

## Vesicle properties and health benefits of milk phospholipids: a review

Zhiguang Huang<sup>a,b,c</sup>, Hui Zhao<sup>a\*</sup>, Wenqiang Guan<sup>a</sup>, Jianfu Liu<sup>a</sup>, Charles Brennan<sup>a,b,c\*</sup>,  
Don Kulasiri<sup>b</sup> and Maneesha S. Mohan<sup>b</sup>

<sup>a</sup>Tianjin Key Laboratory of Food and Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China

<sup>b</sup>Department of Wine, Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, Christchurch 7647, New Zealand

<sup>c</sup>Riddet Research Institute, Palmerston North, New Zealand

\*Corresponding author: Hui Zhao and Charles Brennan, Tianjin Key Laboratory of Food and Biotechnology, School of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China. E-mail: ZhaoHui@tjcu.edu.cn; Charles.Brennan@lincoln.ac.nz

DOI: 10.31665/JFB.2019.5176

Received: December 20, 2018; Revised received & accepted: January 20, 2019

Citation: Huang, Z., Zhao, H., Guan, W., Liu, J., Brennan, C., Kulasiri, D., and Mohan, M.S. (2019). Vesicle properties and health benefits of milk phospholipids: A review. J. Food Bioact. 5: 31–42.

### Abstract

Phospholipids are important ingredients in milk. They serve as bioactive components with processing functionalities, despite representing only a small proportion of total milk lipids. There has been increasing interest in vesicle properties and health effects of milk phospholipids. However, there are limited reports on industry-scale manufacturing of related commercial products. This contribution aims to elucidate the industrial processes of manufacturing milk phospholipid products including phospholipid extraction and fraction as well as summarizing determination assays of milk phospholipids. In addition to industrial production, this review elaborates on application aspects, such as the biological properties of milk phospholipids and their technical importance as delivery vesicles of liposomes and phytosomes. In addition, new insights on large-scale production of milk phospholipids and new applications such as phytosomes and antioxidant properties are discussed.

**Keywords:** Milk phospholipids; Solvent extraction; Liposome; Phytosome; Health effects; Vesicle properties.

### 1. Introduction

Milk contributes about one third of human dietary lipid intake (USDA, 2017). Milk phospholipids have been used as materials for nutrient carriers since the early 2000s. Thompson (2005) first used milk phospholipids to fabricate three kinds of liposome to encapsulate bioactive compounds. Since then, milk phospholipid-based liposomes have been prepared to encapsulate ascorbic acid (Farhang et al., 2012) and lactoferrin (Liu et al., 2013). More recently, milk phospholipid liposomes were applied to improve the storage stability of encapsulates (Gulseren and Corredig, 2013), the encapsulate solubility and encapsulation efficiency (Jin et al., 2016; Liu et al., 2012) and the bioavailability of encapsulate

(Maswadeh et al., 2015), showing better efficiency than soy lecithin (Liu et al., 2012). Furthermore, in terms of biological effects, several review papers have summarized various health benefits of milk phospholipids, with emphasis on therapeutic aspects (Castro-Gómez et al., 2015), infant's gut development and cognitive functions (Ortega-Anaya and Jimenez-Flores, 2018), and physiological functionalities (Verardo et al., 2017).

This contribution aims to summarize the industrial processes of manufacturing milk phospholipids, to update last five-year results on using phytosomes or liposomes to enhance bioavailability of bioactive compounds. It also reports on the recent trends on biological activities of milk phospholipids including antioxidant potential.

## 2. Structure, composition and occurrence

### 2.1. Molecular structure

Milk phospholipids include two subclasses, glycerophospholipids and sphingolipids. Glycerophospholipids consist of a glycerol moiety with two fatty acids (lipophilic) esterified at positions of *sn-1* and *sn-2* and a hydroxyl group at *sn-3* position, linked to a phosphate group and a hydrophilic residue. The structural details of the latter determine the types of glycerophospholipids, namely phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidic acid (PA) (Verardo et al., 2017). The amphiphilic structure (lipophilic tail and hydrophilic head) provides milk phospholipids with emulsification properties.

Sphingolipids consist of sphingosine backbone (2-amino-4-*octadecene-1,3-diol*), linked to a fatty acid through an amide bond and a polar head. Sphingomyelin (SM) is a prominent subclass of sphingolipids, having a phosphocholine head group. A minor constituent of sphingolipids in milk is glycosphingolipid, whose polar group comprises carbohydrate groups (glucose, galactose, and lactose) (Ortega-Anaya and Jimenez-Flores, 2018).

### 2.2. Composition

Milk lipids represent approximately 4% of bovine milk (Bylund, 2015). Among total milk fat, only 0.32–1% represents phospholipid compounds (Le et al., 2015). Thus, it takes 2.5–8 liters of raw milk to produce one gram of phospholipids. Phospholipids are structured, functional lipids (Jala and Kumar, 2018). In all the three phospholipid sources, PC and PE contribute to the major proportion (52, 55 and 90% for milk, soy and egg yolk, respectively) of polar lipids. Compared to soy lecithin and egg yolk lecithin, milk phospholipids have a more balanced distribution in each subclass. SM and PS (24 and 12% in milk phospholipid profile, respectively), being regarded as functional ingredients for brain development (Castro-Gómez et al., 2015; Higurashi et al., 2015), are virtually absent in other sources, such as soy (0 and 0.5%, respectively) and egg yolk lecithin (1.5 and 0%, respectively) (Li, 2014).

Apart from SM and PS profile, milk phospholipids have advantages over the other two sources due to their natural origin, oxidative stability and color compatibility. Milk phospholipids have lower content of polyunsaturated fatty acids (PUFA 7.2–7.9% (Lopez et al., 2008)) than soy lecithin (60.37% (Imran et al., 2015)) and egg lecithin (23.2% (Asomaning and Curtis, 2017)). Unsaturated fatty acids had a proportion of approximately 46.14% for mature bovine milk phospholipids (Zou et al., 2015), and 33–44.8% for two kinds of bovine milk polar lipids fed on maize silage and linseed (Lopez et al., 2008), while for the lecithin of soy and egg yolk, this percentage was 79.58 (Imran et al., 2015) and 54.6 (Asomaning and Curtis, 2017), respectively. Thus, milk phospholipids are more resistant to oxidation than other phospholipids and they also have less color intensity for this kind of fatty acid profile (Ireland, 2014).

In terms of fatty acid profile of phospholipids, bovine milk, soy and egg yolk all have predominant distribution of long chain fatty acids (LCFA 13–21), and the abundance of their LCFA is above 90% (Asomaning and Curtis, 2017; Butina et al., 2017). The top two prominent fatty acids of phospholipids for milk and egg yolk are oleic and palmitic acids, which together account for more than 60%. The principal fatty acids of soy lecithin are linoleic and palmitic acids, contributing to 63.4 and 16.4%, respectively (Lopez

et al., 2014).

### 2.3. Occurrence

In intact raw bovine milk, phospholipids take the form of milk fat globule membrane (MFGM: 0.1–20  $\mu\text{m}$  in diameter, 10–50 nm in thickness (Arranz and Corredig, 2017)). The triple-layer membrane consists of a surface-active inner monolayer enveloping triacylglycerols (TAG) in the center and an outer bilayer in contact with the aqueous phase of milk (Castro-Gómez et al., 2015). The milk fat globule membrane is composed of polar lipids, proteins, glycoproteins, enzymes and minor neutral lipids (Zhao et al., 2019).

In dairy products, the triple-layer membrane structure becomes disrupted during processing and milk phospholipids redistribute into such products as buttermilk (BM) and beta serum powder (BSP, >60% lipid), which is an aqueous dairy stream through phase inversion from an oil-in-water to a water-in-oil emulsion (Fletcher et al., 2006).

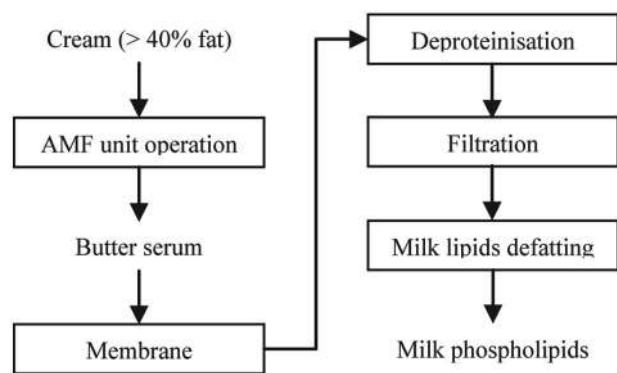
## 3. Industrial manufacturing

### 3.1. Phospholipid extraction from dairy products

Milk phospholipid concentrated streams are related to butter processing, anhydrous milk fat (AMF) or whey fraction. Commercial milk phospholipid products are usually derived from dairy products, such as butter serum AMF, buttermilk, or acid butter whey. The level of phospholipids in these streams can be as high as 11.54, 2.03 and 1.84%, respectively (Le et al., 2015). Butter serum powder represents the highest level of phospholipid concentrate among those dairy streams. Therefore, it is a preferred feed for making milk phospholipid.

