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REVIEW

Composition, Structure, and Bioactive Components in Milk Fat Globule Membrane

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Abstract

A unique biophysical membrane which surrounds the milk fat globules is called the milk fat globule membrane (MFGM). Various researches were studied about origin, composition, structure and bioactive components of MFGM. Bioactive protein components of MFGM play an important beneficiary function such as defense mechanism in new born. Among the bioactive lipid components from MFGM phospholipids showed health enhancing functions. The phospholipids also help in the production of certain dairy product from deterioration. MFGM phospholipids also showed antioxidant activity in some dairy products such as butter and ghee produced from milk of buffalo. Based on the beneficial effects, researchers developed MFGM as functional ingredients in various food products. This current review focuses on health enhancing function of MFGM and its components in various dairy products.

Key words: milk fat globule membrane, bioactive protein, bioactive lipid

Introduction

Bioactive milk component refers to nonessential biomolecules in milk, exhibits the capacity to modulate one or more metabolic processes and also meets the need for mammal newborn (Lopez *et al*., 2010). These components are usually found in different forms in various dairy products. In fermented milk, milk, yogurt, butter and other milk products, these components are in the form of prebiotics, bioactive lipids and bioactive peptides. They have different metabolic activities which aid in curing of many diseases. Some research focus specifically on the bioactive components in milk fat globule membrane (MFGM) which are reported to be highly nutraceutical (Spitsberg, 2005).

MFGM composition and properties are varies from those of either milk or its components. The milk fat globules are more complex emulsion droplets because of their biological membrane, called the MFGM (Lopez *et al*., 2010). It is rich in proteins, enzymes, glycoproteins, phospholipids, sphingolipids, cholesterol and other minor components (Keenan and Patton, 1995; Lopez *et al*., 2010). It also acts as a natural emulsifying agent, which prevents fat globules coalescence in milk and prevents the enzymatic action on milk fat (Heid and Keenan, 2005). Their properties and its structure have various influences on the dairy products. Some of the MFGM components has anti-viral properties in buttermilk (Ochonicky *et al*., 2005). Buttermilk solids which are rich in MFGM showed antioxidant activity (Bilgin *et al*., 2006). Phospholipids of MFGM showed potential physiological effects on brain health (Kidd, 2000), against colon cancer, gastrointestinal pathogens (Spitsberg, 2005), cholesterol binding *in vivo* (Noh and Koo, 2004) and inhibition of tumour growth (Schmelz *et al*, 2003).

Both MFGM proteins and lipids have been proven to possess some unique health benefits and enhance the development of food characteristics in certain dairy foods. Based on the typical bioactive properties, MFGM could open a broad range of new means as health promoters in food and non food outlets. This review views on current research relating to the MFGM composition, structure, bioactive components and its role in various dairy products.

Composition and Structure of MFGM

Composition

Milk lipids are secreted in colloidal assemblies form

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called milk fat globules (MFGs). These particles have a size distribution ranging from about 0.1 to 10 μm in bovine milk, with a mean diameter of around 4 μm. The milk fat globules contain complex compositions and structures which have many properties (Argov *et al*., 2008). The thin membrane encircled around the globules derived from the apical membrane of the lactating cells, called the MFGM (Bianchi *et al*., 2009). The membrane mass of MFGs varies from 2-6% of total mass of fat globules (Lopez, 2010; Singh, 2006). MFGM consists of various components which includes proteins, lipids, enzymes, glycoproteins and other minor components (Danthine *et al*., 2000). The fat content and the size of globule in milk influence the amount and the composition of MFGM. It also depends on many factors such as diet, breed, health and stage of lactation of cows (Keenan, 2001; Keenan and Dylewski, 1995).

