ARTICLE IN PRESS

International Dairy Journal xxx (2010) 1–5

Contents lists available at ScienceDirect

International Dairy Journal

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

Use of ultrafiltration and supercritical fluid extraction to obtain a whey buttermilk powder enriched in milk fat globule membrane phospholipids

Marcelade Rezende Costa [a], Xiomara Elizabeth Elias-Argote [b], Rafael Jiménez-Flores [b], Mirna Lúcia Gigante [c,*]

[a] Universidade Norte do Paraná, Mestrado em Ciência e Tecnologia do Leite, Avenida Paris n.675, CEP 86041-140, Londrina-PR, Brazil
[b] California Polytechnic State University, Dairy Products Technology Center, 1 Grand Avenue, Bldg. 18A, San Luis Obispo, CA 93407-0251, USA
[c] Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Departamento de Tecnologia de Alimentos, Rua Monteiro Lobato n.80, PO Box 6121, CEP 13083-862, Campinas-SP, Brazil

ARTICLE INFO

Article history:
Received 18 August 2009
Received in revised form
15 March 2010
Accepted 22 March 2010

ABSTRACT

Whey buttermilk, a by-product from whey cream processing to butter, is rich in milk fat globule membrane (MFGM) constituents, which have technological and potential health properties. The objective of this work was to produce a dairy ingredient enriched in MFGM material, especially phospholipids, from whey buttermilk. Whey buttermilk was concentrated by ultrafiltration (10×) and subsequently diafiltered (5×) (10 kDa molecular mass cutoff membrane) at 25 °C and the final retentate was spray-dried. The whey buttermilk powder was submitted to supercritical extraction (350 bar, 50 °C) using carbon dioxide. The membrane filtration removed most of the lactose and ash from the whey buttermilk, and the supercritical extraction extracted exclusively non-polar lipids. The final powder contained 73% protein and 21% lipids, of which 61% were phospholipids. This ingredient, a phospholipids-rich dairy powder, could be used as an emulsifier in different food systems.

1. Introduction

The milk fat globule membrane (MFGM) is a surface-active membrane derived from the apical membrane of the secretory cell in the mammary gland (Wiking, Nielsen, Bavius, Edvardsson, & Svennersten-Sjaunja, 2006). It surrounds each of the milk fat globules (MFG) allowing them to remain dispersed in milk. The MFG core is essentially composed of triglycerides, while the MFGM envelope is a true polar lipids bilayer with proteins, enzymes, neutral lipids and other trace elements (Danthine, Blecker, Paquot, Innocente, & Deroanne, 2000). Phospholipids and glycoproteins from the MFGM affect several cell functions, such as growth, molecular transport system, memory processing, stress responses, and central nervous system myelination (Astaire, Ward, German, & Jiménez-Flores, 2003). Moreover, several studies have shown the beneficial effects of these components on human health (Fong, Norris, & Macgibbon, 2007; Kilara & Panyam, 2003; Noh & Koo, 2004; Riccio, 2004; Spitsberg, 2005; Spitsberg & Gorewit, 1997; Wang, Hirmo, Millen, & Wadstrom, 2001). Also, MFGM components, especially the phospholipids, have an important role as emulsifiers in food systems and can be used to improve the features of bread, chocolate, margarine and dairy products (Dewettinck et al., 2008; Singh & Tokley, 1990; Turcot, Turgeon, & St. Gelais, 2001). The application of MFGM polar lipids in other industrial fields would include their potential use in a delivery or carrier system for drugs and other substances (Kisel et al., 2001; Thompson & Singh, 2006).

The technological and potential health properties of the MFGM components have led to an interest in isolating and concentrating these materials from dairy sources. Several methods have been used to reach this goal, especially microfiltration procedures applied to regular buttermilk, the aqueous phase derived from the churning of cream to butter (Astaire et al., 2003; Corredig, Roesch, & Dalgleish, 2003; Morin, Britten, Jiménez-Flores, & Pouliot, 2007; Morin, Jiménez-Flores, & Pouliot, 2004; Roesch, Rincon, & Corredig, 2004; Sachdeva & Buchheim, 1997). The major difficulty in isolation and concentration of the MFGM components from buttermilk is the presence of skim milk solids, especially casein micelles, which restricts the concentration of MFGM, since the microfiltration concentrates both MFGM fragments and casein micelles. Therefore, preliminary treatments, such as washing the cream prior the churning (Morin & Britten, et al., 2007), and adding citrate (Corredig et al., 2003; Roesch et al., 2004), rennet, citric acid and lactic cultures (Sachdeva & Buchheim, 1997) have

* Corresponding author. Tel.: +55 19 3521 3993; fax: +55 19 3289 3617.
E-mail address: mirna@fea.unicamp.br (M.L. Gigante).

