

## Milk Fat Globule Membrane (MFGM): An Ingredient of Dairy Products as Nutraceutical

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### HIGHLIGHTS

- Milk fat globule membrane is a complex biological membrane of lipid-protein, surrounded by the minute fat globules.
- MFGM holds a great potential as nutraceutical with health beneficial properties.
- Functionally important bioactivities associated with milk fat globule membrane protein include immune-stimulating, antimicrobial and antiviral properties.
- MFGM proteins plays a role in a protection against colon cancer and gastrointestinal stress.

### ABSTRACT

Over the recent couple of years, milk fat globule membrane perceived a considerable attention due to its complexity in structure and health beneficial properties. This chapter describes the complex structure of the milk fat globule membrane as potential ingredient of dairy products as nutraceutical. The structure and function of milk fat globule membrane proteins are significant markers of the dietary nature of milk which might be ensnared in an assortment of medical advantages. Utilization of MFGM as nutraceutical depends upon its chemical composition, modifications introduced during processing and individual specific prepared food products. The MFGM and its associated proteins are involved in energy production, signal transduction, metabolic process regulation, cell to cell communication, and boost up immune system. This cross examination gives more bits of knowledge into the dynamic organization of human MFGM proteins, which thus will improve our comprehension of the physiological noteworthiness of MFGM proteins. We present the summary of the advances of research and functions of membrane and its associated proteins that are relevant to health and wellness. Milk fat globule membrane (MFGM) has attained a greater consideration as a potential source of nutraceutical with regards to its lipid-soluble vitamins, phospholipids and essential fatty acids.

### Keywords:

Buttermilk

Human health

Lipid

Milk fat globule membrane

Phospholipids

Protein

Sphingomyelin


## Introduction

Since 17<sup>th</sup> century observations, fat in milk composed mainly of triglycerides, exists as minute globules (0.1 to 15  $\mu$ m). About 99% of cow milk lipids are composed of these minute droplets called as milk fat globule,

originates at the basal region of secretory cells. These lipid droplets are enclosed by a special lipid bilayer membrane and associated proteins, designated the milk fat globule membrane (MFGM). Milk fat globule membrane is a complex biological membrane of lipid-protein, surrounded by the minute fat globules mainly comprised of polar lipids and specific membrane fat globule proteins (Phan et al., 2016a; He et al., 2017; Li et al., 2018). Milk fat globule membrane fractions contains amphiphilic properties which serve as good natural

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emulsifier (Perrechil et al., 2015). In recent scenario, MFGM and its fractions have attained a wide attraction owing to its nutritional aspects, especially in human health viz. antiviral and antibacterial properties (Saeland et al., 2009; Matsumoto et al., 2005; Martin et al., 2004). The proteins related to MFGM have several health promoting properties such as bactericidal, cholesterolemia-lowering factors, suppressors of multiples sclerosis, inhibitors of cancer cell growth, phospholipids as agents against colon cancer, depression, Alzimers's disease and vitamin binders (Guggenmos et al., 2004; Spitsberg, 2005).

Milk fat globule membrane can be separated from milk or from dairy products such as butter, buttermilk enriched in MFGM components. Dairy products are widely consumed as functional food all over the world. The separation of milk into cream and skim milk, the churning of butter and flavor and texture of milk products might be related to properties of milk fat globule surface. Thus, precise use of MFGM and its components can greatly increased the value added to dairy products (Phan et al., 2013b). Human milk is the first food that a newborn baby will receive from its mother. Human milk fat globules are more efficiently digested than lipid droplets in infants in spite of their greater size. Several studies have been reported on the health benefits of human milk and milk fat globules membrane-rich diets (Zavaleta et al., 2011; Kuchta et al., 2012). Antibacterial and antiviral activity of milk fat globules membrane have been attained through lipolysis of milk fat globules which results in liberation of short chain fatty acids and monoglycerides. From these observations, an interesting fact emerged is that milk fat globule is secreted from the lactating cell enclosed by the plasma membrane.

### Origin of membrane

The nature of milk fat globules membrane is best understood by knowing its origin. The functional unit of lactating mammary tissue is the alveolus and spherical arrangement of lactating cells around lumen. The lumen acts as receptacle for the milk secreted by the cells connected by a duct system draining to the outlet(s) at the skin surface.

