Microbial retention and membrane fouling during low temperature microfiltration of skim milk using ceramic membranes

Julia de A. Fritsch, Cosmin Beliciu and Carmen I. Moraru, Food Science Department, Cornell University, Ithaca, NY 14853

Introduction

The non-thermal removal of microorganisms - both vegetative cells and spores - from milk using membrane separation technology has the potential to significantly improve the safety, quality and shelf life of milk and dairy products. Cross flow microfiltration (CMF) has been used in the last years by the Dairy Industry as a means for microbial removal (Saboya and Maubois, 2000). Microfiltration for microbial removal can only be applied to skim milk, because the contaminating microorganisms are in the same size range as the fat globules from whole milk. Skim milk CMF is typically integrated in the commercial processing of milk and performed at temperatures of 50-55^{o}C due to economical considerations. Yet, maximum benefits could be achieved if microorganisms were removed from milk in the early stages of its collection and processing, preferably in the raw milk stage. Technical challenges arise from the fact that due to regulatory provisions such a process must occur at temperatures <7°C, which greatly limits the permeate flux through the membrane and thus the economical attractiveness of the process. Efforts have been made to develop a cold CMF process that removes microorganisms from raw skim milk and yields economically attractive permeate fluxes. The objective of this work was to understand the formation of the fouling layer during the cold CMF of raw skim milk and to develop solutions to minimize fouling and increase the process yield.

Materials and Methods

Microfiltration Experiments. A pilot-scale experimental setup consisting of a ceramic membrane, a tubular heat exchanger and a centrifugal pump was used to microfilter cold raw skim milk from the Cornell Dairy Plant (Ithaca, NY). The tubular ceramic membrane of nominal pore size of 1.4μm (TAMI membrane, GEA Filtration, WI, USA) had a 25mm diameter, 23 channels of 3.5mm hydraulic diameter each, and a total membrane area of 0.35m^2. The operating temperature was maintained between 6-7^{o}C during the experiments by passing the milk through a counter current tubular heat exchanger that used chilled water as cooling medium. Cross-flow velocities ranging from 5.0m/s to 7.0m/s, and constant transmembrane pressures (TMP) of up to 1.3 bar were tested. The permeate flux was determined gravimetrically, according to the formula:

$$Flux = \frac{Permeate\ \ weight}{Filtration\ \ time \times Milk\ \ density \times Membrane\ \ area} \quad (L/m^2h)$$

The results presented are based on the average of minimum 3 replicate experiments. After each run, the membrane was chemically cleaned following the protocol recommended by the membrane manufacturer. The efficiency of cleaning was verified by determining the water flux of the clean membrane prior to every CMF run.

Protein Analyses. Raw skim milk samples and permeate samples were collected after every experiment, then immediately frozen and stored at –80^{o}C prior to chemical analyses. The following analyses were performed: total protein content (Kjeldahl method AOAC 991.22), non-protein-nitrogen content (NPN) (AOAC 998.05 method), and non-casein nitrogen content.

Microbiological Analyses. Aseptic sampling of permeate was performed using a QMI Aseptic collection system (QMI, St. Paul, MN), consisting of an aseptic collection elbow and aseptic collection 2-liter bags. Samples of microfiltered milk (permeate) were diluted in saline blanks and plated using TSA medium. Plates were incubated at 32^{o}C for 48 hours. Standard plate count was used to quantify colony-forming units (CFU/mL) present in the initial raw milk and in the permeate – immediately after the CMF process and during refrigerated storage.

Scanning Electron Microscopy (SEM). Following a 45min experimental run performed at a cross-flow velocity of 7m/s and TMP of 0.93 bar, the ceramic membrane was taken out of the experimental setup and drained. One end of the membrane was frozen by dipping it into a liquid nitrogen tank and than freeze fractured. Pieces of clean and fouled membranes were freeze dried over night, then sputter coated with a 60:40 mixture of Au:Pd, using a Denton Vacuum Desk II Cold Sputter Etch Unit. The sputter-coated samples were then viewed with a Leica Stereoscan 440 SEM (Leica Cambridge Ltd.; Cambridge, England).

