

Review

Nutritional and technological aspects of milk fat globule membrane material

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Abstract

The milk fat globule membrane (MFGM) has gained a lot of attention recently, due to the growing interest in its nutritional and technological properties. The whole membrane as well as the separate lipid and protein components have great potential for new product applications with unique nutritional and technological properties. This review focuses on the nutritional and technological aspects of the MFGM material, but also gives an overview of the gathered information about the composition, structure and isolation methods of the MFGM from different dairy sources.

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1. Introduction

The fat globules in milk consist of a triglyceride core, surrounded by a thin membrane, called the milk fat globule membrane (MFGM). This membrane, about 10–20 nm in cross-section, acts as an emulsifier and protects the globules from coalescence and enzymatic degradation. The MFGM is highly structured and contains unique polar lipids and membrane-specific proteins. Sphingolipids (highly bioactive molecules, mainly present in polar lipids from animal origin) account for up to one third of the MFGM polar lipid fraction. Scientific evidence on the nutritional benefits of these sphingolipids is accumulating. Moreover, it is assumed that the MFGM proteins also possess specific nutritional properties. As such, due to their origin and structure, MFGM polar lipids and proteins could be used as an emulsifier or stabilizer, combining technological and nutritional functionality. This review deals with the composition and structure of the MFGM, MFGM purification techniques on laboratory and industrial scale, the nutritional aspects of MFGM polar lipids and proteins, and finally the technological aspects and applications of MFGM material.

2. Composition and structure

The majority of the MFGM comprises membrane-specific proteins, mainly glycoproteins, and phospho- and sphingolipids. Its gross composition is given in Table 1. Literature findings on the composition of the MFGM material are highly variable due to differences in isolation, purification and analysis techniques.

2.1. Lipids of the milk fat globule membrane

The lipids of the MFGM are primarily polar lipids, although neutral lipids can also occur. The latter are triglycerides, diglycerides, monoglycerides, cholesterol and its esters. It was often mentioned that the MFGM contains a significant amount of high-melting triglycerides (Wooding & Kemp, 1975), although this must be rather attributed to the isolation methods of the MFGM-preparate (Walstra, 1974, 1985), as during isolation from

milk, these MFGM-fragments can easily become contaminated with triglyceride crystals.

The polar lipids of the MFGM consist of phospho- and sphingolipids. These are amphiphilic molecules with a hydrophobic tail and a hydrophilic head group. The glycerophospholipids consist of a glycerol backbone on which two fatty acids (FAs) are esterified. A phosphate residue with different organic groups (choline, serine, ethanolamine, etc.) may be linked on the third hydroxyl group. The characteristic structural unit of sphingolipids is the sphingoid base, a long-chain aliphatic amine, containing two or three hydroxyl groups. A ceramide is formed when the amino group of this sphingoid base is linked with a FA. On this ceramide unit, an organophosphate group can be bound to form a sphingophospholipid (e.g., phosphocholine in the case of sphingomyelin, SM) or a saccharide to form the sphingoglycolipids (glycosylceramides) (Christie, 2003; Fong, Norris, & MacGibbon, 2007; Newburg & Chaturvedi, 1992; Pfeuffer & Schrezenmeir, 2001; Vanhoutte, Rombaut, Dewettinck, & Van der Meeren, 2004; Vesper et al., 1999; Yang, Yu, Sun, & Duerksen-Hughes, 2004). The major types of polar lipids present in the membrane are phosphatidylcholine (PC), 35%; phosphatidylethanolamine (PE), 30%; SM, 25%; phosphatidylinositol (PI), 5%; phosphatidylserine (PS), 3%. Glucosylceramide (GluCer), lactosylceramide (LacCer)

Table 1
Estimated average composition of the milk fat globule membrane (Walstra et al., 2006)

Component	mg 100 g ⁻¹ fat globules	g 100 g ⁻¹ MFGM dry matter
Protein	1800	70
Phospholipids	650	25
Cerebrosides	80	3
Cholesterol	40	2
Monoglycerides	+ ^a	?
Water	+	–
Carotenoids + Vit. A	0.04	0.0
Fe	0.3	0.0
Cu	0.01	0.0
Total	>2570	100

^a +; present, but quantity unknown.

and gangliosides (Gang) are present in trace amounts (Danthine, Blecker, Paquot, Innocente, & Deroanne, 2000; Deeth, 1997).

The short and medium chain length FAs (C4–C14), typically for milk fat, are virtually absent in the phospholipid fraction of milk. In particular, PE is highly unsaturated, followed by PI and PS. PC is rather saturated compared with the other glycerophospholipids. The FA pattern of SM is very uncommon. Although long-chain FAs occur, nearly all of them are saturated ($\approx 97\%$). In addition, the occurrence of C23:0 ($> 17\%$) is remarkable (Bitman & Wood, 1990; Jensen, 2002). This uncommon high degree of saturation gives SM the ability to form with, cholesterol, the so-called ‘lipid rafts’, rigid domains in cellular membranes, with a higher melting point and a higher degree of packing (liquid ordered state) in comparison with the glycerophospholipids and their packing (liquid-disordered state). These rafts are implicated in cellular processes like signal transduction, cell sort, endocytosis and cholesterol trafficking (Brown & London, 2000; van Meer & Lisman, 2002). Similarly, the dietary intake of SM lowers cholesterol absorption in the intestines by lowering the membrane fluidity of the liposomes.

2.2. Proteins of the milk fat globule membrane

Depending on the source, 25–70% of the MFGM consists of proteins (Danthine et al., 2000; Deeth, 1997; Fong et al., 2007; Walstra, Wouters, & Geurts, 2006). These membrane proteins are only present in very small amounts in other milk phases, and account for 1–2% of total milk protein (Ricci, 2004). The reported composition is highly dependent on the isolation and analysis procedures used, since not all proteins are equally connected with the MFGM. Some are integral proteins, some are peripheral proteins, others are believed to be only loosely attached. Upon separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the MFGM material is resolved into 7–8 major bands. However, several minor species are as yet unidentified. Despite the recent efforts undertaken to elucidate their structure and amino acid sequence, little is known about their specific concentration and function.

Major MFGM proteins such as mucin 1 (MUC1) (Pallesen et al., 2001), xanthine dehydrogenase/oxidase (XDH/XO) (Berglund, Rasmussen, Andersen, Rasmussen, & Petersen, 1996; Spitsberg, Matitashvili, & Gorewit, 1995), CD36 (Berglund, Petersen, & Rasmussen, 1996; Greenwalt, 1993; Rasmussen, Berglund, Rasmussen, & Petersen, 1998), PAS 6/7 (Bash, Harold M. Farrell, & Greenberg, 1976; Hvarregaard, Andersen, Berglund, Rasmussen, & Petersen, 1996; Kim, Kanno, & Mizokami, 1992), adipophilin (ADPH) and butyrophilin (BTN) (Nielsen et al., 1999) have been purified and characterized. Furthermore, it is assumed by several authors that parts of the proteose peptone fraction like proteose peptone 3 (PP3), originate from the MFGM (Campagna, Cosette, Molle, & Gaillard, 2001;

Girardet et al., 1995; Nejjar, Pâquet, Aubert, & Linden, 1990; Sorensen & Petersen, 1993a, 1993b; Sorensen, Rasmussen, Moller, & Petersen, 1997).

Bovine MFGM preparations contain many more proteins and enzymes than those discussed above. These components include enzymes, immunoglobulins, proteins derived from the cytoplasm of the secretory-epithelial cells, proteins from milk leukocytes and skim milk constituents. The majority are undoubtedly peripheral proteins loosely adsorbed to the MFGM. However, they could exert important biological functions. A more exhaustive listing of possible protein components of bovine MFGM is given in McPherson and Kitchen (1983), Keenan, Mather, and Dylewski (1999), Mather (2000), Reinhardt and Lippolis (2006) and Fong et al. (2007).

2.3. Structure of the milk fat globule membrane

As viewed from the lipid core outwards, the MFGM consists of an inner monolayer of polar lipids and proteins surrounding the intracellular fat droplet, an electron dense proteinaceous coat located on the inner face of the bilayer membrane and finally a true bilayer membrane of polar lipids and proteins (Fig. 1). Cytoplasmic material can be entrained between the inner coat and the outer double membrane layer resulting in ‘cytoplasmic crescents’ (Danthine et al., 2000; Evers, 2004; Michalski, Michel, Sainmont, & Briard, 2002; Rasmussen, Berglund, Pallesen, & Petersen, 2002).

As the greater part of the membrane of the MFGM is derived from the apical plasma membrane of the secretory cell, the most widely accepted model for this type of membrane would be the fluid mosaic model. This suggests that the phospholipid bilayer serves as a backbone of the membrane, which exists in a fluid state. Peripheral membrane proteins are partially embedded or loosely attached to the bilayer. Trans-membrane proteins extend through the lipid bilayer. Carbohydrate moieties from glycolipids and glycoproteins are orientated outwards. Cholesterol is present in the polar lipid bilayer.