The real synthesis of milk fat globule takes place in the lactating cell, similarly the lactose, principal carbohydrate of milk. The fat droplets move to the apical region, where they become encapsulated in plasma membrane and expelled into the lumen. The electron photomicrographs of milk fat globules at the point of secretion showed that fat droplets have been connected to the cells through a narrow channel of cytoplasm (Bargmann and Knoop, 1959; Wellings, 1959). From this secretion mechanism, it is postulated that milk fat globule has several surfaces. Prior to the secretion, the inner and outer surface of plasma membrane are super-

imposed on the milk fat globule surface at secretion. The cells plasma membrane becomes the outer exposed surface on the droplet, which is considered as membrane "sidedness". The lactating cell has three types of plasma membrane: first in basal region, it is functionally related with the uptake of substrates from circulation, second in lateral region which is mainly involved in communication with adjacent mammary epithelial cells and last in apical region primarily concerned with secretion and repressing the milk fat globule membrane.

### Isolation and purification of membrane

A typical isolation method can be divided into four steps: fat globule separation, cream washing, and release of MFGM from the globules and collection of the MFGM material (Fig. 1) (Mather, 2000; Singh, 2006). 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) or more number of times in 3–15 fold volumes of distilled or deionized water, sucrose saline solution with or without pH buffering, pH buffered sucrose solution isotonic phosphate buffer solution phosphate-saline buffer or simulated milk ultrafiltrate (Ye et al., 2004b). In some cases, detergents 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 et al., 2007). After washing the cream, the MFGM is released from the triglyceride fat core into the aqueous phase by churning, agitation at reduced temperatures or applying cycles of freezing-thawing. Alternatively, MFGM can directly be released from washed cream by the use of polar aprotic solvents (Bingham and Malin, 1992), bile salts or nonionic detergents; in addition these authors reported that 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 (El-Loly, 2011).

Finally, the released MFGM material from buttermilk and/or butter serum is collected by ultracentrifugation, freeze-drying (Rombaut et al., 2006) or microfiltration (Morin et al., 2007). Two fractions, the soluble supernatant and the MFGM pellet, are obtained by ultracentrifugation. Precipitation of MFGM fragments at low pH or by 'salting out' with ammonium sulfate may be applied to MFGM suspensions, after which the MFGM material is separated by centrifugation. They are summarized in Fig. 2.

### Composition of membrane

#### Gross composition

The composition of milk greatly varies in diversity of

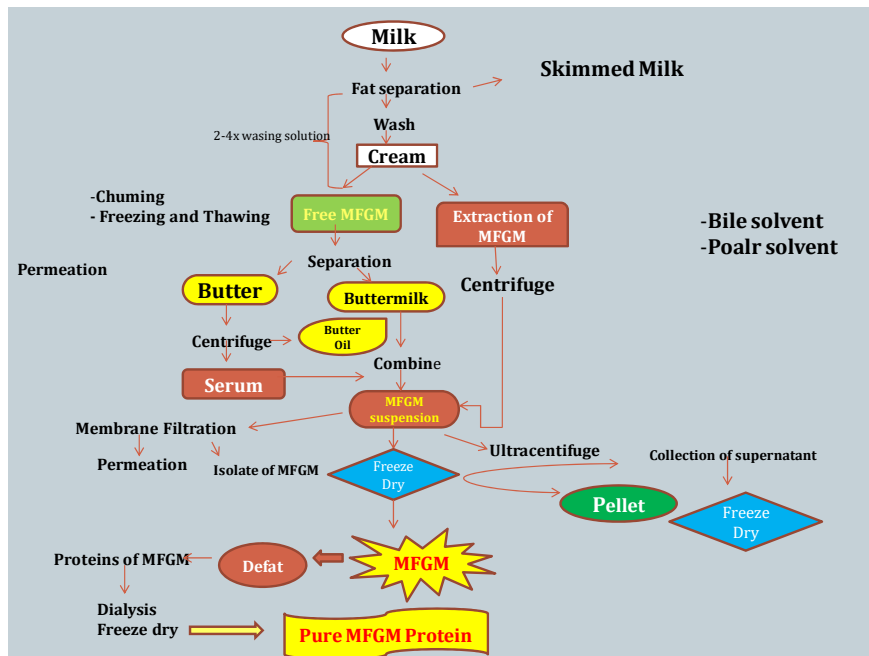


Figure 1. Summary of isolation and purification methods of milk fat globule membrane and its proteins from MFGM rich milk and butter.

