A comparative study of the fractionation of regular buttermilk and whey buttermilk by microfiltration

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Abstract

The use of a ceramic microfiltration (MF) membrane for the fractionation of buttermilk and whey buttermilk obtained from pilot scale churning of cream and whey cream from industrial sources has been studied. Whey buttermilk contained comparable amounts of phospholipids compared to regular buttermilk but its protein content was lower due to the absence of caseins. However, it was found that lipid content of whey cream did vary significantly between lots resulting in important variations in the fat content of whey buttermilk. A twofold MF concentration of regular buttermilk doubled its phospholipids content whereas that of whey buttermilk was increased by 50%. The overall efficiency of the MF processing of regular buttermilk was limited by the amount of caseins retained by the MF membrane. Analysis of the protein profile of permeates and retentates showed that the transmission of milk fat globule membrane proteins by the MF membrane was lower when using whey buttermilk as compared to regular buttermilk possibly indicating the influence of casein micelles in fractionation or some structural differences between both products.

Keywords: Buttermilk; Whey buttermilk; Whey cream; Microfiltration; Milk fat globule membrane

1. Introduction

Buttermilk is a by-product from butter manufacture that finds applications in various food products. Uses of buttermilk in food systems are closely related to its particular composition in emulsifying components such as phospholipids which can act as emulsifiers in salad dressings. However, in most applications, buttermilk is used because of its typical flavor such as in baked goods (O’Connell & Fox, 2000). Growing interest is showing on that particular by product because of its unique composition (Astaire, Ward, German, & Jimenez-Flores, 2003; Corredig & Dalgleish, 1997; Corredig, Roesch, & Dalgleish, 2003; Morin, Jimenez-Flores, & Pouliot, 2004; Sachdeva & Buchheim, 1997). When milk fat globules are broken during churning of cream, the membrane covering the lipid core is excluded from the lipid matrix and recovered in buttermilk along with most of the proteins, lactose and minerals contained in the aqueous phase of cream. The milk fat globule membrane (MFGM) is rich in various proteins and phospholipids which have some potential for both functional and nutraceutical applications.

For example, it was shown that sphingomyelin (SM) could help in the prevention of various diseases including colon cancer (Schmelz, Sullards, Dillehay, & Merrill, 2000) and that phosphatidylcholine (PC) could interfere with the development of hepatic diseases (Niederau,
Various attempts have been made to fractionate buttermilk in order to create an enriched fraction in MFGM components. Surel and Famelart (1995) were the first to report the use of microfiltration (MF) to fractionate buttermilk. The major issue they reported for the use of MF to fractionate buttermilk was the similar size of the casein micelles and the MFGM components present in buttermilk. In order to overcome this problem, Sachdeva and Buchheim (1997) used renneting and acid coagulation of buttermilk to remove caseins prior to fractionation by a combination of MF and ultrafiltration (UF). The authors reported a recovery of 70–77% of the total phospholipids of buttermilk using this process. However, these results were highly dependent of various coagulation factors. Corredig et al. (2003) have reported the use of citrate to disrupt the casein micelles followed by either UF or MF on polysulfone membranes. Their results showed that this approach seems to be effective to concentrate MFGM proteins in the retentate. However, their data did not show the efficiency of this process to recover other MFGM components including phospholipids. The high levels of citrate in the permeate limit the potential use of this process for the recovery of MFGM from buttermilk.