The proteins of MFGM are arranged asymmetrically. Adipophilin (ADPH), which has a very high affinity for triglycerides, is located in the inner polar lipid monolayer. XDH/XO is exposed on the inner face of the monolayer, and is closely connected with BTN, which is a transmembrane protein of the outer layer, and with ADPH. As such, these proteins act as anchorpoints, thereby forming a supramolecular complex that interconnects the inner and outer membrane (Mather & Keenan, 1998). Together with ADPH and XOR/XO, BTN plays an important role in the assembly and the stabilization of the MFGM (Mather, 2000). Other proteins, like PAS 6/7, are located at the outer part of the membrane. Some MFGM proteins, like MUC1, are heavily glycosylated. Carbohydrate moieties appear to be uniformly distributed over the external membrane surface (Danthine et al., 2000; Evers, 2004; Harrison, 2002; Mather, 2000).

The lipids are, like the proteins, asymmetrically arranged. The choline-containing phospholipids, PC and

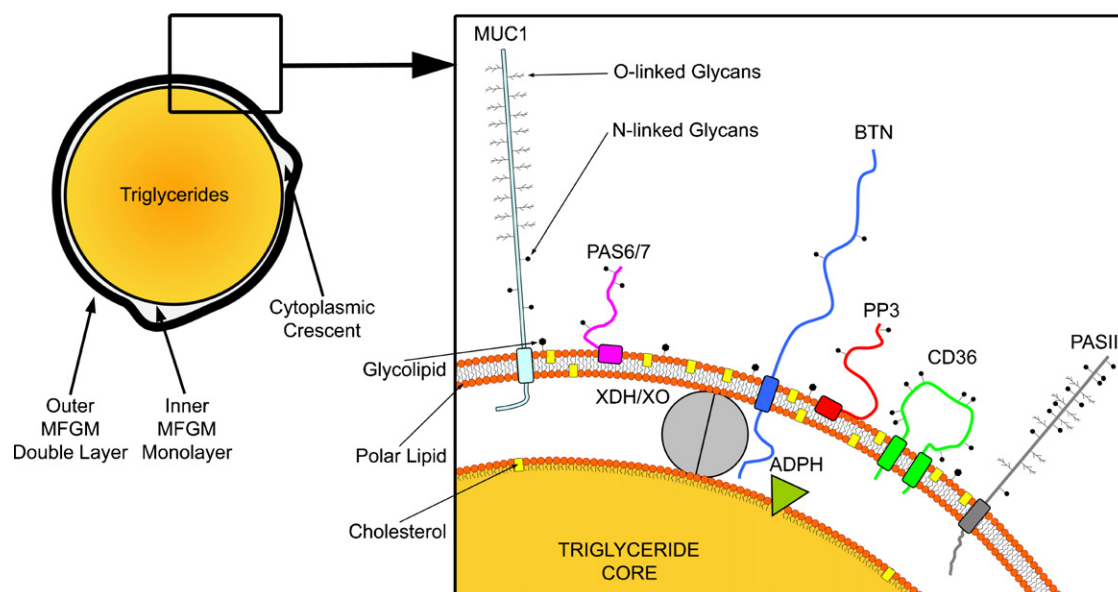


Fig. 1. Structure of the fat globule with detailed arrangement of the main MFGM proteins. The drawing is highly schematic and sizes are not proportional. A double layer of polar lipids is placed on an inner monolayer of polar lipids. Membrane-specific proteins are distributed along the membrane. ADPH is located in the inner polar lipid layer, XDH/XO is located in between both layers. MUC1, BTN, CD36 and PASIII are located in the outer layer. PAS6/7 and PP3 are only loosely attached at the outside of the MFGM. The choline-containing phospholipids, PC and SM, and the glycolipids, cerebrosides and gangliosides, are largely located on the outside of the membrane, while PE, PS and PI are mainly concentrated on the inner surface of the membrane (Danthine et al., 2000; Deeth, 1997; Evers, 2004; Harrison, 2002; Mather, 2000; Mather & Keenan, 1998; Rasmussen et al., 2002).

SM, and the glycolipids, cerebrosides and Gang are largely located on the outside of the membrane, while PE, PS and PI are mainly concentrated on the inner surface of the membrane (Deeth, 1997).

After milk secretion and milking, compositional and structural changes in the MFGM occur, and membrane material is shed into the skimmed milk phase. Factors like age of the cow, bacteriological quality of the milk, stage of lactation and season have an influence on these changes, but are rather insignificant compared with the effects of processing on the MFGM composition. Cold storage leads to specific migration of PL and proteins towards the serum phase, pumping and air inclusion induces serious MFGM damage and losses, a heat treatment causes denaturation of MFGM proteins and a further complexation of BTN and XO (Ye, Singh, Taylor, & Anema, 2002), whilst homogenization leads to a newly formed membrane, mainly consisting of caseins and whey proteins. Factors inducing MFGM changes are discussed in-depth in the review of Evers (2004).

Apart from MFGM fragments, secretory cell fragments (microvilli, cytoplasm, membrane particles) can be secreted into the lumen. This material, which sediments upon centrifugation, comprises only 4% of total milk lipids, is rich in polar lipids and has a similar composition to the MFGM material (Deeth, 1997; Keenan et al., 1999).

3. Content of MFGM in dairy products

Polar lipids in milk, which comprise phospholipids and sphingolipids, are mainly (60–70%) situated in the MFGM. When milk is processed, this biological membrane

Table 2
Polar lipid content of various dairy products during processing (Rombaut & Dewettinck, 2006; Rombaut, Dewettinck, & Van Camp, 2007)

Product	On product (g 100g ⁻¹)	On dry matter (g 100g ⁻¹)
Raw milk	0.03–0.04	0.23–0.32
Skim milk	0.02	0.28
Cream	0.19	0.40
Pasteurized cream	0.14	0.31
Butter	0.14–0.23	0.17–0.26
Buttermilk	0.16	2.03
Butterserum	1.25	11.54
Fresh acid buttermilk quarg	0.31	1.86
Acid buttermilk whey	0.10	1.84
Cheddar cheese	0.15	0.25
Cheddar cheese whey	0.02	0.26

is disrupted and as such is no longer associated with the fat globules. Table 2 shows that during processing polar lipids are preferentially distributed to aqueous phases such as buttermilk and butter serum. In the MFGM, polar lipids and proteins are closely associated so they will probably co-migrate during dairy processing. As seen in Table 2, Fig. 2 and other figures in various references (Corredig, Roesch, & Dalgleish, 2003; Roesch, Rincon, & Corredig, 2004; Rombaut, Dejonckheere, & Dewettinck, 2006, 2007), dairy products rich in polar lipids are also enriched in MFGM proteins. As such, buttermilk and butter serum are suitable as sources for the isolation of MFGM material; the latter is the richest source of MFGM material on dry

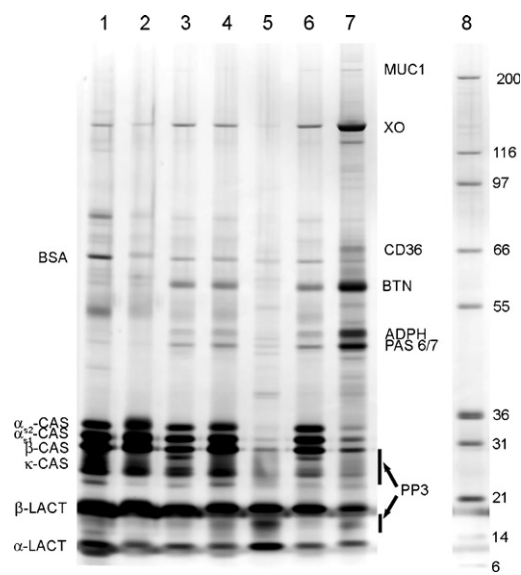


Fig. 2. SDS-PAGE of different dairy products. Names of MFGM proteins are given at the right, the other proteins are named at the left. Separation is performed on gradient (4–12%) polyacrylamide gels with the Xcell Surelock system. Visualization was done with SilverXpress silver stain. On each lane, 250 ng of protein was loaded: (1) raw milk; (2) skimmed milk; (3) acid buttermilk; (4) butter serum, aqueous fraction obtained from churning of cream; (5) acid buttermilk whey, soluble fraction obtained from acidification of sweet-cream buttermilk; (6) acid buttermilk quarg, coagulated fraction obtained from the acidification of the sweet-cream buttermilk; (7) MFGM-isolate; and (8) mark 12 molecular weight standard (Rombaut, 2006).

basis. The technology for the isolation of MFGM fragments from these sources is discussed in the next section.

