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Review

Properties, analysis and purification of milk polar lipids

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Abstract

The phospholipids and sphingolipids in milk are gaining interest due to their nutritional and technological qualities. Sphingolipids and their derivatives are highly bioactive compounds with anti-cancer, bacteriostatic and cholesterol-lowering properties. Several low-value process streams of the dairy industry contain considerable amounts of polar lipid and exert potential for further purification. This review deals with the structure of the main dairy polar lipids, their origin and molecular arrangement, their occurrence in raw milk and other dairy products, their methodology of analysis and purification and their nutritional and technological properties.

Keywords: Polar lipids; Sphingolipids; Phospholipids; Milk fat globule membrane; Milk

Contents

1. Introduction

Polar lipids are the main constituents of natural membranes, occurring in all living organisms. As such, different polar lipid species and concentrations are present in derived food products. Polar lipids from mainly vegetable origin (lecithin) are widely used for their emulsifying and structural improving properties in food matrices. Nowadays, polar lipids derived from animal sources are gaining attention as they are rich in sphingolipids, highly bioactive compounds that have profound effects on cell metabolism and regulation. Several animal sources like brain and marrow are rich in polar lipids, though their use is legally restricted due to possible transmission of prions, which are involved in diseases like

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bovine spongiform encephalitis, scrapie and Creutzfeld-Jacob. This makes milk products an interesting alternative as a source of animal polar lipids. This article reviews the structure, origin and occurrence of polar lipids in dairy products, their biochemical and nutritional relevance, the analysis and purification methodology and technological functionalities of purified fractions.

2. Structure of polar lipids

Phospholipids and sphingolipids (= polar lipids) are amphiphilic molecules with a hydrophobic tail and a hydrophilic head group. The glycerophospholipids consist of a glycerol backbone on which two fatty acids are esterified on positions sn-1 and sn-2. These fatty acids are more unsaturated than the triglyceride fraction of milk. On the third hydroxyl, a phosphate residue with different organic groups (choline, serine, ethanolamine, etc.) may be linked. Generally, the fatty acid chain on the sn-1 position is more saturated compared with that at the sn-2 position. Lysophospholipids contain only one acyl group, predominantly situated at the sn-1 position. The head group remains similar.

The characteristic structural unit of sphingolipids is the sphingoid base, a long-chain (12–22 carbon atoms) aliphatic amine containing two or three hydroxyl groups. Sphingosine (d18:1), is the most prevalent sphingoid base in mammalian sphingolipids, containing 18 carbon atoms, two hydroxyl groups and one double bond. A ceramide is formed when the amino group of this sphingoid base is linked with, usually, a saturated fatty acid. On this ceramide unit, an organophosphate group can be bound to form a sphingophospholipid (e.g., phosphocholine in the case of sphingomyelin) or a saccharide to form the sphingoglycolipids (glycosylceramides). Monoglycosylceramides, like glucosylceramide or galactosylceramide are often denoted as cerebrosides while tri- and tetraglycosylceramides with a terminal galactosamine residue are denoted as globosides. Finally, gangliosides are highly complex oligoglycosylceramides, containing one or more sialic acid groups in addition to glucose, galactose and galactosamine. (Christie, 2003; Newburg & Chaturvedi, 1992; Pfeuffer & Schrezenmeir, 2001; Vanhoutte, Rombaut, Van Der Meeren, & Dewettinck, 2004; Vesper et al., 1999; Yang, Yu, Sun, & Duerksen-Hughes, 2004).

3. Origin of milk polar lipids

The polar lipids in milk are mainly situated in the milk fat globule membrane (MFGM). This is a highly complex biological membrane that surrounds the fat globule, hereby stabilizing it in the continuous phase of the milk, and preventing it from enzymatic degradation by lipases (Danthine, Blecker, Paquot, Innocente, & Deroanne, 2000).

In the secretory cells of the mammary gland, small fat globules originate in the endoplasmatic reticulum as intracellular precursors. Upon release into and transport through the cytoplasm, these fat globule precursors are covered with a monolayer of proteins and polar lipids, and they can coalesce to form larger droplets. The most widely accepted mechanism of milk fat globule secretion is the 'budding' of lipid globules into the alveolar lumen. When the intracellular fat droplets arrive at the apical pole of the secretory cell, they are progressively enveloped by the secretory cell's plasma membrane, which is a true bilayer of polar lipids and proteins. Upon closure of this plasma membrane, the fat globule is released in the alveolar lumen. Cytoplasmatic material can be entrained between the inner coat and the outer double membrane layer resulting in 'cytoplasmatic crescents' (Fox & McSweeney, 1998; Keenan, Dylewski, & Woodford, 1983; Keenan, Mather, & Dylewski, 1988; Mather & Keenan, 1998; Mcpherson & Kitchen, 1983). Hence, as viewed from the lipid core outwards, there is an inner monolayer of polar lipids and proteins surrounding the intracellular fat droplet, an electron dense proteinaceous coat located on the inner face of the bilayer membrane and finally a true bilayer membrane of polar lipids and proteins (Danthine et al., 2000; Evers, 2004; Michalski, Michel, Sainmont, & Briard, 2002).