Lipid composition of MFGM

Various techniques have been used in analysis of lipid composition of bovine MFGM. Among the techniques thin-layer chromatography (TLC) and gas chromatography have been extensively used at early 1970 to late 1980 with poor resolution in MFGM analysis (Bracco *et al*., 1971; Fong *et al*., 2007; Hamzawi and Shahin, 1986; Kuchroo and Narayanan, 1981; Patton and Keenan, 1975; Ray and Singh, 1974; Sharma *et al*., 1987). The MFGM major lipid components are listed in Table 1. Among the total lipids, neutral lipids contribute a major amount which range from 56-80% in the MFGM. Triglycerides are the major section of neutral lipids (62% of total lipids) (Singh, 2006). A most of the triglycerides appear due to the contamination of core lipid during separation of the MFGM from fat globules (Walstra, 1985). The minor neutral lipids are diglycerides (2.1%), esters (0.1-0.8%) and cholesterol (0.2-6.1%) (% of total lipids) (Danthine *et al*., 2000; Vanderghem *et al*., 2010). A very little amounts of monoglyceride (0.4% of the total lipid; Table 1) were found in the MFGM samples. Further, it is not clear that these mono- and diglycerides are original composition of MFGM or derived by the lipolytic action of various enzymes during isolation of MFGM (Fong *et al*., 2007). Extensive amount of sterol and sterol esters were reported with cholesterol accounting for 90% of the total sterols (Jensen and Newberg, 1995).

Table 1. Lipid components of milk fat globule membrane[†]

Constituent class	Total lipids (%)
Triglycerides	62
Diglycerides	2.1
Monoglycerides	0.4
Sterols	0.2-2.0
Free fatty acids	0.6-6.0
Phospholipids[*]	26-31
Sphingomyelin	25
Phosphatidyl choline	36
Phosphatidyl enthanolamine	30
Phosphatidyl inositol	11
Phosphatidyl serine	4
Glucosyl-ceramide and lactosyl-ceramide	6
Lysophosphatidyl choline	2

[*] Phospholipid classes given as % of total Phospholipids
[†] Cited by (Keenan and Dylewski, 1995; Singh, 2006; Fong *et al.*, 2007; Lopez *et al.,* 2010)

Among the total lipids 15-43% accounts for polar lipids such as phospholipids and sphingolipids in MFGM (Danthine *et al*., 2000). Among phospholipids, phosphatidyl choline(PC), phosphatidyl serine (PS) exist in zwittorionic form and some polar lipids such as phosphatidyl inositol (PI) and serine exist in anionic form (Dewettinck *et al*., 2008). Some minor sterols were also detected in MFGM, e.g. desmosterol, lathosterol by using GCMS technique (Fauquant *et al*., 2007).

Protein composition of MFGM

The MFGM protein composition ranged from 1-4% among the total milk protein. Even though these proteins have a low nutritional value (Keenan and Patton, 1995), they have very important roles in defense mechanisms and various cellular processes in the newborn (Cavaletto *et al*., 2008). MFGM protein compositions are varied depending on the source (Danthine *et al*., 2000; Deeth, 1997; Dewettinck *et al*., 2008; Fong *et al*., 2007; Singh, 2006; Walstra *et al.,* 2006).

The MFGM contains complex protein composition which composed of about 40 different proteins and their molecular mass ranges from 15,000 to 240,000 Da (Mather, 2000; Riccio, 2004; Ye *et al*., 2002). The major and minor proteins of MFGM are listed in Table 2. Six of them are glycoproteins including mucin 1 (MUC 1), xanthin dehydrogenase/oxidase (XDH/XO), mucin 15 (MUC 15 or PAS III), CD36 (PAS IV), butyrophilin (BTN) and PAS 6/7 (lactadherin). The two other proteins, adipophilin (ADPH) and the fatty acid binding protein (FABP), are smaller and unglycosylated (Riccio, 2004). The major proteins are identified in past by comparison of electrophoretic mobilty by various staining of the gels. Modern proteomic approaches, including mass spectrometric (MS) analysis, provided rapid and unambiguous information on protein identity and further identification of minor pro-

