} diameter of fat globules, operating pressures needed on a microfluidiser and homogeniser are 35 MPa and 48 MPa, respectively (Mccrae 1994). Microfluidisers are apparently less sensitive to variations in operating pressure than homogenisers (Hardham et al. 2000; Olson et al. 2004). Efficiency in downsizing of MFG in fresh milks and recombined dairy emulsions depends on microfluidising pressure (Hardham et al. 2000; Olson et al. 2004; Truong 2013) as well as the fat content of the product (Olson et al. 2004). Fat globule size was reduced with increasing microfluidising pressure (460 and 304 nm at 50 and 100 MPa, respectively) but recoalescence of newly generated fat globules occurred at pressure above 100 MPa, ultimately resulting in larger fat globules (361 and 383 nm at 150 and 200 MPa, respectively) (Olson et al. 2004). In the formation of milk fat nanoemulsions, it was

also reported that microfluidising pressure (83–123 MPa) was the most influential factor, followed by number of cycles (2–6) and emulsifier concentration (0.5–5.0 % w/w) in achieve a droplet sizes less than 200 nm (Truong 2013). Others have shown that MFG size tends to increase with an increase in milk fat level at the same microfluidising pressure (Olson et al. 2004).

4.3.3 Ultrasonication

Ultrasonic homogenisation is essentially a process of acoustic cavitation caused by the intense mechanical vibrations generated by acoustic power, typically at a frequency of 20 kHz. The disruption of MFG is induced by cavitation forces resulting from the formation of the high pressure gradient generated when bubbles collapse upon sonication (Mason et al. 2006). The efficiency in reduction of MFG size using ultrasonication typically depends on ultrasonic power, exposure time, and treatment temperature. Relatively high ultrasonic power is needed to disrupt MFG (Villamiel and de Jong 2000; Ertugay et al. 2004). A sub-micron range of MFG size (0.5–0.7 μm) can be achieved with an ultrasound power range of 400–450 W (Bermudez-Aguirre et al. 2008; Ertugay et al. 2004; Villamiel and de Jong 2000). Sonication power as low as 20–40 W for 1–10 min produces a similar MFG size range (2–5 μm) as conventional homogenisation (20 MPa). In general, an increase in ultrasonic power, along with longer treatment duration, yields a lower fat globule size (Wu et al. 2000; Ertugay et al. 2004). It was also observed that the globule size and size distribution becomes smaller (0.57–0.95 μm) when milk is pre-heated to 70–75 °C. It was speculated that this might be related to deeper penetration of ultrasound into the system when viscosity of milk decreased with increasing treatment temperature (Villamiel and de Jong 2000). In a continuous flow ultrasonication system, a longer residence time (102.3 s) also led to a 5 % fat globule size reduction (Villamiel and de Jong 2000).

4.4 Effect of Homogenisation on Microstructure of Milk Fat Globules

While homogenisation can be effective in downsizing MFG size to the sub-micron range, it can also alter the structure of milk fat globules as illustrated in Fig. 2.1. During the process of division of the fat globules the MFGM is ruptured and eventually re-adsorbed on to newly formed droplets. Disruption of the original protective membrane of native MFG is also associated with accelerated lipolysis, which is commonly observed in homogenised milk and creams (Goudedranche et al. 2000). Furthermore, reduction in the fat globule size leads to increased globule surface area. It is estimated that only 10–25 % of homogenised milk is coated by intact MFGM (Lopez 2005). In homogenised milk, the greater proportion of newly generated fat globule surface area is not coated by the native membrane material but stabilised by

adsorbed caseins, whey protein and fragments of MFGM (Michalski et al. 2002a). The thickness of protein layer in the MFGM tended to be thinner in microfluidised milks than that of pressure-homogenised milks (Dalgleish et al. 1996). After ultrasound treatment, scanning electron microscopy of fat globules from thermosonicated-treated milk (120 μm amplitude, 63.5 °C for 30 min) appeared to show that MFGM was cracked and disintegrated. Thermosonicated globules smaller than 1 μm exhibited a granular surface, possibly caused by an interaction between the casein micelles and the disrupted MFGM (Bermudez-Aguirre et al. 2008).

It is worth mentioning that homogenisation not only breaks up the MFG but also modifies the structure of casein micelles and whey proteins. High pressure homogenisation disrupts the hydrophobic interactions between β-casein and κ-casein in the supramolecular structure of the micelle, causing dissociation into casein micellar fragments. This also induces unfolding of the protein structure that facilitates the release of ionic calcium. It was reported that β-lactoglobulin, the main component of whey proteins, is denatured by hydrostatic pressure above 100 MPa whereas α-lactalbumin and bovine serum albumin are not susceptible to pressure below 400 MPa (Lopez-Fandino et al. 1996). High pressure homogenization (50–200 MPa) also results in partial unfolding of whey proteins and exposure of the hydrophobic groups, facilitating lipid-protein interaction (Lee et al. 2009).

Taken together, the altered structures might create either detrimental or improved impact on physicochemical properties and functionalities of dairy products using homogenised milks. The effect of reduced globule size by homogenisation and associated changes in dairy products will be discussed later.

References

Abeni F, Degano L, Calza F, Giangiacomo R, Pirlo G. Milk quality and automatic milking: fat globule size, natural creaming, and lipolysis. J Dairy Sci. 2005;88(10):3519–29.

Argov N, Lemay DG, German JB. Milk fat globule structure and function: nanoscience comes to milk production. Trends Food Sci Technol. 2008;19(12):617–23. doi:10.1016/j.tifs.2008.07.006.

Avramis CA, Wang H, McBride BW, Wright TC, Hill AR. Physical and processing properties of milk, butter, and Cheddar cheese from cows fed supplemental fish meal. J Dairy Sci. 2003;86(8):2568–76. doi:10.3168/jds.S0022-0302(03)73851-X.

Banks JM, Clapperton JL, Muir DD, Girdler AK. The influence of diet and breed of cow on the efficiency of conversion of milk constituents to curd in cheese manufacture. J Sci Food Agric. 1986;37(5):461–8. doi:10.1002/jsfa.2740370505.

Bermudez-Aguirre D, Mawson R, Barbosa-Canovas GV. Microstructure of fat globules in whole milk after thermosonication treatment. J Food Sci. 2008;73(7):E325–32. doi:10.1111/j.1750-3841.2008.00875.x.

Carroll SM, DePeters EJ, Taylor SJ, Rosenberg M, Perez-Monti H, Capps V. Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. Anim Feed Sci Technol. 2006;131(3–4):451–73. doi:10.1016/j.anifeedsci.2006.06.019.

Couvreur S, Hurtaud C, Marnet PG, Faverdin P, Peyraud JL. Composition of milk fat from cows selected for milk fat globule size and offered either fresh pasture or a corn silage-based diet. J Dairy Sci. 2007;90(1):392–403.

Czerniewicz M, Kietczewska K, Kruk A. Comparison of some physicochemical properties of milk from Holstein-Friesian and Jersey cows. Pol J Food Nutr Sci. 2006;15(56):61–4.

Dalgleish DG, Tosh SM, West S. Beyond homogenization: the formation of very small emulsion droplets during the processing of milk by a microfluidizer. Neth Milk Dairy J. 1996;50(2): 135–48.

Ertugay MF, Sengul M, Sengul M. Effect of ultrasound treatment on milk homogenisation and particle size distribution of fat. Turk J Vet Anim Sci. 2004;28(2):303–8.

Fauquant C, Briard V, Leconte N, Michalski MC. Differently sized native milk fat globules separated by microfiltration: fatty acid composition of the milk fat globule membrane and triglyceride core. Eur J Lipid Sci Technol. 2005;107(2):80–6. doi:10.1002/ejlt.200401063.

Goudedranche H, Fauquant J, Maubois JL. Fractionation of globular milk fat by membrane microfiltration. Lait. 2000;80(1):93–8.

Hardham JF, Imison BW, French HM. Effect of homogenisation and microfluidisation on the extent of fat separation during storage of UHT milk. Aust J Dairy Technol. 2000;55(1): 16–22.

Hayes MG, Kelly AL. High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. J Dairy Res. 2003;70(3):297–305. doi:10.1017/S0022029903006320.

Hayes MG, Fox PF, Kelly AL. Potential applications of high pressure homogenisation in processing of liquid milk. J Dairy Res. 2005;72(1):25–33. doi:10.1017/S0022029904000524.

Hurtaud C, Faucon F, Couvreur S, Peyraud JL. Linear relationship between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid composition and butter properties. J Dairy Sci. 2010;93(4):1429–43. doi:10.3168/jds.2009-2839.

Juliano P, Kutter A, Cheng LJ, Swiergon P, Mawson R, Augustin MA. Enhanced creaming of milk fat globules in milk emulsions by the application of ultrasound and detection by means of optical methods. Ultrason Sonochem. 2011;18(5):963–73.

Lee SH, Lefevre T, Subirade M, Paquin P. Effects of ultra-high pressure homogenization on the properties and structure of interfacial protein layer in whey protein-stabilized emulsion. Food Chem. 2009;113(1):191–5. doi:10.1016/j.foodchem.2008.07.067.

Leong T, Johansson L, Juliano P, Mawson R, McArthur S, Manasseh R. Design parameters for the separation of fat from natural whole milk in an ultrasonic litre-scale vessel. Ultrason Sonochem. 2014a;21(4):1289–98.

Leong T, Juliano P, Johansson L, Mawson R, McArthur SL, Manasseh R. Temperature effects on the ultrasonic separation of fat from natural whole milk. Ultrason Sonochem. 2014b;21(6): 2092–8.

Logan A, Auldist M, Greenwood J, Day L. Natural variation of bovine milk fat globule size within a herd. J Dairy Sci. 2014a;97(7):4072–82. doi:10.3168/jds.2014-8010.

Logan A, Day L, Pin A, Auldist M, Leis A, Puvanenthiran A, Augustin MA. Interactive effects of milk fat globule and casein micelle size on the renneting properties of milk. Food Bioprocess Technol. 2014b;7(3):3175–85.

Lopez C. Focus on the supramolecular structure of milk fat in dairy products. Reprod Nutr Dev. 2005;45(4):497–511. doi:10.1051/Rnd:2005034.

Lopez C, Briard-Bion V, Menard O, Rousseau F, Pradel P, Besle JM. Phospholipid, sphingolipid, and fatty acid compositions of the milk fat globule membrane are modified by diet. J Agric Food Chem. 2008;56(13):5226–36. doi:10.1021/Jf7036104.

Lopez C, Briard-Bion V, Menard O, Beaucher E, Rousseau F, Fauquant J, Leconte N, Robert B. Fat globules selected from whole milk according to their size: different compositions and structure of the biomembrane, revealing sphingomyelin-rich domains. Food Chem. 2011;125(2):355–68. doi:10.1016/j.foodchem.2010.09.005.

Lopez-Fandino R, Carrascosa AV, Olano A. The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. J Dairy Sci. 1996;79(6):929–36.

Ma Y, Barbano DM. Gravity separation of raw bovine milk: fat globule size distribution and fat content of milk fractions. J Dairy Sci. 2000;83(8):1719–27. doi:10.3168/jds.S0022-0302(00)75041-7.

Martini M, Cecchi F, Scolozzi C, Leotta R, Verita P. Milk fat globules in different dairy cattle breeds part I: morphometric analysis. Ital J Anim Sci. 2003;2:272–4. doi:10.4081/ijas.2003.s1.272.

Martini S, Suzuki AH, Hartel RW. Effect of high intensity ultrasound on crystallization behavior of anhydrous milk fat. J Am Oil Chem Soc. 2008;85(7):621–8.

Martini M, Altomonte I, Pesi R, Tozzi MG, Salari F. Fat globule membranes in ewes' milk: the main enzyme activities during lactation. Int Dairy J. 2013;28(1):36–9. doi:10.1016/j.idairyj.2012.07.002.

Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM. Nanoemulsions: formation, structure, and physical properties. J Phys Condens Matter. 2006;18(41):R635–66. doi:10.1088/0953-8984/18/41/R01.

Mccrae CH. Homogenization of milk emulsions – use of microfluidizer. J Soc Dairy Technol. 1994;47(1):28–31.

Mcpherson AV, Dash MC, Kitchen BJ. Isolation and composition of milk-fat globule-membrane material. 2. From homogenized and ultra heat-treated milks. J Dairy Res. 1984;51(2):289–97.

Menard O, Ahmad S, Rousseau F, Briard-Bion V, Gaucheron F, Lopez C. Buffalo vs. cow milk fat globules: size distribution, zeta-potential, compositions in total fatty acids and in polar lipids from the milk fat globule membrane. Food Chem. 2010;120(2):544–51. doi:10.1016/j.foodchem.2009.10.053.

Mesilati-Stahy R, Argov-Argaman N. The relationship between size and lipid composition of the bovine milk fat globule is modulated by lactation stage. Food Chem. 2014;145:562–70. doi:10.1016/j.foodchem.2013.08.077.

Mesilati-Stahy R, Mida K, Argov-Argaman N. Size-dependent lipid content of bovine milk fat globule and membrane phospholipids. J Agric Food Chem. 2011;59(13):7427–35. doi:10.1021/Jf201373j.

Michalski MC, Cariou R, Michel F, Garnier C. Native vs. damaged milk fat globules: membrane properties affect the viscoelasticity of milk gels. J Dairy Sci. 2002a;85(10):2451–61. doi:10.3168/jds.S0022-0302(02)74327-0.

Michalski MC, Michel F, Sainmont D, Briard V. Apparent zeta-potential as a tool to assess mechanical damages to the milk fat globule membrane. Colloid Surf B. 2002b;23(1):23–30. doi:10.1016/S0927-7765(01)00203-X.

Michalski MC, Leconte N, Briard-Bion V, Fauquant J, Maubois JL, Goudedranche H. Microfiltration of raw whole milk to select fractions with different fat globule size distributions: process optimization and analysis. J Dairy Sci. 2006;89(10):3778–90.

Microfluidics. M-110P Laboratory Models for Continuous High Shear Fluid Processing. M-110P Microfluidizer® Materials Processors. Westwood: Microfluidics International Corporation; 2010.

Mulder H, Walstra P. The fat dispersion. In: Mulder H, Walstra P, editors. The milk fat globule. Emulsion science as applied to milk products and comparable foods. Wageningen: Center for Agricultural Publishing and Documentation; 1974. p. 54–66.

Olson DW, White CH, Richter RL. Effect of pressure and fat content on particle sizes in microfluidized milk. J Dairy Sci. 2004;87(10):3217–23.

O'Mahony JA, Auty MAE, McSweeney PLH. The manufacture of miniature Cheddar-type cheeses from milks with different fat globule size distributions. J Dairy Res. 2005;72(3):338–48. doi:10.1017/S0022029905001044.

Pandolfe WD. Effect of premix condition, surfactant concentration, and oil level on the formation of oil-in-water emulsions by homogenization. J Dispers Sci Technol. 1995;16(7):633–50.

Paquin P. Technological properties of high pressure homogenizers: the effect of fat globules, milk proteins, and polysaccharides. Int Dairy J. 1999;9(3–6):329–35. doi:10.1016/S0958-6946(99)00083-7.