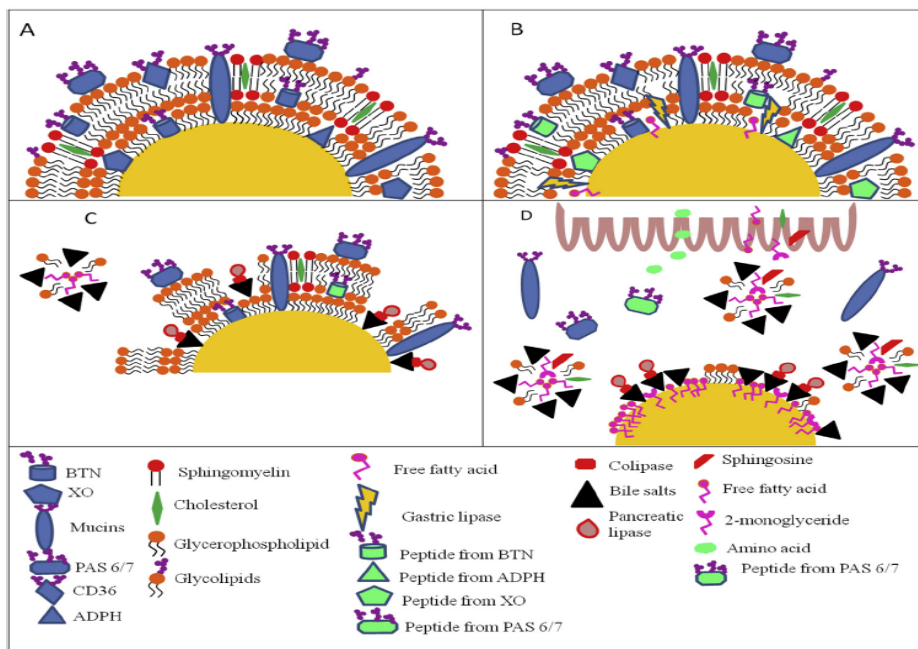


Figure 2. Schematic diagram illustrating the interfacial changes occurring at the surface of the milk fat globule during gastric and intestinal digestion (Gallier, et al., 2014).

**Table 1.** Gross composition of bovine milk fat globule membrane (the glycerides contain mono-, di- and triglycerides).

Component of bovine milk fat globule membrane	Concentration
Proteins	25 to 60% of dry weight
Total lipids	0.5 to 1.1 mg/mg protein
Neutral lipids	56 to 80% of total lipids
Hydrocarbons	1.2% of total lipids
Sterols	0.20 to 5.2% of total lipids
Sterol esters	0.1 to 0.87% of total lipids
Glycerides	53 to 74% of total lipids
Free fatty acids	0.6 to 6.3% of total lipids
Cerebrosides	3.5 nmol/mg protein
Gangliosides	6 nmol/mg protein
Sialic acids	63 nmol/mg protein
Hexoses	0.6 $\mu$ mol/mg protein
Hexosamines	0.3 $\mu$ mol/mg protein

species. Only the milk fat globule membranes which have been extensively studied are bovine. The gross composition of milk fat globule membrane is given in Table 1.

#### *Protein composition of MFGM*

MFGM proteins comprises of 1-4% of total milk protein. MFGM represents a complex system of peripheral and integral proteins (El-Loly, 2011). They play an important role in several metabolic processes and defense systems in the newborn (Cavaletto et al., 2006). The characterization of milk fat globule membrane proteins by polyacrylamide gel electrophoresis exposed the presence of about nine polypeptide chains. Milk fat globule membrane protein exhibits a fraction of milk proteins that plays a protective role in early childhood. Recently, proteomic studies have given in detail the composition of the milk fat globule membrane protein (Table 2). Till date, functionally important bioactivities associated with milk fat globule membrane protein include immune-stimulating, antimicrobial and antiviral properties. The specific bovine milk fat globule membrane protein components like lactadherin, reveal less bioactivity than their human analogues (Kvistgaard et al., 2004).