Table 2. Major and minor protein components of milk fat globule membrane†

Proteins (Abbreviation)	Molecular weight (Da)
Mucin 1 (MUC 1)	160,000-200,000
Xanthin dehydrogenase/oxidase (XDH/XO)	150,000
Periodic acid Schiff (PAS III)	95,000-100,000
Cluster of Differentiation (CD36)	76,000-78,000
Butyrophilin (BTN)	67,000
Adipophilin (ADPH)	52,000
Periodic acid Schiff 6/7 (PAS 6/7)	43,000-59,000
Fatty-acid binding protein (FABP)	210,000
Proteose peptone (PP3)	18,000-30,000
β-glucuronidase inhibitor	Unknown
Helicobacter pylori inhibitor	Unknown
Phosphoproteins	Unknown
MFGM antigens	Unknown

†Cited by (Keenan and Dylewski, 1995; Mather, 2000; Singh, 2006; Dewettinck *et al.,* 2008; Zamora *et al.,* 2009)

teins. Some human MFGM proteins were also identified by modern proteomic approach (Cavaletto *et al*., 2002; Charlwood *et al*., 2002; Quaranta *et al*., 2001). Some MFGM proteins are also reported by mass spectrometric (MS) analysis: two-dimensional gel electrophoresis (2-DE) followed by reversed phase-LC-MS/MS (Fong *et al*., 2007) and various modern techniques (Lippolis, 2006; Vanderghem *et al*., 2008). Keenan *et al*. (2006) reported about 28 different enzymes in cows milk MFGM, e.g. catalase, aldolase. Some of these enzymes may come from the cytoplasmic crescents (Vandeghem *et al*., 2010).

Structure of MFGM

MFGM is composed of trilayer with approximate thickness of 10-50 nm. The inner mono layer of the membrane covers the core lipid, which originated from their endoplasmic reticulum and the triglyeceride rich core are

Table 3. Components of milk fat globule membrane associated with health benefits

Component	Health benefit	Reference
BRCA 1	Inhibition of breast cancer	Spitsberg and Gorewit, 1997a
BRCA 2	Inhibition of breast cancer	Vissak *et al*., 2002
Fatty acid binding protein (FABP)	Cell growth inhibitor	Spitsberg *et al*., 1995; Spitsberg and Gorewit, 2002; Dewettinck *et al*., 2008
Beta-glucuronidase inhibitor	Inhibition of colon cancer	Ito *et al*., 1993
FABP as selenium carrier	Anticancer factor	Bansal and Medina, 1993; Whanger, 2004
Helicobacter pylori inhibitor	Prevention of gastric diseases	Wang *et al*., 2001; Dewettinck *et al*., 2008
Cholesterolemia-lowering factor	Anticholesterolemic	Ito *et al*., 1992
Butyrophilin	Suppression of multiple sclerosis	Mana *et al*., 2004
Vitamin E and carotenoids	Antioxidants Anticancer Anticholesterolemic	Lindmark-Mansson and Akesson, 2000; Jensen and Nielsen, 1996; Sen *et al*., 2006; Dewettinck *et al*., 2008
Vitamin K	Antiosteoporosis Antiatherosclerosis Antihepatocarcinoma	Kaneki *et al*., 2006
Xanthine oxidase	Bactericidal agent	Martin *et al*., 2004; Hancock *et al*., 2002
Phospholipids	Inhibition of colon cancer Anticholesterolemic Suppression of gastrointestinal pathogens Anti-Alzheimer, antidepressant Antistress	Parodi, 2001 Noh and Koo, 2004 Sprong *et al*., 2002 Horrocks and Farooqui, 2004 McDaniel *et al*., 2003
Sphingolipid	Age-related diseases Anticholesterolemic Bactericidal agent Antiatherosclerosis Hydrolysis of triacylglycerols and lipoprotein synthesis in GI tract	Parodi, 2001; Spitsberg, 2005; Sprong *et al*., 2001, 2002, Lopez *et al.,* 2010
Phosphoproteins	Source of organic phosphorus and Ca-phosphate	Spitsberg and Gorewit, 1997 b
MUC I	Immuno-protective role	Mather, 2000; Lopez, 2010