AMF, derived either from fresh cream or butter, contains purified milk fat (>99.8%) with removal of water and non-fat solid (Bylund, 2015). Butter serum AMF consists of highest proportion of phospholipids, with 11.54, 1.25 and 48.4% in terms of dry matter (DM), wet base and lipid base, respectively (Pimentel et al., 2016; Smithers and Augustin, 2013). Buttermilk, a product of churn process, is the serum of butter, containing the most of original milk whey proteins and less fat than butter (Chandan and Kilara, 2010). Buttermilk phospholipids are less abundant than those of butter serum, with 2.03% of dry matter (DM) content. Acid buttermilk whey has a DM-based protein percentage of approximately 84.7%, containing 1.84 and 0.1% phospholipids for dry and wet products, respectively (Smithers and Augustin, 2013). Intact milk fat globule membrane contains 30–70% polar lipids. However, it is generally only regarded as a laboratory source of phospholipids (Holzmüller and Kulozik, 2016; Lu et al., 2016).

Solvent extraction is one of the common methods to isolate milk phospholipids from dairy lipid concentrates. Ethanol is the most used solvent to extract milk lipids, for instance, hot alcohol (90%) extraction at 70 °C rendered around 90% recovery rate (Price et al., 2018). Ethanolic extraction of lipids from proteins resulted in high purity (75%) phospholipids (Burling and Graverholt, 2008). In a laboratory up-scaling test, supercritical carbon dioxide and 20% ethanol was utilized to extract lipids and final product had a purity of  $56.24 \pm 0.07\%$  (Barry et al., 2017). Supercritical carbon dioxide can only dissolve triacylglycerols without phospholipids, but together with near-critical dimethyl ether, it extracts both neutral and polar lipids (Fletcher et al., 2006). Hexane is also a solvent that is occasionally used for lipid extraction (Shulman et al., 2011).



**Figure 1.** Block process flow diagram to illustrate a typical routine of milk phospholipid (PL) isolation and purification.

Phospholipids are acetone-insoluble, but triacylglycerols dissolves in acetone. This selectivity in solubility also provides an approach to purify milk phospholipids (Le et al., 2015; Zou et al., 2015).

To obtain a high purity of phospholipids, lactose and protein (casein and whey protein) need to be isolated from lipids. Proteins can be denatured thermally or in acid solution (pH 4.6) (Ferreiro et al., 2016; Price et al., 2018), the aggregated particles are then sieved by subsequent filtration. Starting with whey protein phospholipid concentrate, ethanol at 60–80 °C denatures proteins, resulting in phospholipid concentration of *ca.* 45.8% in the filtrates (Price et al., 2018). Proteolysis is also a viable way to remove proteins, in which whey and casein break into peptides and amino acids. Then the small molecules enter permeate after ultrafiltration (UF) or microfiltration (MF) operation (Barry et al., 2017; Konrad et al., 2013). Alcalase (E.E. 3.4.21.62), a serine type endoprotease with esterase activity, catalysed amino esters at pH 7.5 and 35–60 °C (Barry et al., 2017), while tryptic and peptic hydrolysis may be carried out at 42 °C for 2–16 h, with pH at 7.7 and 2.0, respectively (Konrad et al., 2013). Lactose is a smaller molecule than lipid and it also goes into permeate (Levin et al., 2016).

The process flow diagrams of industrial milk phospholipid manufacturing are not available due to commercial confidence. However, according to previous research reports, a block diagram was proposed to illustrate the principle of typical industrial production processes of milk phospholipids (Figure 1). Starting from butter serum or buttermilk, milk phospholipid concentrate can be refined by sequential unit operations of delactosing, deproteinising, and defatting (Fletcher et al., 2006; Ireland, 2014; Zou et al., 2015).

Apart from the combined methods of solvents, filtration and centrifugation, milk phospholipids can be synthesized by using lecithin phosphatidylcholine and milk L-serine (WO2005027822). First, the choline group of soy PC is cleaved with Phospholipase D, and replaced with an L-serine group in the presence of calcium salt. The synthesized PS 20/60 (21 and 62% PS, respectively) can acquire an unpleasant taste and may become undrinkable. Thus,

oil capsule was formulated to alter the flavor of PS. PS20/60 are physically unstable, and as they come from impure origin, these PS products (PS 20/60) were restricted by the public health authorities as described in WO2006-128465A1 (Table 1).

### 3.2. Available commercial products and related patents

Among the milk phospholipid portfolio of Fonterra Co-operative Group Ltd, Phospholac 600 consists of approximately 75% phospholipid, representing one of the most concentrated milk phospholipids in large-scale commercial products (Li, 2014). Its Phospholac 500/600/700 and Gangolac 600 have a phospholipid content of 34, 70, 62, and 15%, respectively (Li, 2014; Thompson, 2005). Additionally, Arla Foods amla have commercialized a series of phospholipid rich dairy milk concentrated (PRDMC) products, including Lacprodan<sup>®</sup> MFGM 10 and Lacprodan<sup>®</sup> PL 20/75. Lacprodan<sup>®</sup> MFGM 10 has been claimed to support physiological development of infant gut and provide infants with similar phospholipid benefits as breastfed infants because the fatty acid profile of Lacprodan<sup>®</sup> is similar to that of human milk (Sokol et al., 2015). In addition, PL 20 is made out of serum phase of butter oil product (AMF) with membrane filtration, yielding over 20% phospholipids in total solids, which is a pure-natural nutraceutical with properties that is not discovered in conventional phospholipid sources including soy. PL 75 is a further ethanolic extract from PL 20, with 75% of phospholipids and protein-free. PL 20 and 75 targets infant milk formula and skin care, respectively (Arla, 2018).

As illustrated in Table 2, ethanol has frequently been used to extract milk lipids during industrial processes of manufacturing milk phospholipids. To further purify phospholipids, acetone (or dimethyl ether) is a common solvent to dissolve triacylglycerols. Most industrial milk products are generated from buttermilk (BM) and butter serum powder (BSP), except for some other origins such as whey protein concentrate (WPC) by Morinaga Milk Industrial Co Ltd. Tatua Co-operative Dairy Company produces a milk phospholipid concentrate from beta serum powder, an aqueous dairy ingredient separated from dairy streams comprising more than 60% lipids which has been made by phase inversion from an oil-in-water to a water-in-oil dispersion (Tatua, 2018). For instance, Lipidex, a derivative from beta serum powder by Synlait Milk Limited, contains 5–7% phospholipids and 26.6% fat in total (Moukarzel, 2016). Bovine milk SM (#860063, 25–200 mg) by Avanti has a purity of 99% (Lopez et al., 2014). Lecico Lipamine M20 comprises 20% of phospholipids including sphingomyelin, ceramides and ganglioside. This product has been produced with a special membrane technology, which used only water without using other solvents (Lecico, 2018).

### 3.3. Analysis: sample preparation, fractionation and chromatography

For analysis purposes, milk lipid samples are usually prepared

**Table 1.** Phospholipid composition of three typical dairy products

Product	PL on product (g/100g)	PL on DM (g/100g)	PL on fat (g/100g)	Protein on product (g/100g)	Protein on DM (g/100g)	Reference
Butter serum AMF	1.25	11.54	14.8–48.4	2.91	32.71	(Lopez et al., 2017; Smithers and Augustin, 2013)
Sweet buttermilk	0.16	2.03	4.49–33.1	3.31	32.95	(Smithers and Augustin, 2013)
Acid buttermilk whey	0.1	1.84	25.4	0.99	84.7	(Pimentel et al., 2016)

Table 2. Industrial manufacturing of milk phospholipids

Patent	Patent family	Assignee	Feed	Lipid extract	Defat	DM Purity (%)	Yield (%)
US8471002B2(2013)	EP1814399B1(2016), JP2008515455A(2008), KR20070114108A(2005), CN101090635B(2012), CA2583704C(2012), DK1814399T3(2016)	Fonterra, NZ	BSP, et al	Ethanol, DME <sup>f</sup>	CO <sub>2</sub>	25, 75, 60 (Phospholac 500, 600, 700)	
US8231922B2(2012)	EP1901620A1(2008), JP5455365B2(2008), JP5455365B2(2014), KR101352288B1(2014), CA2611121C(2014), RU2420083C2(2011), WO2006128465A1(2006)	Arla, DK	BM whey	MF		75, 20 (PL 75/20)	
US13205071 (2011)	EP20060728307(2006), RU2480474C2(2013), WO2006114790A3(2007), CA701316521A	Enzymotec Ltd, Israel	Milk PL 12.3%	Ethanol and <i>n</i> -hexane	Acetone, CO <sub>2</sub>	87	
US9700061B2(2017)	EP1880612A1(2008), JP5079805B2(2012), KR101492647B1(2015), CN10149490B(2013), CA2655238A1(2008), WO2008009636A1(2008)	Corman SA, Belgium	Fresh cream	Deproteinize by UF/Df <sup>d</sup>		20	50
JP2018052912A(2018)	JP2017092808A(2017) JP2018052912A(2018)	Megmilk Snow Brand Co Ltd, Japan	Milk PL	Ethanol	CO <sub>2</sub>	60–99	
US5677472A (1997)	EP0689579B1(1999), JP3504267B2(2004), CA2155950A1(1994), DE69420712D1(1999), DK0689579T3(2000)	Svenska Mejerienas Riksforenings Ekonomi AB, Sweden	BM, whey	Ethanol, et al <sup>a</sup>	Acetone et al <sup>b</sup>	70–95	52
US9567356B2(2017)	EP2912044B1(2018), JP2018000944A(2018), CN104768959B(2018), CA2888483A1(2014), DK2912044T3(2018), ES2684310T3(2018), WO2014066623A1(2014)	Cargill Inc, US	BM, Lecithin	Alcohol (C1–3, C8–22)		58.7	
EP2168438A1(2010)	EP20080015710(2008)	Meggle Japan Co Ltd	BSP, BM <sup>c</sup>	High pressure ethanol	CO <sub>2</sub>	70 (PE10, PS 33, PI 27)	3
WO2002034062A1(2002)	PCT/BE2000/000130(2000)	Nv Marc Boone	BM et al	5–20 kDa UF		SM doubled	
JP2005336230A(2005)	JP2004153154A(2004)	Morinaga	WPC80 <sup>e</sup>		CO <sub>2</sub>	22–46	1
WO2007123424A1(2007)	PCT/NZ2007/000087(2007)	Owen John Catchpole	PL, egg yolk	Ethanol	CO <sub>2</sub>		

