Effect of Temperature and Pore Size on the Fractionation of Fresh and Reconstituted Buttermilk by Microfiltration

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ABSTRACT

The objective of this research was to evaluate the effect of temperature (7, 25, and 50°C) and pore size (0.1, 0.8, and 1.4 μm) on the separation of proteins and lipids (neutral lipids and phospholipids) during microfiltration (MF) of fresh or reconstituted buttermilk. Buttermilk was subjected to MF using a pilot-scale unit mounted with ceramic membranes. The MF runs were carried out in a uniform transmembrane pressure (UTP) mode. Changes in processing temperature had no significant impact on protein transmission, whereas increasing temperature reduced both lipid and phospholipid transmission. A maximum concentration factor (CF) for lipids was reached at 25°C, as protein CF remained essentially unaffected by temperature. The use of the smaller pore size (0.1 μm) resulted in low lipid (10%) and protein ($\approx 20\%$) transmission. Larger pore sizes (0.8 and 1.4 μm) resulted in higher levels of protein, lipid, and phospholipid transmission ($>50\%$), but gave high permeation fluxes. Transmission of both proteins and lipids was markedly different when using fresh buttermilk as opposed to reconstituted buttermilk. This study showed that MF temperature, pore size, and buttermilk type influence fractionation but that MF alone cannot achieve optimal separation of lipids and proteins for the production of novel ingredients from buttermilk.

(**Key words:** buttermilk, fractionation, microfiltration)

Abbreviation key: MF = microfiltration, MFGM = milk-fat globule membrane, TMP = transmembrane pressure, VCF = volumetric concentration factor.

INTRODUCTION

Buttermilk is characterized by a fat composition rich in milk fat globule membrane (MFGM) components,

such as phospholipids, sphingolipids, and various glycoproteins (Vyas et al., 2002). When cream is churned, MFGM components are broken down, most of the hydrophilic components are expelled into the aqueous phase, and buttermilk is created. The high concentration of phospholipids, such as sphingomyelin, phosphatidylethanolamine, and phosphatidylcholine found in buttermilk is related to the presence of MFGM fragments that migrated to the aqueous phase. This particularity has created an important interest in buttermilk, as evidenced by the many articles written on that subject (Ramachandra Rao et al., 1995; Sural and Famelart, 1995; Raval and Mistry, 1999; Roesch and Corredig, 2002; Vyas et al., 2002). Currently, buttermilk is mainly used for its emulsifying properties in various food products (O'Connell and Fox, 2000). However, recent advances in dairy science have shown that some components of buttermilk could also be exploited as health ingredients. For example, sphingomyelin, through its bioactive derivatives, has been found to play important roles in transmembrane signal transduction, cell regulation, and apoptosis (Parodi, 1997). Moreover, Dillehay et al. (1994) have found that dietary sphingomyelin could inhibit 1,2-dimethylhydrazine-induced colon cancer in mice. Important bioactive properties of phosphatidylcholine and phosphatidylserine have also been recently reviewed (Kidd, 2002).

Since buttermilk is a unique dairy source of phospholipids, it could be a good substrate for fractionation. Sachdeva and Buchheim (1997) reported a method for the recovery of phospholipids using whey from buttermilk acid or rennet coagulation. Purification of phospholipids by thermocalcic aggregation (Fauquant et al., 1985) followed by membrane processing (Baumy et al., 1990) has already been demonstrated from cheese whey, but the presence of casein micelles in buttermilk makes this approach unsuitable. Buttermilk is a substrate that is highly susceptible to oxidation, which partially explains why it has to be rapidly used or spray-dried (O'Connell and Fox, 2000). The effects of various drying treatments followed by rehydration are not

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known and might be of importance if membrane processing is considered. Data from the recovery of MFGM fragments from buttermilk are still lacking (Jensen, 2002), and before going any further in fractionation, effective separation between lipids and proteins is necessary. Because of its ability to separate particles in suspension (Saboya and Maubois, 2000), microfiltration (MF) could be used as the first step of a process for obtaining a lipid concentrate from buttermilk. However, optimal MF processing conditions for better yield between lipid and protein remain to be defined. This paper reports the effect of temperature and pore size in MF of fresh and reconstituted buttermilk.