The membrane consists roughly of 60% protein and 40% of lipids (Fox & McSweeney, 1998; Keenan et al., 1988). Numerous proteins are associated with the MFGM and are arranged asymmetrically. Some are exposed on the inner face (e.g., xanthin oxidase), some at the outer part of the membrane (e.g., Periodic acid/Schiff 6/7). Others are transmembrane proteins (e.g. butyrophilin) (Danthine et al., 2000; Evers, 2004). For an extensive review of the MFGM proteins, the reader is referred to the article of Mather (2000). The lipids of the MFGM are mainly triaglycerides, cholesterol, phospholipids and sphingolipids in varying proportions (Fox & McSweeney, 1998; Keenan et al., 1983). The lipids are, like the proteins, asymmetrically arranged. The choline-containing phospholipids, phosphatidylcholine (PC) and sphingomyelin (SM), and the glycolipids, cerebrosides and gangliosides, are largely located on the outside, while phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) are concentrated on the inner surface of the membrane (Deeth, 1997).

After milk secretion and milking, compositional and structural changes in the MFGM occur, and membrane material is shed into the skimmed milk phase. Factors like temperature, age, bacteriological quality, stage of lactation and season can influence these changes (Evers, 2004). As such, milk also contains membrane material in the serum phase. This material, which sediments upon centrifugation, comprises only 4% of total milk lipids and has a similar composition to the MFGM. Apart from MFGM fragments, lipoprotein particles, cells and cell fragments from the mammary secretory gland are also associated with this sedimentable fraction (Deeth, 1997; Keenan et al., 1988).

Table 1
Polar lipid content and relative phospholipid and sphingolipid content of raw milk

Reference	Polar lipids (mg 100 g^{-1} product)	Relative sphingolipid content (g 100 g^{-1} polar lipid)			Relative phospholipid content (g 100 g^{-1} polar lipid)				Method
		GluCer	LacCer	SM	PE	PS	PI	PC	
Christie et al. (1987)	23[a]	5.0	2.9	23.6	34.2	2.8	6.2	25.4	HPLC-ELSD
Rombaut et al. (2005)	29	2.7	6.7	18.0	42.0	6.7	4.8	19.2	HPLC-ELSD
Rombaut et al. (2006)	40	2.1	2.8	23.8	31.5	8.8	4.9	26.0	HPLC-ELSD
Bitman and Wood (1990)	20.4[a,b]	—	—	28.7	31.0	8.1	4.1	28.0	TLC-Densitometry
Bitman and Wood (1990)	35.5[a,c]	—	—	34.1	27.8	8.4	4.6	25.1	TLC-Densitometry
Bitman and Wood (1990)	25.1[a,d]	—	—	28.7	31.1	8.5	5.2	26.4	TLC-Densitometry
Bitman and Wood (1990)	12.8[a,e]	—	—	31.4	19.8	1.9	11.8	35.1	TLC-Densitometry
Avalli and Contarini (2005)	9.4	—	—	20.5	32.3	10.5	9.3	27.3	HPLC-ELSD
Fagan and Wijesundera (2004)	24.2[a]	—	—	29.2	38.6	—	—	32.2	HPLC-ELSD
Souci et al. (2000)	35	—	—	28.0	31.1	3.1	0.6	37.3	—
Weihrauch and Son (1983)	34	—	—	26.5	29.4	2.9	5.9	35.3	—
Zeisel et al. (1986)	18.8[a,f]	—	—	—	—	—	—	—	Choline assay

[a]A conversion factor of 1 g mL^{-1} was used.
[b]At lactation day 3.
[c]At lactation day 7.
[d]At lactation day 42.
[e]At lactation day 180.
[f]Conversion factors of 751 and 770 g mol^{-1} were used for, respectively, SM and PC.

4. Occurrence of polar lipids in dairy products

The polar lipid content of raw milk is reported to range between 9.4 and 35.5 mg 100 g^{-1} (Table 1). The major milk phospholipids are PE (19.8–42.0%, w/w), PC (19.2–37.3%, w/w), PS (1.9–10.5%, w/w) and PI (0.6–11.8%, w/w). The major milk sphingolipids are glucosylceramide (GluCer) (2.1–5.0%, w/w), lactosylceramide (LacCer) (2.8–6.7%, w/w) and SM (18.0–34.1%, w/w) (Avalli & Contarini, 2005; Bitman & Wood, 1990; Christie, Noble, & Davies, 1987; Fagan & Wijesundera, 2004; Rombaut, Van Camp, & Dewettinck, 2005, 2006; Souci, Fachmann, Kraut, Scherz, & Senser, 2000; Weihrauch & Son, 1983; Zeisel, Char, & Sheard, 1986). Newburg and Chaturvedi (1992) gave a more detailed glycosphingolipid composition of bovine milk. Their reported values of 0.67 mg GluCer and 1.71 mg LacCer 100 g^{-1} raw milk (using a conversion factor of 713 and 960 g mol^{-1}, respectively) correspond very well with those given in Table 1. Different gangliosides are present in bovine milk, albeit some in trace amounts. The ganglioside content of bovine milk, of which monosialoganglioside 3 (GM_3) and disialoganglioside 3 (GD_3) are the predominant ones, varies between 0.14 and 1.10 mg 100 mL^{-1} (Laegreid, Otnaess, & Fuglesang, 1986; Pan & Izumi, 2000). For more details on structure, naming, function and occurrence, the reader is referred to the reviews of Rueda, Maldonaldo, Narbona, and Gil (1998) and Jensen (2002). Variations in the polar lipid content of raw milk can be ascribed to differences in the method of preparation and analysis, and to environmental factors such as breed of the animal, stage of lactation, season of the year, age, feeding of the cow and treatment of the milk (Bitman & Wood, 1990; Christie et al., 1987; Keenan et al., 1988; Puente, GarciaPardo, Rueda, Gil, & Hueso, 1996).