4. Isolation and purification

4.1. Isolation of MFGM from milk

A typical isolation method can be divided into four steps (Mather, 2000; Singh, 2006): fat globule separation, cream washing, release of MFGM from the globules and collection of the MFGM material. First, cream can be separated from milk by a laboratory centrifuge or in a large scale, bench-top cream separator. Next, the separated cream is washed two (Ye et al., 2002), three (Fong et al., 2007; Kanno & Kim, 1990) or more number of times (Mangino & Brunner, 1975) in 3–15 fold volumes of distilled or deionized water (Kanno & Kim, 1990; Newman & Harrison, 1973), sucrose-saline solution with (Erickson, Dunkley, & Smith, 1964; Mather, Weber, & Keenan, 1977; Nejjar, Pâquet, Godbillion, & Deaut, 1986; Snow, Colton, & Carraway, 1977) or without (Dowben, Brunner, & Philpott, 1967) pH buffering, pH buffered sucrose solution (Khodaparast-Sharifi & Snow, 1989), isotonic phosphate buffer solution (Nielsen & Bjerrum, 1977), phosphate-saline buffer (Innocente, Blecker, Deroanne, & Paquot, 1997), or simulated milk ultrafiltrate (Ye, Singh, Taylor, & Anema, 2004). In some cases, detergents (Diaz-Maurino

& Nieto, 1976; Mather et al., 1977) or dissociating agents (Ye et al., 2002) are added to facilitate the washing. Recently, skim milk ultrafiltrate has also been used as washing solution (Morin, Britten, Jiménez-Flores, & Pouliot, 2007). The number of washes can be reduced if milk is suspended in washing solution prior to separation of the fat globules (Politis, Barbano, & Gorewit, 1992). After washing the cream, the MFGM is released from the triglyceride fat core into the aqueous phase by churning (Diaz-Maurino & Nieto, 1976; Dowben et al., 1967; Fong et al., 2007; Mather et al., 1977; Nielsen & Bjerrum, 1977), agitation (Harrison, Higginbotham, & Newman, 1975) at reduced temperatures or applying cycles of freezing–thawing (Dowben et al., 1967; Khodaparast-Sharifi & Snow, 1989; Snow et al., 1977; Snow, Doss, & Carraway, 1980). Alternatively, MFGM can directly be released from washed cream by the use of polar aprotic solvents (Bingham & Malin, 1992; Dapper, Valivullah, & Keenan, 1987), bile salts (Erickson et al., 1964; Snow et al., 1980), or nonionic detergents (Patton, 1982). Direct extraction normally results in a lower yield, and a certain difference in composition depending on the concentration of the applied chemicals, the time and temperatures of extraction (Patton, 1982). Finally, the released MFGM material from buttermilk and/or butter serum is collected by ultracentrifugation (Anderson & Brooker, 1974; Snow et al., 1977), freeze-drying (Rombaut et al., 2006) or microfiltration (Morin, Britten et al., 2007). Two fractions, the soluble supernatant and the MFGM pellet, are obtained by ultracentrifugation. Precipitation of MFGM fragments at low pH (Fong et al., 2007; Kanno & Kim, 1990) or by ‘salting out’ with ammonium sulfate (Kanno & Kim, 1990; Nielsen & Bjerrum, 1977) may be applied to MFGM suspensions, after which the MFGM material is separated by centrifugation. All the above reviewed methods are laboratory applications for the isolation of MFGM material from untreated milk. They are summarized in Fig. 3.

The percentage of membrane proteins recovered in the supernatant ranges from 20% to 25% of total MFGM protein (Diaz-Maurino & Nieto, 1976; Dowben et al., 1967; Mather, Tamplin, & Irving, 1980; Tomich, Mather, & Keenan, 1976). Delipidation of the prepared MFGM sample with a solvent mixture of chloroform and methanol (2:1, v/v) (Hvarregaard et al., 1996; Wooding & Kemp, 1975) favours the separation of the protein components on SDS-PAGE. However, it should be noted that not all lipids are removed by this solvent mixture (Wooding & Kemp, 1975).

Prior to measuring the activity of MFGM enzymes, extra washing steps of the pellet are often applied to remove most of the contaminating whey protein (Diaz-Maurino & Nieto, 1976; Khodaparast-Sharifi & Snow, 1989; Nielsen & Bjerrum, 1977; Snow et al., 1977, 1980). Solutes used in the washing solution can affect the membrane enzyme activity (Diaz-Maurino & Nieto, 1976; McPherson, Dash, & Kitchen, 1984; Snow et al., 1980) and

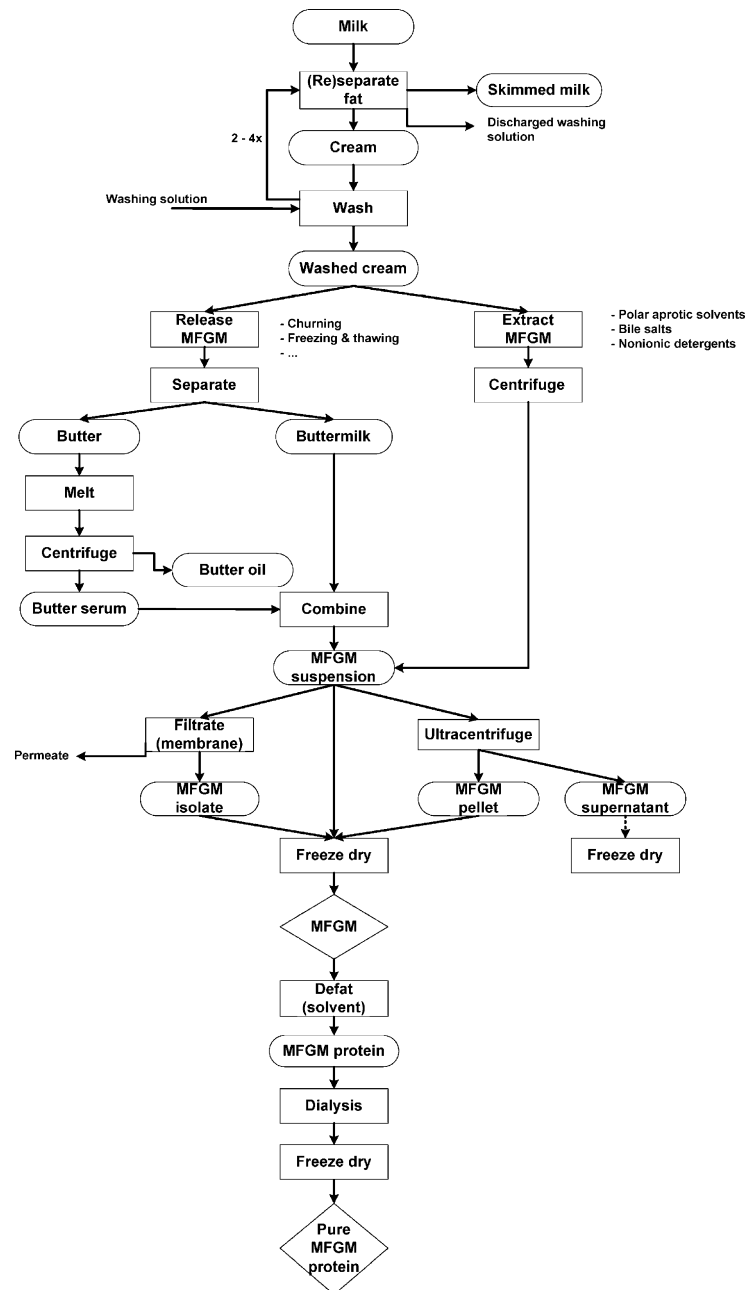


Fig. 3. Summary of isolation methods of MFGM.

dialysis does not always remove the solutes completely (Bash et al., 1976). The selectivity and amount of losses during washing and desorption into buttermilk depends on the affinity for the suspension solution used (Walstra, 1985). Lipid content, protein composition, and enzyme activities of MFGM pellets are different with different collecting methods used (Kanno & Kim, 1990). Using membrane filtration to collect MFGM fragments from buttermilk may result in a loss of small MFGM fragments in the permeate (Morin, Britten et al., 2007). Therefore, collecting the membrane material from both buttermilk and butter serum without separating the supernatant and the pellet—e.g., by lyophilization of the combined

solution (Rombaut et al., 2006)—is necessary to have a representative evaluation of MFGM characteristics. Three washing steps are sufficient to remove virtually all milk serum components (Nejjar et al., 1986; Ye et al., 2004). However, three washes already cause a loss of MFGM components (Anderson & Brooker, 1974; Nejjar et al., 1986). Membrane proteins are highly vulnerable to losses during isolation, especially the loosely bound proteins. Only 4% of phospholipids compared with 16% of the MFGM proteins were lost during the washing process (Anderson & Brooker, 1974). Washing also causes losses of tocopherol, an antioxidant in the MFGM (Erickson et al., 1964).

Bovine serum albumin (BSA) was still detected in isolated MFGM in spite of successive washings (Nejjar et al., 1986). On SDS-PAGE gels, casein, β -lactoglobulin, BSA and lactoferrin were still visible in MFGM material after three washes in three volumes of deionized water (Fong et al., 2007). It was suggested by Morin, Jiménez-Flores, and Pouliot (2007) that skim milk proteins may interact strongly with MFGM even before milk is collected. However, examination by transmission electron microscopy (TEM) showed that casein micelles were uniformly distributed and did not increase in concentration at the MFGM (Lee & Morr, 1992).

A lower yield of the membrane proteins was seen at a washing temperature of 45°C compared with 20°C (Ye et al., 2002). Washing can be done at lower temperatures in laboratory centrifuges than in cream separators. The latter method, through repeated washing and separation of the cream, tends to produce small butter granules at temperatures lower than 40°C (Fong et al., 2007). Coalescence of fat globules may cause losses of membrane material proportional to the reduction of the specific surface area of the fat globules (Walstra, 1985). As suggested by Fong et al. (2007), damage of the globule surface may produce exposed MFGM fragments to which caseins bind, as a result of which the latter cannot entirely be washed out. Proteolytic and microbial inhibitors should be used in the isolation process since plasminogen and its active form, plasmin, are present which can slowly hydrolyze the prepared MFGM proteins (Hofmann, Keenan, & Eigel, 1979; Politis et al., 1992). Bacterial proteinases might be responsible in some cases (McPherson et al., 1984). Other factors, such as breed (Graves, Beaulieu, & Drackley, 2007), feeding (Couvreur, Hurtaud, Marnet, Faverdin, & Peyraud, 2007), lactation stage (Graves et al., 2007), milking frequency (Wiking, Nielsen, Båvius, Edvardsson, & Svennersten-Sjaunja, 2006), mastitis, cooling, freezing, mechanical stress, high-pressure treatment, heat treatment, homogenization and spray-drying (Morin, Jiménez-Flores et al., 2007; Ye, Anema, & Singh, 2007), influence the yield and composition of the isolated MFGM material. The effects of these factors have been reviewed by several authors (Evers, 2004; Huppertz, Fox, de Kruif, & Kelly, 2006; McPherson & Kitchen, 1983; Singh, 2006).