Milk fat globule membrane glycoproteins are responsible for the inhibition of lipase activity on emulsified milk fat and show resistance to proteolysis by Pronase and trypsin (Kobylka and Carraway, 1973). Two milk fat globule membrane glycoproteins, namely, lactadherin and mucin show resistance to pepsin and retain their biological function at low gastric pH (Hamosh et al., 1999). This resistance of glycoproteins may be associated with the presence of carbohydrate

moieties that forms a glycocalyx surrounding the milk fat globules. As well as providing a steric barrier against aggregation and coalescence, the glycocalyx presents a barrier to lipolysis (Gallier et al., 2012). During *in vitro* digestion, the presence of liquid-ordered domains at the milk fat globule surface plays a crucial physiological role in absorption process. By subjecting MFGM fragments to *in vitro* gastric and intestinal digestion, glycosylated MFGM proteins were more resistant to digestion by gastric and intestinal lipolysis than non-glycosylated proteins. Cluster of differentiation 36 (CD36) and PAS III and PAS 6/7 were detected intact or partially digested at the end of gastric digestion. During gastrointestinal digestion, MUC1 glycoproteins showed resistance due to the presence of few intact fragments which might reflect their anti-adhesive properties against harmful microbes. MUC1 has anti-infection activity against rotavirus and common enteropathogenic bacteria and prevents HIV transmission from mother to child. PAS 6/7 and CD36 are also beneficial to the intestinal immune system by binding to pathogens.

The undigested MFGM glycoproteins in infants stool showed the presence of large MUC1 fragments while rats fed on MFGM rich diet, showed reduction in  $\beta$ -glucuronidase activity of colonic enterobacteria. A randomized double-blind clinical trial on 499 infants fed either a MFGM-rich or skim milk-rich complementary food showed that the consumption of MFGM reduced the number of diarrhea episodes in infants (Zavaleta et al., 2011). The lipo-polysaccharide-injected mice with corn oil rich diet or MFGM-emulsified milk fat for 5 weeks showed lower gut permeability and lower levels of pro-inflammatory cytokines (Snow et al., 2010).

MFGM proteins have proven anticancer properties.

**Table 2.** Components of MFGM protein.

MFGM Proteins	Molecular weight (Da)	Location
Butyrophilin (BTN)	67 000	Transmembrane protein
Mucin I (MUC1)	160 000 – 200 000	Outer polar lipid monolayer
Xanthine oxidase (XO)	150 000	Inner polar lipid monolayer
Cluster of differentiation (CD36) or PAS IV	76 000 – 78 000	Outer polar lipid monolayer
Periodic acid schiff III (PAS III)	95 000 – 100 000	Outer polar lipid monolayer
Periodic acid schiff 6/7 (PAS 6/7)	48 000 – 54 000	Outer of MFGM
Adipophilin (ADPH)	52 000	Inner polar lipid monolayer
Breast cancer type 1 (BRCA1)	210 000	
Fatty acid binding protein (FABP)	13 000	Surface of membrane

In *in vitro* conditions, the growth of breast cancer cell lines was inhibited due the presence of FABP (Spitsberg, 2005). BRCA1 and BRCA2 proteins, present in human and bovine MFGM, act as an onco-suppressor. BTN is a suppressor of multiple sclerosis and is involved in lipid secretion. XDH/XO is a potent bactericidal agent which also generates nitrogen and reactive oxygen species (Ward et al., 2006). Milk fat globule membrane *via*. FABP and other functionally active bioactive peptides act as transporter of organic phosphates, liposolubles, selenium, and organic phosphates (Couvreur and Hurtaud, 2007).