An approach using whey cream as a starting material in order to concentrate MFGM components could also be considered. Whey cream is obtained by separation of fat from cheese whey by centrifugation. The whey cream obtained is mainly used to standardize milk fat prior to cheese making but can also be used to produce whey butter (Fox, Guinee, Cogan, & McSweeney, 2000) and cheese making but can also be used to produce whey obtained is mainly used to standardize milk fat prior to fat from cheese whey by centrifugation. The whey cream is obtained by separation of fat from cheese whey by centrifugation. The whey cream obtained is mainly used to standardize milk fat prior to cheese making but can also be used to produce whey butter (Fox, Guinee, Cogan, & McSweeney, 2000) and thus, a by-product, whey buttermilk. Because the objective of the cheese maker is to maximize caseins recovery in the cheese, virtually no caseins can be found in cheese whey, therefore the buttermilk recovered from churning of whey cream is expected to contain very low amount of caseins. This is likely to enhance the membrane separation of MFGM components from the other milk solids. There are currently no uses reported for buttermilk obtained from whey cream butter, thus MFGM isolation can be a promising way of valorization this by-product.

The objective of the present work was to compare microfiltration of whey buttermilk and regular buttermilk using two different filtration modes, namely volumetric concentration (VCMF) and diafiltration (DFMF). The efficiency of separation was compared in terms of selectivity by assessing compositional differences of initial products and fractions (lipids, proteins, phospholipids), and in terms of productivity using microfiltration permeation flux data.

2. Materials and methods

2.1. Buttermilk production

For each trial, 200 l of fresh regular manufacturing cream was purchased from Foster Farms (Modesto, CA) and 200 l of whey cream was graciously donated by Hilmar Cheese Co. (Hilmar, CA). Both cream were churned to butter using a continuous pilot scale butter churn (Egli, Switzerland). Buttermilk was recovered in milk cans after butter fines were removed by filtration through cheese cloth. The buttermilk production was repeated three times with three different lots of both creams.

2.2. Microfiltration

Batches of 22.7 kg (50 lbs) of fresh regular buttermilk were used for the VCMF and DFMF process. The same batch system was used for fresh whey buttermilk. The remaining buttermilk was spray dried using a Niro Filtearl Spray Dryer (Hudson, WI). The microfiltration system used is described elsewhere (Astaire et al., 2003). Two tubular membranes (0.7 m² total surface) were fitted in parallel on the module for all experiments. MF ceramic membranes (Tami Sunflower Design, 0.45 μm pore size, Tami Industries, France) were fitted in US filters (Warrendale, PA) stainless steel housings. All runs were carried out at low temperature (8–10 °C) at a transmembrane pressure of 80–95 kPa. The cross-flow rate was 87.11 min⁻¹ (3.1 m s⁻¹). The VCMF process consisted in buttermilk concentration by MF until a volumetric concentration factor (VCF) of 2X was reached. The DFMF process consisted in continuously adding chilled tap water (4 °C) to the retentate to replace the extracted permeate until reaching a twofold diafiltration (DF) factor (45.4 kg of water added). Samples of retentates and permeates for were collected for composition analysis when 25%, 50%, 75% and 100% of the VCF or DF was reached. Permeate flux (1 h⁻¹ m⁻²) was also measured at fixed intervals during all experiments. The final permeates and retentates from all experiments were spray-dried. All microfiltration trials runs were performed in triplicate.

2.3. Chemical analysis

Cream, butter, liquid buttermilk, samples collected during MF, retentate and permeate powders and buttermilk powders were analyzed for total solids, total protein, total lipids, ash content. Total solids were obtained using direct oven drying method in a forced air oven at 102 °C and ash content was obtained by incineration at 550 °C in a muffle furnace (Marshall, 1992). Protein was obtained using macro-Kjeldahl
method with 6.38 as a nitrogen to protein conversion factor (Marshall, 1992). Protein profile was established for the buttermilk powders, final retentate and permeate powders by SDS-PAGE according to Laemmli (1970) using a Mini-Protean III system (Biorad, Hercules, CA). All samples were diluted to 3.3 mg ml\(^{-1}\) of protein with reducing sample buffer and 15 \(\mu\)l were loaded into a 12% acrylamide SDS-PAGE gel. Gels were stained with Coomassie Blue R-250 (Biorad, Hercules, CA) and proteins have been identified by their molecular weight according to Mather (2000). Lipids were obtained using the Mojonnier ether extraction method (Marshall, 1992). Extracted lipid were then diluted to 10 mg of lipids per ml with 1:2 chloroform–methanol and kept in a freezer (-20\(^\circ\)C) until analysis. Phospholipids were analyzed by HPLC (System Gold, Beckman-Coulter, Mississauga, Ont., Canada) with an electro evaporative light scattering detector (ELSD) (SEDEX 75, SEDERE, France) as described elsewhere (Morin et al., 2004). All reagents were electrophoresis or HPLC grade.