In Table 2, the polar lipid and sphingolipid content is given for various dairy products. Values of polar lipids, either expressed on product, dry matter or total lipid base, differ markedly amongst different products. Even in each product category, a high variation is noticed, which could be ascribed to the different methods of analysis, processing and variable composition of the starting materials. Fat rich products like cream, butter and cheese have a polar lipid content of less than 1%, w/w, of total lipids, while this is much higher for products like skimmed milk, buttermilk, butterserum (= the aqueous phase of butter) and whey. These variations are no doubt due to a fractionation of polar and neutral lipids upon processing. For example, mechanical treatments like heating (Kim & Jimenez-Flores, 1995; Lee & Sherbon, 2002; Ye, Singh, Taylor, & Anema, 2002), homogenization (CanoRuiz & Richter, 1997), aeration and agitation (Evers, 2004) seriously enhance MFGM release into the serum phase. Upon destabilization of the fat globule, like in churning, the membrane fraction is recovered in the buttermilk. Relative differences in polar lipid species are noticed; however, it is incorrect to attribute this solely to processing, as raw milk relative polar lipid proportion is already far from constant (Table 1). Rombaut et al. (2006), however, monitored a complete commercial line of raw milk processed into skimmed milk, cream, butter, buttermilk, buttermilk cheese, acid whey, anhydrous milk fat and butterserum on polar lipids and sphingolipids. Corresponding mass balances were calculated. They found that the polar lipids are preferentially enriched in aqueous phases like skimmed milk, buttermilk

Table 2
Polar lipid content of different dairy products

Sample	Polar lipids			Sphingolipids (mg 100 g^{-1} product)	Method	Reference
	(mg 100 g^{-1} product)	(g 100 g^{-1} dry matter)	(g 100 g^{-1} total lipids)			
Butter	159.32	—	0.20	27.24	HPLC-ELSD	Avalli and Contarini (2005)
Butter	181	0.22	0.22	65	HPLC-ELSD	Rombaut et al. (2005)
Butter	230	0.26	0.27	71	HPLC-ELSD	Rombaut et al. (2006)
Buttermilk[a]	71.84	—	—	18.03	HPLC-ELSD	Christie et al. (1987)
Buttermilk	8.97	—	4.49	1.64	HPLC-ELSD	Avalli and Contarini (2005)
Buttermilk	91	1.15	21.85	19	HPLC-ELSD	Rombaut et al. (2005)
Buttermilk (acid)	160	2.03	33.05	31	HPLC-ELSD	Rombaut et al. (2006)
Buttermilk (reconstituted)	130	1.44	21.66	—	Phosphorous assay	Sachdeva and Buchheim (1997)
Buttermilk Quarg	310	1.86	29.06	74	HPLC-ELSD	Rombaut et al. (2006)
Buttermilk whey	100	1.84	23.66	19	HPLC-ELSD	Rombaut et al. (2006)
Buttermilk whey (rennet)	104	1.55	—	—	Phosphorous assay	Sachdeva and Buchheim (1997)
Buttermilk whey (citric acid)	98	1.52	—	—	Phosphorous assay	Sachdeva and Buchheim (1997)
Butterserum[a,b]	660	—	14.8	—	Phosphorous assay	Mcpherson and Kitchen (1981)
Butterserum[b]	1250	11.54	48.39	379	HPLC-ELSD	Rombaut et al. (2006)
Cheddar	153	0.25	0.47	39	HPLC-ELSD	Rombaut et al. (2005)
Cottage cheese	376	—	5.30	139	—	Weihrauch and Son (1983)
Cream	139	0.31	0.35	32	HPLC-ELSD	Rombaut et al. (2005)
Cream	190	0.4	0.45	49	HPLC-ELSD	Rombaut et al. (2006)
Cream (centrifuged)	95.76	—	0.53	19.54	HPLC-ELSD	Avalli and Contarini (2005)
Cream (natural)	189.20	—	0.86	54.11	HPLC-ELSD	Avalli and Contarini (2005)
Quarg	32	0.25	24.66	10	HPLC-ELSD	Rombaut et al. (2005)
Skimmed milk[a]	9.06	—	—	2.65	HPLC-ELSD	Christie et al. (1987)
Skimmed milk	20	0.28	19.06	6	HPLC-ELSD	Rombaut et al. (2006)
Skimmed milk powder	—	—	—	14.4[c]	HPLC of Sphingoid bases	Ahn and Schroeder (2002)
Swiss cheese	—	—	—	8.42[c]	HPLC of Sphingoid bases	Ahn and Schroeder (2002)
Whey (Cheddar)	18	0.26	5.32	5	HPLC-ELSD	Rombaut et al. (2005)
Whey (Emmenthal)[a]	14	0.22	21.88	4	HPLC-UV	Baumy et al. (1990)
Whey (Emmenthal)[a]	22	0.33	45.21	3	HPLC-ELSD	Theodet and Gandemer (1994)
Whole milk[a]	14.48	—	—	4.89	HPLC-ELSD	Christie et al. (1987)
Yoghurt	—	—	—	2.81[c]	HPLC of Sphingoid bases	Ahn and Schroeder (2002)