Particle Size Analysis. The particle size distribution in the feed, permeate, as well as the fouling layer was determined by dynamic light scattering, using a Brookhaven 90Plus Nanoparticle Size Analyzer (Brookhaven Instruments Corp., Holtsville, NY). Sample collection from the fouled membrane was performed by gently brushing the inside of the membrane channel with a very thin, clean plastic brush and continuously rinsing with 70 mL UF water for 10 times. 10 mL of the rinse were then diluted with 30 mL UF water in order to achieve the count rate required for an accurate determination (~500 kcps). Each reading consisted of a 4 minute-long measurement, with 8 runs of 30s. Since brushing introduced some ceramic particles into the samples, the dust filter algorithm was employed during data analyses.

Results and Discussion

The CMF process was extremely effective in terms of the physical removal of microorganisms from raw skim milk. More than 4 log reduction in the vegetative microflora was achieved by membrane separation: from a microbial load of 5.25±0.3 log CFU/mL in the raw skim milk, a load of only 0.93±0.48 log CFU/mL in the CMF milk (permeate) was obtained. The microbial load of the permeate was monitored during storage under refrigeration, and counts below 1log CFU/mL were maintained even after 38 days (Figure 1).

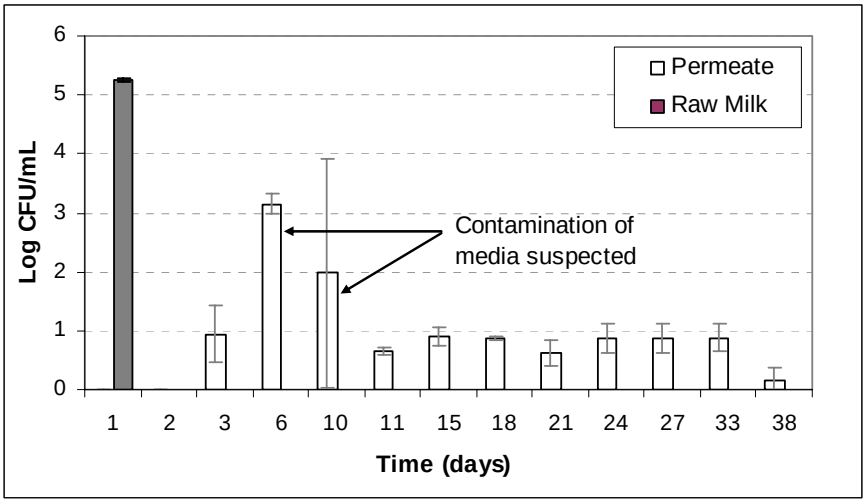

Figure 1: Microbiological quality of microfiltered milk during refrigerated storage

This demonstrates the value of this process for maintaining the microbiological quality of raw skim milk for periods of time that exceed by far the current shelf life of pasteurized milk.

Effect of process parameters. As expected, the main challenge was related to the very low flux obtained during CMF of raw skim milk at low temperature. The flux over time followed a pattern that is common for most membrane separation processes, with a pronounced decline of the permeate flux after the first moments of the process, followed by a slower, but steady decline with time (Figure 2). This is attributed to concentration polarization and fouling effects, and could be counteracted to some extend by a proper selection of the processing parameters.