2.4. Calculations

Transmission (\(Tr\)) of proteins, lipids, phospholipids and ash through the membrane was calculated according to Eq. (1):

\[
\%Tr = \left(\frac{C_p}{C_r}\right)\times 100
\]

(1)

where \(C_p\) is the concentration of a component in the permeate and \(C_r\) the concentration of the same component in the retentate (Cheryan, 1998). Concentration factor (\(CF\)) was also calculated for components using:

\[
CF = \left(\frac{C_f}{C_o}\right)
\]

(2)

where \(C_f\) is the final concentration reached in the retentate and \(C_o\) the concentration in the initial feed.

2.5. Statistical analysis

All statistical analysis was done by ANOVA with Tuckey’s pairwise comparison using Minitab 14.0 software (Minitab Inc., PA). Results were considered statistically different at \(p < 0.05\).

3. Results and discussion

3.1. Analytical data

The composition of cream, butter and initial buttermilk samples are presented at Table 1. No significant differences were found between levels of lipids of both regular and whey cream. A significantly lower protein (\(p < 0.01\)) and ash (\(p < 0.05\)) content for the whey cream was observed. The ash content was also lower in the whey butter and whey buttermilk. The lower amount of caseins in whey cream may explain these differences since caseins contains about 8% of minerals, mainly calcium phosphate (Walstra, Geurts, Noomen, Jellema, & van Boekel, 1999). A high variability in the total fat content of whey cream (32.34 ± 14.31%) was observed. Variations between 30% and 70% have been previously reported (Pointurier & Adda, 1969). This variability may originate from a number of processing characteristics such as the type of cheese that generates whey and by differences of operating conditions of the whey cream separator. This phenomenon induced a higher variability of total lipids content in the whey buttermilk but not in the whey butter. Table 1 also shows that the phospholipids content of whey buttermilk was not significantly different from that of regular buttermilke (\(p = 0.627\)). However, considering the lower total solids in whey buttermilk, phospholipids levels would be representing a more important portion of the solids in whey buttermilk.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Cream</th>
<th></th>
<th>Buttermilk</th>
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<tbody>
<tr>
<td></td>
<td>Whey</td>
<td>Regular</td>
<td>Whey</td>
<td>Regular</td>
</tr>
<tr>
<td>Total solids (%)</td>
<td>65.31 ± 15.23</td>
<td>50.84 ± 2.13</td>
<td>87.41 ± 2.84</td>
<td>87.00 ± 0.70</td>
</tr>
<tr>
<td>Proteins (%)</td>
<td>0.89 ± 0.36(^a)</td>
<td>1.94 ± 0.14(^b)</td>
<td>0.50 ± 0.25</td>
<td>0.66 ± 0.12</td>
</tr>
<tr>
<td>Lipids (%)</td>
<td>32.34 ± 14.31</td>
<td>37.92 ± 3.62</td>
<td>80.84 ± 3.24</td>
<td>84.25 ± 3.14</td>
</tr>
<tr>
<td>Phospholipids (%)</td>
<td>nd(^*)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>(% of total)**</td>
<td>PE</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td></td>
<td>PS</td>
<td>nd</td>
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<td>nd</td>
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<tr>
<td></td>
<td>PC</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>SM</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.25 ± 0.00(^a)</td>
<td>0.43 ± 0.03(^b)</td>
<td>0.08 ± 0.01(^a)</td>
<td>0.14 ± 0.01(^b)</td>
</tr>
</tbody>
</table>

Values in same line for each product with different superscript differs significantly (\(p < 0.05\)). ± Indicates standard deviation.