in contact with membrane through hydrophobic tails of polar lipids of the inner membrane (Keenan and Patton, 1995; Vandeghem *et al*., 2010). Outer bilayer originates from the secretory cell apical membrane, where milk aqueous phase are in contact with the outer most polar hydrophilic lipid groups (Danthine *et al*., 2000; Vandeghem *et al*., 2010). Polar lipids are not homogenously organized in MFGM, some are located in inner surface, such as PE, PS and PI, where other phospholipids like PC, SM and glycolipids are concentrated in bilayer of MFGM (Lopez *et al.,* 2008). The lipid droplets in MFGM aggregate and subsequently form large fat clumps known as butter after MFGM disturbed (Spitsberg, 2005). In the external membrane surface carbohydrate moieties are uniformly distributed (Danthine *et al*., 2000; Lopez *et al.,* 2008). Proteins are like lipids arranged assymetrically, ADPH located inner polar lipid monolayer, XO in inner face monolayer, whereas BTN and ADPH are transmembrane proteins which form supramolecular complex which interconnects inner and outer membrane (Lopez, 2008; Mather and Keenan, 1998) and PAS6/7 located outside bilayer. The much detailed study of model is required since much of the MFGM components yet to be revealed.

Bioactive components of MFGM

MFGM components have been studied for a long time and continue to interest for its bioactive components and its beneficial roles in the human health (Spitsberg, 2005). Some of the health beneficial roles of proteins and phospholipids in bovine MFGM are reported (Riccio, 2004; Spitsberg, 2005) and it is shown in Table 3. Even though many studies reported the beneficial effects of MFGM, some of them reported a few putative effects (Riccio, 2004).

Bioactive MFGM lipids

Polar and complex lipids contained in the MFGM have been reported in various studies for its health strengthen functions (Bourlieu *et al*., 2009; Dewettinck, 2006; German and Dillard, 2006; Rombaut and Singh, 2006; Spitsberg, 2005). Inhibition of the proliferation of carcinoma cell lines was reduced by the bioactivity of phospholipids such as sphingomylin (Berra *et al*., 2002; Schmelz, 2003; Spitsberg, 2005). Some of the lipids are also associated in reduction of stress responses, age-related diseases, development of Alzheimer's disease and apoptosis (Parodi, 2001; Spitsberg, 2005). Inhibition of intestinal absorption of cholesterol and suppression of colon tumors are most effectively done by sphingomyelin (Lemmonnier *et al*., 2003; Noh and Koo, 2004). The vitamin K shows protective actions against hepatocarcinoma and atherosclerosis (Kaneki *et al*., 2006). The vitamins such as tocotrienols possess neuroprotective, anticancer and antioxidant activities (Sen *et al*., 2006).

Bioactive MFGM proteins

Bovine milk MFGM contains about 120 proteins (Reinhardt and Lippolis, 2006). Most of the proteins are involved in membrane/protein trafficking and cell signaling, and some of them are on fat transport and/or metabolism, protein synthesis, general transport and immune functions. Some of them have unknown functions. Their individual functions are listed in Table 4. Beyond their

Table 4. Function of major and minor proteins of milk fat globule membrane in milk

Protein	Function	Reference
Butyrophilin	Glycoprotein consists of two extracellular immunoglobulin-like domains. It has some receptorial function and modulates the encephalitogenic T cell response. Induce oral tolerance and protect against development of multiple sclerosis	Cavaletto *et al*., 2002; Affolter *et al*., 2010
Carbonic anhydrase	Essential factor in the normal growth and development of the gastrointestinal tract of the newborn.	Karhumaa *et al*., 2001; Quaranta *et al*., 2001
Lactadherin	Promotes cell adhesion and is antiviral.	Quaranta *et al*., 2001; Affolter *et al*., 2010
Lactoferrin	Inhibits the classical pathway of complement activation and bacteriostatic action by competing with bacteria for iron.	Smolenski *et al*., 2007
Xanthine oxidase	Involved in lipid globule secretion and defense protein and indispensable for milk fat secretion.	Mather, 2000; Affolter *et al*., 2010
Mucin 1	Involved in protection against the attachment of fimbriated microorganisms.	Affolter *et al*., 2010
Apidophilin and TIP47	Structural role for lipid droplet packaging and storage. TIP47 involved in the trafficking of the mannose-6-phosphate receptor.	Fong *et al*., 2007
Fatty acid binding protein	Regulation of lipid metabolism and inhibition of cell growth	Peterson *et al*., 1998; Affolter *et al*., 2010