MATERIALS AND METHODS

Microfiltration Procedure

Fresh pasteurized buttermilk was purchased from a local butter factory (Agropur, Plessisville, Canada). Dried buttermilk (Parmalat, Victoriaville, Canada) was reconstituted at the total solid level of the fresh buttermilk (8.4%) using deionized water at 40°C under agitation for 1 h and then cooled overnight at 4°C. The MF pilot unit was a TetraPak MSF1 (Lund, Sweden) working in uniform transmembrane pressure mode and equipped with Membralox (Bazet, France) ceramic membranes of 1.4, 0.8, and 0.1 μm average pore size. Each MF run was carried out with 50 kg of fresh buttermilk for the temperature study and 50 kg of reconstituted buttermilk for the pore size study. Temperature effect and pore size effect runs were carried out with different buttermilk sources because of the limited amount of fresh buttermilk that was available for the experiments. The transmembrane pressure (TMP) during the runs was kept constant at 0.5 to 0.7. For the temperature study, the fresh buttermilk temperature was adjusted to 7, 25, and 50°C under agitation in a stainless steel vat for 1 h before MF and the pore size used for this study was 0.8 μm . Temperatures of MF were 49.1 ± 0.9 , 25.1 ± 1.3 , and 7.3 ± 0.5 . In the pore size study, reconstituted buttermilk temperature was adjusted to 50°C under agitation for 1 h in a stainless steel vat. Flux was monitored with a stopwatch and a graduated cylinder. Each experiment was carried out in triplicate. A sample of retentate and permeate from each replicate was collected at the end of each run and frozen (-20°C) until analysis. Transmission rate (%) of proteins, total lipids, and phospholipids was calculated using the relation $T(\%) = (C_p/C_r) \times 100$, where C_p is the concentration of a component in the permeate side and C_r , the concentration of the same component in the retentate side.

Cleaning Procedure

Cleaning of the MF system was achieved by rinsing the plant with deionized water followed by alkaline cleaning (Ultrasil 25, EcoLab, Canada; 1.5% at 75°C /45 min) combined with 200 ppm of chloride. The system was rinsed with warm, deionized water (50 to 60°C) until reaching normal water pH, and then acid detergent (Ultrasil 76, EcoLab, Canada; 0.3% nitric acid) was circulated for 30 min at 50°C . Finally, the system was rinsed with deionized water until reaching normal water pH. The cleaning procedure was repeated until initial water permeation flux was reached.

Composition Analysis

Dry matter, protein, and lipids. Dry matter content of all permeates, retentates, and initial buttermilk sample were obtained using a microwave dryer (μWave , Omnimark, Tempe, AZ). In brief, 3 g of sample was placed on a weighing paper and dried in the microwave oven until a constant weight was reached. The protein content was obtained using Kjeldahl (IDF, 1993) nitrogen determination, with 6.38 as the protein conversion factor. The lipid content was obtained gravimetrically using the Mojonnier extraction method (IDF, 1987). For each experiment, extracted lipids (triplicates) were combined and diluted to 10 mg/mL in chloroform/methanol (2:1) and were stored in a freezer at (-20°C) until analysis.

Phospholipid analysis. Phospholipids from all samples were analyzed by HPLC (Waters 600, Milford, MA) with an evaporative light-scattering detector (SEDEX 75, Sedere, France). The injector was a Rheodyne model 7725i (Cotati, CA), the column was a Zorbax Sil 5 μm (4.6 i.d $\times$ 150 mm, Agilent Technology, Palo Alto, CA). The data were collected and treated using Millennium chromatographic software (Waters). Mobile phases used were A) chloroform/methanol/ammonium hydroxide (80:19.5:0.5) and B) chloroform/methanol/water/ammonium hydroxide (60:34:5.5:0.5), and the binary gradient used is illustrated in Figure 1. Lipid samples were diluted from 10 to 2 mg/mL before injection. Neutral lipids were eluted in the first 6 min of the run and phospholipids were eluted between the 7th and the 30th min. Runs were 41-min long. Fresh and reconstituted buttermilk phospholipids profiles are shown in Figure 2. Components were identified and quantified using calibration curves made with phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, and sphingomyelin standards (Sigma, St. Louis, MO). All solvents and reagents were HPLC grade.

Figure 1. Binary gradient used in phospholipid analysis by HPLC–evaporative light-scattering detector.

Figure 3. Permeation fluxes during microfiltration (0.8 μm) of fresh buttermilk at 50°C (▲), 25°C (■), and 7°C (◆).

