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Evolution of phospholipid contents during the production of quark cheese from buttermilk

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ABSTRACT

We report the evolution of phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylserine (PS), and sphingomyelin (SM) contents during the production of quark cheese from buttermilk by successive ultrafiltration concentration, enrichment with cream, concurrent homogenization and pasteurization, fermentative coagulation, and separation of quark from whey by further ultrafiltration. Buttermilk is richer than milk itself in phospholipids that afford desirable functional and technological properties, and is widely used in dairy products. To investigate how phospholipid content is affected by end-product production processes such as ultrafiltration, homogenization, pasteurization or coagulation, we measured the phospholipids at several stages of each of 5 industrial-scale quark cheese production runs. In each run, 10,000 L of buttermilk was concentrated to half volume by ultrafiltration, enriched with cream, homogenized, pasteurized, inoculated with lactic acid bacteria, incubated to coagulation, and once more concentrated to half volume by ultrafiltration. Phospholipid contents were determined by HPLC with evaporative light scattering detection in the starting buttermilk, concentrated buttermilk, ultrafiltrate, cream-enriched concentrated buttermilk (both before and after concurrent homogenization and pasteurization), coagulate, and quark, and also in the rinsings obtained when the ultrafiltration equipment was washed following initial concentration. The average phospholipid content of buttermilk was approximately 5 times that of milk, and the phospholipid content of buttermilk fat 26 to 29 times that of milk fat. Although phospholipids did not cross ultrafiltration membranes, significant losses occurred during ultrafiltration (due to retention on the membranes) and during the homogenization and pasteurization process. During coagulation, however, phospholipid content rose, presumably as a consequence of the proliferation of the inoculated lactic acid bacteria. In spite of these changes in total phospholipid content, the relative proportions of the phospholipids studied remain fairly stable throughout quark production (PE > PC > SM > PS > PI) and similar to those found in the milk of the region, except that SM content was lower than in milk.

Key words: phospholipids, buttermilk, ultrafiltration, quark production

INTRODUCTION

Traditional buttermilk is the aqueous phase left when cream is churned to butter. It contains all the water-soluble components of cream, including not only proteins, lactose, and minerals (Sodini et al., 2006; Vanderghem et al., 2010), but also glycero- and sphingophospholipids, the main structural components of the membranes delimiting milk fat globules (and, of course, of other biological membranes). The phospholipid content of buttermilk is greater than that of milk because the fragmented membranes of milk fat globules disrupted during churning mostly migrate to the aqueous phase (Corredig et al., 2003; Vanderghem et al., 2010).

As butter is produced from cream with a fat content of around 40% (wt/wt), the world production of buttermilk may be estimated as similar to the world production of butter, which in 2013 was approximately 5.2 million tonnes (FAOSTAT, 2015). Long considered a low-value by-product, buttermilk is now widely used both as the basis of beverages and certain types of cheese, and as an ingredient of other foods that has high emulsifying capacity, adds flavor, and affords the functional and technological properties of its high phospholipid content (Kuchta et al., 1996; Vesper et al., 1999; Spitsberg, 2005; Duivenvoorden et al., 2006; Dewettinck et al., 2008; Contarini and Povolo, 2013). Several of these uses require that buttermilk be subjected to processes such as homogenization, pasteurization, and coagulation. Currently microfiltration and UF have also achieved an important development

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in the dairy industry, including its application to buttermilk (Mistry et al., 1996; Govindasamy-Lucey et al., 2007). However, phospholipid content can be altered by heat treatment (Corredig and Dalgleish, 1998; Ye et al., 2004; Morin et al., 2007), by homogenization (Lee and Sherbon, 2002; Morin et al., 2007), and by filtration (Morin et al., 2004).

In the work reported here, we investigated the evolution of phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, phosphatidylserine, and sphingomyelin contents during successive stages of the production of quark cheese from buttermilk by UF concentration, homogenization, pasteurization, fermentative coagulation, and separation of quark from whey by further UF.