0958-6946/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.idairyj.2010.03.006

been used to remove the casein fraction from the buttermilk before the filtration.

An alternative to avoid the presence of casein in the MFGM concentrates is using raw materials with a naturally lower concentration of this protein, such as butter serum (Rombaut, Camp, & Dewettinck, 2006) and acid whey (Rombaut, Dejonckheere, & Dewettinck, 2007). Another material less explored is the whey buttermilk, the aqueous phase liberated after the churning of whey cream to obtain whey butter, which contains residual triglyceride and water-soluble compounds from milk, including some derived from MFGM fragments (Corredig & Dalgleish, 1997). Whey buttermilk can be considered a suitable source of the MFGM components since it generally contains either low or no detectable amounts of casein (Morin, Pouliot, & Jiménez-Flores, 2006; Sodini, Morin, Olabi, & Jiménez-Flores, 2006).

The production of ingredients that contain high concentrations of MFGM material is normally also associated with high triglyceride content. Supercritical fluid extraction (SFE) has been utilized to extract lipids from foods, such as nuts and spices (Araújo, Machado, França, & Meireles, 2000; Coelho, Oliveira, & Pinto, 1997; Lameira, Coelho, & Mothé, 1997; Nobrega, Monteiro, Meireles, & Marques, 1997; Silva, Mendes, Pessoa, & Queiroz, 2008). More recently, some research groups have successfully used this technology on dairy products. Astaire et al. (2003) applied SFE on buttermilk powders while Yee, Walker, Khalil, and Jiménez-Flores (2008) used this technology on grated Cheddar and Parmesan cheeses to remove triglycerides and cholesterol from the products.

The goal of the present study was to produce a dairy ingredient enriched in MFGM compounds, especially phospholipids, from whey buttermilk by combining the use of membrane filtration (ultrafiltration and diafiltration) and supercritical fluid extraction.

2. Material and methods

2.1. Dairy ingredient production

Three batches of whey cream, around 120 L each, were donated by Hilmar Cheese Company (Hilmar, CA, USA). The product was derived from unsalted whey obtained from renneted cheese processing. The cream was processed at 12 °C using a rotatory churn (Blentech Corporation, Santa Rosa, CA, USA) to obtain whey butter and buttermilk. Buttermilk was recovered in milk cans, after butter fines were removed by filtration through cheese cloth, and stored overnight at 4 °C until membrane filtration.

A pilot plant scale system (R-12 model, GEA-Niro Filtration, Hudson, WI, USA) using two spiral polymeric membranes fitted in parallel on the module (10 kDa molecular mass cutoff, 11.33 m^2 total surface area) was used for buttermilk concentration. The process was carried out at 25 °C, the transmembrane pressure was around 6 bar and feed pump was operated at 35 Hz. The ultrafiltration (UF) was conducted until a ten-fold volumetric concentration factor was reached. Diafiltration (DF) was done adding continuously tap water at 25 °C to the feed tank to replace the removed permeate until reaching a five-fold diafiltration factor (5 × DF). In each step of the filtration, samples of retentates were collected for composition analysis and the permeate flux ($L\ h^{-1}\ m^{-2}$) was measured. The final retentates from all experiments were spray-dried (Niro Filterlab Spray-drier, Hudson, WI, USA) using 35 bar of pressure, and inlet and outlet air temperatures of 185 °C and 95 °C, respectively, to obtain whey buttermilk powders (WBP). All filtration trial runs were performed in triplicate.