MFGM material from milk can be obtained in a shorter time by the method of Patton and Huston (1986), where milk is added with sucrose (5 g 100 mL⁻¹) and fat globules are, by a light centrifugation, passed through an above-situated phosphate-buffered salt solution. The method gave quite comparable results in phospholipid and cholesterol content compared with the washing method and was considered to give less damage to MFGM (Patton & Huston, 1986). Making use of a density gradient, MFGM fragments can be collected as a layer separated from other components after centrifugation of unwashed cream, buttermilk or butter serum against a concentrated sucrose solution. This unwashed method gave a lower yield of MFGM material but similar protein composition

compared to the washing method. The method was found suitable for extraction of MFGM material from milk products, e.g., pasteurized cream and milk (McPherson et al., 1984).

4.2. Isolation of MFGM from industrial sources

The modern food processing industry is focussed on utilizing natural components that improve the nutritional value and create specific functionalities for food products (Innocente et al., 1997). Rich sources of MFGM material, e.g., buttermilk and butter serum, are still considered as low value by-products originating from dairy processing. Many attempts have been performed to isolate MFGM from buttermilk. By using micro- and ultrafiltration, the transmission of components through the membrane was found to depend on filtration conditions such as temperature, pH, pore size and type of membrane material and the type of buttermilk (Morin, Jiménez-Flores, & Pouliot, 2004; Morin, Pouliot, & Jiménez-Flores, 2006; Rombaut, Dejonckheere et al., 2007). The isolation is based on the selective removal of casein, whey proteins, lactose and minerals from the concentrate. The similarity in size of casein micelles and MFGM fragments was reported to be the major obstacle during isolation (Sachdeva & Buchheim, 1997). The latter authors used renneting and acid coagulation (citric acid and fermentation by lactic acid bacteria) to remove caseins prior to concentration of MFGM by a combination of microfiltration and ultrafiltration. Of the total phospholipids in buttermilk, 70–77% was recovered depending on methods applied. Renneting coagulation was found to be the most efficient (Sachdeva & Buchheim, 1997). Using another approach, Corredig et al. (2003) and Roesch et al. (2004) used citrate to dissociate casein micelles followed by microfiltration to collect MFGM material in the retentate. Increasing the number of diafiltration steps with deionized water from 2 to 6 reduced the casein contamination in the retentate from 30% to 6% of total proteins (Corredig et al., 2003). However, this application also causes loss of MFGM material (Rombaut et al., 2006). Here citrate addition was applied to butter serum. These authors reported that 44% of polar lipids was lost during filtration due to blocking and fouling of the filter membrane with MFGM particles. Addition of sodium citrate agent causes dispersion of not only casein micelles but also MFGM fragments (Rombaut et al., 2006). Whey buttermilk (Morin et al., 2006), the aqueous fraction obtained by churning of whey cream which is separated from cheese whey, and acid buttermilk whey (Rombaut, Dejonckheere et al., 2007), the aqueous fraction obtained by acidification of sweet-cream buttermilk, were considered favorable for MFGM isolation by filtration due to absence of casein micelles. As expected, transmission of MFGM proteins through the membrane was lower when using whey buttermilk compared with regular buttermilk (Morin et al., 2006). At optimized conditions, 98% of the polar lipids from the acid buttermilk whey were recovered

in the retentate. Thermocalcic aggregation of the whey before filtering aids to clarify the whey, but also results in low permeate fluxes and high retention of ash and whey proteins (Rombaut, Dejonckheere et al., 2007).

Another strategy for removal of skim milk proteins has been explored, by washing cream with skim milk ultrafiltrate before churning has been studied. Compared with the filtering of buttermilk from unwashed cream, this method caused losses of MFGM material, but improved the permeation flux and gave an isolate with higher MFGM content and lower contamination of skim milk proteins (Morin, Britten et al., 2007). This method, however, may be difficult to apply in industry.

Selective removal of neutral lipids by supercritical fluid extraction (Astaire, Ward, German, & Jiménez-Flores, 2003) or precipitation of polar lipids with acetone after solvent extraction (Baumy, Gestin, Fauquant, Boyaval, & Maubois, 1990) are two of the vast number of methods used to further purify dairy phospholipids from a MFGM isolate. More purified phospholipids can be separated into two application categories, namely for oil-in-water or

water-in-oil emulsions based on their hydrophilic–lipophilic balance (HLB) (Boyd, Drye, & Hansen, 1999).

5. Nutritional aspects of MFGM components

A general overview of the nutritional aspects of the lipid and protein fraction of the MFGM material is given in Tables 3 and 4. Several health-promoting effects have been attributed to the MFGM material, but some researchers also reported a link between the MFGM fraction or individual components and disease. However, the reader should bear in mind that further research is required, as in most cases the evidence for the presumed beneficial or detrimental effects of MFGM components is quite weak or even absent.

5.1. Lipid fraction of the MFGM

5.1.1. Bioactivity of sphingolipids and metabolites

The polar lipid fraction of the MFGM consists of glycerophospholipids and sphingolipids, which may

Table 3
Nutritional aspects of polar lipids of the MFGM and other MFGM components

Component	Nutritional aspects	References
<i>Polar lipids</i>		
Sphingolipids and metabolites	Reduction of the number of aberrant crypt foci and adenocarcinomas	Dillehay et al. (1994)
	Shift in tumor type (malignant → benign)	Schmelz et al. (1996, 2000), Symolon et al. (2004), Spitsberg (2005)
	Anticholesterolemic	Noh and Koo (2003, 2004), Nyberg et al. (2000), Eckhardt et al. (2002)
	Protection of the liver from fat- and cholesterol-induced steatosis	Duivenvoorden et al., 2006
	Suppression of gastrointestinal pathogens	Sprong et al. (2002), Vesper et al. (1999), Pfeuffer and Schrezenmeir (2001)
	Neonatal gut maturation	Oshida et al. (2003)
	Myelination of the developing central nervous system	Oshida et al. (2003)
Sphingosine 1-phosphate	Endogenous modulators of vascular function	Michel et al. (2007)
	Associated with age-related diseases and the development of Alzheimer	Parodi (2001), Spitsberg (2005)
	Mitogenic	Zhang et al. (1990), Zhang et al. (1991)
Phosphatidylserine (PS)	Restore normal memory on a variety of tasks	McDaniel et al. (2003)
	Positive effects on alzheimer patients	Crook et al. (1992), Heiss et al. (1994), Pepeu et al. (1996), Gindin et al. (1998), Hashioka et al. (2004)
Phosphatidylcholine (PC)	Improve exercise capacity of exercising humans	Kingsley (2006)
	Support liver recovery from toxic chemical attack or viral damage	Kidd (2002)
	Protects the human GI mucosa against toxic attack	Anand et al. (1999)
Lysophosphatidylcholine (lysoPC)	Reduction of necrotising enterocolitis	Carlson et al. (1998)
	Bacteriostatic and bactericidal capacity	Van Rensburg et al. (1992)
	Strong gastroprotective role in the duodenal mucosa	Kivinen, Salminen et al. (1992), Kivinen, Tarpila et al. (1992); Kivinen et al. (1995)
<i>Other components</i>		
Vitamin E and carotenoids	Antioxidants	Spitsberg, 2005

Table 4

The major proteins and other components of the milk fat globule membrane with their abbreviation, molecular mass (M), iso-electric point (IEP), function and health aspects

Component	Abbreviation	M (kDa)	IEP	Function	Health aspects	References
Fatty acid binding protein	FABP	13	5–5.5	Transport of fatty acids Regulation of lipid metabolism Increase of lipid droplets in cytoplasm	Cell growth inhibitor Anticancer factor (FABP as selenium carrier) Similar to P2 myelin protein involved in EAN	Spitsberg et al. (1995), Peterson et al. (1998), Kromminga et al. (1990), Bansal and Medina (1993), Whanger (2004), Spitsberg and Gorewit (2002), Riccio (2004)
Butyrophilin	BTN	66–67	5.32	MFG secretion Belongs to Ig	Suppression of MS Induces or modulates EAE Influences pathogenesis of autistic behavior	Mana et al. (2004), Guggenmos et al. (2004), Johns and Bernard (1999), Stefferl, Brehm et al. (2000), Stefferl, Schubart et al. (2000) Vojdani et al. (2002)
Xanthine oxidase	XDH/XO	146 (300)	7.8	Structural, lipid secretion Role in purine metabolism	Bactericidal agent Redox reaction/anti-inflammatory	Martin et al. (2004), Hancock et al. (2002) Spitsberg (2005), Fong et al. (2007)
Mucin 1	MUC1	160–200	< 4.5	Protection from physical damage Protection from invasive pathogens	Protective effect against rotavirus infection	Kvistgaard et al. (2004)
Breast cancer type 1 susceptibility protein	BRCA1			Cancer suppressor	Inhibition of breast cancer	Spitsberg and Gorewit (1997, 1998)
Breast cancer type 2 susceptibility protein	BRCA2			Cancer suppressor Direct regulator of cytokinesis	Inhibition of breast cancer	Vissak et al. (2002) Daniels et al. (2004)
Lactadherin	PAS VI/VII	47	6–6.6	Member of cadherins Ca-dependent adhesive properties Phospholipid binding	Protection from viral infections in the gut Role in epithelialization, cell polarization, cell movement and rearrangement, neurite outgrowth Synaptic activity in the central nervous system	Kvistgaard et al. (2004) Riccio (2004)
Proteose peptone 3	PP3	18–30/14	6.3	Membrane associated Expressed in lactating mammary gland		Fong et al. (2007)
Adipophilin	ADPH	52	7.5–7.8	Uptake and transport of FA/ TAG		Riccio (2004)
Periodic acid Schiff III	PAS III	95–100	< 4.5	Marker of the secretory and ductal epithelium		Riccio (2004)