[a]A conversion factor of 1 g mL^{-1} was used.
[b]The aqueous phase of butter.
[c]A conversion factor of 751 g mol^{-1} was used.

and butterserum. The latter fraction represented 28.4% of the original raw milk polar lipids, whilst only representing 0.9% of the original raw milk mass. Significant differences in relative sphingolipid proportions were observed. Mainly cream, butter and butterserum were relatively enriched in sphingolipids, a phenomenon which is probably related to the asymmetric composition of the MFGM. They concluded that butterserum, with its high polar lipid and sphingolipid content on a dry matter and total lipid base was the most promising raw material for further purification.

5. Analysis of polar lipids

Critical points in the analysis of polar lipids are the method of extraction, separation and detection. Often, little attention is given to the first. Standard methods of lipid extraction of dairy products (e.g., Röse–Gottlieb)

which make use of alkali or acid in combination with heat, can lead to oxidation and hydrolysis of PL. Therefore, cold-extraction procedures like those of Folch, Lees, and Stanley (1956) or Bligh and Dyer (1959) using chloroform–methanol, or like Hara and Radin (1978), using a less toxic mixture of hexane-isopropanol, should be applied. The combination of an apolar solvent with a polar counterpart, preferably an alcohol, is advantageous. The latter is indispensable for the complete release of polar lipids out of their complex MFGM matrix. It acts by dehydration and denaturation of the proteins, and by degradation of the hydrogen bonds between the lipid and protein complexes. The disadvantage is, however, that coextraction of non-lipid contaminants can occur, which can give rise to erroneous results, for example, when calculating the gravimetric difference after evaporation of the solvent, or when measuring total inorganic phosphorous. Evaluations, modifications and alternatives of several methods for the extraction of polar lipids were done by Theodet and Gandemer (1991), Shaikh (1994), Vaghela and Kilara (1995a) and Heitmann, Lissel, Kempken, and Muthing (1996). Prior to analysis, a preconcentration step is often performed using open column chromatography or small solid-phase extraction (SPE) cartridges. The variable and incomplete recovery of these purification methods, however, demands the use of a chemically similar internal standard upon implementation.

After the extraction and possible purification step, various methods for analysis are possible. The total phosphorous of the extract can be measured colorimetrically by formation of blue molybdenum complexes before or after digestion of the matrix (Vandermeeren, Vanderdeelen, & Baert, 1988). Alternatives of total phosphorous content are the use of atomic absorption spectrometry, inductively coupled plasma and fourier transformed infrared spectroscopy. Milk products are, however, rich in organic and inorganic compounds which can be coextracted upon the application of polar solvents, giving rise to serious overestimations of phospholipids. Other disadvantages are that this methodology gives no information of the relative polar lipid classes, and non-phosphorous polar lipids like glycosphingolipids are not taken into account.

Techniques such as thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) do give information concerning the polar lipid classes. TLC is flexible, easy to use, quick and a rather inexpensive method. All types of mobile phases can be used in multiple dimensions, even those that are not compatible with HPLC. Several specific staining sprays exist, which allow identification of unknown lipids. By densitometric scanning after visualization, quantitative results can be obtained, albeit less precise than other methods. For quantification purposes, the method should also be scrupulously validated and standardized, because the staining intensity is time- and matrix-dependent, and saturation can occur very quickly, depending on the polar lipid species. Therefore, TLC is principally used for qualitative and semi-quantitative purposes. Excellent TLC separations of phospholipids and numerous glycosphingolipids can be obtained with, respectively, the methods of Leray, Pelletier, Hemmendinger, and Cazenave (1987) and Dreyfus, Guerold, Freysz, and Hicks (1997).

Numerous HPLC methods are described for the separation of polar lipids of vegetable origin. Nevertheless, these methods are less applicable to dairy (and animal) products, as PS, which is not present in standard lecithin mixes, is poorly separated from other compounds. These methods also do not consider the presence of sphingolipids. Most of the recent chromatographic methods used for the separation of dairy polar lipids are based on the method of Becart, Chevalier, and Biesse (1990), using a buffer at pH >7, with an alkali modifier like triethylamine or ammonium hydroxide on a plain silica column (Avalli & Contarini, 2005; Vaghela & Kilara, 1995b). Although enabling an excellent separation of dairy polar lipid classes, the high pH quickly dissolves the silica packing of the column, hereby seriously reducing column life. A modification of this method was published by Rombaut et al. (2005). Using a gradient of chloroform, methanol and a formic acid–triethylamine buffer at pH 3, GluCer, LacCer, PE, PI, PS, PC and SM were resolved in less than 21 min, while column life was extended to over 1500 runs. This is illustrated in Fig. 1, where a chromatogram of butterserum polar lipids is given. The double peak of SM is caused by the absence or presence of an extra hydroxyl group. For