MATERIALS AND METHODS

Quark Production

Phospholipids were determined during 5 industrial-scale production runs, in each of which the raw material was 10,000 L of buttermilk obtained in the production of butter by the NIZO method from cream with a fat content of 40% that had previously been pasteurized for 30 s at 90°C. Quark cheese was produced in 5 stages, as follows.

1) The starting buttermilk was concentrated to half volume by UF at 45 to 50°C using membranes with a 50 kDa cut-off and a maximum pressure of 800 kPa.
2) Cream with a fat content of approximately 40% was added to the concentrated buttermilk to increase its fat-to-protein ratio.
3) To reduce its microbial burden, denature proteins, and improve its texture, the cream-enriched buttermilk was pasteurized for 5 min at 90 to 95°C, with concurrent homogenization at 70°C and 15 MPa.
4) After cooling to around 30°C, the pasteurized, homogenized, cream-enriched buttermilk was inoculated with *Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*, and *L. lactis* ssp. *lactis* var. *diacetylactis* and incubated to coagulation at pH 4.6.
5) The resulting coagulate was separated into quark and permeate by UF using the same membranes and conditions as in stage 1.

In each run, samples were taken from the starting buttermilk, concentrated buttermilk, cream-enriched concentrated buttermilk (both before and after homogenization and pasteurization), coagulate, and quark, and also from the ultrafiltrate and from the rinsings obtained when the UF equipment was washed with 300 L of water. Pending analysis, all samples were stored in a refrigerator or were frozen and stored at subzero temperatures, as appropriate.

Analytical Methods

Fat. The fat content of buttermilk was determined in accordance with FIL-IDF standard 141C:2000 (FIL-IDF, 2000) by infrared spectrometry in a Milkoscan FT2 apparatus (Foss Electric A/S, Hillerød, Denmark) after warming to 40°C. Quark fat content was determined by the Van-Gulik method in accordance with FIL-IDF 222 (FIL-IDF, 2008). The fat contents of other samples were determined by the Rose-Gottlieb method in accordance with FIL-IDF 1D (FIL-IDF, 1996). All fat determinations were performed in duplicate.

Phospholipids. The phospholipid fraction was considered to consist entirely of phosphatidylethanolamine (**PE**), phosphatidylinositol (**PI**), phosphatidylcholine (**PC**), phosphatidylserine (**PS**), and sphingomyelin (**SM**). Phospholipids were extracted and PE, PI, PC, PS, and SM were determined using slightly modified versions of the methods of Rombaut et al. (2005). Briefly, phospholipids were extracted into chloroform:methanol in proportion 2:1 and the extracts were stored at −42°C in amber vials pending analysis by HPLC. All samples were extracted in duplicate. The HPLC apparatus (Shimadzu, Kyoto, Japan), comprising a degasser, a solvent delivery module, a controller module, a column oven, a Rheodyne manual injector valve, and an interface module, was used with a 150 × 3.0 mm, 3 μm particle diameter Prevail silica column (Grace, Deerfield, IL) and a precolumn with the same packing. Samples were eluted with chloroform:methanol:buffer (buffer being 0.5% formic acid brought to pH 6 with ammonium hydroxide) in proportions of 80:19.5:0.5 for 17 min and 60:33:7 for the next 3 min, after which the mobile phase returned to the initial conditions until the next injection 15 min later. The mobile phase flow rate was 0.5 mL/min and the column oven temperature was 35°C. The injection volume was 20 μL. The detector was a Shimadzu ELSD-LTII low-temperature evaporative light scattering detector operated at 50°C, a nebulizer (N_2) pressure of 350 kPa, and a gain of 3. The phospholipids of interest were identified by comparison of their retention times with those of pure standards. All extracts were run in duplicate.

Statistical Analysis

Fat and phospholipid contents are presented as means ± standard deviations of the 5 runs. Total phospholipid