functions some of the MFGM protein has a beneficial role in health of human. For example, Mucin 1 protects the intestine of human by the prevention of attachment of fimbriated microbes. The MFGM enzyme such as carbonic anhydrase also plays as a vital role in the infant immunity (Quaranta *et al*., 2001). However, some article suggested the possible negative effect of some major protein in MFGM on human health like multiple sclerosis, coronary heart disease (Riccio, 2004). Further research should be focused before exploiting the results.

Bioactive MFGM in dairy products

Bioactive components of MFGM also involve in the development of certain dairy products. MFGM plays an important role in the development of milk based gel (Lopez and Dufour, 2001; Lucey *et al*., 1998; Michalski *et al*., 2002). It also enhances the texture (Lopez *et al*., 2007) and water holding capacity of certain cheese. MFGM also acts as a carbon source for lactic acid bacteria during ripening and increase the flavor in cheese by providing enzymes (Laloy *et al*., 1996; Lopez *et al*., 2007). Texture of ripened cheese was influenced by the microstructure of MFG, and its membrane MFGM enhances water holding capacity in Emmental cheese obtained from the native small MFG (3 μm) than the Camembert cheese which made from large MFG (6 μm). MFGM lysophospholipids also acts as surface-active agents in curds (Lilbaek *et al*., 2006). During the ripening of Cheddar cheese MFGM and its hydrolyzed components act as a carbon source for lactic acid bacteria (Laloy *et al*., 1996; Lopez *et al*., 2007). Intense flavor in cheeses was obtained from the proteolysis and lipolysis of MFGM enzymes.

MFGM also helps in prevention of excessive lipolysis and acts as a barrier which results in the reduction of rancid flavor in cheese (Mayes *et al*., 1994; Vanderghem *et al*., 2010). MFGM phospholipids are of excellent emulsifiers which prevent fat globules from their aggregation and coalescence in dairy emulsions (Sodini, 2006). Some of the phospholipids have antioxidant activity in buffalo butter (Hamzawi, 1990). It also acts an important factor in delaying the deterioration of ghee (clarified butter). Buttermilk added bread dough shows higher water absorption, increased resistance and better sensory score mostly due to the MFGM components (Bilgin et al., 2006; Vanderghem *et al*., 2010).

On the basis of health aspects, production of low fat yogurt, non-fat yogurt and other non-fat milk products result in the reduction of product quality due to the lower level of MFGM. The supplement of MFGM rich fraction results in enhancing the quality of low fat yogurt (Trachoo and Mistry, 1998). By means of MFGM, new yogurt or cheeses can be developed (Vanderghem *et al*., 2010). However, low fat in milk result in the reduction of MFGM which ultimately lower the level of bioactive component. The addition of processed MFGM may also result in different effects compared with the native MFGM (Vandeghem *et al*., 2010).

Conclusion

Recent advances in new methods and greater advancement in techniques result in the identification of bioactive components in utility of MFGM components. These also result in application of MFGM fractions in various food applications. Bioactive MFGM enhances the product quality in certain cheese such as Pizza cheese by reducing the meltability. The removal of fat results in the reduction of MFGM level which ultimately reduced the bioactive component availability in fat-reduced products. There is still a need to provide information about MFGM choices to consumers to aid in the selection of dairy components that contain optimal levels of health-promoting MFGM components. Further advanced research could explore more about the utility of MFGM components as a bioactive food ingredient.

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