Table 4 (continued)

Component	Abbreviation	M (kDa)	IEP	Function	Health aspects	References
Cluster of differentiation	CD36	76–78	<7	Macrophages marker Phagocytosis by neutrophils		Riccio (2004)
<i>Other components</i>						
β -glucuronidase inhibitor					Inhibition of colon cancer	Ito et al. (1993)
<i>Helicobacter pylori</i> inhibitor					Prevention of gastric diseases	Wang et al. (2001)
Phosphoproteins					Organic phosphorus/Ca-phosphate source	Spitsberg and Gorewit (1997)
MFGM antigens					Coronary atherogenic effects	Moss and Freed (2003)

EAE, Experimental autoimmune encephalomyelitis; EAN, Experimental allergic neuritis.

represent the most structurally diverse category of lipids in nature. Sphingolipids are functional ingredients, due to their regulatory properties, in addition to their structural functionality, and their effectiveness at low concentrations (Schmelz, 2000). SM is the major sphingolipid membrane component, which is highly bioactive through its metabolites ceramide, sphingosine and sphingosine 1-phosphate (S1P). The sphingolipid metabolites are lipid secondary messengers involved in trans-membrane signal transduction and regulation, growth, proliferation, differentiation and apoptosis (programmed cell death) of cells (Alessenko, 1999; Colombaioni & Garcia-Gil, 2004; Futerman & Hannun, 2004). Intriguingly, ceramide, sphingosine and S1P have opposite effects upon cellular phenotypes. Ceramide and sphingosine are pro-apoptotic, antimitogenic metabolites that inhibit growth and/or induce cell death (Hannun, 1994; Jajadev et al., 1995; Sweeney, Inokuchi, & Igarashi, 1998), while S1P is a pro-survival and mitogenic derivative, that inhibits apoptosis (Spiegel & Milstien, 2000). Hence, cell fate in response to specific stimuli is determined by the relative balance between ceramide and its metabolite S1P (Kolesnick, 2002; Ogretmen & Hannun, 2004).

SM and cerebroside undergo little cleavage in the stomach, but are hydrolyzed in all subsequent regions of the small intestine and colon of rats and mice (Vesper et al., 1999). The sequential hydrolysis of dietary SM by intestinal alkaline sphingomyelinase and neutral ceramidase results in ceramide and sphingosine by removal of the head groups and the FAs (Duan, Nyberg, & Nilsson, 1995; Nilsson, 1968, 1969). The metabolites are rapidly absorbed by intestinal cells and reincorporated into complex sphingolipids that remain associated primarily with the intestine, or are further degraded to FAs via fatty aldehydes (Schmelz, Crall, Larocque, Dillehay, & Merrill, 1994). However, not all of the ingested sphingolipids are absorbed and a part of the dietary sphingolipids is excreted via the faeces (Nilsson, 1969). Significant, dose-dependent amounts of SM and its

metabolites were found in the intestinal contents, colon and excreted faeces (Nyberg, Nilsson, Lundgren, & Duan, 1997). SM digestion is slow and is affected by luminal factors such as bile salt, cholesterol, and other lipids. SM and its metabolites may influence triglyceride hydrolysis, cholesterol absorption, lipoprotein formation, and mucosal growth in the gut (Nilsson & Duan, 2006).

There is no evidence that consumption of dietary sphingolipids is required for growth under normal conditions, as all cells appear to be capable of de novo sphingolipid synthesis (Merrill, Nixon, & Williams, 1985; Nagiec, Lester, & Dickson, 1996). No nutritional requirements for dietary sphingolipids (Berra, Colombo, Sottocornola, & Giacosa, 2002; Schmelz et al., 1996; Vesper et al., 1999) or deleterious effects have yet been observed in several sphingolipid feeding studies (Dillehay, Webb, Schmelz, & Merrill, 1994; Imaizumi, Tominaga, Sato, & Sugano, 1992; Kobayashi, Shimizugawa, Osakabe, Watanabe, & Okuyama, 1997). Nonetheless, exogenous sphingolipids are required for the growth of mammalian cells with defects in serine palmitoyltransferase (Hanada et al., 1992), the initial enzyme of sphingolipid biosynthesis, which leads to the suggestion that sphingolipids are necessary for normal cell function (Vesper et al., 1999). Furthermore, dietary SM plays an important role in neonatal gut maturation during the suckling period of rats and contributes to the myelination of the developing rat central nervous system (CNS) (Oshida et al., 2003). Michel, Mulders, Jongsma, Alewijnse, and Peters (2007) also suggested that SM metabolites are important endogenous modulators of vascular function, which may contribute to the pathophysiology of some diseases.

5.1.2. Effects of sphingolipids on cancer and bowel-related diseases

Normal intestinal cells undergo rapid turnover, except in cancer in which normal growth arrest and apoptosis is delayed (Duan, 2005). SM might exert an effect on colon

cancer cells, mainly through its metabolites, ceramide and sphingosine, which induce growth arrest, differentiation and/or apoptosis (Merrill, Schmelz, Sullards, & Dillehay, 2001). Sphingolipids were found to inhibit both the early and the late stages of colon carcinogenesis, in tests on mice in which tumorigenesis was caused by an inherited genetic defect or chemically induced by a chemical agent. Sphingolipid supplementation reduced the number of aberrant crypt foci and, with longer feeding, also the number of adenocarcinomas (Dillehay et al., 1994). Moreover, a significant shift in tumor type from the malignant adenocarcinomas to the more benign adenomas was observed (Schmelz et al., 1996; Schmelz, Sullards, Dillehay, & Merrill, 2000; Spitsberg, 2005; Symolon, Schmelz, Dillehay, & Merrill, 2004). Sphingolipids were found to be chemopreventive as well as chemotherapeutic, i.e., tumor reduction was observed when mice were fed SM before and after tumor initiation (Lemonnier et al., 2003). It is important to note that the concentrations of sphingolipids that had a detectable effect (0.025–0.5% of the diet of mice) were close to the estimated human consumption (0.01–0.02% of the diet) in the United States (Vesper et al., 1999). Furthermore, different studies suggest that the enzyme sphingomyelinase, which catalyzes the conversion from SM to ceramide, might have antiproliferative effects on colon cancer cells (Duan et al., 2003), as a decrease in sphingomyelinase expression and activity was observed in human and rodent colon adenomas and carcinomas (Dudeja, Dahiya, & Brasitus, 1986; Hertervig, Nilsson, Nyberg, & Duan, 1997). Similar findings were reported for patients with chronic colitis, who have an increased risk of developing colorectal cancer (Sjoqvist et al., 2002). Dietary sphingolipids may also affect cancers at sites other than the colon, such as breast cancer (Schmelz, Simon, Malayev, & Roberts, 2007), but this is still under investigation.

However, it should be noted that neither human clinical trials nor epidemiologic studies have yet evaluated whether sphingolipids influence human colon cancer (Vesper et al., 1999). Nonetheless, sphingosine and ceramide induced apoptosis in a human adenocarcinoma cell line, HT29 cells (Schmelz et al., 1998). Besides, multiple intestinal neoplasia (Min) mice (Schmelz & Merrill, 2000), which have a genetic defect similar to that found in human familial adenomatous polyposis, showed a reduced tumor number after feeding sphingolipids. These findings suggest that dietary sphingolipids influence human colon cancer risk (Vesper et al., 1999). However, it is important to mention that sphingosine could be mitogenic through its conversion to S1P (Zhang, Buckley, Gibson, & Spiegel, 1990; Zhang et al., 1991).

5.1.3. Sphingolipids and age-related diseases

Sphingolipids are associated with age-related diseases and the development of Alzheimer's disease (Parodi, 2001; Spitsberg, 2005), as sphingolipid signalling may play a role in the progressive loss of cell function during the aging

process. In many tissues, aging results in changes in the SM content (Eisenberg, Stein, & Stein, 1969; Jenkins & Kramer, 1988; Levi, Jameson, & Vandermeer, 1989; Yechiel & Barenholz, 1986). Furthermore, ceramide has been implicated as a mediator of senescence in a cell culture model for aging (Lee & Obeid, 1997; Venable, Lee, Smyth, Bielawska, & Obeid, 1995; Vesper et al., 1999).