Serra M, Trujillo AJ, Quevedo JM, Guamis B, Ferragut V. Acid coagulation properties and suitability for yogurt production of cows' milk treated by high-pressure homogenisation. Int Dairy J. 2007;17(7):782–90. doi:10.1016/j.idairyj.2006.10.001.

Sharma SK, Dalgleish DG. Interactions between milk serum-proteins and synthetic fat globule-membrane during heating of homogenized whole milk. J Agric Food Chem. 1993;41(9):1407–12.

TetraPak. Centrifugal separators and milk fat standardisation. In: Dairy processing handbook. Lund: TetraPak Processing Systems AB; 2009. p. 91–113.

Thiebaud M, Dumay E, Picart L, Guiraud JP, Cheftel JC. High-pressure homogenisation of raw bovine milk. Effects on fat globule size distribution and microbial inactivation. Int Dairy J. 2003;13(6):427–39. doi:10.1016/S0958-6946(03)00051-7.

Timmen H, Patton S. Milk-fat globules – fatty-acid composition, size and in vivo regulation of fat liquidity. Lipids. 1988;23(7):685–9. doi:10.1007/Bf02535669.

Truong T. Physical properties of milk fats in nanoemulsions. The University of Queensland; 2013.

Villamiel M, de Jong P. Influence of high-intensity ultrasound and heat treatment in continuous flow on fat, proteins, and native enzymes of milk (vol 48, pg 472, 2000). J Agric Food Chem. 2000;48(7):472–8. doi:10.1021/Jf0006224.

Wagh A, Walsh MK, Martini S. Effect of lactose monolaurate and high intensity ultrasound on crystallization behavior of anhydrous milk fat. J Am Oil Chem Soc. 2013;90(7):977–87.

Walstra P, Geurts TJ, Noomen A, Jellama A, Van Boekel MAJS. Dairy technology: principles of milk properties and processes. New York: Marcel Dekker, Inc.; 1999.

Walstra P, Wouters JTM, Geurts TJ. Dairy science and technology. 2nd ed. Boca Raton: CRC; 2005.

Wiking L, Bjorck L, Nielsen JH. Influence of feed composition on stability of fat globules during pumping of raw milk. Int Dairy J. 2003;13(10):797–803. doi:10.1016/S0958-6946(03)00110-9.

Wiking L, Stagsted J, Lennart B, Nielsen JH. Milk fat globule size is affected by fat production in dairy cows. Int Dairy J. 2004;14(10):909–13. doi:10.1016/j.idairyj.2004.03.005.

Wiking L, Nielsen JH, Bavius AK, Edvardsson A, Svennersten-Sjaunja K. Impact of milking frequencies on the level of free fatty acids in milk, fat globule size, and fatty acid composition. J Dairy Sci. 2006;89(3):1004–9.

Wu H, Hulbert GJ, Mount JR. Effects of ultrasound on milk homogenization and fermentation with yogurt starter. Innovative Food Sci Emerg Technol. 2000;1(3):211–8.

Chapter 5
Size-Dependent Variations in Fatty Acid Composition and Lipid Content of Native Milk Fat Globules

Compositional differences are found across the wide size range of bovine MFG. However, no clear size-related compositional tends have been reported in the literature, presumably because of complex interactions with the effects of cow genetics, seasonal factors and cow diets as described earlier in Chap. 4. Interpretation of the data from such studies is difficult, as apparent compositional discrepancies might also be derived from differences in sampling and analytical methods (e.g. lipid extraction and particle size measurement), as well as from overlapping size ranges (i.e. the large size fraction may contain some smaller size globules and vice versa).

5.1 Total Fatty Acids

A few authors have reported that the total content of saturated and unsaturated fatty acids in the MFG is size-independent (Lopez et al. 2011; Mesilati-Stahy et al. 2011; Briard-Bion et al. 2008). However, Martini et al. (2006) found that saturated fatty acids were more prevalent in a large MFG size fraction (>6 μm).

5.2 Individual Fatty Acids

5.2.1 Short-Chain FAs

Timmen and Patton (1988) reported that there were fewer short chain FAs (−5.9 %) in small MFG (1–1.5 μm). However, later studies by Briard et al. (2003) and Lopez et al. (2011) demonstrated that the proportion of short-chain FAs remained unchanged between the two size fractions in their studies (0.96–3.26 μm vs. 5.92–7.34 μm;1.6 μm vs. 6.6 μm, respectively).

T. Truong et al., *Effect of Milk Fat Globule Size on the Physical Functionality of Dairy Products*, SpringerBriefs in Food, Health, and Nutrition,
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5.2.2 *Medium-Chain FAs*

Studies using microfiltration to separate discrete small and large MFG fractions have reported a significant increase in medium-chain FAs in small globules (Fauquant et al. 2005; Lopez et al. 2011; Michalski et al. 2005). For example, one small size fraction (2.3–3.7 μm) had higher C12:0, C14:0, and C16:0 at 4.1, 5, and 3.3 % respectively, as compared to a fraction with larger globules within size range of 5.2–8.0 μm (Fauquant et al. 2005). On the other hand, research investigating small- to large-globule secreted milks from segregated cow groups has reported a positive correlation between saturated, medium chain length FAs with increasing MFG size (Martini et al. 2006; Wiking et al. 2004; Mesilati-Stahy et al. 2011).

5.2.3 *Long-Chain FAs*

Previous studies have reported that stearic acid (C18:0) was enriched in large globule size fractions (Briard et al. 2003; Wiking et al. 2004; Michalski et al. 2005; Briard-Bion et al. 2008; Lopez et al. 2011). These results are in agreement with analyses of Timmen and Patton (1988) and Fauquant et al. (2005), who found that stearic acid decreased in small globules (−20.5 and −11.7 %, respectively).

5.2.4 *Unsaturated FAs*

There are contrary observations on effect of MFG size on the relative amounts of individual unsaturated FAs. Palmitoleic acid (C16:1) was found at a higher amounts (+20.2 %) in small globules by Fauquant et al. (2005) and Michalski et al. (2005), who used microfiltration to obtain discrete size fractions; whereas Wiking et al. (2004) reported that palmitoleic acid increased with larger globule size. Larger globules had higher oleic (C18:1) and linoleic (C18:2) acids in their TAG core (Fauquant et al. 2005; Lopez et al. 2011; Wiking et al. 2004). In contrast, smaller proportions of oleic acid were measured in the large globule size fractions studied by Timmen and Patton (1988) and Martini et al. (2006).

5.3 Conjugated Linoleic Acids (CLA)

CLA is considered as a mixture of isomers (either *cis*- or *trans*-) of octadecadienoic acid in which the double bonds are conjugated and separated by a single bond. CLA is reported to be beneficial to human health in relation to immune system improvement, anti-carcinogenic, anti-obesity and anti-diabetic properties (Belury 2002). Bovine milk is one of the primary dietary sources of these bioactive isomers.

To form CLA, isomerisation and biohydrogenisation of unsaturated fatty acids (UFAs) such as oleic acid (C18:1 *cis*-9), linoleic acid (C18:2 *cis*-9, *cis*-12) and α-linoleic acid (C18:3 *cis*-9, *cis*-12, *cis*-15) takes place in the mammary glands, reactions which are driven by Δ^9-desaturase enzyme in the rumen. The main component of CLA, rumenic acid (C18:2, *cis*-9 *trans*-11), which originates from linoleic and α-linoleic acids, accounts for 75–90 % of the total CLA (Bauman et al. 2003). The amount of rumenic acid in bovine milk fat is within range of 0.24–0.72 mg/g of fatty acids with the average value of 4.3 mg/g of fatty acids (Kelsey et al. 2003).

It has been found that total CLA is strongly associated with small MFG (Michalski et al. 2005; Lopez et al. 2011). Also, rumenic acid is more concentrated in small globules (2.9 μm; ca. 87 %) compared to the corresponding large size fraction (4.9–5.7 μm; 82–85 %) (Michalski et al. 2005). Lopez et al. (2011) also found a higher proportion of rumenic acid in small MFG (1.6 μm) than in large MFG (6.5 μm). However, with closer and less distinct size classes, there was no significant difference in the amount of rumenic acid (87 %) between the two size fractions (2.73 versus 4.8 μm) (Michalski et al. 2005). It was also found that the larger the fat globule sizes, the higher the proportion of some CLA isomers such as *trans*-11, *trans*-13, and *trans*-8, *cis*-10 (Michalski et al. 2005).

Some of these differences in fatty acid compositions suggest differences in metabolic activity between the differently sized MFGs. For example, the observation that small fat globules contain higher amount of both rumenic acid and ratios of C14:1/C14:0 and C18:1/C18:0 might indicate that desaturation activity happens at higher level in smaller droplets during milk fat synthesis (Lopez et al. 2011).

5.4 Polar Lipids

Polar lipids typically exist in MFGM, at the surface of the globule. Since the surface area: volume ratio is higher in smaller globules, the relative amount of total polar lipids, compared to triacylglycerols (mainly neutral lipids) is also higher in small MFG. In one study, quantification of total polar lipids from two MFG size fractions obtained by microfiltration of raw milk estimated the concentration of polar lipids in small globules (1.6 μm) to be 8.91 mg/g fat, which was significantly higher than in the larger MFG (6.5 μm; 2.72 mg/g fat). Among the main polar lipids, the small MFG contained relatively more phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) whereas the large MFG were richer in choline-containing polar lipids (sphingomyelin: SM and phosphatidylcholine: PC) (Lopez et al. 2011). These differences were assumed to be associated with milk secretion and the tri-layer structure of MFGM (Fig. 2.1), in which mainly PI, PS and PE are localised in the internal surface while PC and SM are in the external layer (Deeth 1997). It was postulated that since small fat globules are of greater curvature, their dynamic processes at the molecular level are correspondingly higher. As a consequence, membrane material might be lost or desorbed, preventing the incorporation of PC and SM in their MFGM (Lopez et al. 2011). Another possible explanation is

that rearrangement of materials in apical plasma membrane during post-secretion might induce loss of membrane materials. PC and SM may be detached from the membrane and form micro-some like particles through vesiculation (Lopez et al. 2011; Evers 2004). However, in contrast to these findings, others have reported that the concentration of PC was constant across six size classes obtained by gravity-based sedimentation of raw milk (Mesilati-Stahy et al. 2011).

References

Bauman DE, Corl BA, Peterson GP. The biology of conjugated linoleic acids in ruminants. In: Advances in conjugated linoleic acid research, vol. 2. Champaign: AOCS; 2003.

Belury MA. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu Rev Nutr. 2002;22:505–31. doi:10.1146/annurev.nutr.22.021302.121842.

Briard V, Leconte N, Michel F, Michalski MC. The fatty acid composition of small and large naturally occurring milk fat globules. Eur J Lipid Sci Technol. 2003;105(11):677–82. doi:10.1002/ejlt.200300812.

Briard-Bion V, Juaneda P, Richoux R, Guichard E, Lopez C. trans-C18:1 isomers in cheeses enriched in unsaturated fatty acids and manufactured with different milk fat globule sizes. J Agric Food Chem. 2008;56(20):9374–82. doi:10.1021/Jf801333j.

Deeth HC. The role of phospholipids in the stability of milk fat globules. Aust J Dairy Technol. 1997;52(1):44–6.

Evers JM. The milkfat globule membrane - compositional and structural changes post secretion by the mammary secretory cell. Int Dairy J. 2004;14(8):661–74. doi:10.1016/j.idairyj.2004.01.005.

Fauquant C, Briard V, Leconte N, Michalski MC. Differently sized native milk fat globules separated by microfiltration: fatty acid composition of the milk fat globule membrane and triglyceride core. Eur J Lipid Sci Technol. 2005;107(2):80–6. doi:10.1002/ejlt.200401063.

Kelsey JA, Corl BA, Collier RJ, Bauman DE. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. J Dairy Sci. 2003;86(8):2588–97. doi:10.3168/jds.S0022-0302(03)73854-5.

Lopez C, Briard-Bion V, Menard O, Beaucher E, Rousseau F, Fauquant J, Leconte N, Robert B. Fat globules selected from whole milk according to their size: different compositions and structure of the biomembrane, revealing sphingomyelin-rich domains. Food Chem. 2011;125(2):355–68. doi:10.1016/j.foodchem.2010.09.005.

Martini M, Cecchi F, Scolozzi C. Relationship between fat globule size and chemical and fatty acid composition of cow's milk in mid lactation. Ital J Anim Sci. 2006;5(4):349–58.

Mesilati-Stahy R, Mida K, Argov-Argaman N. Size-dependent lipid content of bovine milk fat globule and membrane phospholipids. J Agric Food Chem. 2011;59(13):7427–35. doi:10.1021/Jf201373j.

Michalski MC, Briard V, Juaneda P. CLA profile in native fat globules of different sizes selected from raw milk. Int Dairy J. 2005;15(11):1089–94. doi:10.1016/j.idairyj.2004.11.011.

Timmen H, Patton S. Milk-fat globules – fatty-acid composition, size and in vivo regulation of fat liquidity. Lipids. 1988;23(7):685–9. doi:10.1007/Bf02535669.

Wiking L, Stagsted J, Lennart B, Nielsen JH. Milk fat globule size is affected by fat production in dairy cows. Int Dairy J. 2004;14(10):909–13. doi:10.1016/j.idairyj.2004.03.005.

Chapter 6
Effect of Milk Fat Globule Size on Physical Properties of Milk

Within the wide size range of MFG, the smallest globules are approximately 100-fold smaller in diameter compared to the largest ones. For the same bulk volume of fat, milk, with smaller MFG will have a higher total number of MFG. Within these milks, the smaller MFG will tend to have greater surface curvature, and a larger surface area/volume ratio, compared to larger MFG. These differences can give rise to marked differences in the physical properties of MFG size-differentiated milk and milk fat as summarised in Fig. 6.1.