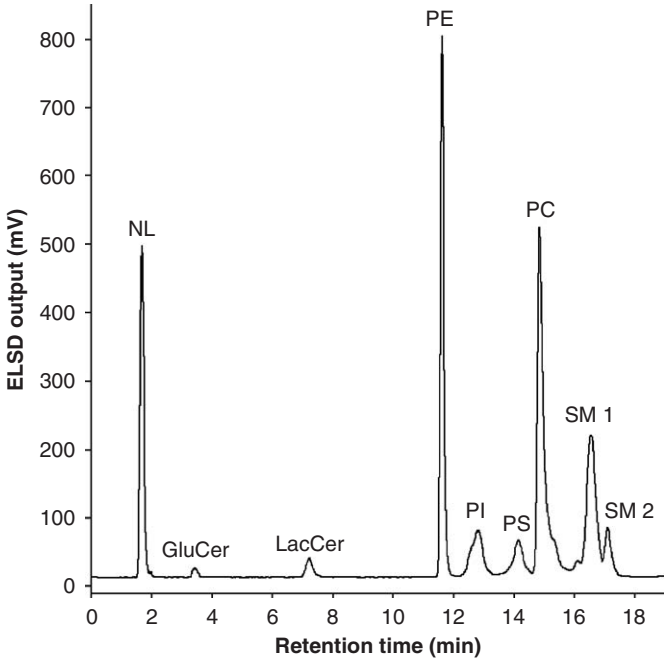

Fig. 1. Chromatogram of butterserum polar lipids obtained by the method of Rombaut et al. (2005). NL: neutral lipids; GluCer: glucosylceramide; LacCer: lactosylceramide; PE; phosphatidylethanolamine; PI: phosphatidylinositol, PS; phosphatidylserine; PC; phosphatidylcholine and SM: sphingomyelin. The double peak of SM is caused by the absence or presence of an extra hydroxyl group.

the specific quantification of sphingolipids in dairy products, Ahn and Schroeder (2002) liberated the sphingoid bases by acid hydrolysis before analysis by HPLC as described by Merrill et al. (1988).

For the chromatographic analysis of fats and oils, the use of an evaporative light scattering detector (ELSD) is generally preferred. The ELSD is an universal, mass sensitive detector, compatible with a broad range of solvents, it allows gradient elution (unlike the refractive index detector), and the signal is virtually independent on the degree of saturation and chain length of an acyl chain (unlike the UV detector). In this type of detector, the solvent and analyte is atomized in a heated tube with the aid of a nebulizing gas. The solvent evaporates, and non-volatile analyte droplets scatter a light beam at the end of the heating tube. This scattering is directly related to the quantity of the analyte. However, the response is highly dependent on the flow of the nebulizing gas, the temperature of the evaporating tube and the flow rate and composition of the mobile phase. Therefore, the working conditions should be reproduced rigorously each time. Otherwise, a recalibration is indispensable when working quantitatively. More detailed information about the principle, advantages, disadvantages and applications of the ELSD are given in the publications of Lafosse, Elfakir, Morinallory, and Dreux (1992), Onken and Berger (1998), Koropchak et al. (2000) and Christie (2005).

Nowadays, ^{31}P-NMR is sometimes proposed as an alternative method for the determination of phospholipid classes. A chromatogram-like ^{31}P-NMR spectrum can be obtained, without performing a physical separation (Murgia, Mele, & Monduzzi, 2003). As the chemical shift of the phosphorous atom is dependent on its molecular environment, unique signals are obtained for the different phospholipid classes, and the peak areas of the ^{31}P-NMR spectrum are directly proportional to the phosphorous concentration. Non-phospholipid contaminants are not detected, so this technique only requires a minimal sample pretreatment. Disadvantages of this technique are the investment costs for high-resolution equipment, the need of high-skilled operators and the restriction to phosphor containing lipids. Therefore, most likely, ^{31}P-NMR will remain a research technique rather than a quality control method. However, as it is an absolute technique, it seems ideally suited to certify the composition of standard solutions whose quality largely determines the accuracy of TLC and HPLC results.

More extensive overviews on polar lipid analysis are given in Christie (1985, 2003) and Vanhoutte et al. (2004).

6. Nutritional value of dairy polar lipids

6.1. Significance of dairy sphingolipids

Sphingolipids are highly biologically active through their metabolites ceramide, sphingosine and sphingosine phosphate. These compounds are secondary messengers involved in transmembrane signal transduction and regulation, growth, proliferation, differentiation and apoptosis of cells. They play a role in neuronal signaling, are linked to age-related diseases, blood coagulation, immunity and inflammatory responses. These functions are extensively reviewed, and are beyond the scope of this article (Cinque et al., 2003; Colombaioni & Garcia-Gil, 2004; Deguchi, Yegneswaran, & Griffin, 2004; Pettus, Chalfant, & Hannun, 2004; Yang et al., 2004). Nowadays, increasing interest is shown for sphingolipids and its derivatives as therapeutics with clear clinical potential (Kester & Kolesnick, 2003; Radin, 2004).