5.1.4. Anticholesterolemic effects of sphingolipids

Sphingolipids are also involved in the intestinal uptake of cholesterol. SM was found to dose-dependently lower the intestinal absorption of cholesterol and fats in rats (Eckhardt, Wang, Donovan, & Carey, 2002; Noh & Koo, 2003; Nyberg, Duan, & Nilsson, 2000). The lowest absorption was observed at equimolar ratios, which falls in the observed SM and cholesterol intakes by humans. The inhibition has been explained by a direct inhibiting effect of the highly saturated long chains of fatty acyl groups of milk SM on the rate of luminal lipolysis, micellar solubilization, and transfer of micellar lipids to the enterocyte (Spitsberg, 2005). The interaction between both molecules is favored by saturation of the SM acyl chain, which explains the greater effect observed with milk SM than with SM derived from eggs (Eckhardt et al., 2002; Noh & Koo, 2004). In addition, the absorption of α -tocopherol is also decreased by both milk and egg SM (Noh & Koo, 2003). Duivenvoorden et al. (2006) reported that dietary sphingolipids play an important role in lowering plasma cholesterol and triacylglycerol (TAG) and protecting the liver from fat- and cholesterol-induced steatosis. They suggested that dietary sphingolipids should be considered as compounds that not only treat or ameliorate the lipid component of cardiovascular disease, but also the inflammatory processes involved in atherosclerosis and insulin resistance. They concluded that dietary sphingolipids hold great potential to treat multiple aspects of the metabolic syndrome, such as dyslipidemia, insulin resistance and cardiovascular diseases.

5.1.5. Bactericidal effect of sphingolipids

Dietary sphingolipids could have protective capacities against bacterial toxins and infection by bacteria or viruses. It is plausible that food sphingolipids can compete for and act as cellular binding sites (Bibel, Aly, & Shinefield, 1992; Fantini et al., 1997), since many bacteria, as well as bacterial toxins and viruses, use glycosphingolipids to bind to cells (Karlsson, 1989). As the adherence of the pathogens to the intestinal mucosa is often the first step in infection, the competition leads to an elimination of pathogens from the intestine, which causes a shift in the bacterial population of the colon (Pfeuffer & Schrezenmeier, 2001; Vesper et al., 1999). When infants are given a formula supplemented with Gang, significantly fewer *Escherichia coli* and more bifidobacteria were observed in their faeces than those of the control group (Rueda, Maldonado, Narbona, & Gil, 1998). Sprong, Hulstein, and Van der Meer (2001, 2002) even reported direct in vitro

bactericidal activities of digestion products of sphingolipids. Ceramide was not bactericidal at the tested concentration of 100 mmol L^{-1} , but lysophingomyelin appeared highly bactericidal against *Campylobacter jejuni*, *Listeria monocytogenes* and *Clostridium perfringens*, and showed moderately lowered viable counts of *E. coli* and *Salmonella enteritidis*. Sphingosine decreased viable counts of all pathogens tested. They also observed a decreased colonization of *L. monocytogenes* in rats fed diets based on sweet buttermilk powder compared with rats fed skim milk diets, which leads to the suggestion that bovine milk sphingolipids may also protect against gastrointestinal infections. Nonetheless, Possemiers, Van Camp, Bolca, and Verstraete (2005) revealed that under simulated intestinal conditions, with the presence of food compounds and digestion products, no significant shifts in bacterial concentrations could be observed.

5.1.6. Phospholipids

Many neuronal effects of ageing in animals are attenuated by PS, and it was also shown to restore normal memory on a variety of tasks. Preliminary findings with humans, though, are limited (McDaniel et al., 2003). At elevated doses of 200 mg per day, clinical trials with patients suffering from Alzheimer's disease showed positive effects (Crook, Petrie, Wells, & Massari, 1992; Gindin, Naor, Stovichek, Dushenat, & Konstantin, 1998; Hashioka, Monji, Han, Nakanishi, & Kanba, 2004; Heiss, Kessler, Mielke, Szeliés, & Herholz, 1994; Pepeu, Pepeu, & Amaducci, 1996). PS supplementation on exercising humans showed that PS might alter neuroendocrine function and positively influenced perceived muscular soreness and well-being. Furthermore, oral supplementation with soybean PS has recently been demonstrated to improve exercise capacity during high-intensity cycling (Kingsley, 2006). Generally, the contribution of dairy products will be of limited significance, since PS is only present in small amounts (Rombaut & Dewettinck, 2006).

PC is believed to clinically support liver recovery from toxic chemical attack (pharmaceuticals, alcohol, mushroom poisoning) or acute or chronic viral (hepatitis) damage (Kidd, 2002). PC also protects the human gastrointestinal mucosa against toxic attack (Anand et al., 1999) and reduces life-threatening necrotizing enterocolitis in hospitalized preterm infants (Carlson et al., 1998). As in the case of SM, PC is a source of choline, which is an essential nutrient for humans. It is believed to promote synthesis and transmission of neurotransmitters important to memory, and might also be involved in brain development (Blusztajn, 1998; Szuhaj & Nieuwenhuyzen, 2003). Furthermore, some phospholipids are digested in the gastrointestinal tract to compounds that might possess antimicrobial activity (van Hooijdonk, Kussendrager, & Steijns, 2000). Pancreatic phospholipase A2 hydrolyzes the FA bound at the sn-2 position, yielding a free FA and a lysophosphoglyceride. Lysophosphatidylcholine displays bacteriostatic and bactericidal capacity

(Van Rensburg, Joone, O'Sullivan, & Anderson, 1992). Sprong et al. (2001) found that Gram-positive bacteria were only moderately vulnerable to lysophosphoglycerides, whereas the Gram-negatives were not sensitive. There is also evidence that milk phospholipids exert a strong gastro-protective role in humans, particularly in the duodenal mucosa (Kivinen, Salminen, Homer, & Vapaatalo, 1992; Kivinen, Tarpila, Kiviluoto, Mustonen, & Kivilaakso, 1995; Kivinen, Tarpila, Salminen, & Vapaatalo, 1992).

5.2. Protein fraction of the MFGM

5.2.1. Anticancer effects

Spitsberg and Gorewit (1997) studied the effect of some bovine mammary gland proteins and some MFGM proteins on cancer cell growth. One of the isolated proteins of bovine MFGM, namely fatty acid binding protein (FABP), has been found to inhibit the growth of some breast cancer cell lines in vitro at extremely low concentrations (Kromminga, Grosse, Langen, Lezius, & Spener, 1990; Peterson et al., 1998; Spitsberg & Gorewit, 2002; Spitsberg et al., 1995). Furthermore, they demonstrated the presence of the onco-suppressor BRCA1 protein in bovine and human MFGM (Spitsberg & Gorewit, 1998). Vissak et al. (2002) detected BRCA1 and BRCA2 in extracts obtained from human and bovine MFGM using affinity chromatography. Both the BRCA2 and BRCA1 proteins are involved in DNA repair processes, although BRCA2 has an additional function as one of the direct regulators of cytokinesis (Daniels, Wang, Lee, & Venkitaraman, 2004).

Dietary bovine MFGM material could prevent colon cancer due to the presence of an inhibitor of β -glucuronidase, which is an enzyme involved in the intestinal degradation of glucuronides. The enzyme glucuronyl transferase neutralizes the toxic compounds in liver cells through the formation of glucuronides, which are subsequently excreted. Some bacteria in the intestine have β -glucuronidases, which degrade the glucuronides. As a result, toxic agents which might be carcinogenic are released, and stimulate the formation of colon cancer. This MFGM component is presumably of protein origin, and inhibits the purified *E. coli* β -glucuronidase in vitro (Ito, Kamata, Hayashi, & Ushiyama, 1993; Spitsberg, 2005). Spitsberg stated the hypothesis that after consumption of MFGM fragments, a certain number of inhibitory peptides could be released and subsequently absorbed in the digestive tract. The absorbed peptides would enter the bloodstream, and after reaching the organs or tissues, they could exert their inhibitory action on the cells undergoing carcinogenic transformation (Spitsberg, 2005; Spitsberg et al., 1995). Nonetheless, this hypothesis has not been further elaborated or tested.

5.2.2. Butyrophilin and multiple sclerosis

Multiple sclerosis (MS) is a progressive autoimmune condition that affects the CNS. It is a chronic neurodegenerative disease of the CNS, in which relentless attacks

of autoimmune-mediated inflammation result in demyelination, loss of oligodendrocytes and axonal degeneration. Although the etiology of MS remains unknown, both genetic and environmental factors are believed to be involved. It has been suggested that milk and dairy products may cause MS, or exacerbate symptoms or progression in MS patients (Butcher, 1976, 1986; Lauer, 1997; Malosse & Perron, 1993; Malosse, Perron, Sasco, & Seigneurin, 1992). Moreover, the major MFGM protein, BTN, shows molecular mimicry with the myelin oligodendrocyte glycoprotein (MOG), a minor component of the myelin membrane in the CNS. MOG is a putative auto-antigen in human MS, which might induce experimental autoimmune encephalomyelitis (EAE), a disease that displays clinical characteristics similar to those seen in human MS, in many experimental animals (Johns & Bernard, 1999; Stefferl, Brehm, & Linington, 2000; Stefferl, Schubart et al., 2000). BTN in milk could act as a molecular mimic of MOG resulting in a cross-reactivity (Guggenmos et al., 2004). Recently, it has been reported that BTN can modulate the encephalitogenic T-cell response to MOG in EAE related to human MS. However, Mana et al. (2004) reported that treatment of C57BL/6 mice with BTN, either before or after immunization with MOG, prevented or suppressed the clinical manifestation of EAE. Therefore, the consumption of dairy products enriched with MFGM could modulate the pathogenic response to MOG in a positive direction. In conclusion, BTN can both trigger the development of EAE, or suppress the disease (Berer, Schubart, Williams, & Linington, 2005; Stefferl, Brehm et al., 2000).