Notes: <sup>a</sup>Ethanol, iso-propanol, *n*-heptane; <sup>b</sup>Acetone, ethyl acetate, and 2-pentanone; <sup>c</sup>Butter serum powder, buttermilk; <sup>d</sup>Ultrafiltration and dia-filtration; <sup>e</sup>Whey protein concentrate 80; <sup>f</sup>Dimethyl ether.

with solvent extraction. The Folch and Bligh extraction method using both chloroform and methanol, a common formula to dissolve milk lipids. Though dichloromethane (DCM, less toxic than chloroform) has recently been introduced to replace chloroform (Claumarchirant et al., 2016), the principal methods of lipid extraction remain to be the Folch extraction (Bourlieua et al., 2018), the Bligh method (chloroform:methanol:water is 1:2:0.8, v/v/v) (Cheema et al., 2017) or the Röse-Gottlieb extraction of ammoniacal ethanolic solution of milk samples with diethyl ether and light petroleum (Barry et al., 2016; Ferreira et al., 2016). Total lipids of samples may be measured using gravimetric determination, Gerber-van Gulik butyrometer, infrared spectral method specified in International Dairy Federation (IDF) (Ferreiro et al., 2016), or gas chromatograph equipped with a flame ionization detector (FID) (Rodríguez-Alcal et al., 2015).

Milk phospholipid fractions are usually further purified by a solid-phase extraction (SPE) before a determination assay of phospholipids and their subclasses, as illustrated in Table 3. Silica gel bonded cartridge is the most used SPE column to fractionate phospholipids from neutral lipids. First, the column is conditioned with hexane, then it is eluted by hexane (C6) and diethyl-ether (DEE) mixture to separate triacylglycerols. After that, another elution with chloroform, methanol and water will bring phospholipids out of the SPE column, which will be collected for solvent evaporation by using rotary evaporation. The final product (phospholipids) after solvent drying is stored at -20 °C before using (Haddadian et al., 2018). In addition, chloroform and methanol have been used as SPE conditioning and elution solvents (Walczak et al., 2016). Some SPE was performed with silica gel plate instead of silica gel bonded cartridges (Zou et al., 2015). Total phospholipids in milk samples can be determined by IDF molybdate assay (Vilamarim et al., 2018), Fourier transform infrared (FTIR) spectroscopy (Kala et al., 2018) or enzymatic method measuring the choline content (Shrestha et al., 2017).

Nuclear magnetic resonance (NMR) using  $^{31}\text{P}$  is a standard assay to quantify milk phospholipids and their subclasses (Hickey et al., 2017; Xu et al., 2015). However, chromatography is the more common assay to determine milk phospholipids. Thin layer chromatography (TLC) is a convenient assay without sophisticated instruments. A formula of TLC elution solvent mixture containing hexane, diethyl ether and acetic acid (80:20:1, v/v/v) has often been applied on a silica gel plate. The fractionated subclasses are then visualized on the plate with iodine vapor (Fuller et al., 2012; Zou et al., 2015).

High-performance liquid chromatography (HPLC) remains the most commonly used method, because it can accurately quantify the total phospholipids and each of their subclasses than TLC. For each HPLC assay, 5–10  $\mu\text{L}$  sample (approximately 5–100  $\mu\text{g}/\text{mL}$ ) is the necessary amount to perform chromatographic analysis (Cheema et al., 2017). As shown in Table 3, HPLC was usually coupled with such detectors as ultraviolet (UV) absorbance, evaporative light-scattering detector (ELSD) and mass spectroscopy (MS). Due to the polarity of milk phospholipid, silica column has often been used to separate the subclasses of milk phospholipids. To further fractionate the species of specified milk phospholipid subclasses, reverse phase (RP) HPLC with C18 column can be employed (Dugo et al., 2013). The binary solvents of chloroform and methanol or acetonitrile and ammonium acetate are frequently used as an elution medium. The change of formula of elution solvents leads to the different detection order of phospholipid subclasses in the chromatogram, as illustrate in Table 3. In some cases, pH of mobile phase was modulated by trimethylamine or ammonia hydroxide (pH 3 and 6, respectively) and formic acid has shown benefits in providing a flat baseline

(Ferreiro et al., 2014).

## 4. Vesicle properties

### 4.1. Liposomes

Milk phospholipid concentrate has good emulsification properties due to its amphiphilic molecular structure. Milk phospholipids can also be used to deliver nutraceuticals and bioactive compounds in food and bio-pharmaceutical industries, achieving better stability, solubility and bioavailability of the encapsulate (Livney et al., 2016). In a recent report, the vesicle properties of milk phospholipids was thoroughly reviewed (Arranz and Corredig, 2017).

Milk phospholipids-based liposomes have been proven to deliver lipophilic or hydrophilic components to improve the bioavailability of encapsulates, in either pharmaceutical or food industries. In the cosmetic area, liposomes have been used to facilitate dermal absorption of active compounds. Milk phospholipids-based liposomes have been applied to co-deliver beta-carotene within the membrane and ascorbic acid in the inner phase (Farhang, 2013). The complexing index increased when milk phospholipid concentration was improved from 5 to 10%, then plateaued at  $26 \pm 0.5\%$  when milk phospholipid concentration was 10–15%. The size of carriers was  $120 \pm 2$  nm using micro-fluidization unit. Due to the limited physical stability, the produced liposomes aggregated and stratified in one day (Farhang et al., 2012). The liposome carriers based on milk phospholipids were shown in Table 4.

### 4.2. Phytosomes

As illustrated in Table 4, phytosome carrier can also deliver bioactive compounds, both lipophilic and hydrophilic, in order to enhance oral bioavailability (Lu et al., 2018). Phytosomes are a durable complexes, with a simple manufacturing process (Gnananath et al., 2017). Complexing reaction of milk phospholipids and encapsulate (molecular ratio 1–5) was realized in either ethanolic or methanolic solution of 55°C. As a result, the bioavailability of encapsulate was enhanced by 3–5-fold (Freaga et al., 2018; Yu et al., 2016), while the solubility of 36-fold increase was evidenced (Telange et al., 2017).

Both milk phytosomes and liposomes are derived from milk phospholipids. Liposomes encapsulate bioactive compounds in either the core of phospholipid globule or in the phospholipid bilayer, whereas phytosomes are different from liposomes because phospholipids conjugate with encapsulates, hence they are more durable and efficient than liposomes (Karimi et al., 2015). Currently, the milk phospholipid-based phytosomes are not yet explored, and it should provide a prospective area to study.

### 4.3. Gastrointestinal digestion and absorption

Milk phospholipids do not hydrolysis in lingual and gastric tract, thereby they can be carriers of bioactive compounds (Castro-Gómez et al., 2015). Their digestion occurs in lumen, the upper part of intestinal gut. Phospholipase A/B/C/D acts on either *sn*-1 or 2 acyl (A), both *sn*-1 and 2 acyl (B), *sn*-3 phosphoric base (C) and *sn*-3 polar head (D), respectively (Gurr et al., 2002)). In human being, pancreatic phospholipase  $A_2$  (EC 3.1.1.4 (Venuti et al., 2017)) can act upon *sn*-2 position of phospholipids, resulting in lysophospholipids and fatty acids. The fatty acid group of lysophospholipids