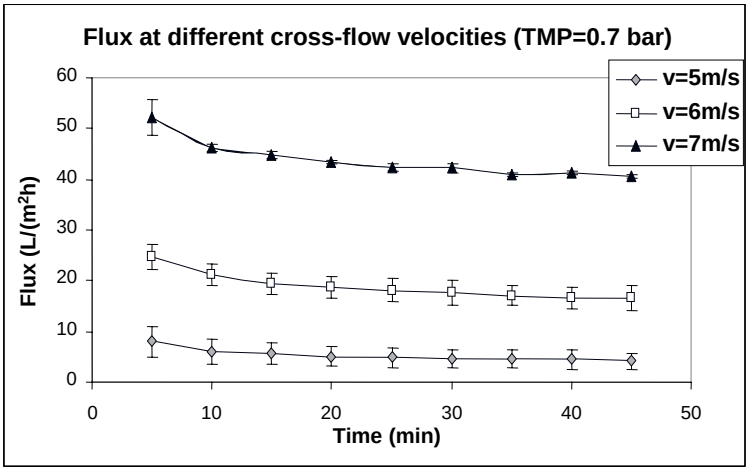

Figure 2: Effect of crossflow velocity on flux

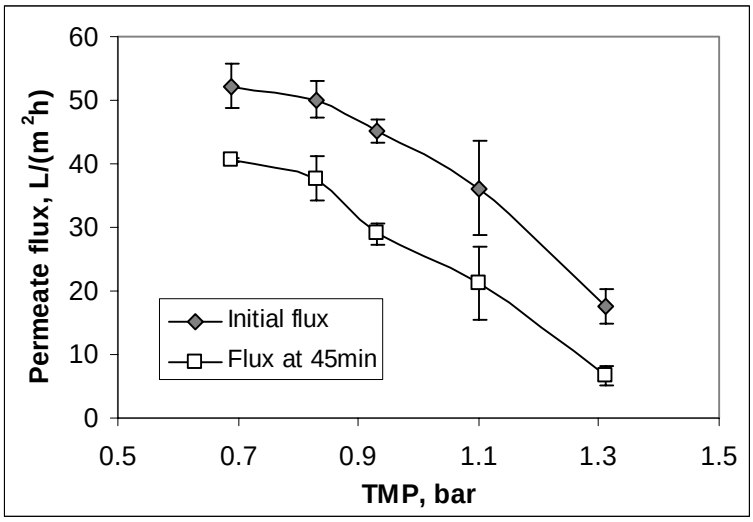

Figure 3: Effect of transmembrane pressure on flux

Cross-flow velocity had the most dramatic influence on flux: after 45min, a flux of 4.2 L/(m^2h) was obtained at 5.0 m/s. The final flux for the 45 min runs increased up to 16.7 L/(m^2h) at 6.0 m/s, and then to 40.5 L/(m^2h) at 7.0 m/s (Figure 2). Since transmembrane pressure (TMP) is the driving force in CMF, it would be expected that the higher the TMP, the larger the permeate flux. Yet, the results of this study demonstrate that higher TMP values resulted in lower fluxes. After 45min, a permeate flux of 6.7 L/(m^2h) was obtained at TMP=1.31 bar, while at TMP=0.69 bar a flux of 40.5 L/(m^2h) was recorded. This behavior was attributed to a more pronounced membrane fouling at high TMP values. It was therefore concluded that the optimal process conditions for cold CMF of skim milk are high cross-flow velocities, which promote turbulent flow and destabilization of the fouling layer, coupled with low TMP.

Besides efficient removal of microorganisms, it is important that after the CMF process milk retains a chemical composition as close as possible to the unfiltered milk. It was observed that the conditions that favored large permeate fluxes also resulted in lower retention of proteins by the membrane, which is the largest concern in terms of depleting the milk from its native components. For instance, for the CMF experiments performed at v=7m/s the flux was the largest and the total protein retention in the microfiltered milk was nearly 100% (Figure 4). This was expected, since those conditions that result in low membrane fluxes are those that promote membrane fouling, which affects both the yield and the selectivity of the membrane. Milk contains two different classes of proteins,

caseins and serum proteins. These have very different molecular sizes and organization, and the membrane most likely preferentially retained the larger proteins, caseins.

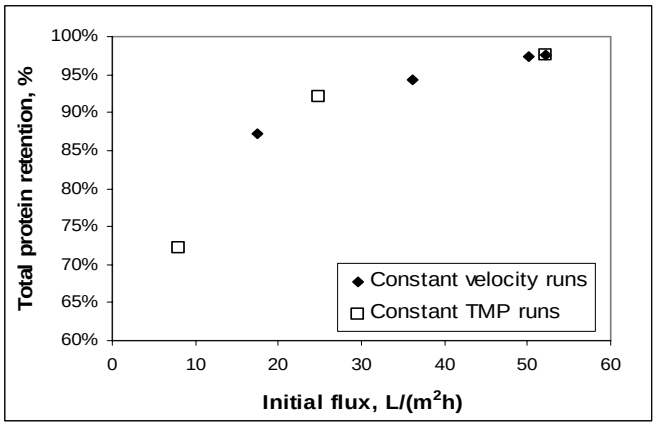

Figure 4: Flux - permeate composition

Table 1: Casein/True protein ratio. TMP=const.