A portion of the powder obtained from each whey cream batch was submitted to supercritical fluid extraction (SFE). The SFE system and components were acquired from Thar Designs, Inc. (Pittsburgh, PA, USA), including: the 500 mL sample vessel, the model P-50 high-pressure pump, the automated back pressure regulator (model BPR-A-200B), and the PolyScience brand water bath and pump unit (model 9505). Circulated deionized water at 3 °C was used for cooling different zones in the SFE apparatus. Carbon dioxide tanks (50-lb) were filled and inspected by A & R Welding Supply (San Luis Obispo, CA, USA). The system conditions were controlled manually by Windows 2000-based software (Hewlett–Packard). Approximately 135 g of each sample were submitted to three extraction cycles using the following conditions: 1500 g of CO_2 at a flow rate of 20 g min^{-1}, extraction pressure of 350 bar, and both extraction and collection temperature of 50 °C. The SFE trials were done in triplicate. The powders submitted to the SFE (SFE-WBP) as well as the WBP were stored at 10 °C until further analysis.

2.2. Analytical procedures

Whey cream, butter and buttermilk, retentates of membrane filtration, and buttermilk powders (WBP and SFE-WBP) were analyzed for gross composition, protein profile and phospholipid content. The profile of the fat removed from the WBP by SFE was determined using thin layer chromatography (TLC). All the analyses were done in triplicate.

Gross composition was determined using AOAC (2003) recommended methods. Nitrogen content was determined by using the Kjeldahl method and the crude protein content was calculated by multiplying the nitrogen content by 6.38. Lipid content was determined by the Mojonnier method, ash content by the gravimetric method of incineration in a furnace at 550 °C, and total solids content by forced air oven drying.

Protein profiles were obtained using the sodium dodecyl sulfate polyacrylamide gel electrophoresis technique (SDS-PAGE) (Laemmli, 1970). The analyses were done in a Mini-Protean II system (Bio-Rad Laboratories Ltd., Hercules, CA, USA). Gels were stained with Coomassie Brilliant Blue (Sigma Chemical Company, St. Louis, MO, USA). Protein classes were determined according to their molecular weight by comparison with a molecular weight standard (Precision Plus Unstained Protein Standard, Bio-Rad Laboratories Ltd.).

Lipids obtained using the Mojonnier ether extraction method were diluted to 2 mg of lipids per mL with 2:1 chloroform–methanol and kept at −18 °C until phospholipid analysis. Phospholipids were analyzed by High Performance Liquid Chromatography (Hitachi HPLC System D-7000) with an electro evaporative light scattering detector (ELSD) Sedex model 55 (SEDERE, Alfortville, France), according to the methodology described by Morin & Jiménez, et al. (2004). The phospholipid standards utilized were phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol, and phosphatidylserine (Sigma–Aldrich, St. Louis, MO, USA).

Lipids removed by SFE were analyzed using the TLC technique following the procedure described by Astaire (2002). The lipid standards were phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin (Sigma–Aldrich). Silica gel glass plates were by Merck (Darmstadt, Germany) and the developing tanks by Kontes Glass Company (Vineland, NJ, USA).

2.3. Statistical analysis

Results were analyzed statistically using Statistica 5.5 software (StatSoft Inc, 2000). The ANOVA procedures were performed and mean comparisons were carried out using Tukey's test. Results were considered significantly different at $P < 0.05$.

Please cite this article in press as: Costa, M.R., et al., Use of ultrafiltration and supercritical fluid extraction to obtain a whey buttermilk..., International Dairy Journal (2010), doi:10.1016/j.idairyj.2010.03.006

3. Results and discussion

3.1. Raw material features and dairy ingredient production

The composition of whey cream, butter, and buttermilk samples are presented in Table 1. The protein, lipid, and ash content of whey cream and buttermilk were closer to the composition obtained by Morin et al. (2006) for regular cream and buttermilk than for the whey derivatives. The differences observed in composition between the studies may be due to variations in the product origins. The whey cream could have been obtained using different processing parameters or even from whey resulting from different types of cheese production. The butter composition was similar in both studies indicating that the initial cream composition has a greater impact on the buttermilk than on the butter composition.