RESULTS AND DISCUSSION

Temperature Effect

Processing. Temperature adjustments during buttermilk MF induced important differences in permeation fluxes, and the volumetric concentration factors (VCF) achieved were 8 $\times$, 6 $\times$, and 1.6 $\times$ for MF at 50, 25, and 7°C, respectively. Flux curves are presented in

Figure 2. Phospholipid profile of fresh (A) and reconstituted (B) buttermilk obtained by HPLC–evaporative light-scattering detector. NL = neutral lipids, PPL = phospholipids, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PC = phosphatidylcholine, SM = sphingomyelin.

Figure 3. High fluxes obtained at 50°C are comparable to those classically obtained in MF of dairy fluids (Surel, 1993; Cheryan, 1998). The increase in the viscosity of buttermilk lowered flux values at 25 and 7°C (Cheryan, 1998). However, fluxes were lower, but more stable, while processing at 7 vs. 50°C. Technical difficulties were encountered at the pilot plant during cold (7°C) processing, which included a temperature increase caused by fluid circulation in the pumps and fluid friction in the membrane resulting from the high shear rate (>6 m/s). This increase of temperature was noticeable after 2 h of processing; therefore, the process had to be stopped at the VFC reached after 2 h.

Composition. Compositional analysis of the different retentates and permeates revealed important differences between lipids and phospholipids concentration, whereas less pronounced differences were observed between protein concentration at the various temperatures. Permeate and retentate composition is presented in Table 1 and component transmissions are provided in Figure 4. Lipids were concentrated in the retentates at 1.07 $\times$, 2.19 $\times$, and 1.61 $\times$ for MF at 7, 25, and 50°C, respectively, in comparison to original buttermilk. Lipid transmission at 25°C was significantly ($P < 0.05$) lower than at 7°C, but not significantly different than at 50°C. However, it appears that lowering temperature from 50°C to 7°C increases the lipid transmission. Hydrophobic interactions, which are known to be stronger at high temperatures, could induce more aggregation between lipid particles and increase particle size at high temperatures, therefore reducing the transmission of those particles. Fewer hydrophobic interactions at low temperature are also known to induce β -casein dissociation (Dalgleish and Law, 1988). The slight decrease in protein concentration in retentates between 7 and 50°C may be related to that phenomenon. However, the pore size used in this part of the experiments (0.8 μm) is probably too large to show im-

Table 1. Composition of fresh buttermilk, permeates and retentates from microfiltration (MF) 0.8 μm at different temperatures. Means are given in % of DM.

	Fresh buttermilk	MF permeates 0.8 μm			MF retentates 0.8 μm		
		7°C	25°C	50°C	7°C	25°C	50°C
Protein (% DM)	30.33	25.91 ^a	28.69 ^a	29.87 ^a	32.59 ^a	35.84 ^b	37.36 ^b
Lipids (% DM)	8.41	4.81 ^a	5.22 ^a	6.05 ^b	9.02 ^a	18.43 ^b	13.56 ^{ab}
PPL (% DM) ¹	2.10	1.95	1.23	1.75	1.94	4.48	3.94
% of PPL ²							
PE	32.4	29.2	37.4	33.1	39.7	30.8	34.0
PI	20.9	13.9	16.3	17.7	16.0	16.1	20.8
PC	30.5	46.2	33.3	36.0	30.4	37.1	31.0
SM	16.2	10.7	13.0	13.1	13.9	16.0	14.2

^{a,b}Subcolumn in the same line values with different superscript differ ($P < 0.05$).

¹PPL = phospholipids; CV (%) calculated on four injections of each standard were 1.63, 2.94, 7.47, and 2.63 for PE, PI, PC, and SM, respectively.

²Percentage of each class into total phospholipids (PE = phosphatidylethanolamine, PI = phosphatidylinositol, PC = phosphatidylcholine, SM = sphingomyelin).

portant differences in protein retention by temperature modifications only as protein concentrations in the permeates at the three temperatures were not significantly different. Phospholipid analysis showed that MF at 25°C and 50°C increased the concentration of all main classes by approximately twofold. The MF at 7°C did not provide the concentration of phospholipids in the retentate. This result shows that phospholipid transmission is closely related to that of total lipids. This result is not unexpected considering the fact that phospholipids in buttermilk are associated with triglycerides and various membrane proteins forming MFGM fragments (Sachdeva and Buchheim, 1997). Furthermore, cold temperature could facilitate phospholipid dissociation of MFGM fragments, enabling higher transmission rates of both phospholipids and MFGM fragments. Nevertheless, process temperature has a considerable impact on MFGM structure (Dufour et al.,

1999), and even if MFGM is present in the form of fragments in buttermilk, it appears that modifying the temperature of processing could induce changes in particles sizes and aggregation, which would lead to variations in transmission levels of both lipids and phospholipids during MF. Temperature combined with other physicochemical modification (pH, hydrolysis, or chelating agents) could be a way to disrupt further the linear correlation between lipid and protein retention that has been reported by Surel and Famelart (1995).