\(^*\) Not detected.

\(^{**}\) PE = phosphatidylethanolamine, PI = phosphatidylinositol, PS = phosphatidylserine, PC = phosphatidylcholine, SM = sphingomyelin.
The main phospholipids that were observed in regular and whey buttermilk were phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylinositol (PI) and phosphatidylserine (PS). The protein content of whey buttermilk was significantly lower ($p < 0.01$) than that of regular buttermilk as explained by the absence of caseins in the whey buttermilk. As opposed to the lipid portion, the protein content of the whey buttermilk did not show any important variability ($0.99 \pm 0.02\%$).

3.2. Microfiltration permeation flux data

Permeation flux has been monitored during all volumetric concentration and diafiltration runs. Fig. 1 shows the average flux curve observed during the trials. Although average flux was significantly higher with while using whey buttermilk ($p < 0.05$), the observed flux decline ($J_o$-$J_f$) for all four experiments were not significantly different. However, it was observed that VCMF induced a more important flux decline than DFMF while being not statistically significant ($p = 0.055$). The lower average flux observed for the regular buttermilk could be caused by the accumulation of caseins micelles at the surface of the membrane creating a secondary layer (cake) inducing more resistance to permeation and therefore lower permeation flux (Vetier, Bennasar, Parodo de la fuente, & Nabias, 1986). This phenomenon could be occurring also with whey buttermilk but the evolution of the concentration polarization (CP) layer composition is likely to be different considering the low amount of caseins. Another possible explanation for the higher flux obtained while using whey buttermilk is the difference in total solids in the initial feed (Table 1). These differences can be expected on the basis of the mass transfer mathematical model predicting flux in the region near the membrane (Cheryan, 1998):

$$J = k \ln(C_g/C_b)$$  \hspace{1cm} (3)

where $J$ is the flux, $k$ the mass transfer coefficient, $C_g$ the gel concentration (concentration at the membrane surface) and $C_b$ the bulk concentration (feed). The more important flux decline observed for VCMF compared to DFMF can also be explained and predicted by the mass transfer model (Cheryan, 1998). In the DFMF mode, the constant water addition in the DFMF process keeps the feed concentration unchanged or slightly reduced. In fact, DFMF induces slightly higher and more stable permeation fluxes.

Surel (1993) reported flux values of around 1201 h$^{-1}$ m$^{-2}$ with a 0.5 μm pore size ceramic membrane in microfiltration of regular buttermilk at 50 °C. The flux values obtained in our experiments are much lower than that ($\approx 20-25$) and this is mainly due to the processing temperature. Our previous results (Morin et al., 2004) indicated that in the case of reconstituted buttermilk, the cold MF process (7 °C) induced permeation fluxes 5× lower than high temperature process (50 °C). The same effect is also reported by Astaire et al. (2003). The main effect of temperature on the feed is on the fluid density, viscosity and most importantly on the diffusivity of solutes. For example Cheryan (1998) reported that diffusivity of proteins decreases of 3–3.5% per °C decrease. The diffusivity has an important effect on the mass transfer coefficient ($k$) of Eq. (3). The mass transfer coefficient is calculated with:

$$k = D/\delta$$ \hspace{1cm} (4)

where $D$ is the diffusivity and $\delta$ is the thickness of the boundary layer where the concentration gradient between the membrane and the bulk is found. The diffusivity ($D$) of the solutes in buttermilk below 10 °C is much lower than at 50 °C thus; the mass transfer coefficient ($k$) is lower which induce lower flux ($J$).