6.1 Physical Stability

Bovine milk, in which fat globules are dispersed in the continuous phase of milk plasma containing casein micelles, serum proteins, sugars and minerals, can be considered as both a colloidal suspension and an oil-in-water emulsion. Within the emulsion, the MFGM maintains the integrity of the lipid droplets and helps to protect them from destabilisation (Walstra et al. 1999). However, as a natural oil-in-water emulsion, milk is thermodynamically unstable and readily subject to various forms of physical instability over time, leading to changes of structural organisation or spatial distribution of MFG. These instability mechanisms include gravitational separation, droplet aggregation, flocculation, coalescence, and partial coalescence, which are governed by three colloidal interactions, i.e. van der Waals attractions, electrostatic repulsion and steric stabilisation (Huppertz and Kelly 2006). Creaming phenomena can be prevented by reducing the emulsion droplet size. Indeed, for nanoemulsions (i.e. below 200 nm) in general, Brownian motion can be sufficient to overcome the influence of gravitational force (Tadros et al. 2004; Mason et al. 2006; McClements and Rao 2011), thereby providing enhanced long-term physical stability. McClements (2011) calculated that creaming becomes negligible if emulsion droplet size is below 10 nm. However, particles in nanoemulsions can be subjected to sedimentation if

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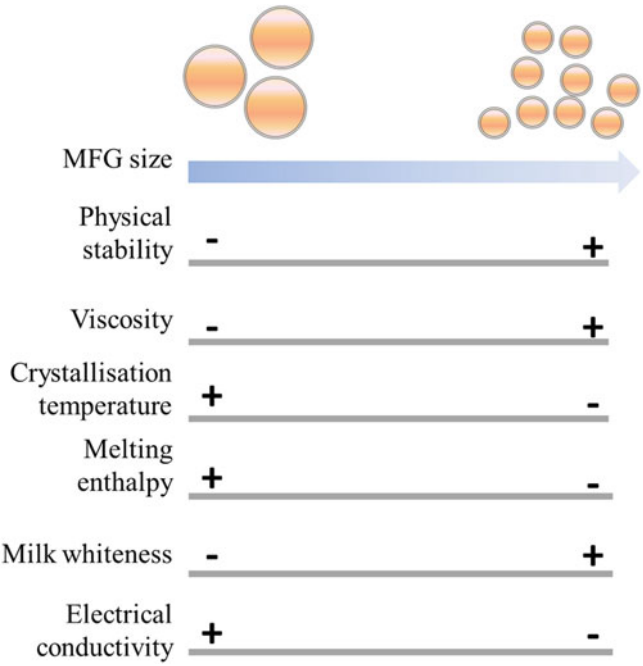

Fig. 6.1 Illustration of impact of milk fat globule size on selected fundamental properties ("+": increased; "−": decreased)

they are coated by a thick adsorbed protein layer to such an extent that their overall density is higher than that of water (McClements 2011). Destabilisation by flocculation and coalescence can also be reduced in nanoemulsions, due to enhanced steric stabilisation (Anton et al. 2008; McClements and Rao 2011; Tadros et al. 2004). Whilst these are well-founded principles with respect to model emulsions, there is little information about the physical stability of native MFG at the nanometric-size scale. However, it is well established that homogenised milks (down to about 0.4 μm) are relatively stable against creaming and it is reasonable to project that the physical stability of MFG may also be enhanced in nanoemulsions.

Milk fat globule size greatly impacts on the formation of milk fat clusters, as in creaming and cold agglutination, which in turn, affects the physical stability of milk and dairy products. In general, the smaller the fat globule size, the more stable it is. Hence, drinking milks are commonly homogenised to reduce the size of milk fat globules (below 1 μm) to achieve the greater stability and shelf-life. Large MFG size accelerates the creaming. Furthermore, large MFG tend to have protruding fat crystals, which facilitate partial coalescence (Walstra 1995). Large MFG size, hence, is undesirable in drinking milks but are preferred in butter manufacture, where small MFG in homogenised cream may lead to inefficiencies in the churning process (Walstra et al. 2005).

Milk fat globule size is also considered to influence the creaming rate in cold raw milk, through its influence on cold agglutination. This occurs in milk due to the presence of agglutinin, a cryoglobulin-lipoprotein complex (Walstra 1995). On cooling raw milk, cryoglobulins are precipitated and coat the MFG, causing them to aggregate and form large floccules, which then rise as a creaming layer. Since surface area is greater with smaller fat globules, more agglutinin is required to envelop them completely, rendering them inherently more stable to cold agglutination (Walstra et al. 2005).

6.2 Viscosity

Viscosity of milk and dairy emulsions is also dependent on MFG size. In homogenised milk, the higher the homogenising pressure, the higher the viscosity. When milk was homogenised from 70 to 245 bar, there was a corresponding increase in viscosity from 7.1 to 15.0 % (Kessler 1981). It was also reported that smaller MFG size causes a slight increase in (apparent) viscosity (Long et al. 2012; Truong et al. 2014a; Kietczewska et al. 2003). A decrease in MFG size of 3.3 % fat milk from 2.7 to 1.0 μm resulted in corresponding higher viscosities (1.8–1.96 mPa s) (Kietczewska et al. 2003). Similar tendencies were noted in dairy-based emulsions (10–36 % milk fat) with much lower size range (0.2–1.3 μm). When emulsion size decreased from 1.2 to 0.2 μm, the apparent viscosities of the dairy-based emulsions increased in the range of 8–15 mPa s at a shear rate of 5.6 s^{-1} (Truong et al. 2014a). For a high fat containing emulsions (36 % milk fat) Long et al. (2012) reported a higher apparent viscosity (0.852 Pa s) for the smaller size emulsion (0.415 μm) compared to the bigger size (1.291 μm; 0.398 Pa s). The increase in emulsion viscosity of milk and dairy emulsions with decreasing MFG size can be partly explained by stronger colloidal repulsion and monodispersed close packing caused by narrow size distribution and smaller particle size (Pal 1996).

6.3 Crystallisation and structural properties

The main components of MFG are TAG, which exist in hundreds of molecular species. The configuration of TAG molecules in their solid state can be described in two dimensions as presented in Fig. 6.2a. The longitudinal stacking (long spacing) is the alignment of repetitive patterns of TAG side-by-side, either in two chain length (2*L*) or higher (triple 3*L*, quartet 4*L* etc. chain length configuration). The cross-sectional view, regarded as the short spacing, is associated with the structural arrangement of the TAG side chains. They are α, β′,and β polymorphs having hexagonal, orthorhombic and perpendicular, and triclinic parallel chain packing, respectively (Chapman 1962).

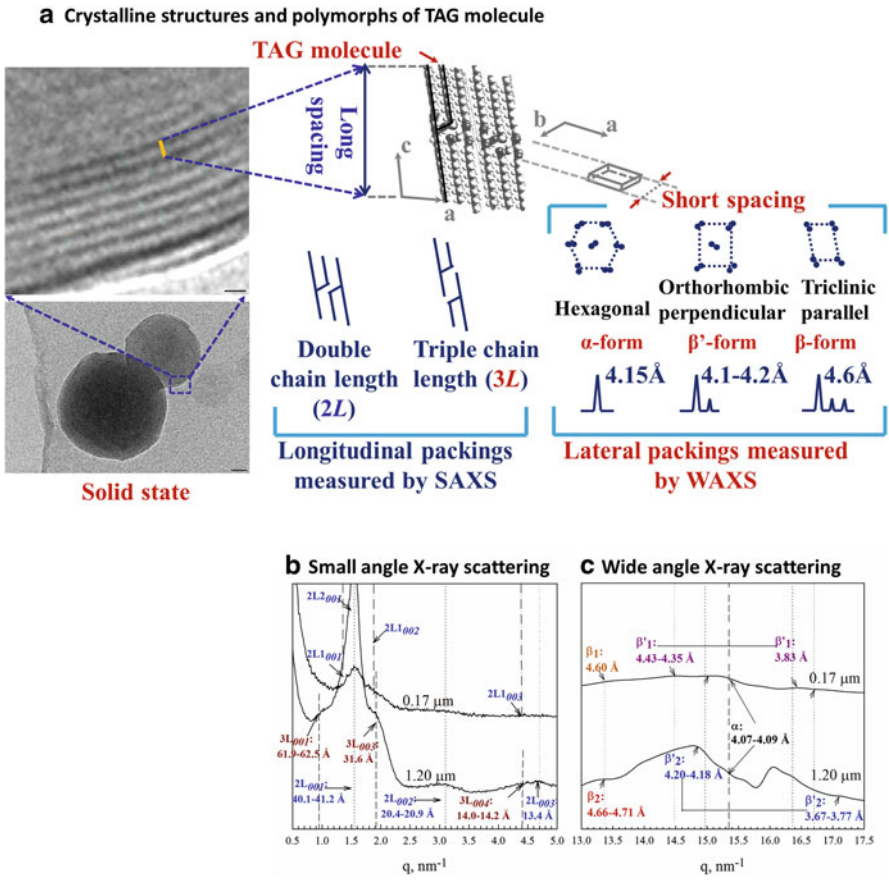

Fig. 6.2 Schematic illustration of TAG crystalline conformation (**a**) and impact of droplet size on crystalline packing longitudinally (**b**) and laterally (**c**) in olein emulsions. The *3L* structure was vanished in the nano-sized emulsion (0.17 μm) versus the control (1.20 μm). The emulsion was prepared from 10 % w/w olein fraction (fractionated from AMF at 21 °C) emulsified with 1 % w/w sodium caseinate (**b** and **c**: Reprinted from Truong et al. (2015), Copyright 2015, with permission from Elsevier)

Each TAG has its own melting point and inter-solubility with other TAGs, which results in very complex overall crystallisation and structural properties of milk fat. Upon crystallisation, individual TAG of milk fat can form different polymorphs, depending on previous thermal conditions applied such as cooling/heating temperature and rate. Previous studies on the crystallisation of bovine MFG in cream (natural and recombined) and milk reported the complexity in crystallisation and structural behaviour of milk fat crystals in the dispersion state (Table 6.1). Typically, milk fat crystals in globules are of mixed types of α, β′ and β depending on rate of cooling (Lopez et al. 2002, 2007).

Table 6.1 Influence of fat globule size on crystalline structures and polymorphs of milk lipids

Composition	Droplet size(s)	Cooling condition[a]	T_C [b]	Longitudinal packing	Lateral packing	References
Native fat globules (small size fraction)	D_{43} 0.93 μm	0.5 °C min^{-1} (60 to −8 °C)	20 °C	2*L* (40.6 Å)		Michalski et al. (2004)
			−8 °C	$3L_1$ (70.7 Å), $3L_2$ (64.2 Å) + 2*L* (39.3 Å)		
	D_{43} 1.75 μm	1.0 °C min^{-1} (60 to −8 °C)	15 °C	3*L* (70.6 Å)	α	
			−8 °C	2*L* + 3*L*	α + sub α	
Native fat globules (large size fraction)	D_{43} 7.15 μm	0.5 °C min^{-1}	20 °C	2*L* (41.1 Å)		
		0.5	−8 °C	2*L* (40.8 Å), $3L_1$ (68.5 Å), $3L_2$ (61.2 Å)		
Fresh cream		25 °C min^{-1}	5 °C	3L + 2L + 3L	α + β′	Fredrick et al. (2011)
Milk fat emulsion (AMF + β-lg)	0.38, 0.45, 0.67, 1.25 μm	1 °C min^{-1}	−7 °C	3*L* (72 Å)	α	Lopez et al. (2002)
Milk fat emulsions	$d_{0.5}$ 0.17, 0.55, 1.45 μm	Steady state	4 °C	3*L* (56.6 Å), 2*L* (39.5 Å)	$\beta_1 + \beta_2 + \beta'_1 + \beta'_2$	Bugeat et al. (2011)
UFA enriched emulsion	0.18, 0.59, 1.67 μm	Steady state	4 °C	2*L* (41.8 Å)	$\beta_1, \beta'_1, \beta'_2$	
Stearin emulsion[c]	Mode 1.2 μm	Steady state after cooling at 1.0 °C min^{-1}	4 °C	2*L* (41.1 Å)	$\beta_1, \beta'_1, \beta'_2$	Truong et al. (2015)
	0.17 μm			2*L* (41.5 Å)	$\beta_1, \beta'_1, \beta'_2$	
	1.2 μm	10 °C min^{-1}	4 °C	2*L* (40.8 Å)	$\beta_1, \beta'_1, \beta'_2$	
	0.17 μm			2*L* (41.1 Å)	$\beta_1, \beta'_1, \beta'_2$	
Olein emulsion[c]	1.2 μm	Steady state after cooling at 1.0 °C min^{-1}	4 °C	3*L* (61.9 Å) + 2*L* (40.5 Å)	$\alpha, \beta'_1, \beta'_2, \beta_2$	
	0.17 μm			2*L* (40.1, 44.3 Å)	$\alpha, \beta'_1, \beta'_2, \beta_1$	
	1.2 μm	10 °C min^{-1}	4 °C	3*L* (62.5 Å) + 2*L* (40.5 Å)	$\alpha, \beta'_1, \beta'_2, \beta_2$	
	0.17 μm			2*L* (40.5, 45.3 Å)	$\alpha, \beta'_1, \beta'_2, \beta_1$	

[a]Cooling condition includes cooling rate and temperature range used
[b]Crystallisation temperature where the crystalline packings were detected
[c]Emulsions prepared from fractionated milk fats (stearin-enriched and olein-enriched)

Fat globule size is also one of the factors influencing the crystallisation and structural characteristics of native MFG (Lopez et al. 2007; Michalski et al. 2004) as summarised in Table 6.1. Using microfiltration to obtain small (1–3 μm) and large (5–7 μm) globule size fractions, Michalski et al. (2004) reported that crystallisation was delayed with smaller MFG (D_{43} 0.93 μm) compared to large MFG (D_{43} 7.15 μm). The latter forms 3*L* structure at higher temperature (13 °C) than the former (9 °C). Also, the large size fraction exhibits more 2*L* structure (2*L*: 40.8 Å) than its smaller counterpart (2*L*: 39.3 Å). However, the authors pointed out that no significant discrepancy was found across the size range of native milk fat globules investigated (0.93–7.14 μm) once the associated cooling rate and thermal history were omitted.

Due to the technical difficulty in separating native MFG into discrete size fractions, few attempts have been made to study the influence of droplet size on crystallisation and structural behaviour of bovine milk TAGs in milk fat emulsion systems. In these systems, anhydrous or fractionated bovine milk fats were used as an oil phase whereas the aqueous phase typically consisted of whey proteins and/or caseins acting as emulsifiers. Generating discrete emulsion droplet size ranges is generally more controllable by varying homogenising pressure and cycles applied to the coarse emulsions during the emulsification process. Furthermore, this method effectively eliminates the compositional variations between small and large MFG in their native form. Using this approach, it was shown that a decrease in droplet size induced a lower crystallisation temperature (Lopez et al. 2002; Truong et al. 2014b), lower solid fat content (Truong et al. 2014b) and smaller melting enthalpy (Bugeat et al. 2011) (Table 6.1). These results were in agreement with studies performed on other fats/oils such as *n*-hexadecane, tripalmitin, tristearin, and trilauroylglycerol (Higami et al. 2003; Bunjes et al. 2000; Dickinson et al. 1991). The tendency of crystallisation temperature to decrease with smaller droplet size can be explained by the increased ratio of droplets to impurities, which are responsible for catalysing or "seeding" crystallisation of individual droplets. An increase in this ratio tends to limit the rate of crystallisation and results in an increasing propensity for supercooling. In nanoemulsions, it is likely that the crystallisation process is further retarded due to the physical constraints of the droplet wall. For example, Bugeat et al. (2011) and Truong et al. (2015) reported the absence of TAG 3*L* structure in bovine milk enriched unsaturated fatty acid (olein fraction) nanoemulsion (approximately 200 nm) compared to micron-sized emulsions of the same composition (Fig. 6.2b and c), suggesting that confinement of emulsion droplet size constrains and retards the crystallisation and fat crystal growth.

