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Original Article

Phospho- and sphingolipid content of selected dairy products as determined by HPLC coupled to an evaporative light scattering detector (HPLC–ELSD)

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Abstract

Dairy phospho- and sphingolipids are gaining interest because of their nutritional and technological properties. As such, 31 commercial dairy products (milk, cream, condensed milk, butter, fermented products, cheeses and whey) were analyzed on their polar lipid (PL) content by high-performance liquid chromatography–evaporative light scattering detector (HPLC–ELSD) after extraction with chloroform/methanol. Values are given for different phospholipids (phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and phosphatidylcholine) and sphingolipids (sphingomyelin, glucosylceramide and lactosylceramide). The total amount of these PLs was considered as total PL content. PL values ranged from 3.6–479.5 mg/100 g product, 0.01–2.19% of the dry matter and <0.1–24.7% of the lipid fraction. These values were in accordance with the scarce values reported in literature. No significant correlation between the PL and fat content was found, so extrapolation of the PL content by measuring the lipid content may result in erroneous data.

Keywords: Dairy products; Polar lipids; Phospholipids; Sphingolipids; HPLC–ELSD

1. Introduction

Phospho- and sphingolipids are amphiphilic molecules with lipophilic acyl chains and a hydrophilic head. Phospholipids consist of a glycerol backbone on which one or two fatty acids and a phosphate residue with different organic groups may be linked. Sphingolipids consist of an a-polar sphingosine backbone on which a fatty acid is bound to form a ceramide, and it can contain a similar organophosphate group like choline (= sphingomyelin, SM) or a mono- or disaccharide (= glycosphingolipids) (Vanhoutte et al., 2004). In dairy products, the polar lipids (PLs) are mainly situated in the milk fat globule membrane (MFGM), stabilizing the fat globule in the milk serum. Important dairy phospholipids are phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylcholine (PC). Important dairy sphingolipids are SM, glucosylceramide (GluCer) and lactosylceramide (LacCer). Lysophosphatidylcholine and phosphatidic acid are only rarely reported in dairy products and are probably attributed to bad sample storage and handling or to activity of phospholipases (Christie et al., 1987; Rombaut et al., 2005).

The applications of PLs are based on their surfactant properties. They are widely used as food additives for their emulsifying, baking enhancing and wetting properties in a

Abbreviations: DM, dry matter; ELSD, evaporative light scattering detector; GluCer, glucosylceramide; LacCer, lactosylceramide; LPC, lysophosphatidylcholine; MFGM, milk fat globule membrane; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, polar lipids; PS, phosphatidylserine; S.D., standard deviation; SM, sphingomyelin; SPH, sphingolipids

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whole range of products (Szuhaj, 1983). In contrast with the PL fraction of plants, dairy products contain a substantial part of sphingolipids, which can be used as raw material for the production of ceramides, applicable in the cosmetic industry (Becart et al., 1990). Besides their technological properties, these sphingolipids are known to exhibit various biological properties and are therefore important in human nutrition. In particular, the capability of dietary sphingolipids to reduce the cholesterol uptake (Eckhardt et al., 2001), and their inhibitory effect on colon cancer (Lemonnier et al., 2003; Vesper et al., 1999) has been studied quite intensively.

Up-to-date data on the PL fraction in dairy products are scarce. The various food composition databases do not mention the content of bioactive components such as SM and other PLs. Only the Souci et al. (2000) database reports sporadically the content of some PLs. Moreover, the rare data on dairy PL concentrations that can be found in literature are barely coherent or even comparable because of the differences in analysis methodology.

High-performance liquid chromatography (HPLC) coupled to an evaporative light scattering detector (ELSD) has become the standard method for the determination of PLs in food matrices, as quantitative and qualitative analysis can readily be obtained at a relatively low cost. The use of an ELSD for lipid analysis is essential, as it is a universal detector, which is compatible with a broad range of solvents and gradient elution (unlike the refractive index detector), and the signal is independent of the degree of saturation and chain-length of an acyl chain (unlike the UV detector).