Pore Size Effect

Processing. The VCF were 4 $\times$ for the 0.1- μm , and 8 $\times$ for 0.8- μm and 1.4- μm membranes. As was the case in the study of the temperature effect, pore size variations during buttermilk MF led to important changes in permeation fluxes (Figure 5). The MF at 1.4 μm was carried at 0.5 bar TMP, whereas MF at 0.8 and 0.1 μm

Figure 4. Transmission of proteins ($\diamond$), lipids ($\blacksquare$), and phospholipids ($\blacktriangle$) during microfiltration (0.8 μm) of fresh buttermilk at different temperatures.

Figure 5. Permeation fluxes during MF of reconstituted buttermilk (50°C) using different pore sizes: ($\diamond$) 0.1 μm at 0.7 bar transmembrane pressure (TMP), ($\blacksquare$) 0.8 μm at 0.7 bar TMP, and ($\blacktriangle$) 1.4 μm at 0.5 bar TMP.

Table 2. Compositions of reconstituted buttermilk, permeates and retentates from MF at different pore sizes. Values are given in % of dry matter (DM).

	Reconstituted buttermilk	MF permeates			MF retentates		
		0.1 μm	0.8 μm	1.4 μm	0.1 μm	0.8 μm	1.4 μm
Protein (% DM)	30.46	11.76 ^b	26.44 ^a	27.53 ^a	54.12 ^a	43.06 ^b	52.68 ^a
Lipids (% DM)	6.06	1.12 ^b	5.21 ^a	5.69 ^a	11.69 ^a	8.49 ^b	9.47 ^b
PPL (% DM) ¹	1.59	0.20	1.24	1.32	3.48	1.61	1.87
% of PPL ²							
PE	28.9	10.0	20.2	22.7	58.3	29.2	32.1
PI	17.0	20.0	15.3	19.0	7.2	19.3	12.8
PC	35.9	50.0	48.4	42.4	23.9	35.4	38.0
SM	18.2	20.0	16.1	15.9	10.6	16.1	17.1

^{a,b}Subcolumn in the same line values with different superscript differ ($P < 0.05$).

¹PPL = phospholipids; CV (%) calculated on four injections of each standard were 1.63, 2.94, 7.47, and 2.63 for PE, PI, PC, and SM, respectively.

²Percentage of each class into total phospholipids. PE = phosphatidylethanolamine, PI = phosphatidylinositol, PC = phosphatidylcholine, SM = sphingomyelin.

were carried at 0.7 bar TMP, and at 50°C in all cases. This explains in part why MF at 0.8 showed higher fluxes than MF at 1.4 μm . The flux pattern for MF at 1.4 μm suggests that we could have obtained higher fluxes with higher TMP. However, an increase in TMP could have led to more fouling since large pore membranes are more susceptible to fouling because of the high amounts of particles at the surface of the membrane (Cheryan, 1998). Low fluxes obtained with MF of reconstituted buttermilk with the 0.1- μm membrane are higher than those reported by others (Surel and Famelart, 1995) with fresh buttermilk on a 0.2- μm membrane mounted on a back-pulse MF unit. Our experiments were done using MF equipment with cocurrent recirculation of permeate (Bactocatch, TetraPak, Lund, Sweden), which gives higher fluxes than a back-pulse module (Cheryan, 1998).

Composition. Pore sizes had an influence on both protein and total lipid levels. Retentate and permeate composition is presented in Table 2. Protein and lipid content was increased in the MF retentate at 0.1 μm by 1.77- and 1.93-fold, respectively, which indicates that the 0.1- μm membrane did not significantly change the correlation between protein and lipid concentration. However, the low proportion of lipids found in MF permeate using the 0.1- μm membrane shows the potential of this membrane. The low transmission of lipids (10%) (Figure 6) indicates that this membrane can effectively concentrate lipids in the retentate side. However, low transmission for protein content was also noticed, indicating that this pore size without any other treatments cannot separate lipids from proteins. This result is not surprising considering that low-pore-diameter membranes ($\approx 0.2 \mu\text{m}$) are often used to produce micellar casein concentrate or native phosphocaseinate (Maubois and Olivier, 1992; Pouliot et al., 1994). Phospholipids were concentrated 3.5 $\times$ in the retentate using the