Table 3. HPLC assays to determine milk phospholipids

Milk sample origin	Lipid extract	PL fraction	Stationary phase	Column size (LxD mm)	Particle size (µm)	Mobile phase	Elution	Volume (mL/min)	Elution time (min)	Oven temperature (°C)	Detector	Identified species in order	Reference
Bovine raw	CM		Silica	100×2.1	1.7	2 (ACN) <sup>f</sup>	gradient	0.3	28	45	Q-TOF-MS <sup>a</sup>	PS, PG, PI, PE, PC, SM, LPC <sup>d</sup>	(Ali et al., 2018)
Cow, ewe, goat skim	DMC		Silica	500×4.5	5	4 (CM) <sup>g</sup>	gradient	0.5–1.4	53	35	ELSD	PE, PI, PS, PC, SM	(Castro-Gómez et al., 2014)
Cow raw	CM		C18	100×2.1	1.7	2 (ACN)	gradient	0.225	21		Q-TOF-MS	PC, SM, LPC	(Cheema et al., 2017)
Cow BM UF	CM		Silica	150×3	3	2 (CM)	gradient	0.5	20	35	ELSD-LTII <sup>b</sup> 50 °C 3.5Bar	PE, PC, SM, PS, PI	(Ferreiro et al., 2014; Ferreiro et al., 2016)
Donkey raw	CM		Silica	150×3	3	2 (AA, MeOH, DCM) <sup>h</sup>	gradient	0.5	17		RP LC-MS	PI, PE, PS, PC, SM	(Contarini et al., 2017)
Cow raw	CM	SPE (CM)	Betasil DIOL	150×4.6	5	3 (C6, IPA FA) <sup>i</sup>	gradient	1.5	19	30	CAD <sup>c</sup> 2.4 Bar	PI, PE, PS, PC, SM, LPC	(Kielbowicz et al., 2013)
Cow raw	CM	SPE (C6, DEE) <sup>k</sup>	Silica	250×4.6	5	2 (CM)	gradient	1	40		ELSD (3.1Bar 50 °C)	PE, PI, PS, PC, SM	(Zheng et al., 2014)
Cow, yak raw	CM	SPE	Silica	8×3 250×3	5	2 (ACN)	gradient	1	24	50	ELSD (90 °C)	PI, PE, PS, PC, SM	(Luo et al., 2018)
Cow raw	CM		Silica	250×4.6	5	2 (ACN)	gradient	0.6	25	30	MS	PS, SM, PE, PC, PI, LacCer <sup>e</sup>	(Liu et al., 2017; Liu et al., 2015)
Cow raw	CM		Silica	150×3	3	2 (CM)	gradient	0.5	35	40	ELSD (85 °C)	PE, PI, PS, PC, SM	(Lopez et al., 2014)
Cow raw	CM		C18	32×0.1 200×0.1	3	2 (ACN)	gradient	0.5	200		MS	PI, PE, PS, PC, SM	(Lu et al., 2016)
HBM <sup>j</sup>	CM		Hypersil APS-2, aminopropyl	150×2.1	3	2 (CM)	gradient	0.25	29	25	MS	PI, PE, PC, PS, SM	(Ma et al., 2017)
Cow raw	CM		Silica	250×4.5	5	2 (CM)	gradient	1–1.5	60	40	ELSD	PE, PI, PS, PC, SM	(Rodríguez-Alcal et al., 2015)
Cow raw	CM	SPE (C6, DEE)	Silica	250×4.6	5	2 (CM)	gradient	1	40		ELSD 50 °C 3.2Bar	PE, PI, PS, PC, SM	(Haddadian et al., 2018)
Cow raw	CM	TLC (C6, DEE)	Silica	150×2.1	3	2 (CM)	gradient	1	55	40	ELSD	PE, PI, PS, PC, SM	(Zou et al., 2015)
HBM	CM	TLC (C6, DEE)	Silica	250×4.6	5	3 (MeOH, ACN) <sup>l</sup>	Isocratic	1		30	UV205nm	PE, PC, SM	(Wu et al., 2019)
Cow	CM		Silica	150×3	3	2 (DCM, MeOH)	gradient		26	40	CAD 2.4Bar	PA, PI, PE, PS, PC, SM	(Barry et al., 2016)
Cow whey	CM		Silica	150×4.6	3	2 (CM)	gradient	0.5	27		ELSD 40 °C 1.8Bar	PE, PI, PS, PC, SM	(Torkamani et al., 2016)

<sup>a</sup>Quadrupole time of flight mass spectrometry; <sup>b</sup>evaporative light-scattering detector, low temperature; <sup>c</sup>charged aerosol detector; <sup>d</sup>lysophosphatidylcholine; <sup>e</sup>lactosylceramide; <sup>f</sup>acetone nitrile and ammonium acetate; <sup>g</sup>chloroform and methanol; <sup>h</sup>acetic acid, methanol; <sup>i</sup>hexane, iso-propanol, di-chloromethane, formic acid; <sup>j</sup>human being milk; <sup>k</sup>hexane, diethyl ether; <sup>l</sup>methanol, phosphoric acid, and acetonitrile (32, 0.6, 67.4 % in volume, respectively).

**Table 4. Milk phytosomes and liposomes as bioactive compound carriers**

Encapsulate	Phospholipid	Vesicle	Bioavailability	Reference
Celastrol (CST)	Soy PC	Phytosomes	4–5-fold increase	(Freaga et al., 2018)
Apigenin	Soy PC	Phytosomes	Up to 82%	(Telange et al., 2017)
Berberine (BER)	Soy PC	Phytosomes	3-fold increase	(Yu et al., 2016)
18 $\beta$ -glycyrrhetic acid	Soy lecithin	Phytosomes	Extended storage to 30–90 days	(Djekic et al., 2016)
Curcumin	Milk PL	Liposomes: Sonication	More efficient and stable than soy lecithin	(Jin et al., 2016)
Lactoferrin (LF)	Milk PL	Liposomes: Ethanol injection	Gastric stable and slow intestinal hydrolysis	(Liu et al., 2013)
Tea phenolic	Milk PL	Liposomes: Microfluidization	More efficient than soy lecithin	(Gulseren and Corredig, 2013)
$\beta$ -carotene and ascorbic acid	Milk PL	Liposomes Microfluidization	Poor physical stability upon storage	(Farhang, 2013)
Silybin	Milk PL	Reverse phase evaporation (RPE)	10-fold increase	(Siegel et al., 2014)

can be further cleaved by lysophospholipase (EC 3.1.1.5) (Winrow et al., 2003). Moreover, the pancreatic lysophospholipase of human being is most likely non-specific phospholipase, but carboxyl ester hydrolase (EC 3.1.1.1) (Duan and Borgström, 1993). In addition, sphingomyelinase (alk-SMase, EC 3.1.4.12) acts on phosphoric di-ester bond of sphingomyelin, generating ceramide and phosphocholine (Nilsson and Duan, 2019). Ceramide will be further split by mucosal ceramidase (N-CDase EC 3.5.1.23) (Mao and Obeid, 2008). The lipolysis products then cross the border of epithelial cells (mucosa) and enter the enterocyte to synthesize new phospholipids, which are then incorporated into chylomicrons (CM). After that, in approximately five hours postprandial, CM will enter into the lymph and blood circulation. Apart from absorption of hydrolysate of phospholipids (lyso-PLs and fatty acids), approximately 20% of phospholipids are passively absorbed in the intestinal lumen (Castro-Gómez et al., 2015). In addition, indigenous phospholipid excretion into bile is 10–20 g per day (Cohn et al., 2010), which was much higher than endogenous phospholipids (2–8 g phospholipid ingestion per day) (Lecomte et al., 2015). Therefore, phospholipids are not essential lipids though they are critical.

## 5. Health impacts

The nutraceutical value of milk polar lipids has previously been reviewed, including the efficacy for modification of the trajectory recession of cerebral structure in old age (Reddan et al., 2018), the roles in the growth of infant brain and gut (Ortega-Anaya and Jimenez-Flores, 2018), the effects of immune-mediated anti-carcinogenic effects and anti-inflammatory activity (Verardo et al., 2017), and the relevance to hepatoprotection and cardiovascular diseases (Castro-Gómez et al., 2015). Moreover, milk phospholipids consequently reduced the waist circumference of the participants in this trial, compared with soy lecithin in a clinical trial, although the blood lipid concentrations of the attendants in the trial was not altered (Weiland et al., 2016). In addition, the effects of Lacprodan® PL-20 on supporting infant intestinal maturation (Arla, 2019) and a healthy microbiota (Nejrup et al., 2017) have been clinically demonstrated. Furthermore, buttermilk and krill oil phospholipids were associated with the improvement of synaptic

signalling in aged rats (Tomé-Carneiro et al., 2018).

### 5.1. Neurocognitive effects

The nutritional value of milk polar lipids includes gut development (SM), neurocognitive development (SM), liver protection (PC), bacteria inhabitation (lyso-phospholipids), maintaining homeostasis (PE), cell signalling (PI) and memory restoration (PS), as reviewed in previous reports (Gallier et al., 2014; Le et al., 2014; Le et al., 2015). It has been documented that milk phospholipids can enhance the neurocognitive development in the trials. For example, the research has shown that sphingolipid supplementation improved the myelination of central nervous system and was responsible for the normal brain weight of rat infants (Castro-Gómez et al., 2015). L-serine is essential for the synthesis of sphingolipids and phosphatidylserine (PS) in particular types of central nervous system neurons (Hirabayashi and Furuya, 2008). Additionally, the cognitive performance benefits of dietary milk phospholipid have been evidenced with the clinical trial (Boyle et al., 2019), the rats model (Schipper et al., 2016), and the piglet model as well (Liu et al., 2014).

Present results appertaining the cognitive functions of milk phospholipids, from either *ex vivo* models or *in vivo* models are illustrated in Table 5. Most tests conferred the benefits of milk phospholipids on brain function, however one examination showed that it might be due to the combined effects of membrane proteins and polar lipids (Timby et al., 2014). In terms of commercial application, milk phospholipids are well-recognized ingredients for infant milk formula (IMF), which represent the world's fastest growing functional food in recent years (Ireland, 2014).

### 5.2. Skin care

Skin parameter enhancement examination has been performed in both *in vivo* and *ex vivo*, yielding positive results except for a non-effectiveness report under the set conditions (Keller et al., 2014), as illustrated in Table 5. Some of these benefits appear to be related to phospholipids, altering the hydration of skin and therefore increasing elasticity and resilience.