As technological and nutritional claims for dairy PLs are emerging, detailed PL composition of various dairy products is of great importance. We present in this paper the detailed composition of individual phospho- and sphingolipids of 31 commercial dairy products.

2. Materials and methods

2.1. Materials

All products (31), except those of raw milk and whey, were bought in two of Belgium's major supermarkets. Product selection was based on fat and dry matter content and differences in processing conditions (e.g., heat treatment for milks, acidification for buttermilk, churning method for butter, and ripening time for cheeses). National and international brands were selected. Mozzarella and Cheddar whey were a bulk tank sample from the production of one day and obtained from Milcobel, Langemark, Belgium. The raw milk of Holstein breed cows was taken from a stirred cooling tank at a local farm two hours after morning milking. The samples were stored for max 24 h at $<4\,^{\circ}C$ prior to analysis. Analyses were carried out on one sample of each of the 31 different products.

Chloroform and methanol used for sample extraction were of 99+ grade and purchased from Chem-Lab NV (Zedelgem, Belgium). Chloroform, methanol and formic acid used as mobile phase were of HPLC grade and obtained from Acros Organics (Geel, Belgium). Triethylamine (HPLC-grade) was obtained from Sigma-Aldrich NV (Bornem, Belgium).

A milk phospholipid standard was provided by Spectral Services GmbH (Köln, Germany). The standard contained LPC, LPE, PA, PC, PE, PI, PS and SM from dairy origin. Standards of GlucCer and LacCer of bovine origin were obtained from Sigma-Aldrich NV (Bornem, Belgium).

2.2. Chemical analyses

2.2.1. Polar lipid analysis

Extraction and chromatographic analysis of PLs was carried out according to the method of Rombaut et al. (2005). Each analysis was performed in duplicate.

Solid samples were first ground using a mixer. Five grams of sample was exactly weighed, diluted with deionised water to 20 mL and thoroughly blended with an ultraturrax. To the slurry, 80 mL of chloroform:methanol 2:1 (vol:vol) was added and the mixture was transferred into a separatory funnel. After shaking for 2 min, the mixture was allowed to stand and separate and the clear lower chloroform layer was released. This step was repeated two times with 40 mL of 20:1 (vol:vol) chloroform:methanol, added to the upper (water) phase. In a fourth step, 40 mL of 86:14:1 (vol:vol:vol) chloroform:methanol:water with 1 N HCl and 0.9 weight% NaCl was used. This lower phase was released and washed with a 0.9 weight% NaCl solution, until neutral pH was reached. Following, the four lower phases were pooled and evaporated, using a rotary vacuum evaporator at $35\,^{\circ}C$. The crude lipids were redissolved in exactly 10.0 mL of chloroform:methanol 88:12 (vol:vol), transferred into a capped test tube and stored at $-32\,^{\circ}C$ until HPLC analysis.

PL analysis was performed on a Thermo Finnigan Surveyor HPLC coupled to an Alltech ELSD 2000 Evaporative Laser Light Scattering Detector (Alltech Associates Inc., Lokeren, Belgium). N_2 was used as the nebulizing gas at a flow of 1.4 L/min, and a nebulizing temperature of $85\,^{\circ}C$. The column was a 150×3.2 mm Prevail Silica with 3 μm particle diameter (Alltech Associates Inc., Lokeren, Belgium). A precolumn with the same packing and internal diameter was used. The elution program was a linear gradient of chloroform:methanol:buffer (1 M formic acid, neutralized to pH 3 with triethylamine) 87.5:12:0.5 (vol:vol:vol) at $t = 0$ min to 28:60:12 (vol:vol:vol) at $t = 16$ min. The mobile phase was brought back to the initial conditions at $t = 17$ min and the column was allowed to equilibrate until the next injection at $t = 21$ min. The flow was maintained at 0.5 mL/min, which resulted in a backpressure of 55–90 bar. The injection volume was 25 μl. The samples and the column were thermostatized at 20 and $40\,^{\circ}C$, respectively.