3.3. Transmission of components during MF

Changes in transmission of proteins, total lipids and ash through the MF membrane were monitored at four different points during each MF run (Fig. 2). It can be observed that protein transmission decreases significantly throughout the DFMF process with both regular ($p < 0.01$) and whey buttermilk ($p < 0.01$). Protein
transmission also decreased significantly in the VCMF process with whey buttermilk. This indicates the occurrence of establishment of CP layer, possibly composed of proteins which could induce the formation of a secondary layer at the membrane’s vicinity. A significant decrease of transmission of ash was observed in the DFMF of regular buttermilk ($p < 0.01$). This can be attributed to the low transmission of casein micelles since this result is not observed in the case of whey buttermilk where minerals could be mostly free in solution.

Transmission of lipids through the 0.45 $\mu$m MF membrane was stable and low ($<10\%$) throughout all experiments with both whey and regular buttermilk. Phospholipids transmission (not shown) was also followed during the whole process but no clear trend could be observed in the case of whey buttermilk as transmission values were highly variable throughout the process. A possible reason for that is the fact that whey cream contains important portion of aggregates heterogeneous in size. This mixture of globules may rupture into a wide range of membrane fragments sizes in the churning process. Furthermore, phospholipids found in whey buttermilk could be originating from the starter used in the cheese process (Unemoto & Sato, 1973) and these phospholipids could be on a completely different aggregated form. The phospholipids transmissions in the case of regular buttermilk (also not shown) did vary to a lesser extent. The membrane fragments in both products could also be drastically influenced by the pumping and the high shear forces at the surface of the membrane.

The average transmission of components has been calculated and is shown in Table 2. Significant effect of the MF mode on lipid transmission was observed ($p < 0.01$) and the effect of the buttermilk type was also significant on lipid transmission ($p < 0.01$). The DFMF process induced a higher transmission of lipids as compared to VCMF process. Since higher and more stable fluxes were observed for DFMF (Fig. 1), lower fouling of the MF membrane allowed higher transmission of lipid. When using whey buttermilk, lipids were transmitted to a lower level as compared to regular buttermilk. Although differences being smaller, they remained highly significant ($p < 0.01$) and the main reason for this could be a different type of aggregation of lipids in the case whey buttermilk but also the presence of casein micelles in the CP layer in the case of regular buttermilk. Also it is known that the fat recovered in the whey cream is partly composed of free lipids (Fox et al., 2000).

![Fig. 2. Transmission of lipids (■), proteins (▲) and ash (♦) through the VCMF (a) and DFMF (b) process as function of the VCF or DF reached for regular buttermilk (—) and whey buttermilk (---). * Indicates significant ($p < 0.05$) transmission decrease throughout the process.](image)

<table>
<thead>
<tr>
<th>Component</th>
<th>Whey buttermilk</th>
<th>Regular buttermilk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCMF</td>
<td>3.02 ± 0.96$^a$</td>
<td>19.76 ± 14.66$^{ab}$</td>
</tr>
<tr>
<td>DFMF</td>
<td>4.73 ± 2.03$^a$</td>
<td>9.88 ± 5.18$^a$</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>21.68 ± 18.21</td>
<td>11.85 ± 6.67</td>
</tr>
<tr>
<td>PE</td>
<td>10.28 ± 6.77</td>
<td>12.74 ± 3.81</td>
</tr>
<tr>
<td>PC</td>
<td>34.12 ± 24.56</td>
<td>34.10 ± 26.08</td>
</tr>
<tr>
<td>SM</td>
<td>21.68 ± 18.21</td>
<td>34.10 ± 26.08</td>
</tr>
<tr>
<td>Proteins</td>
<td>102.66 ± 7.64$^b$</td>
<td>67.93 ± 2.84$^a$</td>
</tr>
<tr>
<td>Ash</td>
<td>33.00 ± 4.79$^a$</td>
<td>18.31 ± 2.79$^a$</td>
</tr>
</tbody>
</table>

Values in the same line with different superscript differs significantly ($p < 0.05$).