Table 5. Health effects of milk phospholipids

Functionality	Dietary supplementary	Model	Result	Reference
Cognitive	PL PUFA	<i>In vivo</i> : Healthy elderly	Cognitive function was activated	(Konagai et al., 2013)
Cognitive	Lacprodan® PL-20	<i>In vivo</i> : Healthy elderly	Set protocols to assess Lacprodan® PL-20	(Scholey et al., 2013)
Cognitive	MFGM	<i>Ex vivo</i> : Rats	MFGM altered brain lipid	(Moukartzel et al., 2018)
Cognitive	Lacprodan® PL-20	<i>Ex vivo</i> : Neonatal piglet	Spatial ability was enhanced	(Liu et al., 2014)
Cognitive	MFGM	<i>Ex vivo</i> : Suckling rat pups	Related genes expression was promoted	(Brink and Lönnerdal, 2018)
Cognitive	Milk SM	<i>In vivo</i> : Low birth weight infants	SM activated prefrontal cortex of the brain, yield better score on visual evoked potential, attention, and memory	(Tanaka et al., 2013)
Cognitive	Ganglioside	<i>In vivo</i> : 6-months infants	Cognitive score increased	(Gurnida et al., 2012)
Cognitive	MFGM	<i>In vivo</i> : Infant and Toddler	The diet led to similar cognitive score to breastfed infants but showed higher score to pure polar lipids fed infants	(Timby et al., 2014)
Cognitive	Milk PL	<i>In vivo</i> : 54 healthy, non-obese adult men	Cognitive performance was improved under conditions of psychosocial stress but failed to moderate cortisol response	(Boyle et al., 2019)
Cognitive	Milk PL coated dietary lipid	<i>Ex vivo</i> : Healthy male mice	T-maze test: Increased to 87% from 74% in short-term memory tests; while same in long-term memory	(Schipper et al., 2016)
Skincare	Milk PL	<i>Ex vivo</i> : Dog with allergic skin disorders	Enteric improvement and better skin conditions	(Karasawa et al., 2017)
Skincare	Milk PL	<i>In vivo</i> : Healthy adults aged 20 to 39 year	Skin elasticity in the region below the eye increased	(Higurashi et al., 2015)
Skincare	Milk PL	<i>In vivo</i> : Atopic dermatitis patients	Not effective	(Keller et al., 2014)
Skincare	Milk PL	<i>Ex vivo</i> : Mice	Modulated epidermal covalently bound ceramides associated with formation of lamellar structures and alleviated skin inflammation	(Morifuji et al., 2015)
Anti-inflammatory	MFGM rich diet	<i>Ex vivo</i> : Mice	Attenuated the inflammatory response to a systemic LPS challenge; cut gut permeability.	(Snow et al., 2010)
Anti-inflammatory	Milk SM diet	<i>Ex vivo</i> : high-fat-fed mice	Altered distal gut microbiota and lowered serum LPS	(Norris et al., 2016)
Anti-inflammatory	Milk SM diet	<i>Ex vivo</i> : high-fat-diet-induced mice	Suppressed metabolic indicator of obesity	(Norris et al., 2017)
Anti-inflammatory	Milk PL extract	<i>In vitro</i> : MA-104 cells of embryonic African green monkey kidney	Polar lipids displayed effects of anti-rotavirus activity by focus-forming unit (FFU) assay	(Fuller et al., 2012)
Anti-inflammatory	MFGM	<i>Ex vivo</i> : Rat pups	Protective and replenishing effects on neonatal intestinal epithelium caused by clostridium difficile toxin; milk PL deficiency led to defect of GI development	(Bhinder et al., 2017)



### 5.3. Anti-inflammatory in gastrointestinal development

Milk phospholipids have proven to be able to modulate inflammatory reaction and to protect against gastrointestinal leakiness, as illustrated in Table 5. Animal models and cell models have shown that the polar lipids fraction from MFGM affects infant gastrointestinal development. Milk phospholipids diet decreased gut permeability (Snow et al., 2010), altered distal gut microbiota and reduced serum lipopolysaccharide (LPS) (Norris et al., 2016), inhibit infectivity of rotavirus (Fuller et al., 2012), and regulate the neonatal gut microbiome and promote intestinal development (Bhinder et al., 2017).

### 5.4. Antioxidant activity

Milk phospholipids act as both antioxidants and a pro-oxidants and sometimes are used to alleviate food oxidation. Anti-oxidative activity of phospholipids might be due to such mechanisms as metal-chelation, alteration of the location of other antioxidants, and regeneration of other primary antioxidants. However, phospholipids can also act as primary antioxidants and pose significant antioxidant activity to biological membranes (i.e. meats), owing to their unsaturated fatty acids and negative charge (Cui and Decker, 2016). Phospholipid supplementation to soybean oil significantly retarded the oxidative process, extending oxidative stability index (OSI) from 7.62 to 12.96 h. However, phosphatidylcholine addition caused trimethylamine (TMA, fishy off-odor) generation (Jiang et al., 2016). Marine lecithin (i.e. krill oil) consists of a natural antioxidant (astaxanthin) and phospholipids bound LC-PUFA, which inhibits oil peroxidation during its shelf life (Ben-Dror et al., 2018). Alpha-tocopherol enhanced the oxidative stability of marine phospholipid emulsions (Lu, 2013).

## 6. Conclusion

In this review, milk vesicle properties and health impacts were addressed. As an emerging material of vesicles in nutraceutical and bio-pharmaceutical, milk phospholipids show advantages over lecithin of soy and egg yolk in encapsulation efficiency. Recently, various kinds of liposomes have been fabricated for enhancing the solubility and bioavailability of encapsulates. Phytosomes, more stable carriers than liposomes, should provide a further area to study. In recent reports, milk phospholipids have been proven to support cognitive development owing to their balanced distribution in phosphatidylserine and sphingomyelin, which was almost absent in soy and egg yolk lecithin. Apart from brain function, milk phospholipids have a role in skin care, due to their more saturated fatty acids, which lead to milky-white color and stability.

In conclusion, milk phospholipids have prospective applications in nutritional delivery, infant formula and cosmetic for their vesicle properties and biological functionalities. As potential alternatives to traditional polar lipids from egg yolk and soy, milk phospholipids need to be efficiently produced in large-scale. Ethanol extraction remains the most used lipid extraction process in dairy industry. Defat with supercritical carbon dioxide or acetone are frequently used to further refine phospholipids from lipids.

## References

Arla. (2018). Arla Foods Ingredients: Ingredients for the next generation.

- In "Dedicated pediatric nutrition from a leading global dairy company", Vol. 2018. Arla Foods Ingredients Group P/S, [www.arlafoodsingredients.com](http://www.arlafoodsingredients.com). Accessed 24 Dec. 2018.
- Arla. (2019). Phospholipids & MFGM: Supporting infant cognitive development and gastrointestinal health. In "Pediatric ingredients: Phospholipid and milk fat globules", Vol. 2019. Arla Foods Ingredients, <https://www.arlafoodsingredients.com/our-ingredients/pediatric-nutrition-ingredients/phospholipids-mfgm/> Accessed Jan 18 2019.
- Arranz, E., and Corredig, M. (2017). Invited review: Milk phospholipid vesicles, their colloidal properties, and potential as delivery vehicles for bioactive molecules. *J. Dairy Sci.* 100: 4213–4222.
- Asomaning, J., and Curtis, J.M. (2017). Enzymatic modification of egg lecithin to improve properties. *Food Chem.* 220: 385–392.
- Barry, K.M., Dinan, T.G., and Kelly, P.M. (2017). Pilot scale production of a phospholipid-enriched dairy ingredient by means of an optimised integrated process employing enzymatic hydrolysis, ultrafiltration and super-critical fluid extraction. *Innov. Food Sci. Emerg. Technol.* 41: 301–306.
- Barry, K.M., Dinan, T.G., Murray, B.A., and Kelly, P.M. (2016). Comparison of dairy phospholipid preparative extraction protocols in combination with analysis by high performance liquid chromatography coupled to a charged aerosol detector. *Int Dairy J.* 56: 179–185.
- Ben-Dror, G., Numa, R., and Dee-Noor, S.S. (2018). Marine lecithin preparations with enhanced oxidation resistance. US Patent Application Publication. In: Aker Biomarine Antarctic, S.N. (Ed.). Norway, Vol. US2018/0125898A1: p. 15.
- Bhinder, G., Allaire, J.M., Garcia, C., Lau, J.T., Chan, J.M., Ryz, N.R., Bosman, E.S., Graef, F.A., Crowley, S.M., Celiberto, L.S., Berkman, J.C., Dyer, R.A., Jacobson, K., Surette, M.G., Innis, S.M., and Vallance, B.A. (2017). Milk fat globule membrane supplementation in formula modulates the neonatal gut microbiome and normalizes intestinal development. *Sci. Rep.* 7: 45–74.
- Bourliewa, C., Cheillan, D., Blota, M., Daira, P., Trauchessec, M., Ruet, S., Gassi, J.-Y., Beaucher, E., Robert, B., Leconte, N., Bouhalla, S., Gaucheron, F., Gésan-Guizou, G., and Michalski, M.-C. (2018). Polar lipid composition of bioactive dairy co-products buttermilk and butterserum: Emphasis on sphingolipid and ceramide isoforms. *Food Chem.* 240: 67–74.
- Boyle, N.B., Dye, L., Arkbage, K., Thorell, L., Frederiksen, P., Croden, F., and Lawton, C. (2019). Effects of milk-based phospholipids on cognitive performance and subjective responses to psychosocial stress: A randomized, double-blind, placebo-controlled trial in high-perfectionist men. *Nutrition* 57: 183–193.
- Brink, L.R., and Lönnerdal, B. (2018). The role of milk fat globule membranes in behavior and cognitive function using a suckling rat pup supplementation model. *J Nutr Biochem* 58.
- Burling, H., and Graverholt, G. (2008). Milk - A new source for bioactive phospholipids for use in food formulations. *Lipid Technol* 20: 229–231.
- Butina, E.A., Gerasimenko, E.O., Bugaets, I.A., and Dubrovskaya, I.A. (2017). Comparative analysis of the physiological value of lecithins obtained from different types of raw materials. *J. Pharm. Sci. & Res.* 9: 2493–2497.
- Bylund, G. (2015). Dairy processing handbook. Tetra Pak Processing Systems AB, S-221 86 Lund, Sweden.
- Castro-Gómez, M.P., Rodríguez-Alcalá, L.M., Calvo, M.V., Romero, J., Mendiola, J.A., Ibañez, E., and Fontecha, J. (2014). Total milk fat extraction and quantification of polar and neutral lipids of cow, goat, and ewe milk by using a pressurized liquid system and chromatographic techniques. *J. Dairy Sci.* 97: 6719–28.
- Castro-Gómez, P., García-Serrano, A., Visioli, F., and Fontecha, J. (2015). Relevance of dietary glycerophospholipids and sphingolipids to human health. *Prostaglandins Leukot. Essent. Fatty Acids* 101: 41–51.
- Chandan, R.C., and Kilara, A. (2010). Dairy ingredients for food processing. John Wiley & Sons, Iowa USA.
- Cheema, M., Smith, P.B., Patterson, A.D., Hristov, A., and Hart, F.M. (2017). The association of lipophilic phospholipids with native bovine casein micelles in skim milk: Effect of lactation stage and casein micelle size. *J. Dairy Sci.* 101: 8672–8687.
- Claumarchirant, L., Cilla, A., Matencio, E., Sanchez-Siles, L.M., Castro-Gomez, P., Fontecha, J., Alegría, A., and Lagarda, M.J. (2016). Addition