The phospholipids represented 5.65, 5.31 and 12.40% of the total lipids (Table 1) in the whey cream, butter and buttermilk, respectively. This demonstrates that this class of lipids distributes preferentially to the aqueous phase. When whey cream is churned, separating whey butter and buttermilk, the aqueous phase retains triglyceride residues and the majority of all whey cream water-soluble compounds, including MFGM material. This fact leads to a higher phospholipid concentration, as a percentage of the total lipids, in the whey buttermilk (12.40%) than in whey butter (5.31%). The observed phospholipid concentration in the whey buttermilk was higher than the 4.5% phospholipid concentration observed by Morin et al. (2006). The total phospholipids content in the whey buttermilk (2.0% of total solids) was higher than the values in former studies (Morin et al., 2006; Rombaut et al., 2006; Sachdeva & Buchheim, 1997), which reported concentration from 1.3 to 1.8%.

The proportion of the different phospholipid classes in the whey butter remained the same as in the whey cream (Table 1). In contrast, the whey buttermilk showed a higher proportion of phosphatidylcholine (44%) with a lower proportion of phosphatidylinositol (7.1%) and phosphatidylserine (8.0%) in relation to the total phospholipids content. Phosphatidylethanolamine and sphingomyelin represented 17.7 and 22% of the phospholipids in the whey buttermilk. Different proportions of phospholipids were observed in whey buttermilk (Morin et al., 2006) and in regular buttermilk (Britten, Lamothe, & Robitaille, 2008; Morin et al., 2006). A study (Rombaut et al., 2006) revealed that phosphatidylethanolamine, phosphotidylinositol, and phosphatidylserine were inversely correlated with phosphatidylcholine and sphingomyelin concentrations in buttermilk, as observed in this study. According to the authors, this phenomenon was probably related to the specific location of polar lipids in the MFGM. Sphingomyelin and phosphatidylcholine were found at the outside of the MFGM with the other phospholipids on the inside (Danthine et al., 2000; Deeth, 1997).

Table 1
Composition (mean ± standard deviation) of the whey cream, butter and buttermilk.

Components (%)	Whey cream	Whey butter	Whey buttermilk
Total solids	44.68 ± 1.28	85.95 ± 0.76	8.05 ± 0.32
Proteins[a]	3.59 ± 0.28	0.55 ± 0.08	24.89 ± 2.02
Lipids[a]	86.11 ± 0.79	96.93 ± 0.93	16.27 ± 2.06
Phospholipids[a]	4.86 ± 0.45	5.14 ± 0.48	2.01 ± 0.16
Ash[a]	3.94 ± 0.51	0.08 ± 0.01	7.01 ± 0.47
Total phospholipids[b]	5.65 ± 0.52	5.31 ± 0.51	12.40 ± 0.80
Phosphatidylcholine[b]	1.90 ± 0.26	1.77 ± 0.24	5.71 ± 1.50
Phosphatidylethanolamine[b]	1.00 ± 0.06	0.94 ± 0.05	2.11 ± 0.68
Sphingomyelin[b]	1.23 ± 0.18	1.16 ± 0.17	2.69 ± 0.17
Phosphatidylinositol[b]	0.87 ± 0.02	0.84 ± 0.02	0.88 ± 0.04
Phosphatidylserine[b]	0.64 ± 0.18	0.61 ± 0.18	1.00 ± 0.25

[a] Values given are on a dry matter basis.
[b] Values given are a percentage of total lipids.

As expected during the UF/DF processing, lipids and proteins were concentrated in the retentate while other whey buttermilk components, especially ash and lactose, were removed in the permeate side (Fig. 1). Until the second DF, the lipid and protein concentration behaved similarly, however, after that the lipids continued to be concentrated and a reduction in the total protein content, calculated based on the nitrogen content, was observed. This means that part of the nitrogen compounds, such as some whey proteins, were being retained/adsorbed on the membrane or lost in the permeate, probably small water-soluble non-protein-nitrogen compounds.

The whey buttermilk composition changed significantly ($P < 0.05$) after applying ultrafiltration and diafiltration. Although nitrogenous compounds have been lost during the diafiltration, the whole filtration process increased the whey buttermilk total protein content around 91% and the lipids around 200%, while the ash content was reduced by 74%. The ultrafiltration did not change the initial lipid to protein ratio (1:1.5) of the whey buttermilk (Table 1). On the other hand, during the diafiltration a greater increase in the lipid content than the protein content occurred, which resulted in a 1:1 ratio of these components in the final (5DF) retentate (47.66 ± 5.67% of protein and 48.48 ± 6.05% of lipids on dry matter basis).