Regarding morphology, milk fat that is crystallised within size-differentiated MFGs exhibits different crystal arrangements and shape, as visualised under polarised light microscopy, depending on MFG size (Lopez et al. 2002). Depending on the microscopic technique used, four main types of crystals in MFG have been proposed based on (1) birefringence of the crystals under polarized light microscopy (Walstra 1967) and (2) location of crystal shell within the fat globule as observed with freeze-fracturing and electron microscopy (Precht 1988). The four main types of crystals observed in cream are: O (tiny crystals interiorly located,

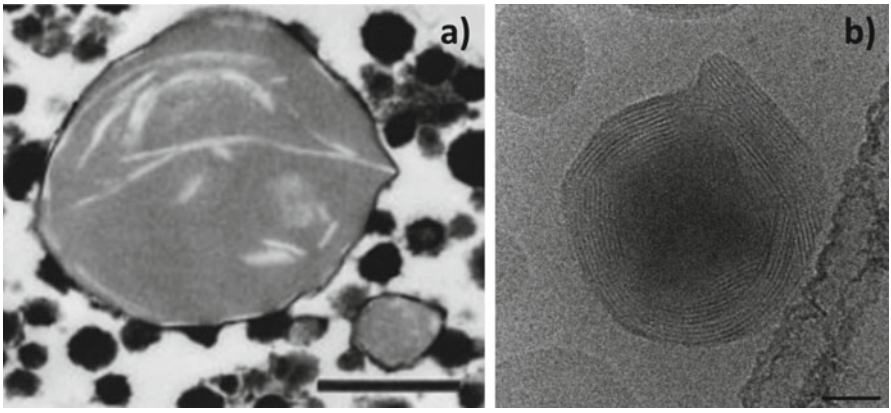

Fig. 6.3 (**a**) Presence of needle-like fat crystals inside a bovine MFG captured by Transmission Electron Microscopy (Reprinted from Goff (1997), Copyright (1997), with permission from Elsevier), bar scale: 0.5 μm; and (**b**) Arrangement of TAG lamellar layers in both interior and free surface of the stearin-enriched nanoparticle (230 nm) after cooling at very slow rate 0.1 °C min^{-1} as visualised under cryogenic Transmission Electron Microscopy (Reprinted from Truong et al. (2015), Copyright (2015), with permission from Elsevier), bar scale 50 nm

showing no birefringence), N ("needle-type": birefringent areas of needle crystals), L ("Layer-type": needle crystals tangentially located at the outer layer) and M ("mixed type": combination of L and N types) (Walstra 1967). As illustrated in Fig. 6.3a, needle-fat crystals were detected in interior part of native MFG (Goff 1997). Based on these categories, Lopez et al. (2002) reported that during rapid cooling from 60 to −8 °C, it is likely that the fat crystals in the smallest fat globules were very small and attributed to type O (very weak birefringence). The largest fat globules held needle-shaped crystals (type N). When native MFG were cooled at slower rate (0.5 °C min^{-1}) the largest globules had a combination of type N and M (layered + needle-shaped crystals) whilst spherulite-shaped crystals were present in the smallest globules (Lopez et al. 2002). A mono-molecular layer about 5 mm in thickness was found to surround the MFG boundary in concentric layers upon crystallization (Precht 1988). For a dairy-based emulsion system, the higher resolution of transmission electronic microscopy revealed that upon very slow crystallisation of TAG (0.1 °C min^{-1}), TAG layers appeared to be arranged into a straight orientation (Fig. 6.3b). The influence of droplet size on crystal morphologies was also demonstrated by Truong et al. (2015) (Fig. 6.4). Here it was apparent that the bent crystals aligned tangentially to the curved interface of micron-sized emulsion droplets were absent from the nanoemulsion droplets. It was suggested that these more typical crystals were unable to form, due to the physical confinement and extreme curvature of the nano-sized droplets (200 nm) (Fig. 6.4). Instead, the crystal lattice within the nano-sized droplets tended to arrange into a straight orientation, causing a deformation of droplets or protruding fat droplet surface (Figs. 6.3b and 6.4).

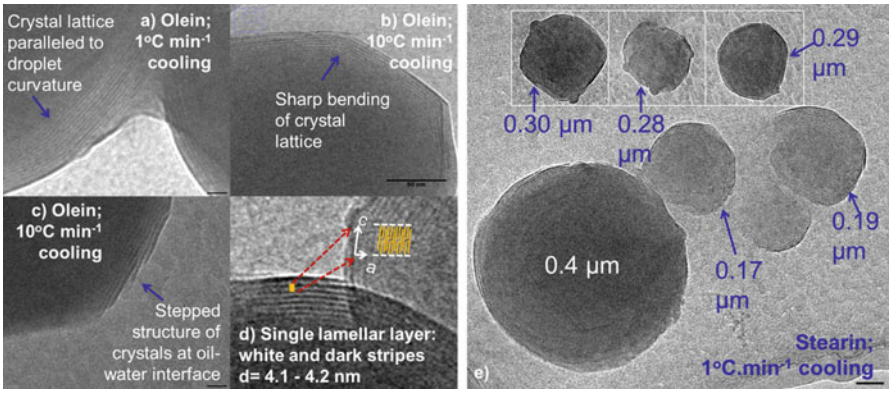

Fig. 6.4 Cryogenic Transmission Electron Microscopy micrographs present different arrangements of TAG lamellar layers externally in olein (**a**–**c**) and stearin (**d**) nanoemulsion (200 nm) at 4 °C after being cooled at different cooling rates (**b** and **c**: Reprinted from Truong et al. (2015), Copyright (2015), with permission from Elsevier). Single lamellar layer (*white* and *dark thick lines*) about 4.1–4.2 nm, corresponding to the length of TAG longitudinal packing (red arrows), stacked along the periphery of particle (**d**). Polydispersity and various morphologies at different particle sizes in the stearin nanoemulsion (**e**)

6.4 Optical Properties

Turbidity of milk is governed by light scattering from milk components, notably fat globules and casein micelles (Goulden 1958; Walstra et al. 2005). Light scattering is stronger with MFG than casein micelles in milk due to a high polydispersity of fat globule size (Walstra et al. 2005). Both fat globule size and fat concentration contribute to light scattering (Goulden 1958; Walstra et al. 2005). Hence, optical properties have been utilised to indirectly estimate MFG size in homogenised milks using spectroturbidimetry (Ashworth 1951; Goulden 1958) and more recently, light scattering (McCrae and Lepoetre 1996; Michalski et al. 2001). On investigating measurement of the optical properties of milk as a means of obtaining reliable MFG size distribution estimates using laser light scattering, Michalski et al. (2002) observed that there was no significant difference in size distribution of natural fat globules using both the corrected (1.458 at 633 nm- and 1.460 at 466 nm-wavelength) and true refractive indices (1.470 and 1.460 at 466 and 633 nm wavelength, respectively). However, using the corrected refractive indices of milk fat improves the peak selectivity in the submicron size range of homogenised milk. These findings appear to indicate at least a small degree of influence of particle size on optical properties of MFG.

The colour and opacity of milk is attributable to both light scattering and absorbance of visible light (Walstra et al. 2005). It is known that homogenised milk appears whiter than raw milk. Sonicated milk, having mean diameter of globule size below

1 μm, is reported to have a significantly higher level of luminosity (L^*: 92.37 ± 0.20) compared to raw milk (87.82 ± 0.18). This was apparently due to an increase in scattering of visible light with smaller fat globules (Fox and McSweeney 1998).

6.5 Electrical Conductivity

Milk generally exhibits good electrical conductivity, largely due to the presence of dissociated soluble salts. The presence of milk fat reduces electrical conductivity, due to the poor conductivity of the fat itself, as well as the immobilisation of conducting ions by MFG. Increasing fat content generally leads to a decrease in electrical conductance. It has been observed that at the same level of fat, commercial full fat milk which has a smaller fat globule size, had a higher conductance (5.05 ± 0.03 mS), than that of raw milk (4.85 ± 0.03 mS) (Mabrook and Petty 2003). In a separate study, when milk was homogenised, conductance properties such as impedance and admittance of homogenised milk remained unchanged in larger particle size emulsions (1.5–5 μm; Banach et al. 2008). However, a marked decrease in homogenised milk impedance was observed in milks with a smaller fat globule size (1.07 μm, homogenised at 20 MPa). This observed difference was attributed to the greater disruption and disintegration of casein micelles under the higher homogenisation pressures. It was assumed that during the mechanical size reduction process, part of the colloidal calcium phosphate was dissociated from the micelles and solubilised, leading to an increase in calcium and phosphate contents in milk serum. This contributed to the imbalance of mineral salts in milk, altering its electrical conductivity (Banach et al. 2008). Given that the fat globule membrane in homogenised milks is not of a highly conductive nature, it appears that any differences seen in conductivity between homogenised milks of different emulsion particle size are more likely to be due to the effects of homogenisation on micelle disruption than any direct effect of emulsion droplet size.

References

Anton N, Benoit JP, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates - a review. J Control Release. 2008;128(3):185–99. doi:10.1016/j.jconrel.2008.02.007.

Ashworth US. Turbidity as a means for determining the efficiency of homogenization. J Dairy Sci. 1951;34(4):317–20.

Banach JK, Żywica R, Kiełczewska K. Effect of homogenization on milk conductance properties. Pol J Food and Nutr Sci. 2008;58(1):107–11.

Bugeat S, Briard-Bion V, Perez J, Pradel P, Martin B, Lesieur S, Bourgaux C, Ollivon M, Lopez C. Enrichment in unsaturated fatty acids and emulsion droplet size affect the crystallization behaviour of milk triacylglycerols upon storage at 4 degrees C. Food Res Int. 2011;44(5):1314–30. doi:10.1016/j.foodres.2011.01.003.

Bunjes H, Koch MHJ, Westesen K. Effect of particle size on colloidal solid triglycerides. Langmuir. 2000;16(12):5234–41.
Chapman D. The polymorphism of glycerides. Chem Rev. 1962;62:433–56.
Dickinson E, Mcclements DJ, Povey MJW. Ultrasonic investigation of the particle-size dependence of crystallization in N-hexadecane-in-water emulsions. J Colloid Interface Sci. 1991;142(1):103–10.
Fox PF, McSweeney PL. Dairy chemistry and biochemistry. London: Blackie Academic & Professional; 1998.
Fredrick E, Van de Walle D, Walstra P, Zijtveld JH, Fischer S, Van der Meeren P, Dewettinck K. Isothermal crystallization behaviour of milk fat in bulk and emulsified state. Int Dairy J. 2011;21(9):685–95. doi:10.1016/j.idairyj.2010.11.007.
Goff HD. Instability and partial coalescence in whippable dairy emulsions. J Dairy Sci. 1997;80(10):2620–30.
Goulden JDS. Some factors affecting turbimetric methods for the determination of fat in milk. J Dairy Res. 1958;25(2):228–35.
Higami M, Ueno S, Segawa T, Iwanami K, Sato K. Simultaneous synchrotron radiation X-ray diffraction - DSC analysis of melting and crystallization behavior of trilauroylglycerol in nanoparticles of oil-in-water emulsion. J Am Oil Chem Soc. 2003;80(8):731–9.
Huppertz T, Kelly AL. Physical chemistry of milk fat globules. In: Fox PF, McSweeney PLH, editors. Advanced dairy chemistry volume 2: lipids, vol. 2. 3rd ed. New York: Springer; 2006.
Kessler HG. Emulsifying _ Homgenizing. In: Food engineering and dairy technology. Germany: Verlag A. Kessler; 1981. p. 119–38.
Kietczewska K, Kruk A, Czerniewicz M, Warminska M, Haponiuk E. The effect of high-pressure homogenization on changes in milk colloidal and emulsifying systems. Pol J Food Nutr Sci. 2003;12/53(1):43–6.
Long Z, Zhao MM, Zhao QZ, Yang B, Liu LY. Effect of homogenisation and storage time on surface and rheology properties of whipping cream. Food Chem. 2012;131(3):748–53.
Lopez C, Bourgaux C, Lesieur P, Bernadou S, Keller G, Ollivon M. Thermal and structural behavior of milk fat - 3. Influence of cooling rate and droplet size on cream crystallization. J Colloid Interface Sci. 2002;254(1):64–78. doi:10.1006/jcis.2002.8548.
Lopez C, Bourgaux C, Lesieur P, Ollivon M. Coupling of time-resolved synchrotron X-ray diffraction and DSC to elucidate the crystallisation properties and polymorphism of triglycerides in milk fat globules. Lait. 2007;87(4–5):459–80. doi:10.1051/Lait:2007018.
Mabrook MF, Petty MC. Effect of composition on the electrical conductance of milk. J Food Eng. 2003;60(3):321–5. doi:10.1016/S0260-8774(03)00054-2.
Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM. Nanoemulsions: formation, structure, and physical properties. J Phys Condens Matter. 2006;18(41):R635–66. doi:10.1088/0953-8984/18/41/R01.
McClements DJ. Edible nanoemulsions: fabrication, properties, and functional performance. Soft Matter. 2011;7(6):2297–316. doi:10.1039/c0sm00549e.
McClements DJ, Rao J. Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit Rev Food Sci Nutr. 2011;51(4):285–330. doi :10.1080/10408398.2011.559558.
McCrae CH, Lepoetre A. Characterization of dairy emulsions by forward lobe laser light scattering - application to milk and cream. Int Dairy J. 1996;6(3):247–56. doi:10.1016/0958-6946(95)00008-9.
Michalski MC, Briard V, Michel F. Optical parameters of milk fat globules for laser light scattering measurements. Lait. 2001;81(6):787–96.
Michalski MC, Michel F, Geneste C. Appearance of submicronic particles in the milk fat globule size distribution upon mechanical treatments. Lait. 2002;82(2):193–208. doi:10.1051/Lait:2002004.
Michalski MC, Ollivon M, Briard V, Leconte N, Lopez C. Native fat globules of different sizes selected from raw milk: thermal and structural behavior. Chem Phys Lipids. 2004;132(2):247–61. doi:10.1016/j.chemphyslip.2004.08.007.
Pal R. Effect of droplet size on the rheology of emulsions. AIChE J. 1996;42(11):3181–90.

Precht D. Fat crystal structure in cream and butter. In: Garti N, Sato K, editors. Crystallization and polymorphism of fats and fatty acids. New York: Marcel Dekker; 1988. p. 305–61.
Tadros T, Izquierdo R, Esquena J, Solans C. Formation and stability of nano-emulsions. Adv Colloid Interface Sci. 2004;108:303–18. doi:10.1016/j.cis.2003.10.023.
Truong T, Bansal N, Bhandari B. Effect of emulsion droplet size on foaming properties of milk fat emulsions. Food Bioprocess Technol. 2014a;7(12):3416–28. doi:10.1007/s11947-014-1352-4.
Truong T, Bansal N, Sharma R, Palmer M, Bhandari B. Effects of emulsion droplet sizes on the crystallisation of milk fat. Food Chem. 2014b;145:725–35. doi:10.1016/j.foodchem.2013.08.072.
Truong T, Morgan GP, Bansal N, Palmer M, Bhandari B. Crystal structures and morphologies of fractionated milk fat in nanoemulsions. Food Chem. 2015;171:157–67. doi:10.1016/j.foodchem.2014.08.113.
Walstra P. On the crystallization habit in fat globules. Neth Milk Dairy J. 1967;21(3/4):166–91.
Walstra P. Physical chemistry of milk fat globules. In: Fox PF, editor. Advanced dairy chemistry vol. 2: lipids. London: Chapman & Hall; 1995. p. 131–78.
Walstra P, Geurts TJ, Noomen A, Jellama A, Van Boekel MAJS. Dairy technology: principles of milk properties and processes. New York: Marcel Dekker, Inc.; 1999.
Walstra P, Wouters JTM, Geurts TJ. Dairy science and technology. 2nd ed. Boca Raton: CRC; 2005.