5.2.3. Butyrophilin and autism

Autism is a chronic neurodevelopmental disorder characterized by social and language impairments and stereotyped, repetitive patterns of behaviour (Bailey, Phillips, & Rutter, 1996; Kolevzon, Gross, & Reichenberg, 2007). As in the case of MS, its etiology is still unknown and it may have a variety of causes including genetic, environmental, immunological and neurological factors (Riccio, 2004). Structural abnormalities have been identified in areas of the autistic brain, with a pattern suggesting that a neurodevelopmental abnormality may have occurred (Purcell, Jeon, Zimmerman, & Peysner, 2001; Rodier, Ingram, Tisdale, Nelson, & Romano, 1996). Immunological research has suggested that the immune system plays an important role in the development of autism (Ashwood & Van de Water, 2004; Gupta, Aggarwal, & Heads, 1996; Korvatska, Van de Water, Anders, & Gershwin, 2002; Krause, He, Gershwin, & Shoenfeld, 2002; Pardo, Vargas, & Zimmerman, 2006; Singh, Warren, Odell, Warren, & Cole, 1993; Stigler, 2006). Moreover, Vojdani et al. (2002) detected antibodies against nine different neuronspecific antigens in the sera of children with autism. The antibodies could bind with different encephalitogenic molecules, which have sequence homologies with the milk protein BTN. These results suggest a role for antibodies against

brain cross-reactive food antigens and infectious agents in the pathogenesis of autistic behaviour.

5.2.4. Direct antibacterial effects of MFGM proteins

The role of XDH/XO as an antimicrobial agent in the gastrointestinal tract has been reviewed recently (Harrison, 2006; Martin, Hancock, Salisbury, & Harrison, 2004). XDH/XO is expressed in different cells of the gastrointestinal lining, and its antimicrobial function is related to production of reactive oxygen species, superoxide and hydrogen peroxide in the gut. It may also catalyze the reduction of inorganic nitrite to nitric oxide, and in the presence of oxygen to peroxynitrite, which both show bactericidal properties. Pathogenic bacteria interact with epithelial membranes of the digestive tract, but can also bind to similar receptors on the MFGM. The latter can act as a decoy to avoid that bacteria interact with their primary target site. At the same time, these bacteria are subjected to the antimicrobial effects of XDH/XO, since XDH/XO is a major component of the MFGM. Chromatographically purified XDH/XO was shown to inhibit the growth of *Staphylococcus aureus*, *E. coli* and *Sal. enteritidis*, through the effect of hydrogen peroxide formation or the stimulation of the lactoperoxidase system in milk (Harrison, 2004, 2006; Martin et al., 2004).

Lactophorin, a 23-residues cationic peptide derived from bovine milk component-3 of proteose peptone (PP3), and presumably part of the MFGM, displays growth-inhibitory activity against some Gram-positive (*Streptococcus thermophilus*) and Gram-negative (*Salmonella*) bacteria, but no activity was observed against *E. coli*. In the peptide concentration range tested (lower than 200 μM), no hemolytic activity could be noticed. The interaction of lactophorin with natural lipid membranes possibly causes the inhibitory effect, through its pore-forming ability. However, no relationship between pore-forming and antibacterial peptide concentrations was found (Campagna, Mathot, Fleury, Girardet, & Gaillard, 2004).

5.2.5. Antiadhesive effects of the MFGM

Some forms of stomach diseases, such as chronic type B gastritis, peptic ulcer disease and stomach cancer, can etiologically be attributed to the colonization of stomach mucosa with *Helicobacter pylori* (Atherton, 2006; Cover & Blaser, 1992; Fox & Wang, 2007), which can cause in vitro hemagglutination. Mucins separated from human gastric juices inhibit sialic acid-specific hemagglutination of *H. pylori*, an activity that is significantly reduced after removal of sialic acids from the mucins. Delipidated bovine MFGM material shows inhibitory potencies similar to that of these gastric mucins, while low molecular mass components from milk like glyco-macropptide (GMP) and sialyl (α -2,3)-lactose show much less inhibitory activity. These results suggest that the high molecular mass mucin-like components from bovine MFGM are most important for the inhibitory potency of the MFGM (Hirno et al., 1998). Further proof that the lipid part of the

MFGM is less important than the glycoconjugate fraction for activity against *H. pylori* was obtained in the study of Wang, Hirno, Willen, and Wadstrom (2001). Bovine MFGM and defatted MFGM fractions both inhibited hemagglutination of *H. pylori* at similar concentrations, and reduced its binding to HeLa S3 cell monolayers. Moreover, both non-defatted MFGM preparations showed healing rates of 20% in a *H. pylori*-infected BALB/cA mice model at a dose of 400 mg kg⁻¹ body weight day⁻¹. Gastric colonization by *H. pylori* was significantly decreased in all mice treated with bovine milk glycoproteins (Wang et al., 2001).

Human lactadherin inhibits the Wa rotavirus infection, currently the major cause of severe dehydration diarrhoea in infant mammals, of Caco-2 cells in a dose-dependent manner. Contrary to human lactadherin, bovine lactadherin failed to induce an antiviral effect with the Wa virus, which might have been related to differences in protein structure and attached oligosaccharides. In the same study, it was demonstrated that the neuraminidase-sensitive RRV strain was effectively inhibited by MUC1 derived from bovine MFGM, while no effect was seen on the neuraminidase-resistant Wa infection process. Similar findings were also observed with human PAS 6/7, but not with the bovine variant, which is slightly different in its glycosylated end structure (Kvistgaard et al., 2004). MUC 1 may further play an immunoprotective role by binding to and sequestering pathogenic micro-organisms (*E. coli*), and as such preventing them to colonize the intestinal tract (Peterson et al., 1998).

5.2.6. MFGM proteins and coronary heart disease

Based on epidemiological analysis, Moss and Freed (2003) reported the presence of antibodies against the MFGM proteins. CHD death is also associated with circulating antibodies against MFGM proteins, raising the possibility that MFGM antigens have both biochemical and immunological coronary atherogenic effects (Riccio, 2004).

6. Technological aspects of MFGM components

6.1. Functionalities and applications of MFGM

Because of their amphiphilic nature and original function in stabilizing the fat globules in whole milk, MFGM fragments are considered to be efficient, natural emulsifiers (Corredig & Dalgleish, 1997, 1998c; Kanno, Shimomura, & Takano, 1991). Early work was done by Kanno and co-workers, who investigated the emulsifying properties (foam and emulsion stability, emulsion capability and whippability) of MFGM isolates by reconstituting the milk fat globules (MFGs). The amounts of MFGM material (20–80 mg MFGM material g⁻¹ fat) significantly affected the properties of the reconstituted emulsions. The physicochemical properties of milk fat emulsions stabilized with bovine MFGM isolates were also studied. A more or less

linear decrease in average globule diameters with increasing membrane concentration was observed (Kanno, 1989; Kanno et al., 1991). The stability of an emulsion against aggregation and coalescence of pure milk fat (25%) and MFGM material (2%) was found to be similar to that of natural milk cream. However, commercial buttermilk was found to have an emulsifying capacity and stability inferior to that of non-fat dried milk, which does contain very little MFGM (Wong & Kitts, 2003). Similar findings were reported by Corredig and Dalgleish (1997), where even more concentrated MFGM isolates, prepared by adding citrate followed by high-speed centrifugation to collect the membrane material, were reported to be inferior in emulsifying properties compared with industrial buttermilk, from which they were prepared. A monomodal droplet size distribution was observed for 10% soybean oil emulsions with an MFGM isolate concentration of >8%, whilst this was already observed at a 1% buttermilk isolate concentration. This type of distribution is an important parameter for emulsion stability, as a bimodal or multimodal distribution would point out aggregation and coagulation having occurred (Corredig & Dalgleish, 1997). In later work they discovered that the stability of oil-in-water emulsions with MFGM material depends on the heat treatment of the cream (Corredig & Dalgleish, 1998b) and that MFGM material isolated from raw cream, which did not undergo any heat treatment, had a good emulsifying capacity (Corredig & Dalgleish, 1998b). The emulsions stabilized by this isolate were stable and the absorbed MFGM material at the interface could not be displaced by surfactants or caseins and β -lactoglobulin added to the emulsions. Both the heat treatment and the churning process used in the industrial manufacture of buttermilk significantly affects the behaviour of the membrane, due to extensive denaturation of the membrane proteins and the association of whey proteins (β -lactoglobulin) with the MFGM (Houlihan, Goddard, Nottingham, Kitchen, & Masters, 1992). The pasteurization temperature had no effect on the emulsifying properties of the whole buttermilk, while temperatures higher than 65 °C resulted in loss of emulsifying capacity of the MFGM isolate, as the amount of membrane associated β -lactoglobulin augments with an increasing temperature, especially if it exceeds 65 °C (Corredig & Dalgleish, 1998a). These results suggest that not the MFGM components, but rather the ratio between the casein, whey protein, and MFGM content in buttermilk determine its functional properties (Wong & Kitts, 2003). Sodini, Morin, Olabi, and Jimenez-Flores (2006) reported that commercial sweet, sour, and whey buttermilks have better emulsifying properties and a lower foaming capacity compared to milk and whey. Furthermore, among the three, whey buttermilk was found to have the best emulsifying properties and the lowest foaming capacity, possibly due to a higher ratio of phospholipids to protein in whey buttermilk compared with the other two (Sodini et al., 2006). Roesch et al. (2004) tested 10% soybean oil emulsions with 0.25% commercial MFGM