The permeate flux during the filtration started at 45 L h^{-1} m^{-2}, dropped approximately 26% in the ultrafiltration step and increased gradually in the diafiltration phase, reaching 36 L h^{-1} m^{-2} at the end of the process. The average flux for the whole filtration process was 31 L h^{-1} m^{-2}.

After the membrane filtration, the whey buttermilk concentrate was spray-dried and submitted to the supercritical fluid extraction (SFE). The SFE process removed approximately 34 g of lipids from each 100 g of sample (47.26 g of lipids), using 97 g of carbon dioxide to extract each gram of this fat. This extraction represented a reduction of 72% in the quantity of total lipids originally present in the whey buttermilk powder. As expected, the extraction using supercritical CO_2 selectively removed non-polar lipids from the matrix powder (Fig. 2). The removed fat did not contain any polar lipids, as can be seen in the chromatogram (Fig. 2, lines 7 and 8), consequently their concentration increased in the powders after the SFE procedure (lines 4–6) when compared with the non-treated whey buttermilk powders (lines 1–3).

According to Astaire et al. (2003), triglycerides have different solubilities in supercritical CO_2 depending on the type of chain and molecular weight. Although the fatty acid concentration was not evaluated in this work, results obtained by these authors allow us

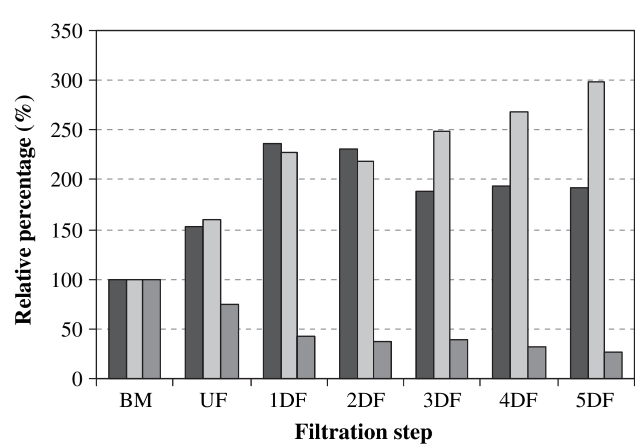

Fig. 1. Relative composition (■ lipids, □ proteins, ■ ash) of the membrane filtration retentates. BM: whey buttermilk; UF: ultrafiltration; DF: diafiltration.

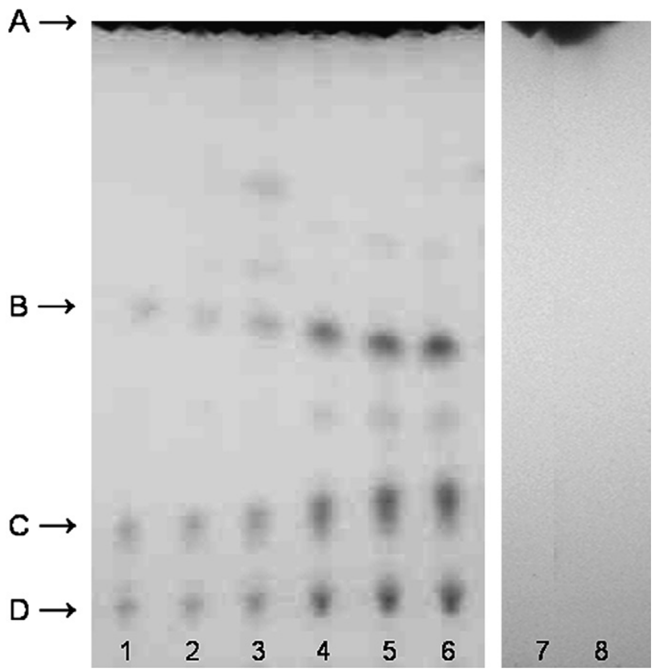

Fig. 2. Thin Layer Chromatography polar lipids profile. (A): Non-polar lipids; (B) Phosphatidylethanolamine; (C) Phosphatidylcholine; (D) Sphingomyelin. Lines: 1–3: whey buttermilk powders (WBP); 4–6: supercritical fluid extraction-treated whey buttermilk powders (SFE-WBP); 7–8: fat removed by SFE.

to assume that most of the non-polar lipids removed by SFE were probably triglycerides of short and medium chain fatty acids ($\leq$C18:1). Selective removal of these lipids and the permanence of lipids containing polyunsaturated fatty acids can be considered positive effects of the extraction using supercritical CO_2, since polyunsaturated fatty acids have been considered lipids with human health benefits (Haug, Høstmark, & Harstad, 2007).