Chapter 7
Effect of Milk Fat Globule Size on Functionalities and Sensory Qualities of Dairy Products

Milk fat globules, along with casein micelles and whey protein, are three main supramolecular constituents of bovine milk. The characteristic physical and chemical properties of the wide variety of dairy products that can be made from milk can, to a large extent, be related back to the fundamental properties of each of these constituents in the product mix (Fig. 7.1). Thus the chemical composition and physical properties of milk fat globules, many of which are size-dependent, can be expected to impact on the physical functionality, texture and flavour of a range of fat-containing dairy products, such as liquid milk, cheese, yoghurt, ice cream and butter (Fig. 7.2). Depending on their size and native state along with their interaction with other components of milk (mainly casein), utilisation of differentiated-size MFG can be exploited to improve the quality of dairy products. The following section describes the impact of MFG size on the physical functionality and sensory quality of selected fat-structured dairy products.

7.1 Milk

Variation in MFG size influences the heat stability, foaming properties, gelation behaviour and sensory perception of milks (Whiteley and Muir 1996; Borcherding et al. 2008; Michalski et al. 2002; Goudedranche et al. 2000; Fibrianto 2013).

7.1.1 Heat Stability

Homogenisation is known to have a detrimental impact on heat stability of milk. In concentrated milk, it was found that provided that homogenisation takes place before the pre-warming or concentration step, heat stability is greater when fat

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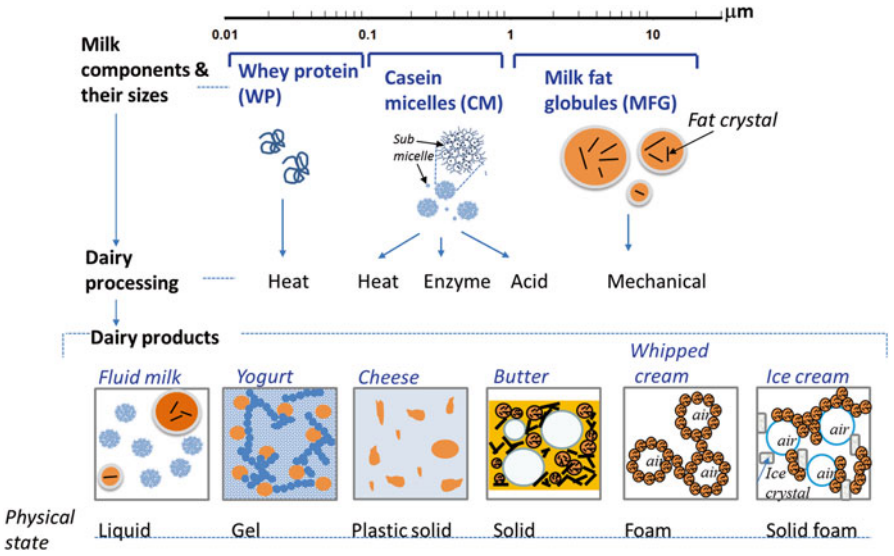

Fig. 7.1 Schematic illustration of the three main components of milk and microstructure of fat-containing dairy products (Redrawn with modifications from Aguilera and Stanley 1999)

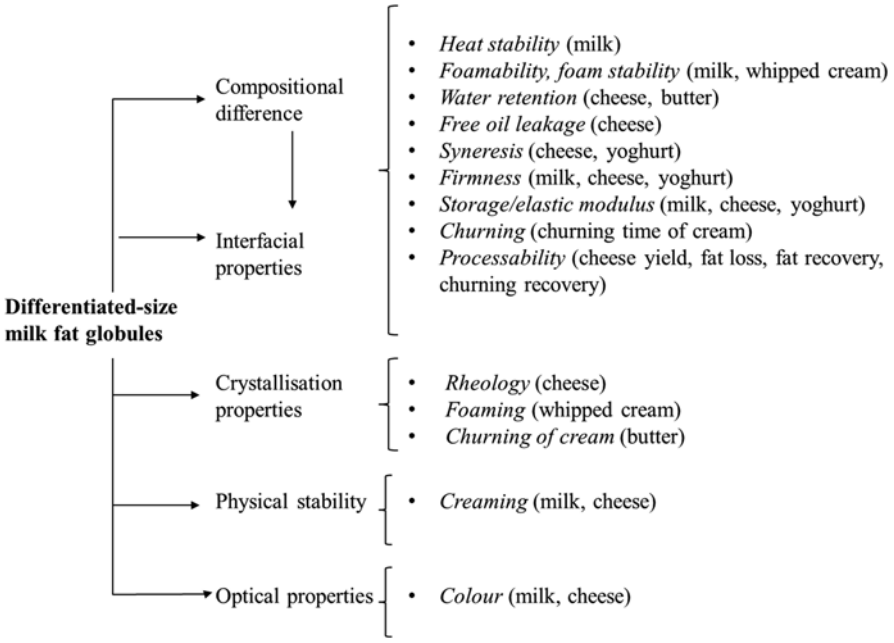

Fig. 7.2 Possible influence of MFG size on fundamental properties and subsequent effects on functionalities of milk and selected fat-structured dairy products

globule size is below 0.4 μm (D_{32}). This was reflected by a sharp increase in heat coagulation time at 120 °C (Whiteley and Muir 1996). For fat globule sizes above 0.4 μm, heat stability appeared to be independent of fat globule size regardless of homogenisation treatment (using homogeniser and microfluidiser). Also, heat stability was reduced with increasing homogenisation pressures if homogenisation was carried out after the concentration step (Whiteley and Muir 1996). This suggests that associated changes in protein conformation during severe homogenisation may be contributing to the heat stability rather than any direct effect of fat globule size.

7.1.2 Foaming

Regarding foaming properties of whole milk, Borcherding et al. (2008) pointed out that it is unlikely that MFG size would have a significant impact on foam formation and stability at 50 °C. The foaming properties at 50 °C of whole milk are predominantly a function of milk protein fractions in the skim milk phase. However, when foaming temperature is lower at 20–30 °C, MFG size remarkably affected the foaming properties (Borcherding et al. 2008). However, it should be noted that the physical state of MFG was not addressed in this study. As milk fat is in liquid state at 50 °C but partially crystalline at 20 and 30 °C, this may also have had some influence on the different foaming properties observed at the different temperatures.

7.1.3 Gelation

The role of MFG size in gelation properties of milk depends on how fat globules are interacting with the protein network. The integrity of MFGM is a key factor for participation of MFG in the gel structure. According to Michalski et al. (2002), native MFG with intact MFGM is non-interactive with the casein network. Once the MFG surface is modified by partially damaged membrane due to mechanical treatment and adsorption of casein and whey proteins, as in homogenised and recombined milks, respectively; fat globules become interacting particles in the gel structure. Hence, MFG can act as either structural breakers or promoters depending on their physical state and interactions with the protein network (Michalski et al. 2002; Logan et al. 2015).

7.1.3.1 Rennet Gelation

Milk can form gels by acidification and/or rennet addition. In rennetted gels, the protein network is induced by fusion of casein strands when κ-casein is chopped off from the casein micelle surface. Rennetted-casein gel pores having typical size of 4–15 μm are formed such that fat globules may be trapped within them (Mellema et al. 2000).

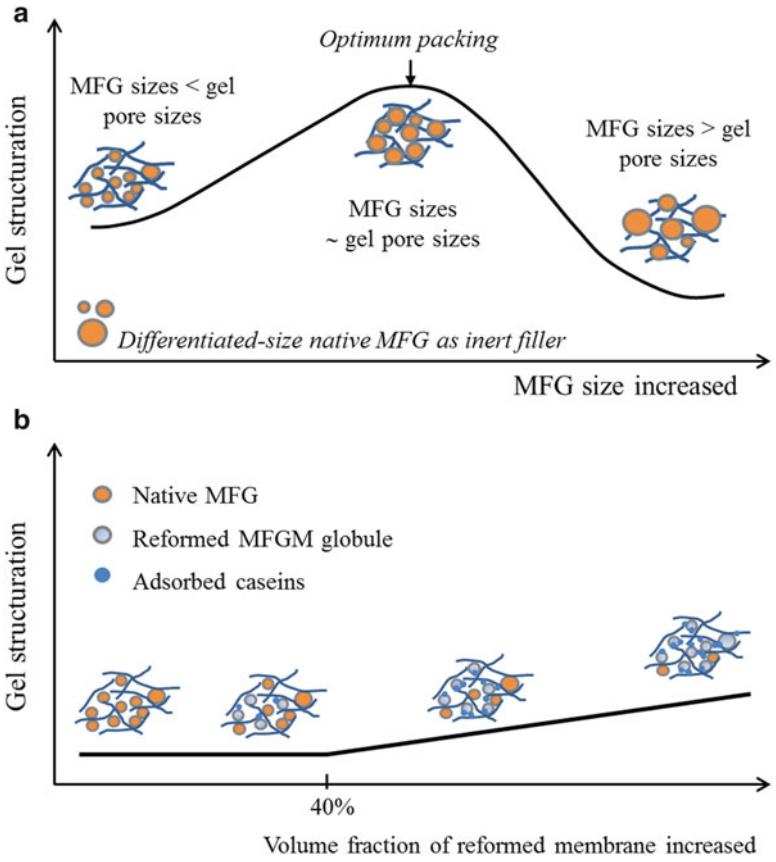

Fig. 7.3 Schematic illustration of inclusion of differentiated-size native (**a**) and recombined fat globules (**b**) in the protein network (Modified from Michalski et al. 2002; Logan et al. 2015)

Thanks to imaging techniques such as electron microscopy and confocal laser scanning microscopy, it has been revealed that the effect of MFG size on gel structure and subsequent gel integrity is related to arrangement of the gel network and typically to the rennet gel pore sizes (Michalski et al. 2002; Logan et al. 2015). For rennet gel formed in fresh milk, it was postulated that if the size of MFG is comparable to the pore sizes, the gel structure is supported, in turn, resulting in a firmer gel (Fig. 7.3a). Milk fat globules larger than the pore size tended to be excluded and eventually weakened the gel integrity (Logan et al. 2015). Rennet gels can be supported with comparatively smaller fat globules fitting into the large pore provided that these fat globules positively interact with the protein matrix, which is not the case for the native MFG. Thus, smaller fat globules may be regarded as inert fillers in some cases (Logan et al. 2015; Michalski et al. 2002). In homogenised and recombined milks, the modified fat globule surfaces become more interactive with the protein network. In this case, effect of MFG size on rennet gel properties is more pronounced when percentage of damaged membrane is more than 40 % (Fig. 7.3b). Regardless of

composition of the fat globule surface, it was observed that there was an increase in G′ with smaller fat globules (i.e. increased globule surface area) in rennet gels (Michalski et al. 2002) (Table 7.1).

7.1.3.2 Acid Gelation

Acid-induced gel structure is built up by linkage of aggregated casein particles in clusters, strands, and chains. Void spaces (pores) of 1–30 μm are found in acid gels. Upon acidification and subsequent gelation steps, smaller fat globules seem to promote fat inclusion into the casein matrix due to the greater surface area that provides more interconnections with other particles such as milk proteins, denatured whey and casein (Cho et al. 1999). Contrary to rennet gel, acid gel prepared from native MFG (i.e. non-interacting particles), exhibit weaker G′ with smaller fat globule size (Michalski et al. 2002). Again, this might be related to the optimum packing between acid gel pores and fat globule size. For recombined and homogenised globules, contrasting results have been reported on the impact of fat globule size on acid gels. For instance, Cobos et al. (1995) reported that increased homogenising pressure, producing a smaller fat globule size, had no impact on acid gel structure. Conversely, other studies have reported a positive impact of reduce fat globule size on acid-induced gels. For example, acid gels containing small fat globules had more interconnection with casein particles, in turn, reinforcing the gel network (Xiong et al. 1991). Ji et al. (2011) also found a slight increase in final G′ from 340 to 430 Pa with decreased fat globule size within size range of 0.2–2 μm regardless of heat treatment before and after homogenisation. Reduction of fat globule size also reinforce the acid gel network, reflecting by an increase in both yield strain and yield stress with smaller fat globule size. The author argued that downsizing of fat globules by homogenisation also alters the protein adsorption behaviour and distribution in plasma phase and surface of fat globules; gelation properties are also governed by the protein composition, number and size of interacting casein particles (Ji et al. 2011). Hence, MFG-size dependent gelation needs to be examined in association with other interacting particles and the volume fractions of interacting particles (fat globules and casein) forming the gel network. There is also little information on how the physical state of emulsified milk fats influences gelation as gels can be formed at different temperatures, e.g. 30 °C and 4 °C in rennet and acid gels, respectively (Michalski et al. 2002).

7.1.4 Sensory Properties

Fat particle size and its distribution is one of numerous factors that contributes to fat perception in dairy foods, along with viscosity and perception of volatile flavour (Tepper and Kuang 1996; Richardson and Booth 1993; Fibrianto 2013). It was reported that at low viscosity (0.12 P), there was no difference in perception of creaminess between unthickened homogenised (0.5 μm) and non-homogenised milks. When the milks were thickened to the same viscosities (~18.6 Pa s)

Table 7.1 Effect of fat globule size on rennet and acid gels

Sample	MFG size	Remarks	References
Rennet-induced gels			
Raw milk; microfiltrated	D_{32}	-G′ ↑ with MFG ↓	Michalski et al. (2002)
	-LFG: 4.6 μm		
	-SFG: 2.8 μm		
Raw milk; homogenised	D_{32}: 1.6–2 μm	-G′ ↑ with MFG ↓	
Reconstituted skim milk	D_{32}: 1.9–3.9 μm	-G′ ↑ with MFG ↓	
Raw milk; microfluidised (14–35 MPa)	LFG: 390 nm	-SFG: coagulation time, curd firming rate ↓	Tosh and Dalgleish (1998)
	SFG: 313 nm	-Microstructure is more fragile with MFG size ↓	
Reconstituted milk (16 % v/v washed cream, 2 % wt/vol WPI)	265–240 nm	-Unchanged coagulation time, firming rate, microstructure	
Fresh milk (cow selection for both MFG and CM)	D_{43}:	-Gel firmness: LFG > SFG at the similar CM size	Logan et al. (2015)
	-LFG: 4.6–5.15 μm		
	-SFG: 3.5–3.6 μm		
	-LCM: 181–206 nm		
	-SCM: 157–163 nm		
Fresh milk (natural CM and fractionated MFG)	D_{43}:	-LFG-LCM had the longest onset gelation	Logan et al. (2014)
	-LFG: 3.88–5.84 μm	-Gel firmness: LFG > SFG at the similar CM size	
	-SFG: 2.76–3.85 μm		
	-LCM: 181–203 nm		
	-SCM: 153–159 nm		
Acid-induced gels			
Fresh cheese model (20 % AMF, 18.6 % low heat SM)	d_{vs}: 0.53–0.76 μm	- Firmness and G′ ↑ with MFG size ↓	Sanchez et al. (1995)
		- Unclear relationship between MFG size and G″	
4 % Melted AMF, skim milk; homogenised at 50–850 bar	D_{32}: 0.2–2 μm	- Yield stress and strain ↑ with MFG size ↓	Ji et al. (2011)
Raw milk; microfiltrated	D_{32}	-G′ ↑ with MFG ↓	Michalski et al. (2002)
	-LFG: 4.6 μm		
	-SFG: 2.8 μm		
Raw milk; homogenised	D_{32}: 1.6–2 μm	- G′ ↑ with MFG ↓	
Reconstituted skim milk	D_{32}: 1.9–3.9 μm	- G′ ↓ with MFG ↓	

homogenised milk (0.5 μm) was judged to be significantly creamier than non-homogenised milk (Richardson and Booth 1993). It was postulated that the small and evenly distributed fat globule size in homogenised milk contributes to the smooth coating whereas adequate viscosity provides sensation of thickness, giving rise to the overall perception of creaminess in liquid milk. A similar trend was noted (Goudedranche et al. 2000), in that the perception of creaminess was greater for smaller fat globule milk (prepared by microfiltration) than for the control milk. Further interpretation is difficult, however, as neither of these studies presented size distribution data (Richardson and Booth 1993; Goudedranche et al. 2000).