In addition to the antiviral, antibacterial, anti-inflammatory, and anticancer properties of MFGM proteins (Dewettinck et al., 2008), MFGM phospholipids and, in particular, sphingomyelin have many health benefits, including gut health and a protective role against colon cancer. Phospholipids are involved in cell proliferation, molecular transport, memory, stress responses, Alzheimer's disease, myelination of the developing central nervous system, maturation of the neonatal gut, and protection against gastric ulceration (Kuchta et al., 2012). They are also antioxidants and antimicrobial and antiviral agents. Gangliosides have been reported to inhibit food borne pathogens (Ward et al., 2006). Lipids derived from whey cream and buttermilk provides resistance against rotavirus. Hydrolytic products of phosphatidylcholine and sphingomyelin are lipid secondary messengers such as ceramide, diacylglycerol, sphingosine, ceramide-1-phosphate, and sphingosine-1-phosphate and are involved in cellular processes of shut down in cancer such as apoptosis, cell growth, and differentiation. In addition, a change from adenocarcinomas to benign adenomas was reported after feeding milk sphingomyelin and glycosphingolipids to mice. Sphingomyelin possesses chemotherapeutic, anti-cholesterolemic, and chemopreventive effect. It binds to cholesterol and reduces its level during absorption in the intestinal tract. Also the phospholipids

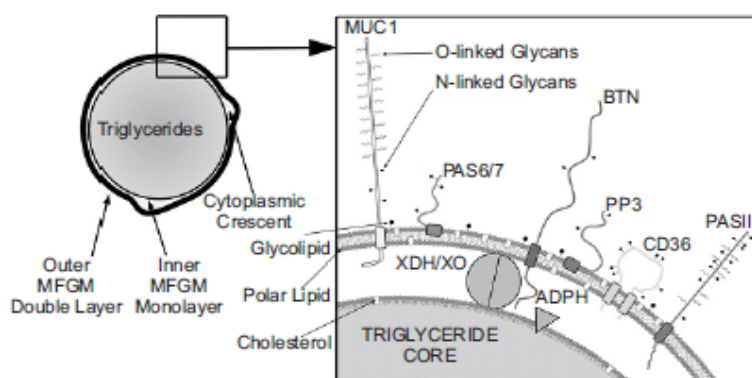
prevent skin damage caused by ultraviolet rays.

#### *Lipid composition*

The amount of neutral lipid content of milk fat globule membrane is highly variable. Glycerides are the most commonly occurring components of total lipid of membrane (Table 1). Triglycerides constitute approximately 90% of the glycerides with mono- and diglycerides accounts for remaining. Triglycerides constitutes a higher ration of long chain fatty acids compared with bulk milk triglycerides, commonly referred to as high melting triglycerides. From pre-cooled milk, milk fat globule membrane prepared at 0 to 4°C show thicker coat along one side of membrane and these are tightly bound so that they can resist the action of acetone for fixation during electron microscopy. While in uncooled milk prepared at 37°C, the level of high melting triglycerides is very low. Newman and Harrison (Newman and Harrison, 1973) studied the micro-electrophoretic properties milk fat globule membrane subjected to various treatments and they concluded that surface of membrane is ionogenic and have little amount of neutral lipids (Newman and Harrison, 1973). The reports confirm that high melting triglycerides are mainly localized to inner side of the membrane.

In the milk fat globule membrane, hydrocarbons and free fatty acids like  $\beta$ -carotene and squalene are also present as lipid fractions. Sterols contribute a considerable proportion of membrane lipids (Keenan and Huang, 1972; Kobylka and Carraway, 1973). About 80% of the total sterol is abundantly present in the free form and rest is considered as sterol ester. Cholesterol and its esters constitute the dominant sterol while other sterols found in milk are dihydrolanosterol and lanosterol (Schwartz et al., 1968). The sterol esters of milk fat globule membrane and milk are exclusive as they have higher amount of odd carbon chins of saturated and unsaturated





**Figure 3.** Structure of the milk fat globule with detailed arrangement of major MFGM proteins (Dewettinck et al., 2008).

fatty acids as compared to other lipid classes of milk. Milk fat globule membrane also constitutes membrane phospholipids fraction that constitutes sphingomyelin, phosphatidylethanolamine and phosphatidylcholine. Low amount of serine phosphatides and inositol are also present. Phospholipids distribution pattern is similar to bovine membrane as observed in human (Bracco et al., 1972) and rats (Keenan et al., 1972). About 60% of the milk phospholipids are associated with milk fat globule membrane and rest is occupied in skimmed milk membrane fraction.