0.1- μm membrane, and the low transmission level observed for phospholipids (5.8%) is once again consistent with lipid transmission and probably results from associations between lipids and phospholipids, as well as reconstituted buttermilk. Lipid concentration was not as effective and not significantly different using both 0.8- and 1.4- μm membranes as concentration factors reached were 1.4 $\times$ and 1.56 $\times$, respectively. Proteins were transmitted more effectively using those pore sizes, indicating that the main mechanism for protein transmission is size exclusion. A lower protein transmission was noticed at 1.4 μm compared with that at 0.8 μm , which indicates deviation from Ferry's law. This filtration law indicates that for a particle of a given diameter, an increase in membrane pore size leads to higher transmission. The observed deviation from this law could be attributed to membrane fouling (i.e., gel layer formation at membrane surface). The range of pore sizes tested did not make it possible to determine a critical pore size in which differences in transmission

Figure 6. Transmission of proteins (♦), lipids (■), and phospholipids (▲) during MF of reconstituted buttermilk at different pore sizes.

of both lipid and protein occurred, most likely somewhere between 0.1 and 0.8 μm . Only two pore sizes in that range are provided by the manufacturer (0.2 and 0.5 μm), however. Large-pore membranes (0.8 and 1.4 μm) give high fluxes and a good transmission of proteins, but the high transmission of lipids remains a problem. Despite the low fluxes obtained, the 0.1- μm membrane is the only membrane that provided an important concentration of lipids and phospholipids. The only way to enhance protein and lipid separation with this membrane would be to reduce the size of protein particles (casein micelles) through chemical treatments (EDTA or citrate) or enzymatic cleavage. Citrate addition induces demineralization (calcium) of casein micelles and can induce a decrease in micelle sizes (Johnston and Murphy, 1992), but solubilization of all colloidal calcium in order to disrupt casein micelles would require a concentration four times greater than that found in natural milk (Surel and Famelart, 1995).

Effect of Buttermilk Source

Composition. From our previous results, it appears that, under the same MF conditions (0.8 μm and 50°C), results from fresh and reconstituted buttermilk separation were markedly different (Tables 1 and 2, Figures 4 and 6). Proteins were more concentrated in the MF retentate from reconstituted buttermilk than retentates from fresh buttermilk. Moreover, the transmission level of proteins using fresh buttermilk reached around 80%, as opposed to 60% with reconstituted buttermilk.

Processing history is the only obvious difference between fresh and reconstituted buttermilks. The various treatments for making buttermilk powder (mainly evaporative concentration and spray drying) are likely to induce some aggregation of proteins that would have an impact on MF of reconstituted buttermilk. Lipid concentration in retentate was more effective with fresh buttermilk (1.61 $\times$) than with reconstituted buttermilk (1.4 $\times$). Transmission of lipids through the membrane was significantly ($P < 0.05$) higher in MF of reconstituted buttermilk (61% vs. 44%). The impact was even greater on phospholipid transmission. In MF of reconstituted buttermilk, 78% of phospholipids were transmitted through the membrane, whereas only 45% were transmitted using fresh buttermilk. This result shows that the correlation between lipid and phospholipid transmission is broken in reconstituted buttermilk, but not in the case of fresh buttermilk. The reason for these differences is not clear, but they could be induced by the additional processing steps in the case of reconstituted buttermilk. Those treatments could induce rearrangement or rupture of a part of the MFGM frag-

ments, which in turn could reduce lipid particle size. Microscopic observation of both fresh and reconstituted buttermilk could shed light on the effect of drying buttermilk on protein aggregation and lipid particle changes.

CONCLUSIONS

Buttermilk is a complex dairy fluid. Knowledge of its colloidal properties is still scarce. However, it remains that temperature changes in buttermilk MF induce important changes in both flux behavior and permeate and retentate composition. Higher protein transmission coupled with low fat transmission was observed at 25°C. Pore size also affects buttermilk fractionation, with better results obtained using the 0.1- μm membrane, despite the high retention of proteins. The drying of buttermilk seems to affect both lipid and protein and results suggest that processing with fresh buttermilk could lead to a better separation between lipids and proteins. Microscopic analysis of buttermilk under various conditions is required to further our understanding of colloidal properties of both dried and fresh buttermilk. A combination of processes (hydrolysis, temperature treatments) could be helpful in controlling separation, and work is in progress to evaluate the potential of this type of process combination.

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