isolate (60% wt/wt proteins) from buttermilk, obtained by citrate addition followed by microfiltration. These emulsions showed good stability to creaming and resulted in a narrow particle size distribution. Similar emulsions prepared with conventional buttermilk concentrate showed extensive flocculation. Different studies on the functionality of MFGM material may have inconsistent results, as they possibly depend on various factors, such as the dairy sources for MFGM isolation, the intensity and frequency of heat treatment (different processing for milk, cream and butter) as well as the preparation conditions of the emulsions. The denaturation of MFGM proteins, the complexation between MFGM proteins and lipid fractions, and the association of whey proteins to the MFGM, caused by heat treatment, may decrease the solubility of the MFGM isolate (Corredig and Dalgleish, 1998b). Hence, it may be necessary to completely hydrate the MFGM isolate before using it in emulsion preparations. The temperatures during the preparation of the emulsions may also affect the functional properties (Innocente et al., 1997).

Several MFGM isolate applications, which are mainly based on their emulsifying properties, have been reported. The polar lipids are amphiphilic molecules with a hydrophobic tail and a hydrophilic head group, which largely contribute to the emulsifying capacity of the membrane. They are applied as a baking improver to ameliorate fat dispersion and antistaling, as additives to chocolate to reduce viscosity and prevent crystallization, as wetting enhancer to improve the wetting stabilization of instant products, and as stabilizer of margarine to prevent spattering and browning (Göbel & Franzke, 1978; Szuhaj, 1983; Vanhoutte et al., 2004; Vannieuwenhuyzen, 1976, 1981). The application of polar lipids in other industrial fields includes uses as a drug delivery carrier and as fat liquoring for leather fatting, etc. (Guo, Vikbjerg, & Xu, 2005; Kisel et al., 2001; Vannieuwenhuyzen, 1981). In contrast with the polar lipid fraction of plants, dairy products contain a substantial part of sphingolipids, which can be used as raw material for the production of ceramides, applicable in the cosmetic industry (Becart, Chevalier, & Biesse, 1990). Due to its major role in maintaining the water-retaining properties of the epidermis, ceramide is of great commercial potential in cosmetic and pharmaceutical industries, such as in hair and skin care products (Zhang, Hellgren, & Xu, 2006).

Thompson and Singh (2006) produced liposomes, a type of vesicle formed through the self-assembly of amphiphilic molecules, from a phospholipid-rich MFGM fraction using a microfluidization technique. The composition of the MFGM phospholipid material is very different from that of the commonly used soy- or egg-derived phospholipids, which influences the structure and properties of the liposomes (Thompson, Haisman, & Singh, 2006; Thompson, Hindmarsh, Haisman, Rades, & Singh, 2006). As hydrophobic molecules can be incorporated in the lipid bilayers and hydrophilic molecules become entrapped in the aqueous core, it should be possible to exploit the

unique composition of the MFGM phospholipids in the delivery and protection of sensitive compounds. There are many potential applications for liposomes in the food industry, ranging from protecting sensitive ingredients, to increasing the efficacy of food additives and to confining undesirable flavours (New, 1990; Singh, 2006).

6.2. The MFGM and dairy products

In a milk-based gel, interactions between fat and milk proteins occur via the MFGM. Changes in membrane composition, decrease of the MFG size and disruption of the MFGM along with the formation of a new modified composition occur through processing treatments such as heat, homogenization, and applied stress. These changes will alter the interaction, hence also the functional properties of the final products. A native fat globule may act as an inert filler (structure breaker) in milk-based gels (Michalski, Cariou, Michel, & Garnier, 2002), while the newly formed MFGM (mainly casein and some serum proteins) in homogenized fresh milk or recombined milk would cross-link (structure promoter) with the protein network and reinforce it in both rennet and acid gels (Lopez & Dufour, 2001; Lucey, Munro, & Singh, 1998; Michalski, Cariou et al., 2002). Heat treatment induces disulfide bridges formation between κ -casein and β -lactoglobulin (Dalgleish, 1990) which is in turn absorbed to MFGM (Cano-Ruiz & Richter, 1997; Houlihan et al., 1992; Ye et al., 2004).

The microstructure of the MFG is of great importance to the texture of ripened cheeses. Depending on resistance of MFG to disruption caused by processing, fat can be present as small fat globules surrounded by the MFGM, clusters of fat globules with partly disrupted MFGM or pools of TAGs filling voids in the protein matrix (Lopez, Camier, & Gassi, 2007; Michalski et al., 2007). The reader is referred to the article of Lopez et al. (2007), in which the influence of the MFGM on the microstructure and the flavour of a ripened Emmental cheese is discussed. Small fat losses were found during the pressing of the cheese curds (Lopez et al., 2007). A higher MFGM retention will be obtained in cheese curds, made from smaller fat globules, since they are more resistant to disruption caused by processing and since they also have a higher MFGM content. The MFGM possesses a high water-holding capacity (Goudéranche, Fauquant, & Maubois, 2000). This explains why Emmental produced from native small MFG ($\sim 3\mu\text{m}$) had 5.0% more moisture on non-fat basis than the cheese made from large MFG ($\sim 6\mu\text{m}$) after 52 days of ripening, and 2.2% more moisture in the case of Camembert cheese after 40 days of ripening (Michalski et al., 2004; Michalski, Gassi, Famelart, Leconte, & Camier, 2003). The binding of β -lactoglobulin to the MFGM caused by heat treatment is also another reason for the increase in cheese yield (Molina, Álvarez, Ramos, Olano, & López-Fandiño, 2000). Lysophospholipids, which are released from the MFGM by phospholipase treatment before the

pressing of the curds, act as surface-active agents and help to emulsify water and fat during processing, leading to their increased retention (Lilbæk et al., 2006). Serum (moisture) captured by the MFGM can serve as a reservoir where enzymes can act and enhance flavor development. However, this statement still needs experimental evidence. Hydrolyzed MFGM components may be a source of carbon for some lactic acid bacteria, residing at the interface region of the para-casein matrix and the fat globule surface, which is suggested to interact with the MFGM in Cheddar cheese during ripening (Laloy, Vuillemand, Soda, & Simard, 1996; Lopez et al., 2007). Proteolysis caused by starter proteases and proteolysis and lipolysis by MFGM enzymes may lead to a richer and more intense flavour in cheeses with higher MFGM contents (Laloy et al., 1996; Lopez et al., 2007; Ma & Barbano, 2000; Michalski et al., 2003). The larger fat globule surface area is likely to enhance aroma perception due to a greater contact surface of fat in the mouth (Michalski et al., 2003). Due to the high amount of unsaturated FAs, MFGM phospholipids are susceptible to oxidation and may provoke a soapy-rancid flavor (Erickson et al., 1964; Lopez et al., 2007).

Addition of sweet buttermilk results in a significant decrease in free oil and an increase in cheese yield, but higher added levels could adversely affect the texture, melt, and sensory properties of Pizza cheese (Govindasamy-Lucey, Lin, Jaeggi, Johnson, & Lucey, 2006; Mistry, Metzger, & Maubois, 1996). In experiments on the production of reduced fat Mozzarella cheese, Poduval and Mistry (1999) found that the addition of ultrafiltered buttermilk increased the moisture content and contributed to a further reduction of the free oil content during homogenization. For the production of non-fat and low fat yoghurt, the reduction on total solids causes adverse effects on textural and sensory properties, such as severe syneresis, a lack of the typical flavour and mouthfeel. Many attempts have been carried out to improve these defects by using buttermilk powder and ultrafiltered buttermilk (Trachoo & Mistry, 1998). Confocal microscopy results on yoghurt with sweet buttermilk showed that membrane-like material may function as bridges, which are linking the protein matrix together and this is expected to improve the yoghurt texture (Trachoo, 2003).

As a conclusion, by means of the MFGM material, new cheeses or yoghurts with different functional and nutritional properties can be produced. This perspective could also bring economical profits by increasing the product yield or using low value by-products from the dairy industry, such as buttermilk. However, the use of processed MFGM material may result in different effects compared with native MFGM. This has to be taken into account when developing products with desired and controlled characteristics.

7. Conclusions and perspectives

In the last few years, our knowledge on the composition and properties of the MFGM increased significantly. It is

now recognized that the MFGM is highly complex in structure and composed of different protein and lipid components with specific technological and nutritional properties. As such, MFGM material and MFGM-components have been isolated and characterized as valuable ingredients for incorporation into new food products. However, MFGM are also sensitive to modification during isolation and processing, and care should be taken to standardize the composition and characteristics of the membrane to maintain its unique properties during application in food products. Further work is needed on the quantification of MFGM components in various dairy products, and on the optimization of food-grade downstream production processes for MFGM components that can be applied in the food industry. Higher quantities of well-characterized MFGM-components are also needed to further evaluate the technological, nutritional and bioactive properties of these valuable components.

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