All organs appear to be capable of de novo sphingolipid biosynthesis, and there is no evidence that consumption of dietary sphingolipids is required for growth under normal conditions (Vesper et al., 1999). Upon digestion, sphingolipids undergo sequential cleavage to ceramide and sphingosine in the regions of the small intestine and colon, and these are subsequently absorbed by intestinal cells and degraded to fatty acids, or reincorporated into sphingolipids (Schmelz, Crall, Larocque, Dillehay, & Merrill, 1994). However, not all of the ingested sphingolipids are absorbed. Nyberg, Nilsson, Lundgren, and Duan (1997) found significant, dose-dependent amounts of SM, ceramide and sphingosine in the intestinal contents, colon and excreted feces. As such, these biologically active compounds might exert an effect on colon cancer cells, in which normal growth arrest and apoptosis is delayed (Duan, 2005). In tests on mice in which tumorgenesis was chemically induced by a chemical agent, or caused by an inherited genetic defect, sphingolipids were found to inhibit both the early and the late stages of colon carcinogenesis, even at concentrations between 0.025 and 0.1 g $100\,g^{-1}$ of the diet. Furthermore, there was a significant shift in tumor type, from the malignant adenocarcinoma to the more benign adenomas (Schmelz, 2004; Schmelz et al., 1996; Schmelz, Sullards, Dillehay, & Merrill, 2000; Symolon, Schmelz, Dillehay, & Merrill, 2004). The effects of sphingolipids were found to be chemopreventive as well as chemotherapeutic, i.e., tumor reduction was observed when mice were fed SM before and after tumor initiation (Lemonnier et al., 2003). Interestingly, the concentrations of sphingolipids used in these tests (0.025–0.5% of the diet) were close to the estimated consumption in the United States (Vesper et al., 1999). More evidence was given by Hertervig, Nilsson, Nyberg, and Duan (1997), as they found a decrease of the sphingomyelinase activity in human colon adenomas and carcinomas of 50% and 75%, respectively. Similar findings were reported for patients with chronic colitis, who have an increased risk of developing colorectal cancer (Sjoqvist et al., 2002). All these studies clearly demonstrate the importance of sphingolipid-rich foods or supplements as it could be an important mediator in the prevention of colon cancer and bowel-related diseases.

Sphingolipids are also involved in the intestinal uptake of cholesterol. In several experiments, SM was found to

significantly lower the cholesterol absorption in rats. Supplementation of 0.1%, 0.5% and 5% milk SM to the feed resulted in a 20.4%, 53.8% and 85.5% reduction of cholesterol absorption, respectively (Eckhardt, Wang, Donovan, & Carey, 2002). This decrease was found to be higher for SM from milk than from other sources (Noh & Koo, 2004) and occurred in a dose-dependent manner (Noh & Koo, 2003). Similar results with rats were obtained by Nyberg, Duan, and Nilsson (2000). When cholesterol was dissolved in soybean oil, and administration occurred in absence of SM, absorption was found to be 68%, while the absorption was only 9% when cholesterol was administered in a 1:1 molar ratio with milk SM. Interestingly, there was a mutual effect on absorption, as 38% of sphingolipid metabolites was recovered in the faeces in the presence of cholesterol, while only 16% was found in the absence of any sterol. These effects were attributed to the fact that SM, which shows a very high affinity for cholesterol, decreases its micellar solubilization, thereby decreasing the cholesterol monomers for uptake by the enterocyte. The very tight packing of saturated SM and cholesterol in micelles results in slower desorption of cholesterol and other lipids. The interaction between both molecules is favored by saturation of the SM acyl chain, which explains the greater effect observed with milk SM than with SM derived from eggs (Eckhardt et al., 2002; Noh & Koo, 2004). Interestingly, the lowest absorption was observed at equimolar ratios, which falls in the observed SM and cholesterol intakes by humans. Thus, a moderate daily intake of SM could lower cholesterol absorption in humans (Noh & Koo, 2004).

Many bacteria, as well as bacterial toxins and viruses, use glycosphingolipids to bind to cells (Karlsson, 1989). It is plausible that food sphingolipids can compete for and act as cellular binding sites. As such, pathogens are prevented to adhere to the intestinal mucosa, which is often the first step in infection, and can hereby easily eliminated from the intestine (Pfeuffer & Schrezenmeir, 2001; Vesper et al., 1999). This can cause a shift in the bacterial population of the colon. Rueda, Sabatel, Maldonaldo, Molina-Font and Gil (1998) reported that newborn infants, given an infant formula supplemented with gangliosides, had significantly fewer *Escherichia coli* and more bifidobacteria in faeces than the control group. Sprong, Hulstein, and Van der Meer (2001) even reported in vitro bactericidal effects of sphingolipid digestion products on several pathogenic bacteria. However, recent research by Possemiers, Van Camp, Bolcaa, and Verstraete (2005) revealed that under simulated intestinal conditions, with the presence of food compounds and digestion products, no significant shifts in bacterial concentrations could be observed.

6.2. Significance of other polar lipids present in dairy products

In animals, PS has been shown to attenuate many neuronal effects of ageing, and to restore normal memory on a variety of tasks. Preliminary findings with humans, though, are limited (McDaniel, Maier, & Einstein, 2003). Positive effects were found in clinical trials with patients suffering from Alzheimer's disease, however, at elevated doses of 200 mg day^{-1}. As PS is only present in small amounts in dairy products (Table 1), the contribution of dairy products will be of limited significance. Therefore, the reader is referred to the review of Pepeu, Pepeu, and Amaducci (1996) for more details.