### Role in digestion

In the small intestine, phospholipase  $A_2$  enzyme is present, which hydrolyze the glycerophospholipids (Fig. 2). It is sensitive to deficiency and defects and is mainly active in the phase coexistence region in lateral structure. Thus, the presence of liquid-ordered domains in the milk fat globule membrane in lateral organization and either at surface of milk fat globules or in liposomes, may assist the digestion of phospholipids in the intestine. Fatty acids and lysophospholipids are released through the hydrolysis of phospholipids by phospholipase  $A_2$  and the latter acts as efficient surfactants which lyse the Gram-positive bacteria (Sprong et al., 2012). Through the action of sphingomyelinase, sphingomyelin is hydrolyzed by the bile and intestinal epithelium in the intestines and colon (Kuchta et al., 2012). In the intestinal lumen, an alkaline ceramidase is able to convert ceramide into sphingosine present in the intestinal epithelium. In lipid rafts, the interaction of cholesterol and sphingomyelin reduces the digestion of sphingomyelin as and high concentration of bile salts in the duodenum. During digestion, all sphingolipids are not digested, some undigested sphingomyelin may reach the colon where they are being hydrolyzed and absorbed. Forceful feeding of native milk

fat globules in rats could delay the appearance of plasma triacylglycerols compared with non-emulsified milk fat (Michalski et al., 2006). In rats, dietary sphingolipids increased the hepatic cholesterol and decreased the total plasma cholesterol by 30%.

### Biological significance of MFGM proteins

About 25-70% of the membrane consists of proteins; majority of them are membrane-specific proteins, mainly glycoproteins (Walstra et al., 2006; Fong et al., 2007). MFGM glycoproteins confer protection against various bacteria and viruses. The MFGM proteins are arranged asymmetrically. MFGM proteins can be utilized as stabilizers or emulsifiers for nutritional functionality in food industry (Danthine et al., 2000; Keenan & Mather, 2002; Lopez et al., 2006; Zamora et al., 2009). Isolation and characterization procedures determine the composition of proteins (Table 2). Some are peripheral, some are integral and others may be loosely attached. A wide range of proteins are associated with MFGM have been purified and characterized (Keenan and Dylewski, 1995; Mather, 2000) such as, PAS 6/7 (Kim et al., 1992; Hvarregaard et al., 1996), xanthine dehydrogenase/ oxidase (XDH/XO) (Berglund et al., 1996), CD36 (Rasmussen et al., 1998), mucin 1 (MUC1) (Pallesen et al., 2001) and adidophilin and butyrophilin (Nielsen et al., 1999) ADPH and XDH/XO are located at the inner polar lipid monolayer. BTN is a trans-membrane protein of the outer layer, and with ADPH (Fig. 3). These proteins act as anchorpoints, thereby forming a supramolecular biological complex that connects the inner and outer membrane (Mather & Keenan, 1998). Bovine MFGM contains several other protein components which includes; immunoglobulins, skimmed milk components, milk leukocytes proteins, and plasma proteins of secretory-epithelial cells; majority of them are peripheral proteins loosely bound to MFGM

surface. They also exhibit essential biological functions. SDS-PAGE could be used to characterize protein pattern of milk fat globule membrane proteins. Zamora et al. (2009) characterized the goat MFGM proteins and found higher level of xanthine oxidase (XO) protein compared with cow milk suggesting a high heterogeneity among samples.

### Health promoting benefits of MGM proteins

MFGM associated proteins, phospholipids and membrane glycoprotein plays a vital role during absorption, reduces blood cholesterol level, boost up immune system, and also anti-cancerous properties. High proportion of sphingomyelin and sphingolipids in MFGM protects against colonic cancer, thus exhibit beneficial properties in the gut.

