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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

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

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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).

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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

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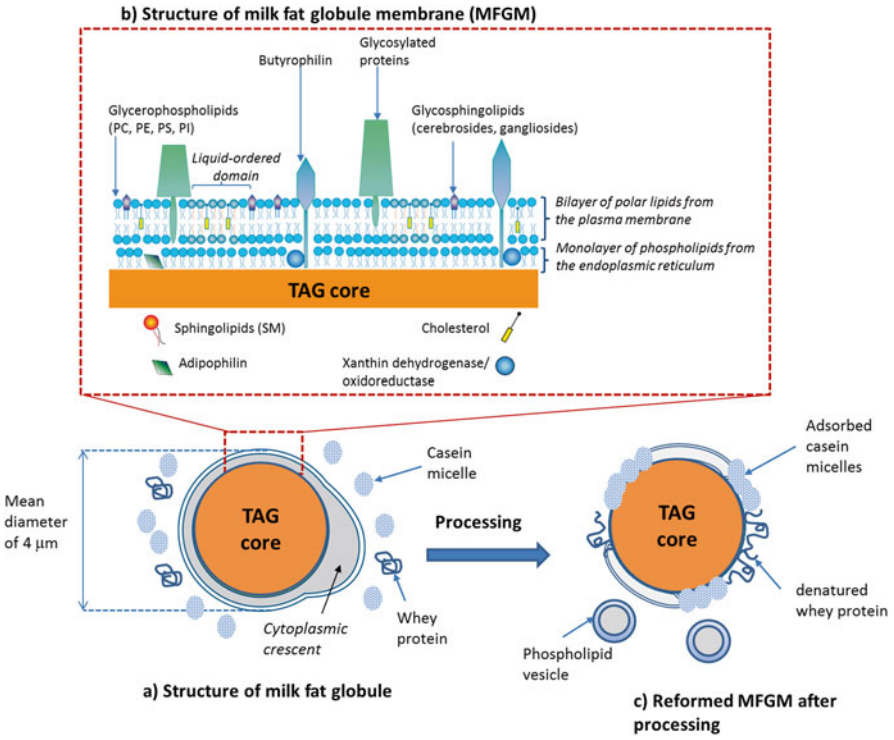

Fig. 2.1 Schematic illustrations of bovine MFG (**a**) and its tri-layer membrane (**b**) (not to scale) (Redrawn with modifications from Lopez et al. 2011; Lopez et al. 2008; Waninge et al. 2004). Heating, mixing and homogenisation of milk can disrupt the intact MFGM resulting in adsorption of casein 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

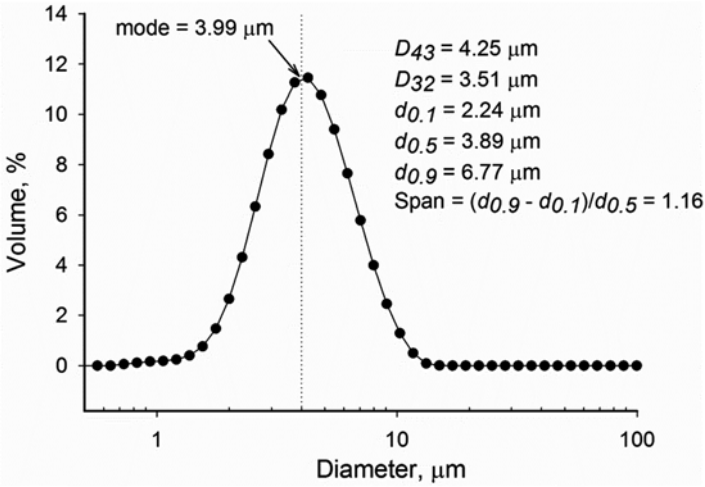

Fig. 2.2 A typical size distribution of raw bovine MFG measured by laser light scattering at 25 °C using the general model (distribution of spherical particles is unknown). Common expressions of mean diameters of MFG size: mode, volume-weighted mean D_{43}; surface-weighted or Sauter mean D_{32}; $d_{0.1}$, $d_{0.5}$ 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).

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