From a broader food emulsion perspective, it has been reported that particle size plays little role in fat perception (Mela et al. 1994; Vingerhoeds et al. 2008). For example, there was only a slight reduction in creaminess of 10 % sunflower oil emulsions with increasing droplet size (D_{32} 0.41–8.10 μm) (Vingerhoeds et al. 2008). Replacement of sunflower oil by milk fat (10 % milk fat emulsion) did not impact on perception of creaminess as comparing between small (D_{32} 0.8 μm) and large (D_{32} 2.21 μm) droplet size. In both emulsion systems, neither odour nor taste/aftertaste attributes were droplet-size dependent (Vingerhoeds et al. 2008). However, significant differences in mouth-feel, lumpiness and dryness were noted between the small and large milk fat emulsions. In contrast, Kilcast and Clegg (2002) reported an increase in creaminess with increasing droplet size (D_{43} 0.5–2.8 μm) in artificial creams. The authors argued that crystalline state (i.e. proportion of solid milk fat), rather than droplet size, is responsible for the perception of dryness and creaminess. However, no thermal properties of the milk fat emulsion and artificial creams were presented to support the hypothesis.

Particle size effects were also revealed when studying a dairy-based emulsion model (2–8 % fat, D_{43} 0.2–1.5 μm) manufactured from anhydrous milk fat and skim milk powder having similar composition to the commercial milk products (Fibrianto 2013). Textural perception, particularly thickness and chalkiness, of the low fat emulsion (4 % w/v) was remarkably impacted by the presence of fat aggregates having mean diameter above 5 μm. The larger fat aggregates also contributed to the powdery feeling of the milk fat emulsions. Note that all the milks in this study had similar flow behaviour and viscosity as per rheological measurement. Using tribology, it was found that presence of fat aggregates (>5 μm) altered the coefficient of friction of 4%w/v milk fat emulsion at boundary friction of 5 mm s^{-1} (Fibrianto 2013). It was postulated that along with fat concentration, the particle size distribution is an additional factor governing fat perception. It was further noted that tribological parameters may provide a more distinct and consistent interpretation of textural parameters than viscosity parameters.

7.2 Cheese

Cheese can be classified on basis of fat content (skim, low-, medium-, full-, and high-fat), moisture level (soft, semi-soft, semi-hard, hard, and extra hard) and curing characteristics (ripened, mould ripened, and unripened). For example,

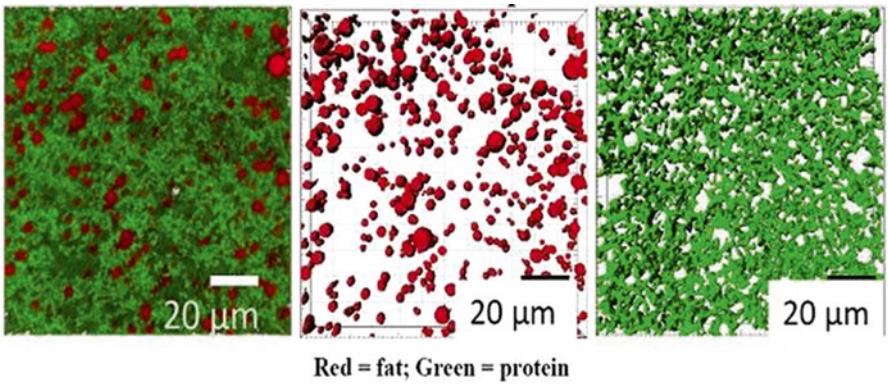

Fig. 7.4 Microstructure of full fat Cheddar cheese prepared from ultrafiltered milk retentate as visualised under confocal laser scanning microscopy (Ong et al. 2013)

Emmental and Cheddar are categorised as hard cheeses. Semi-hard cheese includes Gouda and Mozzarella. Camembert and cottage cheeses can be regarded as semi-soft and (unripened/fresh) soft cheeses, respectively. In very general terms, cheese microstructure consists of a protein matrix made up of linked and aggregated casein micelles, interacting with each other via electrostatic and hydrophobic forces, in which MFG, water and salts are dispersed. In this structure, native MFG can be physically entrapped as inert fillers within the protein network (Fig. 7.4). If the native MFGM is degraded or partially replaced with other milk protein, for example by homogenisation prior to cheese-making, the modified globule surface may then bond with the cheese protein network, mainly through hydrophobic interactions (Everett and Olson 2000; Lucey et al. 2003). Many aspects of initial milk composition have been shown to influence cheese yield and quality, including some effects attributed to MFG size (Lucey et al. 2003). In this book, the effect of MFG size on product properties and functionalities is discussed based on the state of MFG, i.e. native or homogenised MFG, as follows.

7.2.1 Cheese Made from Native Milk Fat Globules

7.2.1.1 Water Binding and Enzyme Activity

Studies performed on large and small native MFG fractions showed that the small globule fraction might be beneficial for some cheese quality attributes. Small MFG have greater surface area: volume ratio and this is reflected in the relatively higher proportion of MFGM material (e.g. phospholipids) usually present in small MFG fractions (as discussed in Chap. 5). Thus, water binding capacity of small MFG fractions is greater and enzyme activity (proteolysis, lipolysis) is enhanced. This in turn results in higher moisture content and softer texture in Emmental and

Camembert cheeses manufactured from small MFG fractions (Michalski et al. 2003, 2007). In the cheese matrix, proteolytic enzymes modulate the interaction between casein micelles and associated availability of calcium ions (Lucey et al. 2003). Greater proteolysis has been observed in small fat globule-containing Camembert, Emmental and Cheddar cheeses (Jana and Upadhyay 1992; Michalski et al. 2003, 2004a). In aged and hard cheese, lipolysis is essential to develop cheese flavour (Ma and Barbano 2000). Greater lipolysis has been associated with smaller MFG in Italian cheese and Camembert cheese (Ma and Barbano 2000; Michalski et al. 2003). This might be related to greater proportion of MFGM with smaller fat globules, which provides more specific sites for lipolytic activity (Michalski et al. 2003). However, in Emmental cheese made from native MFG, it was reported that after 52 days of ripening, large fat globule-containing cheese (5.6 μm) had greater lipolysis (334.1 ± 32.7 mg free fatty acid (FFA) per 100 g of fat) than the cheese manufactured from a smaller size fraction (3.3 μm; 91.8 mg FFA/100 g fat). A similar trend was observed in miniature Cheddar cheese, where the level of FFA in large fat globule cheese (4.68 μm) after 120 days of ripening was much higher than in the small globule counterpart (3.45 μm). It was assumed that lipolytic agents (lipases and esterases of lactic acid bacteria) and change in crystallisation of fat in association with fat globule size might be responsible for the differences seen in liberation of free fat upon ripening (O'Mahony et al. 2005). Compared to the small fat globules, crystalline structures formed in large fat globules induced greater propensity to disrupt the milk fat globule membrane (O'Mahony et al. 2005). Thus, structure of large fat globules is more prone to lipolysis.

7.2.1.2 Mechanical Strength, Rheology and Sensory Properties

Native MFG are likely to be non-interactive with the protein matrix and the smaller fat globules appear to be better retained than the large ones. A greater number of small fat globules may introduce weak points throughout the protein matrix and the casein network in small fat globule-containing cheese appears to be composed of thinner strands (Michalski et al. 2002). Mini Swiss, Camembert, Emmental and fresh cheeses produced from small MFG fractions become softer, with lower Young's modulus and fracture stress values (Goudedranche et al. 2000; Michalski et al. 2003, 2004a, 2007). In Emmental cheese, small fat globules (3.31 μm) were found to improve stretching and elasticity, compared to large fat globules (5.60 μm). The difference in rheological properties might be related to mouth-feel and texture of small fat globule cheeses, which were judged to be smoother, more elastic and more melting than large fat globule and control cheeses (Michalski et al. 2003; Goudedranche et al. 2000). Compositional differences such as moisture in non-fat substance (MNFS), total solids and pH were found in Camembert and Emmental cheeses prepared from native small and large fat globule fractions. These features might also be associated with the differences in rheological and functional properties observed in the cheeses (Michalski et al. 2003, 2004a). Another example of the apparent interaction of MFG size effects with other composition effects is low-fat

Cheddar cheese manufactured from either high- or low mineral retentate powder. The sensory quality of low-fat Cheddar cheese produced from low mineral retentate powder and a large fat globule fraction was better than the corresponding small fat globule cheese in terms of colour, flavour and texture (St-Gelais et al. 1997).

7.2.2 Cheese Made from Homogenised Milk Fat Globules

7.2.2.1 Oil Leakage and Syneresis

Homogenisation is applied in some cheese making processes to reduce the formation of free oil in the cheese, enhance texture attributes and increase cheese yield, notably for soft, high-moisture cheeses (Table 7.2). This is associated with a reduction in size, increase in surface area and increase in reactivity of the fat globules. The presence of small fat globules in homogenised milks is known to reduce oil leakage in cheese, due to their higher emulsion stability (Rowney et al. 2003; Rudan et al. 1998; Tunick 1994; Lelievre et al. 1990). Water retention is also higher in some cheese curds made with small fat globules, resulting in higher cheese moisture content (Lemay et al. 1994; Zamora et al. 2007; Michalski et al. 2004a) and more syneresis (Emmons et al. 1980; Green et al. 1983). In recombined, white brined cheese, the apparent viscosity increased with decreasing fat globule size (Avsar 2010). These effects are largely attributable to size reduction and surface modification of the fat globules upon homogenisation (Green et al. 1983). For instance, small fat globules are more dispersed within the cheese curd. Also, incorporation of casein into the homogenised fat globule membrane may result in less casein being available to participate in the cheese protein matrix, leading to a weaker structure (Green et al. 1983). High water retention by small fat globules was also associated with a remarkable increase in lipolysis (1156 mg FFA/100 g of fat) of Emmental cheese (Michalski et al. 2004a).

7.2.2.2 Firmness of Cheeses

Regarding the firmness of cheese, contrasting results were noted in literature when applying small fat globules via homogenisation. Firmness of cheese curd or finished products was reduced with decreasing fat globule size in studies of Emmons et al. (1980), Ghosh et al. (1994) and Lemay et al. (1994). On the contrary, size reduction of fat globules by homogenisation made cheese firmer in studies of Gavarlc et al. (1989) and Tunick et al. (1993). At maturity, it was also reported that the storage modulus G′ of small fat globule-containing cheese (~1 μm) increased significantly compared to its larger counterparts (~2.7 μm) (Schenkel et al. 2013). In other studies, small fat globule size did not alter the texture of recombined white-brined cheese (Avsar 2010), nor that of Cheddar cheese (Lemay et al. 1994). It is likely that the contrasting results on mechanical properties of cheese can be attributed to differences in cheese types and corresponding manufacturing conditions.

Table 7.2 Selected studies on effect of fat globule size on cheese making process and quality of cheese

Cheese type	MFG size	Remarks	References
Native milk fat globules			
Cheddar (low-fat); 5 % low (LMR) or high mineral content (HMR) retentate milk powder	D_{vs}:	**SFG**:	St-Gelais et al. (1997)
	LFG: 2.4 µm	+*Processability*: cheese yield ↑ (SFG-HMR), fat loss in whey ↓ in LFG cream	
	SFG: 1.6 µm	+*Sensory properties*: texture, flavour, colour ↑ with LFG	
		-*Unchanged*: biological activities	
Camembert; microfiltrated native MFG	D_{43}:	**SFG**:	Michalski et al. (2003)
	SFG: 3 µm	+ *Processability*: cheese yield ↑, fat recovery ↓	
	LFG: 6 µm	+ *Enzyme activity*: proteolysis ↑, lipolysis ↓	
		+ *Physicochemical properties*: moisture ↑, whey ↓, rigidity and firmness ↓,	
		+*Sensory properties*: melting ↑, elasticity ↑, yellowness ↓, smooth ↑	
		+*Unchanged*: manufacture time and pH decrease	
Emmental; microfiltrated native MFG	D_{43}:	**SFG**:	Michalski et al. (2004a)
	SFG: 3.3 µm	+ *Processability*: cheese yield ↑, fat recovery ↓	
	LFG: 5.6 µm	+ *Enzyme activity*: proteolysis ↑, lipolysis ↓	
		+ *Physicochemical properties*: moisture ↑, fracture stress ↓, firmness ↓, melting temperature ↓ → stretching ↑, melting ↑, elasticity ↑, extrusion force ↓	
		+*Unchanged*: oiling-off	
		+*Compositional differences between SFG- & MFG-cheeses*: MNFS, total solids, pH	
Reformed milk fat globules			
Emmental; homogenised milk	D_{43}:	+*Enzyme activity*: lipolysis ↑	Michalski et al. (2004a)
	2.1 µm		
Cheddar; homogenised milk (HM, concentrated)	CTRL: 4.2 µm	**HM**:	Green et al. (1983)
	HM: 2.4–2.7 µm	+*Physicochemical properties*: rennet time ↓, firmness ↑, syneresis ↓	

(continued)

Table 7.2 (continued)

Cheese type	MFG size	Remarks	References
Cheddar; microfluidised milk	710–1020 nm	+*Processability*: fat loss in whey ↓, cheese yield ↑, fat recovery ↑	Lemay et al. (1994)
		+ *Physicochemical properties*: moisture ↑, whiteness ↑	
		+*Unchanged*: cheese firmness, protein and casein recoveries	
Mozzarella; homogenised milk (HM)	D_{43}:	**HM**:	Rudan et al. (1998)
	CTRL: 2.73 µm	+ *Processability*: fat loss to whey ↑, free oil release ↓	
	HM: 0.84 µm	+*Enzyme activity*: proteolysis (soluble N) ↑	
		+*Physicochemical properties*: whiteness ↑	
		+*Unchanged*: protein content of whey, moisture, protein, MNFS, meltability, mechanical strength (hardness, cohesiveness, springiness)	
Mozzarella; homogenised milk (HM)	Number-weighted:	**HM**:	Rowney et al. (2003)
	CTRL: 4.9 µm	+ Moisture ↑ Free oil ↓, viscosity ↑	
	HM: 3.9 µm		
Semi-hard cheese; homogenised milk (HM)	$d_{0.5}$:	**SFG**:	Schenkel et al. (2013)
	LFG: 2.8 µm	+Extractable fat ↓	
	SFG: 1.1 µm	+Flowability ↓, stretchability ↑	
		+$G'_{20\,°C}$ ↑ and $G'_{80\,°C}$ ↑, max. loss tangent ↓	
		+Unchanged: MNFS, pH, proteolysis	

7.2.2.3 Thermo-Physical Properties

Some differences in the thermo-physical properties of cheese have also been associated with differences in fat globule size. Compared to large fat globules (~2.7 μm), small fat globules (~1 μm) were reported to result in lower flowability and stretchability in semi-hard cheese (Schenkel et al. 2013). It was shown that at 4–6 °C microstructure of the 4-week ripening cheese made from large fat globules had greater non-stabilised globular fat. The author suggested that large fat globules are more prone to destabilisation of fat as compared to small fat globules (Schenkel et al. 2013). The higher degree of fat destabilisation in large fat globule cheese apparently promoted the lubrication between the protein strands, in turn, facilitating the mobility of associated protein layers and overall higher flowability in large fat globule-containing cheese (Schenkel et al. 2013).