PC is believed to support liver recovery from damage by pharmaceutical and mushroom poisoning, alcohol and the hepatitis virus (Kidd, 2002). It is, next to SM, a source of choline, which is classified as an essential nutrient for humans. It is thought to promote synthesis and transmission of neurotransmitters important to memory, and it might be involved in brain development itself (Blusztajn, 1998).

Proteins, associated with the MFGM, also exert health-improving properties (Spitsberg, 2005). This might be important, as upon purification, often complete membrane fragments are purified, including MFGM proteins.

7. Purification

Products with a high polar lipid content on either a dry matter or a total lipid base (e.g., buttermilk, -serum and whey) are most promising for further purification, because water can be easily removed by filtration, evaporation or drying. However, separation of neutral and polar lipids is virtually impossible without the use of solvents for extraction and fractionation. A combination of coagulation, filtration, centrifugation and conventional or supercritical extraction techniques have been used for purification. As can be noticed in Table 3, depending on the raw material and the techniques used, different purities are obtained. The polar lipids in dairy products are mainly present as MFGM fragments and lipoprotein particles. As such, they are easily separated from the serum phase by means of tangential micro- and ultrafiltration techniques, sometimes in combination with stepwise addition of water, for further washing out of undesirable components like lactose, whey-proteins and minerals (= diafiltration). Buttermilk is often used as a raw material by many authors. In this case, upon purification of polar lipids by cross flow membrane filtration, the casein micelles, which represent a major part of the dry matter, can cause difficulties as their particle diameter is comparable with that of MFGM fragments. In order to separate them by membrane filtration, the casein micelle diameter should be reduced, allowing it to be recovered in the permeate. The addition of sodium citrate, which dissociates the casein micelle into submicelles (Corredig, Roesch, & Dalgleish, 2003), or an enzymatic treatment with trypsin and chymotrypsin, which destroys the casein micelle (Roesch & Corredig, 2002), decreased the casein retention upon microfiltration and resulted in a phospholipid-enriched retentate. However, in both studies, no actual polar lipid

Table 3
Polar lipid concentration after purification by different techniques

Source	Purification method	Polar lipid concentration				Reference
		Source		Purified product		
		(g 100 g^{-1} dry matter)	(g 100 g^{-1} total lipids)	(g 100 g^{-1} dry matter)	(g 100 g^{-1} total lipids)	
Buttermilk	Casein precipitation by citric acid addition + ultrafiltration (M_r cut-off 10 kDa) + micro/diafiltration (0.2 μm pore size)	1.44	21.67	13.98	21.04	Sachdeva and Buchheim (1997)
Buttermilk	Casein precipitation by rennet addition + ultrafiltration (M_r cut-off 10 kDa) + micro/diafiltration (0.2 μm pore size)	1.44	21.67	15.18	23.15	Sachdeva and Buchheim (1997)
Buttermilk	Micro/diafiltration (0.8 μm pore size) + ultrafiltration (M_r cut-off 10 kDa)	—	—	0.96	31.02	Astaire, Ward, German, and Jimenez-Flores (2003)
Buttermilk	Micro/diafiltration (0.8 μm pore size) + ultrafiltration (M_r cut-off 10 kDa) + supercritical CO_2 fluid extraction	—	—	1.97	83.15	Astaire, Ward, German, and Jimenez-Flores (2003)
Buttermilk	Microfiltration (0.8 μm pore size)	2.10	24.97	4.48	24.31	Morin et al. (2004)
Buttermilk whey	Thermocalcic aggregation + centrifugation	1.84	23.66	7.95	29.46	Rombaut et al. (2006)
Buttermilk whey	Ultrafiltration (M_r cut-off 8 kDa)	1.84	23.66	3.31	24.69	Rombaut et al. (2006)
Emmenthal whey	Thermocalcic aggregation + microfiltration	0.33	45.21	1.10	37.93	Theodet and Gandemer (1994)
Emmenthal whey	Microfiltration (1.4 μm pore size) + ultrafiltration (M_r cut-off 40 kDa) + thermocalcic aggregation + micro/diafiltration (0.25 μm pore size) + enzymatic hydrolysis + ultra/diafiltration (M_r cut-off 10 kDa)	0.22	21.88	16.79	35.81	Baumy et al. (1990)

concentrations were mentioned. Morin, Jimenez-Flores, and Pouliot (2004) investigated the influence of pore size and temperature on the microfiltration of buttermilk. They succeeded in doubling the polar lipid concentration, but concluded that microfiltration alone cannot achieve optimal separation of lipids and proteins. Sachdeva and Buchheim (1997) removed the caseins by acid and rennet coagulation, prior to ultra-, micro- and diafiltration. Although 21% of polar lipids were lost during curd-making, they achieved a 10-fold increase in polar lipid concentration, with a final concentration of 15.18 g $100\,g^{-1}$ dry matter on dry matter base. Only small differences between acid and rennet coagulation were observed (Table 3).