3.2. Powder characterization

The composition of the whey buttermilk powder (WBP) and the whey buttermilk powder after the SFE (SFE-WBP) are compared in Table 2. As a consequence of the non-polar lipid removal by SFE, there was a significant ($P < 0.05$) reduction of the total lipid content by 57% and an increase in protein (53%), ash (55%), and phospholipids (67%). A greater reduction in the lipid content (73%) was observed by Astaire (2002) using SFE in regular buttermilk retentate powders. However, the sample was mixed in biosilicate (flux-calcined diatomaceous earth) and the temperature of extraction was 77 °C. The procedure in the present work used 50 °C as the extraction temperature to avoid denaturation of proteins, especially whey proteins. Moreover, the samples were not mixed in biosilicate, since this material would impair the utilization of this powder as a food ingredient. SFE did not influence the proportion of the phospholipids in the powder, since this process did not remove any of them. However, the lipid to protein ratio was reduced from 1:1 to 1:3.5 while the phospholipid to lipid ratio increased from 1:6.6 to 1:1.7. The whole filtration (UF/DF) and SFE processing resulted in an increase of 500% in the phospholipid content, in comparison to the original whey buttermilk on dry matter basis.

The SDS-PAGE protein profiles (data not shown) revealed that all samples had three distinct protein groups: milk fat globule membrane proteins, caseins and immunoglobulin G light chains, and the main whey proteins β-lactoglobulin and α-lactalbumin. The samples of whey buttermilk, whey buttermilk powder, and whey buttermilk powder after SFE showed a lower proportion of proteins in the molecular weight range of caseins and Ig-G light chains and a greater proportion of MFGM and whey proteins, especially after the SFE step. Different protein profiles have been previously observed for regular buttermilk powders, but casein has generally been found as the main protein component (Morin et al., 2006; Sodini et al., 2006).

Table 2
Composition (mean ± standard deviation) of the whey buttermilk powders composition, prior and after the supercritical extraction.[a]

Components (%)	WBP	SFE-WBP
Total solids	98.42 ± 0.90[a]	96.87 ± 0.74[a]
Proteins[b]	47.39 ± 3.19[b]	72.69 ± 2.79[a]
Lipids[b]	47.26 ± 3.71[a]	20.56 ± 3.84[b]
Phospholipids[b]	7.17 ± 3.53[b]	11.95 ± 4.34[a]
Ash[b]	2.29 ± 0.13[b]	3.21 ± 0.24[a]
Total phospholipids[c]	14.81 ± 6.61[b]	60.59 ± 11.13[a]
Phosphatidylcholine[c]	6.26 ± 3.32[b]	26.68 ± 5.46[a]
Phosphatidylethanolamine[c]	3.19 ± 1.50[b]	13.46 ± 2.51[a]
Sphingomyelin[c]	3.48 ± 1.71[b]	14.52 ± 3.00[a]
Phosphatidylinositol[c]	0.94 ± 0.10[b]	2.74 ± 1.41[a]
Phosphatidylserine[c]	0.93 ± 0.40[b]	3.19 ± 2.11[a]

[a] Abbreviations are: WBP, whey buttermilk powder; SFE-WBP, whey buttermilk powder after the supercritical fluid extraction. Values in a row with different superscript letters are significantly different ($P < 0.05$).
[b] Values are on dry matter basis, except for total solids.
[c] Values are a percentage of total lipids.

4. Conclusions

The procedure adopted in this present study, which included the use of whey buttermilk as raw material and the combination of ultrafiltration/diafiltration and supercritical fluid extraction, allowed the production of a dairy powder enriched in MFGM phospholipids and proteins. This phospholipids-rich ingredient could be useful in different food products formulations, such as ice cream, powdered soup mix or bakery delicacies and also as a material to study or isolate the MFGM components by other techniques.