7.3 Yoghurt

The size of MFG embedded in the microstructure of fat-containing yoghurt appears to have some influence on the physical functionality and sensory properties of the product.

7.3.1 Water Retention and Associated Gel Strength and Syneresis

Previous studies have suggested that a complex range of particle interactions is critical in determining microstructure and gelation properties of yoghurts (Riener et al. 2009; Ciron et al. 2010; Serra et al. 2007). Interacting with the protein matrix, fat globules act either as structure breakers or structure promoters when they are present in inert or active modes, respectively. As discussed earlier, shear processing of MFG can result not only in reduction of globule size, with proportionally increased surface area, but also in increased reactivity of the globule membrane through membrane disruption and inclusion of casein and whey proteins. In some products, such as yoghurt, the overall effect of this can be to strengthen the gel network and increase water retention. For example, thermosonicated yoghurt, having smaller fat globule size about 0.4 μm, tended to retain double the amount of water in the matrix than conventionally produced yoghurt at the same fat content (Riener et al. 2009). The microstructure of low-fat yoghurt prepared from microfluidised MFG also showed small fat globules embedded in and inter-connecting with the protein network, forming larger gel particles; however, the improvement on texture and water retention of microfluidised yoghurt was only slightly higher than its conventional counterpart (Ciron et al. 2010). The small MFG size (D_{32} 0.12–0.16 μm) in high pressure treated yoghurt (at 200–300 MPa) was also shown to improve gel strength, gel firmness and decrease syneresis when compared to yoghurt conventionally manufactured from skim milk powder (Serra et al. 2007).

7.3.2 Texture and Firmness

To the best of our knowledge, the sole reported study of native MFG size on yoghurt texture was undertaken by Goudedranche et al. (2000). Yoghurts produced from small (below 2 μm) and large milk fat globule (above 2 μm) fractions exhibited differences in firmness and mouth-feel texture. Small fat globule-formed yoghurts had lower shear stress and appeared to possess smoother and finer texture. Conversely, the mouth-feel texture of large fat globule-containing yoghurts was assessed as firmer and grainy (Goudedranche et al. 2000). These observations applied for both low-fat and full-fat yoghurts, highlighting the influence of fat globule size. Apart from this study, there is very little information on impact of MFG size on yoghurt properties.

7.4 Aerated Dairy Systems

The microstructure of aerated dairy products such as whipped cream and ice cream is very complex, comprising both multi-phase and multi-component systems. For instance, microstructure of ice cream consists of agglomerated fat, ice crystals and a viscous serum phase building up a solid-like matrix in which air cells are embedded. In whipped cream, foam is structured by coalescence of fat globules holding the air cells, forming a three dimensional network (Goff 1997). One of many influential variables in foam formation and foam stability in these aerated dairy products is fat globule size. Other factors include processing conditions, physicochemical characteristics of ingredients, interactions of ingredients, and spreading behaviour of fat globules at the interface of air and water, to name a few (Goff 1997).

7.4.1 Ice-Cream

Few attempts have been made to vary the fat globule size in ice cream mix and evaluate the physical and functional properties of the resultant ice-cream after freezing. It was reported that homogenisation at low pressure (below 50 MPa) is effective on altering fat globule size in ice cream mix, whereas high pressure homogenisation (above 50 MPa) tends to affect the continuous phase (Koxholt et al. 2001; Biasutti et al. 2013; Hayes et al. 2003; Olson et al. 2003). It was also found that, following homogenisation at 0–30 MPa, foam structures formed by fat particles within the size range of 0.44–0.85 μm melted down faster than foams formed by larger particles (0.85–3.33 μm) (Koxholt et al. 2001). These different effects on meltdown might be attributed to the greater drainage of small fat globules out of the foam lamellae, compared to the larger fat globules, which may be more easily retained within the foam structure (Koxholt et al. 2001).

7.4.2 Whipped Cream and Whippable Dairy Emulsions

In contrast to the observations on size-dependent foam stabilisation of ice cream as previously presented, it has been reported that foam stability of whipped cream containing small fat globules was better than control product (Michalski et al. 2006). It was reported that after a week of storage, the foam formed in small fat globule whipped cream was highly resistant to collapse (12 %) compared to the large fat globule (29 %) and control (25 %) whipped creams. The MFG fractions in this study were obtained by microfiltration of raw milk, which has been claimed to preserve the MFGM integrity (Michalski et al. 2006). Unlike the homogenisation process used in the ice cream studies, it is unlikely that the colloidal phase and MFGM would have been modified by microfiltration. These two apparently conflicting sets of observations on two similar dairy foam systems may also reflect differences in globule preparation and processing.

The main application for whippable dairy emulsions is in low fat whipping cream. In these products, milk fat (the dispersed phase) is mixed with an aqueous phase typically comprising dairy emulsifiers to form dairy-based emulsions. The emulsifiers can be caseins, whey proteins, and/or small molecular surfactants, which are formulated to regulate the air bubble surface adsorption behaviour. The surfactant should also promote partial coalescence of fat particles by their destabilisation during whipping but prevent phase separation on subsequent storage. In such a system, it was reported that a 20 % milk fat emulsion containing 0.25–0.5 % milk proteins and initial droplet size of 0.8–0.9 μm was sufficient to resist foam collapse and drainage during whipping, as well as creaming upon storage (Goff 1997). Increasing the amount of emulsifiers up to 2 % led to a lower emulsion droplet size (about 0.6 μm). However, a stable foam was not facilitated because the emulsion itself too stable to be transformed during whipping (Goff 1997). In other food systems, a range of particle types have been used to build up foam structures, including fat particles (Eisner et al. 2007; Murray et al. 2011), silica particles (Kostakis et al. 2007) and hydrophobic starch particulates (Murray et al. 2011), to name a few. It has been reported that *n*-tetradecane droplets (30 % oil volume; average size of 0.6 μm) in protein-stabilised emulsions can behave as separated and stable entities at the air-water interface and enhanced foam stability (Murray et al. 2011). Hence, the intact fat particles can also be adsorbed into the lamella of air bubbles during whipping. The smaller particles will have higher tendency to be adsorbed onto the lamella than bigger particles. If there is no destabilisation of fat, foaming will be governed by the size of the independent fat particles in the emulsions (Truong et al. 2014a). In a study using a range of micron- to nano-scale size (0.2–1.2 μm) milk fat emulsions (10 % w/w fat), it was found that the droplet size influenced the crystallisation temperature, solid fat content and viscosity of the whipped dairy emulsions. As a result, foam formation and stability were altered in different ways as a function of emulsion droplet size (Truong et al. 2014a). There was a slight increase in overrun with reduced fat particle size in stearin emulsion stabilised by whey protein concentrate. However, disruption of foam structures occurred fastest with nano-sized fat particles, leading

to shorter half-life of the foam (Truong et al. 2014a). Since loss of structural integrity during foam formation with nano-sized droplets is related to the properties of emulsion, the effect of particle size on foaming of whippable dairy emulsions is not straightforward. The particle size effect needs to be examined on the systems having similar properties. In addition, manipulation of wetting characteristics of the emulsion droplets to promote the interaction with foam lamellae may open up the possibility for gaining better foaming properties in low fat dairy emulsions.

7.5 Butter

Butter is made by churning cream, which facilitates phase inversion from oil-in-water to a water-in-oil emulsion. Milk fat globules in the cream, typically 2.5 μm in diameter, are destabilised and the fat disperses as the continuous phase in the microstructure of butter. Physicochemical properties of the initial cream, including MFG size, have great influence on the processability and functionality of butter manufactured by this process (Table 7.3).

7.5.1 Churning

Churning time is a critical factor in the processability of cream and butter. Hurtaud et al. (2010) reported a shorter (45 min) churning time with small fat globules (3.49 μm; obtained from cows fed with an extruded linseed modified diet) compared to the large globules of control cream (4.18 μm; churning time of 54.5 min). In contrast, small globules (1.8 μm) secreted from cows fed with fish meal modified diet induced longer churning times than with cream from control milk (2.3 μm) (Avramis et al. 2003) (Tables 4.2 and 7.3). However, interpretation and comparison of these studies is complicated by the milk fat compositional changes that were also induced as a result of the different cow diets and may also have an effect on

Table 7.3 Effect of milk fat globule size on processing and quality of butter

MFG size	Processing effect	Butter quality	References
SFG fraction < 2 μm	Similar churning abilities	*Sensory*:	Goudedranche et al. (2000)
LFG fraction > 2 μm		**SFG**: greasy and oily	
Control: 2.31 μm	**SFG**: longer churning time		Avramis et al. (2003)
Cow fed with fish meal: 1.84 μm (SFG)			
Control: 4.18 μm	**SFG**: shorter churning time	**SFG**:	Hurtaud et al. (2010)
Cow fed with linseed: 4.07–4.75 μm (SFG)		Fat loss and moisture ↑	
		Fat content and iodine value ↓	
		Firmness ↓, brightness, yellow index ↓, spreadability ↑	

churning time. The percentage of fat recovery in the butter from cream, or churning efficiency, is another critical factor in butter manufacture. Churning efficiency is known to be inefficient with homogenised cream (Walstra et al. 2005). However, when comparing different class sizes of native MFG in cream prepared by microfiltration the churning abilities were shown to be similar for both small (below 2 μm) and large (above 2 μm) fat globules fractions (Goudedranche et al. 2000). Other studies have shown that small fat globules in cream can be responsible for greater fat loss during churning. This is apparently caused by the greater stability and reduced embedding of small MFG in the matrix. The fat content in buttermilk (the phospholipid-rich, milk-like by-product of churning) was reported to be threefold increased (17.6 %) in small fat globules (3.49 μm) compared to the larger fat globules in the control cream before churning (4.18 μm) (Hurtaud et al. 2010). Smaller MFG are also associated with greater water retention, due to the higher proportion of hydrophilic MFGM (Michalski et al. 2004b), resulting in higher moisture content of the final product (Hurtaud et al. 2010; Goudedranche et al. 2000). Enrichment of unsaturated fatty acids in cream also causes higher proportion of water in butter (Hurtaud et al. 2010; Goudedranche et al. 2000).

7.5.2 Firmness, Texture and Mouth-Feel

Butter made from cream containing small MFG (obtained from cows fed with unsaturated fatty acid enriched meal) was softer, more spreadable and had better mouthfeel and melting properties than the control butter (Avramis et al. 2003; Hurtaud et al. 2010). However, these changes in rheological and sensory properties might also be linked to the differences in the physical state of milk fat associated with compositional differences fat globule size. The same study reported that, at the same measuring and storage temperatures, liquid fat tended to be more concentrated in smaller fat globules, presumably due to its enrichment with unsaturated fatty acids. In a contrasting study, butter manufactured from small fat globules (less than 2 μm) fractionated by microfiltration was judged to be greasy, oily and less acceptable than the control butter (Goudedranche et al. 2000).

A few studies have reported the influence of droplet size on thermal, crystalline and microstructural properties of cream and/or dairy emulsions (Lopez et al. 2002; Bugeat et al. 2011; Truong et al. 2014b; Truong et al. 2015). However, there is little information available on the relationship between globule size, crystallisation behaviour and processability/quality of butter. In another aspect, a patented process involving high shear mixing of cooled, reformed cream reported that microstructure of the resulting softer and more spreadable butter consisted of a fine and homogeneous crystal structure (Gnanasambandam and Bedi 2012). This suggests that the utilisation of sub-micron sized globules, which typically crystallise into fine structures, might be beneficial to produce highly spreadable butter. In addition, Ronholt et al. (2012) found that presence of small, residual fat globules provides more contact points in the continuous fat phase of butter, resulting in a crystal network that

was stronger and less brittle compared to the crystal network without fat globules. Thus it may be possible to further improve the stability, texture and spreadability of butter by manipulating fat globule size as a means of indirectly controlling both crystallisation and structure development in the end product. Further research is needed to explore the feasibility of such an approach.

References

Aguilera JM, Stanley DW. Microstructural principles of food processing and engineering, vol. 2. Gaitherburg: Aspen Publishers Inc.; 1999.

Avramis CA, Wang H, McBride BW, Wright TC, Hill AR. Physical and processing properties of milk, butter, and Cheddar cheese from cows fed supplemental fish meal. J Dairy Sci. 2003;86(8):2568–76. doi:10.3168/jds.S0022-0302(03)73851-X.

Avsar YK. Milk fat globule size, powder hydration time, para-kappa-casein content and textural properties of recombined white-brined cheese produced by direct recombination system. J Food Process Preserv. 2010;34:223–40. doi:10.1111/j.1745-4549.2008.00335.x.

Biasutti M, Venir E, Marino M, Maifreni M, Innocente N. Effects of high pressure homogenisation of ice cream mix on the physical and structural properties of ice cream. Int Dairy J. 2013;32(1):40–5. doi:10.1016/j.idairyj.2013.03.007.

Borcherding K, Hoffmann W, Lorenzen PC, Schrader K. Effect of milk homogenisation and foaming temperature on properties and microstructure of foams from pasteurised whole milk. Lwt Food Sci Technol. 2008;41(10):2036–43. doi:10.1016/j.lwt.2007.11.020.

Bugeat S, Briard-Bion V, Perez J, Pradel P, Martin B, Lesieur S, Bourgaux C, Ollivon M, Lopez C. Enrichment in unsaturated fatty acids and emulsion droplet size affect the crystallization behaviour of milk triacylglycerols upon storage at 4 degrees C. Food Res Int. 2011;44(5):1314–30. doi:10.1016/j.foodres.2011.01.003.

Cho YH, Lucey JA, Singh H. Rheological properties of acid milk gels as affected by the nature of the fat globule surface material and heat treatment of milk. Int Dairy J. 1999;9(8):537–45. doi:10.1016/S0958-6946(99)00123-5.

Ciron CIE, Gee VL, Kelly AL, Auty MAE. Comparison of the effects of high-pressure microfluidization and conventional homogenization of milk on particle size, water retention and texture of non-fat and low-fat yoghurts. Int Dairy J. 2010;20(5):314–20. doi:10.1016/j.idairyj.2009.11.018.

