International Dairy Journal 52 (2016) 26–34

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Preparation and characterisation of a milk polar lipids enriched ingredient from fresh industrial liquid butter serum: Combination of physico-chemical modifications and technological treatments

Jean Yves Gassi, Marielle Blot, Eric Beaucher, Benoit Robert, Nadine Leconte, Bénédicte Camier, Florence Rousseau, Claire Bourlieu, Julien Jardin, Valérie Briard-Bion, Sophie Lambert, Geneviève Gésan-Guiziou, Christelle Lopez, Frédéric Gaucheron*

INRA and Agrocampus Ouest, UMR1253, Science et Technologie du Lait et de l'OEuf, 65 rue de St-Brieuc, 35042 Rennes Cedex, France

ARTICLE INFO

Article history:
Received 10 June 2015
Received in revised form
27 August 2015
Accepted 28 August 2015
Available online 11 September 2015

ABSTRACT

Milk polar lipids (PLs) are poorly utilised as ingredients for human consumption, despite their interesting nutritional and functional properties. The objective of this study was to valorise by-products by developing a technological process able to provide a milk PL-enriched ingredient from industrial fresh liquid butter serum. The process comprised the following successive steps: skimming, heat treatment, acid precipitation of caseins, concentration and purification of whey butter serum by ultrafiltration and diafiltration. The proposed process yields a recovery of 62% of PLs present in the initial butter serum. The final ingredient contained 31.5%, 26% and 34% of PLs, proteins and triacylglycerols (on a dry matter basis). The identified proteins were caseins, whey proteins and proteins from the milk fat globule membrane. This process will allow the preparation of a milk PL-rich ingredient for food applications.

1. Introduction

Most of the phospholipids used in the food industry originate from vegetable and egg sources (i.e., soya, colza, sunflower lecithins, and egg lecithin). Polar lipids (PLs) from milk are gaining interest for their nutritional and functional properties (Dewettinck et al., 2008). The main milk PLs are the glycerophospholipids (phosphatidylethanolamine, PE; phosphatidylcholine, PC; phosphatidylserine, PS; and phosphatidylinositol, PI) and the sphingolipids (mainly sphingomyelin, SM). As compared with other sources of phospholipids (lecithins from soya, colza, sunflower and egg), milk PLs are a rich source of SM.

PLs play fundamental roles in the human organism, especially as the constituents of cell membranes, and from results obtained from both in vitro and in vivo studies, they have been demonstrated to impact upon human health [see reviews on dietary phospholipids (Küllenberg, Taylor, Scheinder, & Massing, 2012) and on milk phospholipids (Contarini & Povolo, 2013; Dewettinck et al., 2008)]. Authors have reported that dairy PLs can reduce cardiovascular diseases, inflammation and gastro-intestinal infections, stress conditions, cancer, cholesterol absorption, nervous system myelination and neurological development (Contarini & Povolo, 2013; Dewettinck et al., 2008). Milk PL-enriched ingredients also have very interesting technological functions such as foaming and emulsifying properties with, for example, reduction of particle size in emulsions, modification of texture, and moisture retention (Dewettinck et al., 2008; Vanderghem et al., 2010).

Milk PLs are located in the milk fat globule membrane (MFGM) together with cholesterol and different proteins (Fong, Norris, & MacGibbon, 2007; Lopez, 2011; Singh, 2006). Milk PLs can be recovered from the by-products of cheese-making and cream transformation (Sodini, Morin, Olabi, & Jiménez-Flores, 2006; Vanderghem et al., 2010). Thus, upon churning of cream for butter production, the MFGM is damaged and MFGM fragments are released into the buttermilk. The melting of butter to produce anhydrous milk fat leads to the release of butter serum that contains milk PLs. MFGM fragments released upon processing contain PLs (glycerophospholipids and sphingolipids), neutral lipids (mainly high melting point saturated triacylglycerols; TAG), proteins, glycoproteins (e.g., butyrophilin, mucins), enzymes (e.g., xanthine oxidase/dehydrogenase), cholesterol and other minor compounds (Dewettinck et al., 2008; Lopez, 2011). Rombaut and Dewettinck (2006) reported that buttermilk and butter serum contain about 2 and 8 g L^{-1} of PLs, respectively.

* Corresponding author. Tel.: +33 2 23 48 57 50.
E-mail address: frederic.gaucheron@rennes.inra.fr (F. Gaucheron).