- of milk fat globule membrane as an ingredient of infant formulas for resembling the polar lipids of human milk. *Int Dairy J.* 61: 228–238.
- Cohn, J.S., Kamili, A., Wat, E., Chung, R.W., and Tandy, S. (2010). Dietary phospholipids and intestinal cholesterol absorption. *Nutrients* 2: 116–127.
- Contarini, G., Pelizzola, V., Scurati, S., and Povolola, M. (2017). Polar lipid of donkey milk fat: Phospholipid, ceramide and cholesterol composition. *J. Food Compos. Anal.* 57: 16–23.
- Cui, L., and Decker, E.A. (2016). Phospholipids in foods: prooxidants or antioxidants? *J. Sci. Food Agric.* 96: 18–31.
- Djekic, L., Krajišnik, D., Mičić, Z., and Čalića, B. (2016). Formulation and physicochemical characterization of hydrogels with 18b-glycyrrhetic acid/phospholipid complex phytosomes. *J Drug Deliv Sci Technol* 35: 81–90.
- Duan, R.-D., and Borgström, B. (1993). Is there a specific lysophospholipase in human pancreatic juice? *Biochim Biophys Acta* 1167: 326–330.
- Dugo, P., Fawzy, N., Cichello, F., Cacciola, F., Donato, P., and Mondello, L. (2013). Stop-flow comprehensive two-dimensional liquid chromatography combined with mass spectrometric detection for phospholipid analysis. *J. Chromatogr A* 1278: 46–53.
- Farhang, B. (2013). Encapsulation of bioactive compounds in liposomes prepared with milk fat globule membrane-derived phospholipids. *Food Science. The University of Guelph, Guelph, Ontario, Canada.*
- Farhang, B., Kakuda, Y., and Corredig, M. (2012). Encapsulation of ascorbic acid in liposomes prepared with milk fat globule membrane-derived phospholipids. *Dairy Sci. & Technol.* 92: 353–366.
- Ferreiro, T., Gayoso, L., and Rodríguez-Otero, J.L. (2014). Milk phospholipids: Organic milk and milk rich in conjugated linoleic acid compared with conventional milk. *J. Dairy Sci.* 98: 9–14.
- Ferreiro, T., Martínez, S., Gayoso, L., and Rodríguez-Otero, J.L. (2016). Evolution of phospholipid contents during the production of quark cheese from buttermilk. *J. Dairy Sci.* 99: 4154–4159.
- Fletcher, K., Catchpole, O., Grey, J.B., and Pritchard, M. (2006). Beta-serum dairy products, neutral lipid-depleted and/or polar lipid-enriched dairy products, and processes for their production: US8471002. US Patent. In: USPTO (Ed.). Fonterra Co-operative Group Ltd, NZ.
- Freaga, M.S., Saleh, W.M., and Abdallah, O.Y. (2018). Self-assembled phospholipid-based phytosomal nanocarriers as promising platforms for improving oral bioavailability of the anticancer celastrol. *Int. J. Pharm.* 535: 18–26.
- Fuller, K.L., Kuhlenschmidt, T.B., Kuhlenschmidt, M.S., Jiménez-Flores, R., and Donovan, S.M. (2012). Milk fat globule membrane isolated from buttermilk or whey cream and their lipid components inhibit infectivity of rotavirus in vitro. *J. Dairy Sci.* 96: 3488–3497.
- Gallier, S., Laubscher, A., and Jiménez-Flores, R. (2014). The milk fat globule membrane: Structure, methodology for its study, and functionality. *Food structures, digestion and health Elsevier Inc.* 107–142.
- Gnananath, G. (2017). Phospholipid complex technique for superior bioavailability of phytoconstituents. *Adv Pharm Bull* 7: 35–42.
- Gulseren, I., and Corredig, M. (2013). Storage stability and physical characteristics of tea-polyphenol-bearing nanoliposomes prepared with milk fat globule membrane phospholipids. *J. Agric. Food Chem.* 61: 3242–3251.
- Gurnida, D.A., Rowan, A.M., Idjradinata, P., Muchtadi, D., and Sekarwana, N. (2012). Association of complex lipids containing gangliosides with cognitive development of 6-month-old infants. *Early Hum. Dev.* 88: 595–601.
- Gurr, M.I., Harwood, J.L., and Frayn, K.N. (2002). *Lipid biochemistry*, 5th edition. Blackwell Science, Oxford, UK.
- Haddadian, Z., Eyres, G.T., Bremer, P., and Everett, D.W. (2018). Polar lipid composition of the milk fat globule membrane in buttermilk made using various cream churning conditions or isolated from commercial samples. *Int Dairy J* 81: 138–142.
- Hickey, C.D., Diehl, B.W.K., Nuzzo, M., Millqvist-Feurby, A., Wilkinson, M.G., and Sheehana, J.J. (2017). Influence of buttermilk powder or buttermilk addition on phospholipid content, chemical and bio-chemical composition and bacterial viability in Cheddar style-cheese. *Food Res Int* 102: 748–758.
- Higurashi, S., Haruta-Ono, Y., Urazono, H., Kobayashi, T., and Kadooka, Y. (2015). Improvement of skin condition by oral supplementation with sphingomyelin-containing milk phospholipids in a double-blind, placebo-controlled, randomized trial. *J. Dairy Sci.* 98: 1–7.
- Hirabayashi, Y., and Furuya, S. (2008). Roles of l-serine and sphingolipid synthesis in brain development and neuronal survival. *Prog Lipid Res* 47: 188–203.
- Holzmüller, W., and Kulozik, U. (2016). Technical difficulties and future challenges in isolating membrane material from milk fat globules in industrial settings – A critical review. *Int Dairy J.* 61: 51–66.
- Imran, M., Revol-Junelles, A.-M., Paris, C., Guedon, E., Linder, M., and Desobry, S. (2015). Liposomal nanodelivery systems using soy and marine lecithin to encapsulate food biopreservative nisin. *LWT - Food Sci. Technol* 62: 341–349.
- Ireland, E.R. (2014). *Developing a better buttermilk solution.* Engineering Management. University of canterbury, Canterbury, New Zealand.
- Jala, R.C.R., and Kumar, C.G. (2018). Designer and functional food lipids in dietary regimes: Current trends and future prospects. *Alternative and Replacement Foods Elsevier*, 283–316.
- Jiang, X., Jin, Q., Wu, S., and Wang, X. (2016). Contribution of phospholipids to the formation of fishy off-odor and oxidative stability of soybean oil. *Eur J Lipid Sci Technol* 118: 603–611.
- Jin, H.H., Lu, Q., and Jiang, J.G. (2016). Curcumin liposomes prepared with milk fat globule membrane phospholipids and soybean lecithin. *J. Dairy Sci.* 99: 1780–1790.
- Kala, R., Samková, E., Pecová, L., Hanuš, O., Sekmokas, K., and Riukienė, D. (2018). An Overview of Determination of Milk Fat: Development, Quality Control Measures, and Application. *Acta Univ. Agric. et Silv. Mendel. Brun.* 66: 1055–1064.
- Karasawa, K., Morifuji, M., Yamaji, T., Matsuda, H., and Tanaka, A. (2017). Supplementation with fermented milk containing milk phospholipids improves the enteric environment and skin conditions of dogs with allergic skin disorders. *J Vet Sci* 3: 14–18.
- Karimi, N., Ghanbarzadeh, B., Hamishehkar, H., Keivani, F., Pezeshki, A., and Gholian, M.M. (2015). Phytosome and liposome: The beneficial encapsulation systems in drug delivery and food application. *Appl. food biotechnol* 2: 17–27.
- Keller, S., Le, H.-Y., Rödiger, C., Hipler, U.-C., Kertscher, R., Malarski, A., Hunstock, L.-M., Kiehltopf, M., Kaatz, M., Norgauer, J., and Jahres, G. (2014). Supplementation of a dairy drink enriched with milk phospholipids in patients with atopic dermatitis e A double blind, placebo-controlled, randomized, cross-over study. *Clin. Nutr.* 333: 1010–1016.
- Kielbowicz, G., Micek, P., and Wawrzęczyk, C. (2013). A new liquid chromatography method with charge aerosol detector (CAD) for the determination of phospholipid classes. Application to milk phospholipids. *Talanta* 105: 28–33.
- Konagai, C., Yanagimoto, K., Hayamizu, K., Han, L., Tsuji, T., and Koga, Y. (2013). Effects of krill oil containing n-3 polyunsaturated fatty acids in phospholipid form on human brain function: a randomized controlled trial in healthy elderly volunteers. *Clin Interv Aging* 8: 1247–57.
- Konrad, G., Kleinschmidt, T., and Lorenz, C. (2013). Ultrafiltration of whey buttermilk to obtain a phospholipid concentrate. *Int Dairy J.* 30: 39–44.
- Le, T.T., Camp, J.V., and Dewettinck, K. (2014). Milk fat globule membrane material: Isolation techniques, health-beneficial properties, and potential applications. *Studies in Natural Products Chemistry In: Attar-Rahman, F. (Ed.). Elsevier B. V., Oxford, UK, Vol. 41: 347–382.*
- Le, T.T., Phan, T.T.Q., Van Camp, J., and Dewettinck, K. (2015). Milk and dairy polar lipids: Occurrence, purification, and nutritional and technological properties. *Polar lipids.* In: Ahmad, M.U., and Xu, X. (Ed.). AOCs (Elsevier), Urbana, Illinois, USA, 91–143.
- Lecico. (2018). *Milk phospholipids.* Vol. 2018. Lecico GmbH <http://www.lecico.de/en/products/milk-phospholipids>. Accessed 24 Dec. 2018.
- Lecomte, M., Bourlieu, C., Meugnier, E., Penhoat, A., Cheillan, D., Pineau, G., Loizon, E., Trauchessec, M., Claude, M., Menard, O., Geloën, A., Laugerette, F., and Michalski, M.C. (2015). Milk polar lipids affect in vitro digestive lipolysis and postprandial lipid metabolism in mice. *J. Nutr.* 145: 1770–1777.
- Levin, M.A., Burrington, K.J., and Hartel, R.W. (2016). Whey protein phospholipid concentrate and delactosed permeate: Applications in caramel, ice cream, and cake. *J. Dairy Sci.* 99: 6948–6960.
- Li, Z. (2014). Encapsulation of bioactive Salmon protein hydrolysates with chitosan-coated liposomes. Master of Science. Dalhousie University,