± Indicates standard deviation.
and these may have a greater tendency to aggregate with time and maybe form vesicles in combination with MFGM fragments. Moreover, from the lipid profile of regular buttermilk, others have found that buttermilk contained around 74% of long chain (≥14 carbons) fatty acids (Scott, Duncan, Sumner, Waterman, & Kaylegian, 2003) whereas this number was close to 90% for commercial whey (Boyd, Drye, & Hansen, 1999). Surel (1993) has shown that long chain fatty acids are better retained by MF (0.2 μm) as compared to short chain fatty acids. The slight difference in transmission of lipids in the regular and whey buttermilk could be related to their relative content of long-chain fatty acids. The presence of casein micelles in the CP layer during MF of regular buttermilk could induce a more porous layer therefore inducing lipids to permeate more easily. Transmission of lipids in the order of 60% has been reported with regular buttermilk using a 0.5 μm ceramic membrane at 50 °C (Surel & Famelart, 1995). The low temperature used in this experiment might have prevented transmission of lipids through the membrane which is in agreement with others (Astaire et al., 2003). Previous work showed that transmission of lipids was slightly higher at low temperature (Morin et al., 2004) but this was not observed in the present work. A possible explanation is the fact that the initial buttermilk used in this trial was produced in the pilot plant and excess of lipids (8% vs. 12%) was not removed by centrifugation like it would normally happen when industrial buttermilk is used, as it was with our previous work. Increasing the amount of lipids increase the propensity to aggregation (Walstra et al., 1999) and creating therefore vesicles or particles that are rejected by the membrane.

Despite transmission of phospholipids did vary throughout the process and no clear trend could be observed for both products, the average transmission was calculated and differences were noted in total phospholipids for regular buttermilk (Table 2). The DFMF process using regular buttermilk induced more phospholipids transmission as compared to the VCMF process. This result is an indication that the occurrence of CP layer and possibly the fouling of the membrane during VCMF could alter the ability of phospholipids to permeate through the MF membrane. No significant differences between phospholipids profiles (PE, PC, and SM) were observed in the four treatments, given that variations between the three replicates were observed. As the transmission of total phospholipids was increased, all the main classes of phospholipids showed an increase. Therefore, phospholipids classes tends to permeate equally the membrane showing that MF, in the conditions tested, does not show specificity for any class of phospholipids but more on the total amount that permeates.

Protein transmission has been found to be significantly affected by the MF type \(p < 0.05\) and by the buttermilk type \(p < 0.01\). However, the most observable effect was the type of buttermilk. While using whey buttermilk, we achieved a higher transmission of proteins through the MF membrane, compared to regular buttermilk. By observing the SDS-PAGE profile of the permeates from all experiments (Fig. 3), it appears that the type of proteins permeating the MF membrane in the case of whey buttermilk are whey proteins and a non-identified protein (most likely IgG light chain) at around 29 Kda to a lesser extent (Farrell et al., 2004). Almost no MFGM proteins can be observed in the permeates which shows that MF allowed concentration of MFGM components, at least based on the MFGM proteins. Furthermore, this result indicates that MFGM proteins might exist in a different state in whey buttermilk has compared with regular buttermilk. The different processing steps of cheese making and whey cream separation might induce significant changes in their structure which prevent them to permeate through a MF membrane of 0.45 μm. Protein profile of permeates and retentates from DFMF and VCMF for both buttermilks do not appear to be any different, showing that the type of MF mode did not affect the protein composition of the permeates and retentates but rather the total protein content. The protein profile of the regular buttermilk