#### *Anticancer effects*

The impact of bovine mammary gland proteins and some MFGM proteins on malignant cell development recommended applying MFGM as a nourishment supplement for counteracting the development of cancer cell growth, particularly breast cancer. The fatty acid binding protein, one of the isolated proteins from bovine MFGM, suppresses the growth of breast cancer cell lines *in vitro* at very low concentration (Spitsberg and Gorewit, 2002). The presence of BRCA1 protein in human and bovine MFGM acts as onco-suppressor. Recently, by using affinity chromatography, BRCA1 and BRCA2 have been extracted from human and bovine MFGM (Vissak et al., 2002). The protein sequence of bovine BRCA1 reveals 72.5% sequence homology with human BRCA1 protein and conservation of the amino acids involved in structure and function of BRCA1 protein (Krum et al., 2003). The bovine C-terminal domain is truncated by sequence of 7 amino acids compared to human protein; the bovine BRCA1 protein displays a similar cell cycle-nuclear expression pattern.

The C-terminal domain of BRCA1 proteins of both bovine and human has a comparable function in restricting the phosphopeptides significant in DNA damage responses (Krum et al., 2003; Rodriguez et al., 2003). Both BRCA1 and BRCA2 proteins function as one of the immediate regulators of cytokinesis and also involved in DNA repair mechanism (Daniels et al., 2004). The prevention of cancer cell growth using MFGM as a nourishment supplement depends on the concept that after the intake of MFGM, a specific number of inhibitory peptides gets released and absorbed in the intestinal tract. After absorption, the retained peptides enter the blood circulatory system, reach at their target organs or tissues and exert their inhibitory activity on the cells experiencing cancer-causing change.

#### *Anticholesterolemic effects*

Milk fat globule membrane has a direct inhibitory effect on reduction of blood cholesterol level. During digestion, the excess of cholesterol directly binds to MFGM components such as phospholipids and their derived substances, for example, sphingomyelin which ultimately controls the lipolysis, solubilization and its absorption. Howard and Marks (1979) observed a fascinating fact on serum cholesterol in a group of human volunteers fed on butter rich diet and in another having fat rich cream but in equal proportion. The group fed on cream did not show increase in serum cholesterol as much as that of the group fed on butter because of the significant differences in their diets. This was supported by the fact that MFGM is an essential part of cream, and suspected as the principle factor in bringing down the serum cholesterol level. Afterward, Ito et al. (1992) proved this concept. There is a direct inhibitory action of bovine MFGM on hypercholesterolemia in the rodents. In the intestine, the binding of cholesterol to MFGM induces inhibition and reduces cholesterol level. But the effect of other substances of MFGM like phospholipids, on serum cholesterol was not explained. Noh and Koo (2004) observed that membrane phospholipid; sphingomyelin is a potential inhibitor of intestinal retention of cholesterol in rats. This inhibition has been explained by a direct inhibitory impact of highly saturated long chains fatty acyl groups of sphingomyelin on micellar solubilization, rate of luminal lipolysis and movement of micellar lipids to the enterocyte. The nutraceutical properties of MFGM are due to the presence of phospholipids, which comprises about 30% of the total membrane lipids. Phospholipids are further classified into three main classes: sphingomyelin, phosphatidyl ethanolamine, and phosphatidyl choline, constitute 19.2 to 23.0, 27.0 to 35.0, and 25.7 to 41.1 to 23.0% of total MFGM phospholipids, respectively (Kanno, 1990). The phospholipids regulate several metabolic functions including memory, molecular transport system, growth and development, development of Alzheimer's disease, stress responses, myelination in the central nervous system, colon cancer, and most important absorption in the digestive tract (Astaire et al., 2003; Oshida et al., 2003; Horrocks and Farooqui, 2004).

### Conclusion

Milk fat globule membrane, a complex biological membrane is a potential ingredient for dairy products. Incorporation of MFGM proteins (as nutraceuticals) can greatly enhance the value-added to the dairy products. MFGM proteins also play an important role in various cellular processes and defense mechanisms in the newborn. Its other health promoting effects includes onco-suppressor, immune-stimulator, antibacterial,

antiadhesive, biochemical and immunological coronary atherogenic effects.

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## Competing Interests

The authors declare no conflict of interest.

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