- Halifax, Nova Scotia.
- Liu, H., Logan, A., Cocks, B.G., and Rochfort, S. (2017). Seasonal variation of polar lipid content in bovine milk. *Food Chem.* 237: 865–869.
- Liu, H., Radlowski, E.C., Conrad, M.S., Li, Y., Dilger, R.N., and Johnson, R.W. (2014). Early supplementation of phospholipids and gangliosides affects brain and cognitive development in neonatal piglets. *J Nutr* 144: 1903–1909.
- Liu, W., Ye, A., Liu, C., Liu, W., and Singh, H. (2012). Structure and integrity of liposomes prepared from milk- or soybean-derived phospholipids during in vitro digestion. *Food Res Int* 48: 499–506.
- Liu, W., Ye, A., Liu, W., Liu, C., and Singh, H. (2013). Stability during in vitro digestion of lactoferrin-loaded liposomes prepared from milk fat globule membrane-derived phospholipids. *J. Dairy Sci.* 96: 2061–2070.
- Liu, Z., Moateb, P., Cocks, B., and Rochfort, S. (2015). Comprehensive polar lipid identification and quantification in milk by liquid chromatography-mass spectrometry. *J Chromatogr B* 978-979: 95–102.
- Livney, Y.D., Ruimy, E., Ye, A.M., Zhu, X., and Singh, H. (2016). A milkfat globule membrane-inspired approach for encapsulation of emulsion oil droplets. *Food Hydrocoll* 2016: 1–9.
- Lopez, C., Blot, M., Briard-Bion, V., Cirie, C., and Graulet, B. (2017). Butter serums and buttermilks as sources of bioactive lipids from the milk fat globule membrane: Differences in their lipid composition and potentialities of cow diet to increase n-3 PUFA. *Food Res Int* 100: 864–872.
- Lopez, C., Briard-Bion, V., and Ménard, O. (2014). Polar lipids, sphingomyelin and long-chain unsaturated fatty acids from the milk fat globule membrane are increased in milks produced by cows fed fresh pasture based diet during spring. *Food Res Int* 58: 59–68.
- Lopez, C., Briard-Bion, V., Menard, O., Rousseau, F., Pradel, P., and Besle, J.-m. (2008). Phospholipid, sphingolipid, and fatty acid compositions of the milk fat globule membrane are modified by diet. *J. Agric. Food Chem.* 56: 5226–5236.
- Lu, H.F.S. (2013). Physico-chemical properties, oxidative stability and non-enzymatic browning in marine phospholipid emulsions and their use in food applications. Søborg: DTU Food National Food Institute, Denmark.
- Lu, J., Argov-Argama, N., Anggrek, J., Boeren, S., Hooijdonk, T.v., Vervoort, J., and Hetting, K.A. (2016). The protein and lipid composition of the membrane of milk fat globules depends on their size. *J. Dairy Sci.* 99: 4726–4738.
- Lu, M., Qiu, Q., Luo, X., Liu, X., Sun, J., Wang, C., Lin, X., Deng, Y., and Song, Y. (2018). Phyto-phospholipid complexes (phytosomes): A novel strategy to improve the bioavailability of active constituents. *Asian J. Pharm.* 000: 1–10.
- Luo, J., Huang, Z., Liu, H., Zhang, Y., and Ren, F. (2018). Yak milk fat globules from the Qinghai-Tibetan Plateau: Membrane lipid composition and morphological properties. *Food Chem.* 245: 731–737.
- Ma, L., MacGibbon, A.K.H., Mohamed, H.J.B.J., Loy, S., Rowan, A., McJarow, P., and Fong, B.Y. (2017). Determination of phospholipid concentrations in breast milk and serum using a high performance liquid chromatography-mass spectrometry multiple reaction monitoring method. *Int Dairy J.* 71: 50–59.
- Mao, C., and Obeid, L.M. (2008). Ceramidases: regulators of cellular responses mediated by ceramide, sphingosine, and sphingosine-1-phosphate. *Biochim Biophys Acta* 1781: 424–434.
- Maswadeh, H.M., Aljarbou, A.N., Alorainy, M.S., Alsharidah, M.S., and Khan, M.A. (2015). Etoposide incorporated into camel milk phospholipids liposomes shows increased activity against fibrosarcoma in a mouse model. *BioMed Res Int* 2015: 1–11.
- Morifuji, M., Obaa, C., Ichikawa, S., Ito, K., Kawahataa, K., Asamia, Y., Ikegami, S., Itoh, H., and Sugawarab, T. (2015). A novel mechanism for improvement of dry skin by dietary milk phospholipids: Effect on epidermal covalently bound ceramides and skin inflammation in hairless mice. *J Dermatol Sci* 78: 224–231.
- Moukarzel, S. (2016). The complexity of understanding human milk components and infant brain development. Human Nutrition. University of British Columbia, Vancouver, Canada.
- Moukarzel, S., Dyer, R.A., Garcia, C., Wiedeman, A.M., Boyce, G., Weinberg, J., Keller, B.O., Elango, R., and Innis, S.M. (2018). Milk fat globule membrane supplementation in formula-fed rat pups improves reflex development and may alter brain lipid composition. *Sci Rep* 8: 15277.
- Nejrurp, R.G., Licht, T.R., and Hellgren, L.I. (2017). Fatty acid composition and phospholipid types used in infant formulas modifies the establishment of human gut bacteria in germ-free mice. *Sci Rep* 7: 3975.
- Nilsson, Å., and Duan, R.-D. (2019). Pancreatic and mucosal enzymes in choline phospholipid digestion. *Am J Physiol Gastrointest Liver Physiol* 316: G425–G445.
- Norris, G.H., Jiang, C., Ryan, J., Porter, C.M., and Blesso, C.N. (2016). Milk sphingomyelin improves lipid metabolism and alters gut microbiota in high fat diet-fed mice. *J Nutr Biochem* 30: 93–101.
- Norris, G.H., Porter, C.M., Christina Jiang, C.L., and Millar, C.N.B. (2017). Dietary sphingomyelin attenuates hepatic steatosis and adipose tissue inflammation in high-fat-diet-induced obese mice. *J. Nutr. Biochem.* 40: 36–43.
- Ortega-Anaya, J., and Jimenez-Flores, R. (2018). Symposium review: The relevance of bovine milk phospholipids in human nutrition—Evidence of the effect on infant gut and brain development. *J. Dairy Sci.* 102: 1–11.
- Pimentel, L., Gomes, A., Pintado, M., and Rodríguez-Alcalá, L.M. (2016). Isolation and Analysis of Phospholipids in Dairy Foods. *J Anal Methods Chem* 2016.
- Price, N., Fei, T., Clark, S., and Wang, T. (2018). Extraction of phospholipids from a dairy by-product (whey protein phospholipid concentrate) using ethanol. *J. Dairy Sci.* 101: 8778–8787.
- Reddan, J.M., White, D.J., Macpherson, H., Scholey, A., and Pipingas, A. (2018). Glycerophospholipid supplementation as a potential intervention for supporting cerebral structure in older adults. *Front. Aging Neurosci.* 10: 1–26.
- Rodríguez-Alcalá, L.M., Castro-Gomez, P., Felipe, X., Noriega, L., and Fontecha, J. (2015). Effect of processing of cowmilk by high pressures under conditions up to 900 MPa on the composition of neutral, polar lipids and fatty acids. *LWT - Food Sci. Technol* 62: 265–270.
- Schipper, L., van Dijk, G., Broersen, L.M., Loos, M., Bartke, N., Scheurink, A.J., and van der Beek, E.M. (2016). A postnatal diet containing phospholipids, processed to yield large, phospholipid-coated lipid droplets, affects specific cognitive behaviors in healthy male mice. *J Nutr* 146: 1155–1161.
- Scholey, A.B., Camfield, D.A., Hughes, M.E., Woods, W., Stough, C.K.K., White, D.J., Gondalia, S.V., and Frederiksen, P.D. (2013). A randomized controlled trial investigating the neurocognitive effects of Lacprodan® PL-20, a phospholipid-rich milk protein concentrate, in elderly participants with age-associated memory impairment: the phospholipid intervention for cognitive ageing reversal (PLICAR): study protocol for a randomized controlled trial. *Trials* 14: 404.
- Shrestha, P., Davis, D.A., Veeranna, R.P., Carey, R.F., Viollet, C., and Yarchoan, R. (2017). Hypoxia-inducible factor-1 alpha as a therapeutic target for primary effusion lymphoma. *PLoS Pathogens* 13: 1–20.
- Shulman, A., Zuabi, R., Dror, G.B., Twito, Y., Pelled, D., and Herzog, Y. (2011). Polar lipid mixtures, their preparation and uses. US Patent. In: USPTO (Ed.). Enzymotec Ltd, IL, Vol. US9814252.
- Siegel, A.B., Narayan, R., Rodriguez, R., Goyal, A., Jacobson, J.S., Kelly, K., Ladas, E., Lunghofer, P.J., Hansen, R.J., Gustafson, D.L., Flaig, T.W., Tsai, W.Y., Wu, D.P., Lee, V., and Greenlee, H. (2014). A phase I dose-finding study of silybin phosphatidylcholine (milk thistle) in patients with advanced hepatocellular carcinoma. *Integr Cancer Ther* 13: 46–53.
- Smithers, G.W., and Augustin, M.A. (2013). Advances in dairy ingredients. John Wiley & Sons, Inc. and the Institute of Food Technologists, Iowa, USA.
- Snow, D.R., Ward, R.E., Olsen, A., Jimenez-Flores, R., and Hintze, K.J. (2010). Membrane-rich milk fat diet provides protection against gastrointestinal leakiness in mice treated with lipopolysaccharide. *J. Dairy Sci.* 94: 2201–2212.
- Sokol, E., Ulven, T., Faergeman, N.J., and Ejsing, C.S. (2015). Comprehensive and quantitative profiling of lipid species in human milk, cow milk and a phospholipid-enriched milk formula by GC and MS/MS(ALL). *Eur J Lipid Sci Technol* 117: 751–759.
- Tanaka, K., Hosozawa, M., Kudo, N., Yoshikawa, N., Hisata, K., Shoji, H., Shinohara, K., and Shimizu, T. (2013). The pilot study: Sphingomyelin-fortified milk has a positive association with the neurobehavioural