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**Fig. 3.** SDS-PAGE (12%) profile of whey buttermilk (1), whey buttermilk VCMF permeate (2), whey buttermilk VCMF retentate (3), whey buttermilk DFMF permeate (4), whey buttermilk DFMF retentate (5), regular buttermilk (6), regular buttermilk VCMF permeate (7), regular buttermilk VCMF retentate (8), regular buttermilk DFMF permeate (9), Regular buttermilk DFMF retentate (10). MW: molecular weight marker, XO: xanthine oxidase, BTN: butyrophilin, PAS 6/7: periodic acid Schiff 6/7, CN: caseins, β-Lg: beta-lactoglobulin. Proteins have been identified according to Mather (2000).
permeates shows important amount of caseins but these caseins remains the principal proteins in the retentate suggesting that increasing the VCF or the DF could help to reduce the amount in the retentates. However, some MFGM proteins were observed in the permeates suggesting that important amount of these proteins could also be lost in the permeate if a higher VCF or DF is reached.

Ash transmission was also significantly affected by both the type of MF ($p < 0.05$) and buttermilk type ($p < 0.01$). Dialfiltration is known to induce more transmission of ash in milk UF (Cheryan, 1998) and this is also observed in microfiltration of buttermilk in the present work. The bound minerals to the casein micelles reduce the level of transmission of ash in the case of regular buttermilk.

### 3.4. Concentration factors

Concentration factors of all components in retentate were calculated in order to illustrate the overall efficiency of the MF separations (Table 3). Concentration factor were calculated in dry basis by comparison of the concentration of the components in the final retentate powder as compared to the initial liquid buttermilk collected during the churning of the creams. The CF for both lipids and proteins was significantly affected by the type of MF ($p < 0.01$) but also by the type of buttermilk ($p < 0.05$). The continuous dilution involved in DFMF allowed to “wash out” solutes and solids of size smaller than the MF membrane pores more effectively resulting in higher CF for retained components simply because they end up representing a higher proportion of the retentate total solids. Results show that while using regular buttermilk, the calculated final CF for proteins and lipids are very close. This is in agreement with many reports of problems in separation of lipids and proteins because of the similar size of these two components (Corredig et al., 2003; Morin et al., 2004; Sachdeva & Buchheim, 1997; Surel & Famelart, 1995). However, while using whey buttermilk, this problem does not occur since CF of lipids is higher than that of protein. Moreover, the increase in the CF of proteins could be partly due to an increase in MFGM proteins bound to the retained phospholipids (Fig. 3). The CF reached for phospholipids were definitely higher when using regular buttermilk while not being statistically different ($p = 0.064$) because of the variability in the total phospholipids amount in whey buttermilk. This result is an indication that the phospholipids in whey buttermilk might be less aggregated which could prevent them to be as well retained by the MF membrane as in the case of regular buttermilk. Also, the most likely different composition of the CP layer at the surface of the membrane could modify phospholipids transmission if caseins are present in the case of regular buttermilk, but could also be a facilitator if a higher percentage of lipids are found as it could be the case in whey buttermilk. The affinity of phospholipids for the boundary layer could play an important role in their retention by MF which would show the importance of controlling the parameters of the process in order to use this inevitable boundary layer to our advantage.

### 4. Conclusions

Our results show that using whey buttermilk as a starting material for concentration of MFGM components by microfiltration helps minimizing separation problems associated by the presence of caseins and therefore helps creating an MFGM concentrate of increasing purity. However, this study highlighted the important variability in the lipid portion of whey cream which results in variation of lipid and phospholipids content in buttermilk. This work is the first, to our knowledge, to attempt fractionation of whey buttermilk and before making any attempt to further develop that kind of process, attention should be given to standardize the whey cream prior to butter making as this problem was not observed in regular buttermilk from industrial manufacturing cream. DFMF helped to increase the separation efficiency (flux) but not the selectivity of fractionation. The added processing time, but furthermore, the added volumes of water to process may limit the feasibility of this process. Results obtained while using regular buttermilk were similar to those reported in the literature and modification of the product before microfiltration is definitely needed in order to improve separation. Work is already in progress to develop a novel modification approach.

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