Acknowledgements

This study was supported by Dairy Management Inc.(DMI), California Dairy Research Foundation (CDRF) and Agriculture Research Initiative (ARI) through research funds, and by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through scholarships. The authors would like to acknowledge the Dairy Products Technology Center (Cal Poly) staff and students that helped in this work.

References

Araújo, M. E., Machado, N. T., França, L. F., & Meireles, M. A. A. (2000). Supercritical extraction of pupunha (*Guilielma speciosa*) oil in a fixed bed using carbon dioxide. *Brazilian Journal of Chemical Engineering, 17*, 297–306.
Association of Official Analytical Chemists. (2003). *Official methods of analysis* (17th ed.). Washington, DC, USA: AOAC.
Astaire, J. C. (2002). *Microfiltration and supercritical fluid extraction of buttermilk to concentrate biological lipid messengers*. PhD Thesis, California Polytechnic State University, Faculty of the Agricultural Sciences Department, USA.
Astaire, J. C., Ward, R., German, J. B., & Jiménez-Flores, R. (2003). Concentration of polar MFGM lipids from buttermilk by microfiltration and supercritical fluid extraction. *Journal of Dairy Science, 86*, 2297–2307.
Britten, M., Lamothe, S., & Robitaille, G. (2008). Effect of cream treatment on phospholipids and protein recovery in butter-making process. *International Journal of Food Science and Technology, 43*, 651–657.
Coelho, L. A. F., Oliveira, J. V., & Pinto, J. C. (1997). Modelagem e simulação do processo de extração supercrítica do óleo essencial de alecrim. *Ciência e Tecnologia de Alimentos, 17*, 446–448.

Corredig, M., & Dalgleish, D. G. (1997). Isolates from industrial buttermilk: emulsifying properties of material derived from the milk fat globules membrane. *Journal of Agriculture and Food Chemistry, 45*, 4595–4600.

Corredig, M., Roesch, R. R., & Dalgleish, D. G. (2003). Production of a novel ingredient from buttermilk. *Journal of Dairy Science, 86*, 2744–2750.

Danthine, S., Blecker, C., Paquot, M., Innocente, N., & Deroanne, C. (2000). Progress in milk fat globule membrane research: a review. *Lait, 80*, 209–222.

Deeth, H. C. (1997). The role of phospholipids in the stability of milk fat globules. *The Australian Journal of Dairy Technology, 52*, 44–46.

Dewettinck, K., Rombaut, R., Thienpont, N., Le, T. T., Messens, K., & Van Camp, J. (2008). Nutritional and technological aspects of milk fat globule membrane material. *International Dairy Journal, 18*, 436–457.

Fong, B. Y., Norris, C. S., & Macgibbon, A. K. H. (2007). Protein and lipid composition of bovine milk-fat-globule membrane. *International Dairy Journal, 17*, 275–288.

Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition – a review. *Lipids in Health and Disease, 6*, 25–40.

Kilara, A., & Panyam, D. (2003). Peptides from milk proteins and their properties. *Critical Reviews in Food Science and Nutrition, 43*, 607–633.

Kisel, M. A., Kulik, L. N., Tsybovsky, I. S., Vlasov, A. P., Vorob'yov, M. S., Kholodova, E. A., et al. (2001). Liposomes with phosphatidylethanol as a carrier for oral delivery of insulin: studies in the rat. *International Journal of Pharmaceutics, 216*, 105–114.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Lameira, C. P., Coelho, G. L. V., & Mothé, C. G. (1997). Extração de lipídeos da amêndoa de castanha de caju com CO_2 supercrítico. *Ciência e Tecnologia de Alimentos, 17*, 405–407.

Morin, P., Britten, M., Jiménez-Flores, R., & Pouliot, Y. (2007). Microfiltration of buttermilk and washed cream buttermilk for concentration of milk fat globule membrane components. *Journal of Dairy Science, 90*, 2132–2140.

Morin, P., Jiménez-Flores, R., & Pouliot, Y. (2004). Effect of temperature and pore size on the fractionation of fresh and reconstituted buttermilk by microfiltration. *Journal of Dairy Science, 87*, 267–273.

Morin, P., Pouliot, Y., & Jiménez-Flores, R. (2006). A comparative study of the fractionation of regular buttermilk and whey buttermilk by microfiltration. *Journal of Food Engineering, 77*, 521–528.