- development of very low birth weight infants during infancy, randomized control trial. *Brain Dev.* 35: 45–52.
- Tatua. (2018). Specialty nutritional ingredients: Phospholipid. Vol. 2018. Tatua Co-operative Dairy Company, <https://www.tatua.com/specialty-nutritionals-ingredients/phospholipids/>. Accessed 24 Dec. 2018.
- Telange, D.R., Patil, A.T., Pethe, A.M., Fegade, H., Anand, S., and Dave, V.S. (2017). Formulation and characterization of an apigenin-phospholipid phytosome (APLC) for improved solubility, in vivo bioavailability, and antioxidant potential. *Eur J Lipid Sci Technol* 108: 36–49.
- Thompson, A.K. (2005). Structure and properties of liposomes prepared from milk phospholipids Massey. University, Palmerston North, New Zealand.
- Timby, N., Domellof, E., Hernell, O., Lonnerdal, B., and Domellof, M. (2014). Neurodevelopment, nutrition, and growth until 12 mo of age in infants fed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: A randomized controlled trial. *Am. J. Clin. Nutr.* 99: 860–868.
- Tomé-Carneiro, J., M, C.C., Burgos-Ramos, E., Tomas-Zapico, C., Garcia-Serrano, A., Castro-Gomez, P., Venero, C., Pereda-Perez, I., Baliyan, S., Valencia, A., Fontecha, J., Davalos, A., and Visioli, F. (2018). Buttermilk and krill oil phospholipids improve hippocampal insulin resistance and synaptic signaling in aged rats. *Mol. Neurobiol.* 55: 7285–7296.
- Torkamani, A.E., Juliano, P., Fagan, P., Jimenez-Flores, R., Ajlouni, S., and Singh, T.K. (2016). Effect of ultrasound-enhanced fat separation on whey powder phospholipid composition and stability. *J Dairy Sci* 99: 4169–4177.
- USDA. (2017). “Dietary guidelines for Americans 2015–2020. 8th Edition,” U.S. Department of Health and Human Services and U.S. Department of Agriculture, <https://health.gov/dietaryguidelines/2015/guidelines/> Accessed Jan 14 2019.
- Venuti, E., Shishmarev, D., Kuchel, P.W., Dutt, S., Blumenthal, C.S., and Gaskin, K.J. (2017). Bile salt stimulated lipase: Inhibition by phospholipids and relief by phospholipase A2. *J Cyst Fibros* 16: 763–770.
- Verardo, V., Gomez-Caravaca, A.M., Arraez-Roman, D., and Hettinga, K. (2017). Recent advances in phospholipids from colostrum, milk and dairy by-products. *Int. J. Mol. Sci.* 18: 1–23.
- Vilamarim, R., Bernardo, J., Videira, R.A., Valentão, P., Veiga, F., and Andrade, P.B. (2018). An egg yolk’s phospholipid-pennyroyal nootropic nanoformulation modulates monoamine oxidase-A (MAO-A) activity in SH-SY5Y neuronal model. *J Funct Foods* 46: 335–344.
- Walczak, J., Pomastowski, P., Bocian, S., and Buszewski, B. (2016). Determination of phospholipids in milk using a new phosphodiester stationary phase by liquid chromatography-matrix assisted desorption ionization mass spectrometry. *J Chromatogr A* 1432: 39–48.
- Weiland, A., Bub, A., Barth, S.W., Schrezenmeier, J., and Pfeuffer, M. (2016). Effects of dietary milk- and soya-phospholipids on lipid-parameters and other risk indicators for cardiovascular diseases in overweight or obese men – two double-blind, randomised, controlled, clinical trials. *J Nutr Sci* 5: 1–9.
- Winrow, C.J., Hemming, M.L., Allen, D.M., Quistad, G.B., Casida, J.E., and Barlow, C. (2003). Loss of neuropathy target esterase in mice links organophosphate exposure to hyperactivity. *Nature genetics* 33: 477.
- Wu, K., Gao, R., Tian, F., Mao, Y., Wang, B., Zhou, L., Shen, L., Guan, Y., and Cai, M. (2019). Fatty acid positional distribution (sn-2 fatty acids) and phospholipid composition in Chinese breast milk from colostrum to mature stage. *Br. J. Nutr.* 121: 65–73.
- Xu, S., Walkling-Ribeiro, M., Griffiths, M.W., and Corredig, M. (2015). Pulsed electric field processing preserves the antiproliferative activity of the milk fat globule membrane on colon carcinoma cells. *J. Dairy Sci.* 98: 2867–74.
- Yu, F., Li, Y., Chen, Q., He, Y., Wang, H., Yang, L., Guo, S., Meng, Z., Cui, J., Xue, M., and Chen, X.D. (2016). Monodisperse microparticles loaded with the self-assembled berberine-phospholipid complex-based phytosomes for improving oral bioavailability and enhancing hypoglycemic efficiency. *Eur. J. Pharm. Biopharm.* 103: 136–148.
- Zhao, L., Du, M., Gao, J., Zhan, B., and Mao, X. (2019). Label-free quantitative proteomic analysis of milk fat globule membrane proteins of yak and cow and identification of proteins associated with glucose and lipid metabolism. *Food Chem.* 275: 59–68.
- Zheng, H., Jiménez-Flores, R., and Everett, D.W. (2014). Lateral lipid organization of the bovine milk fat globule membrane is revealed by washing processes. *J. Dairy Sci.* 97: 5964–5974.
- Zou, X., Guo, Z., Jin, Q., Huang, J., Cheong, L., Xu, X., and Wang, X. (2015). Composition and microstructure of colostrum and mature bovine milk fat globule membrane. *Food Chem.* 185: 362–370.