http://dx.doi.org/10.1016/j.idairyj.2015.08.012
0958-6946/

Although buttermilk and butter serum are sources of interesting components from a functional and nutritional point of view, they are considered as low-value products and are mainly spray-dried to be used in animal feed. Only a limited amount of buttermilk is used in the food industry as ingredients for human consumption. The economic valorisation of these products could be improved by developing technological processes to concentrate and purify milk PLs without the use of organic solvents. For the last 20 years, industrial and academic research teams have focused on the development of processes to selectively fractionate buttermilk components, and particularly to recover and concentrate MFGM fragments and milk PLs from buttermilk and other products, such as butter serum and cheese whey. The main challenge for these technological studies is to separate MFGM fragments and milk PLs from the other components (TAG, proteins, lactose and minerals). The different steps involved in the recovery of MFGM materials have been reviewed (Dewettinck et al., 2008). Table 1 reports the main technological studies carried out by various authors with the objectives, raw materials (buttermilk and butter serum), methods of concentration, and the concentration of milk PLs in the final product. These authors used membrane filtration, such as microfiltration, to selectively separate the components according to size; MFGM fragments (1 to several μm long) were recovered in the retentate and proteins (casein micelles: 200 nm in diameter) were partitioned into the permeate fraction. However, the similarity in size of casein micelles and small MFGM fragments or PL vesicles was reported to be a major obstacle during isolation. Acid and rennet coagulation of caseins followed by the concentration of milk PLs from the whey using membrane techniques have been successfully investigated. These authors also used chemical treatments, such as the addition of sodium citrate to dissociate the casein micelles, calcium to precipitate MFGM fragments (thermocalcic aggregation), and enzymatic treatments to hydrolyse proteins. Ultrafiltration (UF), sometimes in combination with diafiltration, is used to remove lactose and minerals and to concentrate milk PLs in the final product. Supercritical fluid extraction has been reported as a possible method to increase the milk PLs/TAG ratio by the selective removal of neutral lipids. These methods use organic solvent to extract milk PLs to produce ingredients with high amount of PLs that are devoid of proteins (PLs > 50%; e.g., PC700 product produced by Fonterra, Auckland, New Zealand, and Lacprodan PL-75 product produced by Arla Foods, Viby, Denmark), but the use of ingredients from organic solvent-free processes is preferred for food applications. These technological processes used fresh or reconstituted buttermilk as starting materials (Table 1); however, there are very few studies using butter serum, which contains the highest concentration of milk PLs. Table 1 shows that the PL concentrations in the final products varied considerably in a range from 1 to 20% of PLs on a (DM) basis. The highest purity (39.4%) was reported in a patent of Dalemans (2008) who reported experiments carried out with pasteurised cream, but their protocol of purification and the obtained results were not detailed.

The objective of this work was to prepare and characterise a PL-enriched ingredient manufactured from fresh liquid industrial butter serum using a combination of physico-chemical modifications and technological treatments. The process was carried out without use of organic solvents. At each step in the process, samples were withdrawn and analysed by classical methods for DM, fat, protein, calcium, and ash. Special attention was paid to the amount and nature of the main PLs present at different steps in the process. Mass ratios between constituents (PLs/DM, PLs/fat, PLs/protein) were calculated, and proportions of SM, PE, PS, PC and PI were determined. Qualification of the residual proteins by electrophoresis and nanoliquid chromatography coupled to tandem mass spectrometry was also carried out in the final PL-enriched ingredient and compared with the initial butter serum.

2. Materials and methods

2.1. Industrial fresh liquid butter serum

Industrial butter serum was provided by a local dairy factory (Brittany, France). In the factory, cream was concentrated to a fat content of 420 g kg^{-1} and heated at 60–65 °C for 20 s. After the cream churning, the butter produced was melted at 60–65 °C and centrifuged to be transformed into anhydrous milk fat. The butter serum was the by-product of the centrifugation, i.e., the aqueous phase contained in butter. The butter serum was stored at 4 °C and used less than 24 h after collection.

2.2. Preparation of the milk PLs enriched ingredient from butter serum

The ingredient enriched in milk PLs was manufactured by a combination of physico-chemical modifications and technological treatments (Fig. 1). These operations were carried out in the Dairy Platform of STLO laboratory (INRA, Rennes, France; http://www6.rennes.inra.fr/plateforme_lait_eng/). Each unit operation is described below in detail. Experiments were carried out in triplicate with about 400 kg of butter serum of different batches.

2.2.1. Butter serum

The butter serum was skimmed at 60 °C and 5000 × *g* using a cream separator (type 500; Elecrem, Fresnes, France). Skimmed butter serum was heated at 92 °C for 3 min using a tubular pasteuriser Actijoule (Actini, Evian-les-Bains, France).

2.2.2. Coagulation at pH 4.6 with lactic acid and draining

Lactic acid (90% purity; VWR International, Lutterworth, Leicestershire, UK) was slowly added to the heated skimmed butter serum to reach a final pH of 4.6 at 40 °C. The volume of added acid was about 570 mL for 100 kg of heated skimmed butter serum. Suspension containing curd and acid whey were slowly stirred for 20 min to maintain a homogeneous casein precipitate. The 200 kg mixtures were distributed in four nylon bags (diameter 50 cm, height 100 cm and pore size 250 μm – Sogebul, Poligny, France) for draining. The bags containing the suspensions were stored at 26 °C overnight and turned three times to facilitate the recovery of the acid whey from the butter serum.

2.2.3. Ultrafiltration and diafiltration of acid whey filtrate

An ultrafiltration pilot unit (TIA, Bollène, France) equipped with tubular ceramic membranes (Membralox, type P1940; Pall-Exekia, Bazet, France) with pore size of 0.02 μm and an effective membrane area of 2.16 m^2 was used. For each filtration experiment, 120 kg of acid whey filtrate of butter serum was treated. The temperature was 49 °C and transmembrane pressure was maintained at a constant 0.4 MPa during filtration. The acid whey filtrate of butter serum was batch concentrated to a volume reduction factor of 2.5 before diafiltration. Reverse osmosis water was added to the retentate for diafiltration in a continuous mode up to a diavolume of 4. A final volume reduction factor of 4 was carried out from the initial acid whey filtrate of butter serum bulk.

2.2.4. Freeze-drying

The concentrate recovered after ultrafiltration/diafiltration was freeze-dried at −20 °C over 72 h (CIRP CS 10-0.8 lyophiliser; Serail, La Coudray Saint Germer, France). Powder was stored in plastic bags at −20 °C under vacuum before chemical analyses.