Nobrega, L. P., Monteiro, A. R., Meireles, M. A. A., & Marques, M. A. O. M. (1997). Comparison of ginger (*Zingiber officiale* roscoe) oleoresin obtained with ethanol and isopropanol with that obtained with pressurized CO_2. *Ciência e Tecnologia de Alimentos, 17*, 408–412.

Noh, S. K., & Koo, S. L. (2004). Milk sphingomyelin is more effective than egg sphingomyelin in inhibiting intestinal absorption of cholesterol and fat in rats. *Journal of Nutrition, 134*, 2611–2616.

Riccio, P. (2004). The proteins of the milk fat globule membrane in the balance. *Trends in Food Science and Technology, 15*, 458–461.

Roesch, R. R., Rincon, A., & Corredig, M. (2004). Emulsifying properties of fractions prepared from commercial buttermilk by microfiltration. *Journal of Dairy Science, 87*, 4080–4087.

Rombaut, R., Camp, J. V., & Dewettinck, K. (2006). Phospho- and sphingolipid distribution during processing of milk, butter and whey. *International Journal of Food Science and Technology, 41*, 435–443.

Rombaut, R., Dejonckheere, V., & Dewettinck, K. (2007). Filtration of milk fat globule membrane fragments from acid buttermilk cheese whey. *Journal of Dairy Science, 90*, 1662–1673.

Sachdeva, S., & Buchheim, W. (1997). Recovery of phospholipids from buttermilk using membrane processing. *Kieler Milchwirtschaftliche Forschungsberichte, 49*, 47–68.

Silva, C. F., Mendes, M. F., Pessoa, F. L. P., & Queiroz, E. M. (2008). Supercritical carbon dioxide extraction of macadamia (*Macadamia integrifolia*) nut oil: experiments and modeling. *Brazilian Journal of Chemical Engineering, 25*, 175–181.

Singh, H., & Tokley, R. P. (1990). Effects of preheat treatments and buttermilk addition on the seasonal variations in the heat stability of recombined evaporated milk and reconstituted concentrated milk. *Australian Journal of Dairy Technology, 45*, 10–16.

Sodini, I., Morin, P., Olabi, A., & Jiménez-Flores, R. (2006). Compositional and functional properties of buttermilk: a comparison between sweet, sour, and whey buttermilk. *Journal of Dairy Science, 89*, 525–536.

Spitsberg, V. L. (2005). Bovine milk fat globule membrane as a potential nutraceutical. *Journal of Dairy Science, 88*, 2289–2294.

Spitsberg, V. L., & Gorewit, R. C. (1997). In vitro phosphorylated bovine milk fat globule membrane proteins. *Journal of Nutritional Biochemistry, 8*, 181–189.

StatSoftInc. (2000). *STATISTICA for Windows [Computer program manual]*. Tulsa, OK, USA: StatSoft, Inc.

Thompson, A. K., & Singh, H. (2006). Preparation of liposomes from milk fat globule membrane phospholipids using a microfluidizer. *Journal of Dairy Science, 89*, 410–419.

Turcot, S., Turgeon, S. L., & St. Gelais, D. (2001). Effect of buttermilk phospholipids concentrations in cheese milk on production and composition of low fat Cheddar cheese. *Lait, 81*, 429–442.

Wang, X., Hirmo, S., Millen, R., & Wadstrom, T. (2001). Inhibition of *Helicobacter pylori* infection by bovine milk glycoconjugates in a BALB/cA mouse model. *FEMS Immunology and Medical Microbiology, 20*, 275–281.

Wiking, L., Nielsen, J. H., Bavius, A. K., Edvardsson, A., & Svennersten-Sjaunja, K. (2006). Impact of milking frequencies on the level of free fatty acids in milk, fat globule size, and fatty acid composition. *Journal of Dairy Science, 89*, 1004–1009.

Yee, J. L., Walker, J., Khalil, H., & Jiménez-Flores, R. (2008). Effect of variety and maturation of cheese on supercritical fluid extraction efficiency. *Journal of Agricultural and Food Chemistry, 56*, 5153–5157.

Please cite this article in press as: Costa, M.R., et al., Use of ultrafiltration and supercritical fluid extraction to obtain a whey buttermilk..., International Dairy Journal (2010), doi:10.1016/j.idairyj.2010.03.006