In the manufacturing of whey protein concentrates by ultrafiltration techniques, whey should be clarified prior to filtration. Otherwise the residual fat, consisting mainly of small fat globules, liposomes and MFGM particles, would lead to rapid fouling of the membranes. This can be achieved by 'thermocalcic aggregation', which is a sequestration of polar lipid components by calcium addition and moderate heating (Fauquant, Vieco, Brule, & Maubois, 1985; Pierre et al., 1992). After coagulation, the MFGM aggregates can be separated by settling, filtration or centrifugation. Although this method is actually developed for the clarification of whey, it can be of interest as the resulting deposit contains merely all polar lipids. Theodet and Gandemer (1994) achieved a polar lipid concentration of 1.1 g $100\,g^{-1}$ dry matter by thermocalcic aggregation of Emmenthal whey, followed by microfiltration. A similar rate of increase was observed by Rombaut et al. (2006), yet by using buttermilk whey instead of Emmenthal whey, a concentration of 7.95 g $100\,g^{-1}$ dry matter was obtained. The same buttermilk whey (untreated) was ultrafiltered on an 8 kDa molecular mass (M_r) cut-off membrane, which resulted only in a concentration of 3.31 g $100\,g^{-1}$ dry matter, probably due to the low volume concentration ratio (2.5).

By combining different techniques, much higher degrees of purification can be obtained. Baumy, Gestin, Fauquant, Boyaval, and Maubois (1990) developed a concentrate of 16.79 g $100\,g^{-1}$ dry matter starting with whey and combining membrane techniques, thermocalcic aggregation, enzymatic hydrolysis and demineralization. Subsequent hexane-isopropanol extraction resulted in a polar lipid concentration of 36.8 g $100\,g^{-1}$ dry matter, whereas subsequent acetone precipitation yielded 76.7 g $100\,g^{-1}$ dry matter.

From Table 3, it can be noticed that the coagulation and filtration techniques do not seriously alter the polar lipid/total lipid ratio. For this, solvent extraction and fractionation techniques are necessary. Supercritical CO_2 (SCO_2) is excellently suited as an extraction solvent due to its low critical parameters, relatively low cost, non-toxicity, chemical inertness, and the resulting products are free from organic solvent residues. Astaire et al.(2003) succeeded in removing the triglycerides present in microdiafiltrated buttermilk retentate powder using SCO_2, hereby increasing the polar lipid content from 31.02 to 83.15 g $100\,g^{-1}$ total lipids. Most likely, the inclusion of a second SCO_2 extraction step, with addition of a modifier like ethanol, would exclusively remove the polar lipids, hereby resulting in a 100% pure fraction. This was carried out on ground soybeans by Montanari, Fantozzi, Snyder, and King (1999).

8. Technological functions of dairy polar lipids

Because of their origin and amphiphilic nature, dairy polar lipids, as well as the MFGM proteins, are thought to have good emulsifying capacities. As such, MFGM isolates could be used as an emulsifier or fat replacer in a whole range of products (e.g., mayonnaises, margarines, recombined butter, instant milk powder, cosmetics and pharmaceuticals). Early work was done by Kanno and co-workers who investigated the emulsifying properties (foam and emulsion stability, emulsion capability and whippability) of MFGM isolates (Kanno, 1989; Kanno, Shimomura, & Takano, 1991). The stability of an emulsion of pure milk fat (25%) and MFGM material (2%) was found to be similar to that of natural milk cream. Corredig and Dalgleish (1997) examined the emulsifying properties of MFGM and buttermilk isolates derived from commercial buttermilk. They found the MFGM isolates to be inferior compared with the complete buttermilk fraction. A monomodal droplet size distribution (i.e., a parameter for emulsion stability, as a bimodal or multimodal distribution would point out coagulation) was observed for 10% soybean oil emulsions with an MFGM isolate concentration of $>8\%$, whilst this was already observed at a 1% buttermilk isolate concentration. As these results were inconsistent with previous research, they repeated the tests, but with buttermilk and MFGM isolates originating from heated and non-heated cream (Corredig & Dalgleish, 1998). The pasteurization temperature had no effect on the emulsifying properties of the buttermilk isolate, while temperatures higher than 65 °C resulted in loss of emulsifying capacity of the MFGM isolate. In the case of buttermilk isolate, the caseins apparently masked the change in MFGM functionalities. In a recent study using 10% soybean oil emulsions, the use of a 0.25% commercial MFGM isolate from buttermilk, obtained by citrate addition followed by microfiltration, showed good stability to creaming and resulted in a small particle size distribution. Similar emulsions prepared with conventional buttermilk concentrate showed extensive flocculation (Roesch, Rincon, & Corredig, 2004).

9. Conclusions

The polar lipids in milk and dairy products are a fraction with nutritional and technological potential. Sphingolipids in particular are shown to be highly active compounds upon ingestion. Whey, buttermilk and butterserum, with

their high polar lipid content on dry matter base, and their high polar lipid/total lipid ratio, are the most suitable sources for further purification. Filtration and coagulation techniques, combined with enzymatic treatment and (supercritical) solvent extraction, seem convenient but should be optimized in order to obtain the desired polar lipid concentration and product characteristics. New functional foods or food ingredients, rich in dairy polar lipids, and with specific technological and nutritional properties, could be derived from these product streams. However, to date, only limited data are available on the polar lipid content in dairy products, the influence of processing and environmental variables on the polar lipid concentration and relative distribution. Information on the contribution of dairy products to the daily phospho- and sphingolipid intake is also scarce. More research should be undertaken in these fields, as this would lead to a better knowledge of the raw materials available, more standardized and optimized purification processes and a deeper insight in the contribution of dairy products in maintaining intestinal health.

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