Cobos A, Horne DS, Muir DD. Rheological properties of acid milk gels.2. Effect of composition, process and acidification conditions on products from recombined milks using the microfluidizer. Milchwissenschaft. 1995;50(11):603–6.

Eisner MD, Jeelani SAK, Windhab EJ. Stability of foams containing proteins, fat particles and nonionic surfactants. Chem Eng Sci. 2007;62(7):1974–87. doi:10.1016/j.ces.2006.12.056.

Emmons DB, Lister EE, Beckett DC, Jenkins KJ. Quality of protein in milk replacers for young calves.5. Effect of method of dispersing fat on curd formation and whey syneresis. J Dairy Sci. 1980;63(3):417–25.

Everett DW, Olson NF. Dynamic rheology of renneted milk gels containing fat globules stabilized with different surfactants. J Dairy Sci. 2000;83(6):1203–9.

Fibrianto K. Contribution of anhydrous milk fat to oral processing and sensory perception of liquid milks. The University of Queensland; 2013.

Gavarlc DD, Carlc M, Kalab M. Effects of protein-concentration in ultrafiltration milk retentates and the type of protease used for coagulation on the microstructure of resulting gels. Food Microstruct. 1989;8(1):53–66.

Ghosh BC, Steffl A, Hinrichs J, Kessler HG. Rennetability of whole milk homogenized before or after pasteurization. Milchwissenschaft. 1994;49(7):363–7.

Gnanasambandam R, Bedi A. Composition and methods for soft butter. US Patent. 2012.
Goff HD. Instability and partial coalescence in whippable dairy emulsions. J Dairy Sci. 1997;80(10):2620–30.
Goudedranche H, Fauquant J, Maubois JL. Fractionation of globular milk fat by membrane microfiltration. Lait. 2000;80(1):93–8.
Green ML, Marshall RJ, Glover FA. Influence of homogenization of concentrated milks on the structure and properties of rennet curds. J Dairy Res. 1983;50(3):341–8.
Hayes MG, Lefrancois AC, Waldron DS, Goff HD, Kelly AL. Influence of high pressure homogenisation on some characteristics of ice cream. Milchwissenschaft. 2003;58(9–10):519–23.
Hurtaud C, Faucon F, Couvreur S, Peyraud JL. Linear relationship between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid composition and butter properties. J Dairy Sci. 2010;93(4):1429–43. doi:10.3168/jds.2009-2839.
Jana AH, Upadhyay KG. Homogenisation of milk for cheesemaking. Aust J Dairy Technol. 1992;47:72–9.
Ji YR, Lee SK, Anema SG. Effect of heat treatments and homogenisation pressure on the acid gelation properties of recombined whole milk. Food Chem. 2011;129(2):463–71. doi:10.1016/j.foodchem.2011.04.099.
Kilcast D, Clegg S. Sensory perception of creaminess and its relationship with food structure. Food Qual Prefer. 2002;13(7–8):609–23. doi:10.1016/S0950-3293(02)00074-5.
Kostakis T, Ettelaie R, Murray BS. Enhancement of stability of bubbles to disproportionation using hydrophilic silica particles mixed with surfactants or proteins. R Soc Chem. 2007;302:357–68. doi:10.1039/9781847557698-00357.
Koxholt MMR, Eisenmann B, Hinrichs J. Effect of the fat globule sizes on the meltdown of ice cream. J Dairy Sci. 2001;84(1):31–7.
Lelievre J, Shaker RR, Taylor MW. The role of homogenization in the manufacture of Halloumi and Mozzarella cheese from recombined milk. J Soc Dairy Technol. 1990;43(1):21–4.
Lemay A, Paquin P, Lacroix C. Influence of microfluidization of milk on Cheddar cheese composition, color, texture, and yield. J Dairy Sci. 1994;77(10):2870–9.
Logan A, Day L, Pin A, Auldist M, Leis A, Puvanenthiran A, Augustin MA. Interactive effects of milk fat globule and casein micelle size on the renneting properties of milk. Food Bioprocess Technol. 2014;7(3):3175–85.
Logan A, Leis A, Day L, Øiseth SK, Puvanenthiran A, Augustin MA. Rennet gelation properties of milk: influence of natural variation in milk fat globule size and casein micelle size. Int Dairy J. 2015;46:71–77.
Lopez C, Bourgaux C, Lesieur P, Bernadou S, Keller G, Ollivon M. Thermal and structural behavior of milk fat - 3. Influence of cooling rate and droplet size on cream crystallization. J Colloid Interface Sci. 2002;254(1):64–78. doi:10.1006/jcis.2002.8548.
Lucey JA, Johnson ME, Horne DS. Invited review: perspectives on the basis of the rheology and texture properties of cheese. J Dairy Sci. 2003;86(9):2725–43. doi:10.3168/jds.S0022-0302(03)73869-7.
Ma Y, Barbano DM. Gravity separation of raw bovine milk: fat globule size distribution and fat content of milk fractions. J Dairy Sci. 2000;83(8):1719–27. doi:10.3168/jds.S0022-0302(00)75041-7.
Mela DJ, Langley KR, Martin A. Sensory assessment of fat-content - effect of emulsion and subject characteristics. Appetite. 1994;22(1):67–81. doi:10.1006/appe.1994.1006.
Mellema M, Heesakkers JWM, van Opheusden JHJ, van Vliet T. Structure and scaling behavior of aging rennet-induced casein gels examined by confocal microscopy and permeametry. Langmuir. 2000;16(17):6847–54. doi:10.1021/La000135i.
Michalski MC, Cariou R, Michel F, Garnier C. Native vs. damaged milk fat globules: membrane properties affect the viscoelasticity of milk gels. J Dairy Sci. 2002;85(10):2451–61. doi:10.3168/jds.S0022-0302(02)74327-0.
Michalski MC, Gassi JY, Famelart MH, Leconte N, Camier B, Michel F, Briard V. The size of native milk fat globules affects physico-chemical and sensory properties of Camembert cheese. Lait. 2003;83(2):131–43. doi:10.1051/Lait:2003003.

Michalski MC, Camier B, Briard V, Leconte N, Gassi JY, Goudedranche H, Michel F, Fauquant J. The size of native milk fat globules affects physico-chemical and functional properties of Emmental cheese. Lait. 2004a;84(4):343–58. doi:10.1051/Lait:2004012.

Michalski MC, Ollivon M, Briard V, Leconte N, Lopez C. Native fat globules of different sizes selected from raw milk: thermal and structural behavior. Chem Phys Lipids. 2004;132(2):247–61. doi:10.1016/j.chemphyslip.2004.08.007.

Michalski MC, Leconte N, Briard-Bion V, Fauquant J, Maubois JL, Goudedranche H. Microfiltration of raw whole milk to select fractions with different fat globule size distributions: process optimization and analysis. J Dairy Sci. 2006;89(10):3778–90.

Michalski MC, Camier B, Gassi JY, Briard-Bion V, Leconte N, Famelart MH, Lopez C. Functionality of smaller vs control native milkfat globules in Emmental cheeses manufactured with adapted technologies. Food Res Int. 2007;40(1):191–202. doi:10.1016/j.foodres.2006.09.011.

Murray BS, Durga K, Yusoff A, Stoyanov SD. Stabilization of foams and emulsions by mixtures of surface active food-grade particles and proteins. Food Hydrocoll. 2011;25(4):627–38. doi:10.1016/j.foodhyd.2010.07.025.

O'Mahony JA, Auty MAE, McSweeney PLH. The manufacture of miniature Cheddar-type cheeses from milks with different fat globule size distributions. J Dairy Res. 2005;72(3):338–48. doi:10.1017/S0022029905001044.

Olson DW, White CH, Watson CE. Properties of frozen dairy desserts processed by microfluidization of their mixes. J Dairy Sci. 2003;86(4):1157–62.

Ong L, Dagastine RRK, Kentish SE, Gras SL. Microstructure and composition of full fat Cheddar cheese made with ultrafiltered milk retentate. Foods. 2013;2(3):310–31.

Richardson NJ, Booth DA. Effect of homogenization and fat content on oral perception of low and high viscosity model creams. J Sens Stud. 1993;8:133–43.

Riener J, Noci F, Cronin DA, Morgan DJ, Lyng JG. The effect of thermosonication of milk on selected physicochemical and microstructural properties of yoghurt gels during fermentation. Food Chem. 2009;114(3):905–11. doi:10.1016/j.foodchem.2008.10.037.

Ronholt S, Kirkensgaard JJK, Pedersen TB, Mortensen K, Knudsen JC. Polymorphism, microstructure and rheology of butter. Effects of cream heat treatment. Food Chem. 2012;135(3):1730–9. doi:10.1016/j.foodchem.2012.05.087.

Rowney MK, Hickey MW, Roupas P, Everett DW. The effect of homogenization and milk fat fractions on the functionality of Mozzarella cheese. J Dairy Sci. 2003;86(3):712–8. doi:10.3168/jds.S0022-0302(03)73651-0.

Rudan MA, Barbano DM, Gu MR, Kindstedt PS. Effect of the modification of fat particle size by homogenization on composition, proteolysis, functionality, and appearance of reduced fat Mozzarella cheese. J Dairy Sci. 1998;81(8):2065–76.

Sanchez C, Maurer K, Hardy J. Influence of fat globule size on the rheological properties of a model acid fresh cheese. In: Dickinson E, Lorient D, editors. Food macromolecules and colloids. London: CRC; 1995. p. 512–6.

Schenkel P, Samudrala R, Hinrichs J. Thermo-physical properties of semi-hard cheese made with different fat fractions: influence of melting point and fat globule size. Int Dairy J. 2013;30(2):79–87. doi:10.1016/j.idairyj.2012.11.014.

Serra M, Trujillo AJ, Quevedo JM, Guamis B, Ferragut V. Acid coagulation properties and suitability for yogurt production of cows' milk treated by high-pressure homogenisation. Int Dairy J. 2007;17(7):782–90. doi:10.1016/j.idairyj.2006.10.001.

St-Gelais D, Passey CA, Hache S, Roy P. Production of low-fat Cheddar cheese from low and high mineral retentate powders and different fractions of milkfat globules. Int Dairy J. 1997;7(11):733–41. doi:10.1016/S0958-6946(97)00084-8.

Tepper BJ, Kuang T. Perception of fat in a milk model system using multidimensional scaling. J Sens Stud. 1996;11(3):175–90. doi:10.1111/j.1745-459X.1996.tb00040.x.

Tosh SM, Dalgleish DG. The physical properties and renneting characteristics of the synthetic membrane on the fat globules of microfluidized milk. J Dairy Sci. 1998;81(7):1840–7.

Truong T, Bansal N, Bhandari B. Effect of emulsion droplet size on foaming properties of milk fat emulsions. Food Bioprocess Technol. 2014a;7(12):3416–28. doi:10.1007/s11947-014-1352-4.

Truong T, Bansal N, Sharma R, Palmer M, Bhandari B. Effects of emulsion droplet sizes on the crystallisation of milk fat. Food Chem. 2014b;145:725–35. doi:10.1016/j.foodchem.2013.08.072.

Truong T, Morgan GP, Bansal N, Palmer M, Bhandari B. Crystal structures and morphologies of fractionated milk fat in nanoemulsions. Food Chem. 2015;171:157–67. doi:10.1016/j.foodchem.2014.08.113.

Tunick MH. Effects of homogenization and proteolysis on free oil in Mozzarella cheese. J Dairy Sci. 1994;77(9):2487–93.

Tunick MH, Malin EL, Smith PW, Shieh JJ, Sullivan BC, Mackey KL, Holsinger VH. Proteolysis and rheology of low-fat and full-fat Mozzarella cheeses prepared from homogenized milk. J Dairy Sci. 1993;76(12):3621–8. doi:10.3168/jds.S0022-0302(93)77703-6.

Vingerhoeds MH, de Wijk RA, Zoet FD, Nixdorf RR, van Aken GA. How emulsion composition and structure affect sensory perception of low-viscosity model emulsions. Food Hydrocoll. 2008;22(4):631–46. doi:10.1016/j.foodhyd.2007.02.011.

Walstra P, Wouters JTM, Geurts TJ. Dairy science and technology. 2nd ed. Boca Raton: CRC; 2005.

Whiteley AJ, Muir DD. Heat stability of homogenised concentrated milk.1. Comparison of microfluidiser with a valve homogeniser. Milchwissenschaft. 1996;51(6):320–3.

Xiong YL, Aguilera JM, Kinsella JE. Emulsified milkfat effects on rheology of acid-induced milk gels. J Food Sci. 1991;56(4):920–5. doi:10.1111/j.1365-2621.1991.tb14606.x.

Zamora A, Ferragut V, Jaramillo PD, Guamis B, Trujillo AJ. Effects of ultra-high pressure homogenization on the cheese-making properties of milk. J Dairy Sci. 2007;90(1):13–23.

Chapter 8
Conclusions

This book has highlighted the importance of both native and emulsified MFG size as a pivotal processing and functionality parameter in many fat-structured dairy products. Naturally, bovine milk fat globules exist in different size classes spanning from 0.1 to 10 μm that are synthesised in different pathways in mammary glands. Selection of differentiated-sized milk fat globules can be partly achieved through breeding, dietary supplement and lactation stage as well as size fractionation techniques such as gravity separation and microfiltration. Many of these approaches preserve the integrity of MFGM but need to be improved in order to provide more discrete size fractions. However, interesting variations in both chemical composition and functionality have been associated with MFG size-classified milks and creams, which have led to continued research interest in this area.

Downsizing of milk fat globules can also be achieved by mechanical homogenisation, which is commonly used in the dairy industry. Whilst this is a more practical approach and can reduce globule sizes to the nano-scale (below 0.2 μm), it yields emulsified globules that are qualitatively quite different from the native MFG, due to destruction of the original MFGM and incorporation of caseins and whey proteins into the reformed globule membrane. Nevertheless this method has been used successfully to produce a wide range of size-classified milk fat emulsions that differ in composition and functionality; indeed this approach has now been incorporated into many industrial processes as a means of improving the processability and functionality of dairy fat-based foods and ingredients.

Recent advances in microscopy, spectroscopy and data analysis have revealed further fascinating insights into how milk fat globules influence and participate in the complex microstructure of dairy products such cheese, yoghurt and ice cream. Fat droplet size is clearly of importance in this aspect of functionality and research in this field is now starting to provide explanations for empirical observations that have been known in dairy manufacturing for many years. The elucidation of the significance of fat droplet size in ice cream manufacture is a good example of the use of advanced technologies to unravel a highly complex problem.

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As more sophisticated techniques become available for dairy scientists to probe these complex systems, it is suggested that the next significant advances may come from the relatively new research area which is trying to relate processability and functionality to the complex interactions between milk fat crystallisation and microstructural behaviour (such as variations in crystal polymorphism and morphology) and the size, structure and composition of emulsified fat globules. This may be particularly important in the development of new, low-fat, nutrient-fortified dairy products, where lipid compositions may vary widely from traditional dairy products and present the manufacturer with new challenges in relation to functionality and processability.

Further drivers for this type of research are likely to come from pharmaceutical and nutritional research, where there is interest in using molecular and supramolecular structuring technologies to manipulate the bioavailability, delivery and controlled release of drugs and functional food ingredients.