Cross-flow velocity	% CN/TP milk	% CN/TP MF milk	Diff.
5 m/s	76.14%	71.73%	4.41%
6m/s	76.14%	75.47%	0.67%
7m/s	75.03%	74.06%	0.97%

This is confirmed by the data presented in Table 1, which shows that at low crossflow velocities the casein/true protein ratio was much lower in the permeate than in the unfiltered milk, indicating a preferential retention of casein by the membrane. It is interesting that for the experiments performed at varying TMP and v=7m/s the reduction in the casein/true protein ratio was below 1% in all cases, even at high TMP and low fluxes.

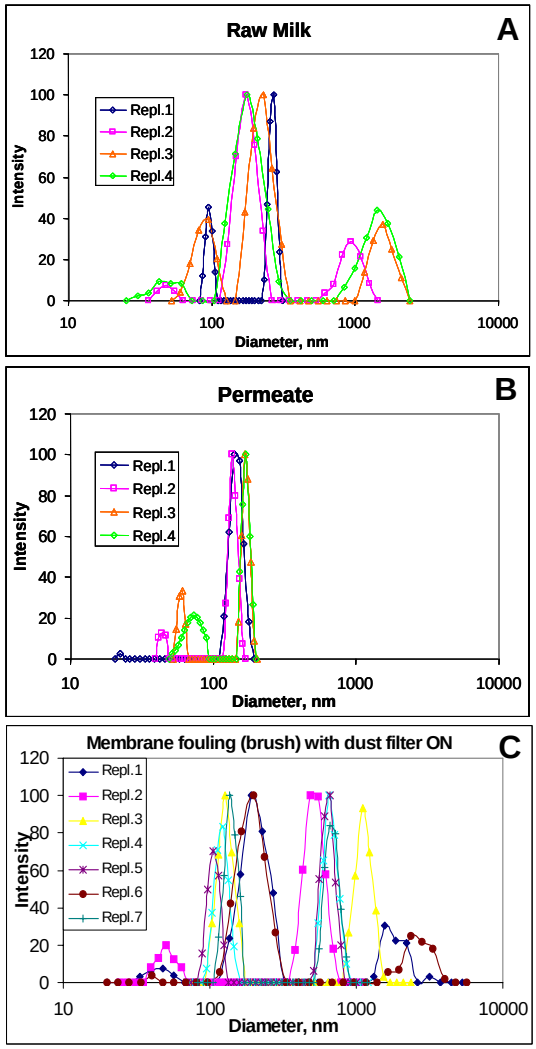

Figure 5: Particle size distribution

The preferential retention of milk components by the membrane was confirmed by particle size analysis. Particle size analyses performed on the feed (raw skim milk) revealed a trimodal distribution with a small peak in the 50-100nm range and two pronounced peaks in the 100 – 500nm and 500 – 2000nm ranges (Figure 5A). The first two peaks were associated with the native milk proteins, while the third one most likely corresponded to a combination of residual fat globules (the skim milk had a fat content <0.1%), bacteria and somatic cells. In permeate only the first two peaks were retained, due to the separation of the larger particles by the membrane (Figure 5B). Interestingly, although the membrane pore size was 1.4μm, the real cut-off was around about 0.2μm (200nm), indicating that the separation was in reality controlled by the fouling layer and not by the membrane itself. Correlating the particle size data with the chemical composition of the permeate, it appeared that a proportion of casein was retained by the membrane. When analyzing the particle size distribution in the fouling material collected from the membrane surface (Figure 5C), it was observed that the distribution also included the particles in the 500 – 2000nm range observed in the initial raw milk (Figure 5A), but also larger particles, not found in the unfiltered milk. This suggested the formation of a new structural organization at the membrane surface.

Characterization of the fouling layer. One technique that can be used to visualize membrane fouling is SEM. Most of the existing microscopic studies of membrane fouling for have been performed on polymeric membranes, although the membranes that are most commonly used for milk applications are ceramic membranes, presumably because of the difficulty of visualizing ceramic membranes. The SEM imaging carried out in this study allowed the visualization of fouled ceramic membranes, and some interesting aspects were observed. First of all, as clearly observed in Figure 6B, a dense fouling layer covers the surface of the membrane, but can also be observed in the internal structure of the membrane. This gel layer significantly altered the membrane pore size, and practically became the dynamic membrane that further controlled the separation process. Also important to note is that microorganisms, residual fat globules or somatic cells did not seem to play a role in fouling, as their presence in the fouling layer was not observed (Figure 6).

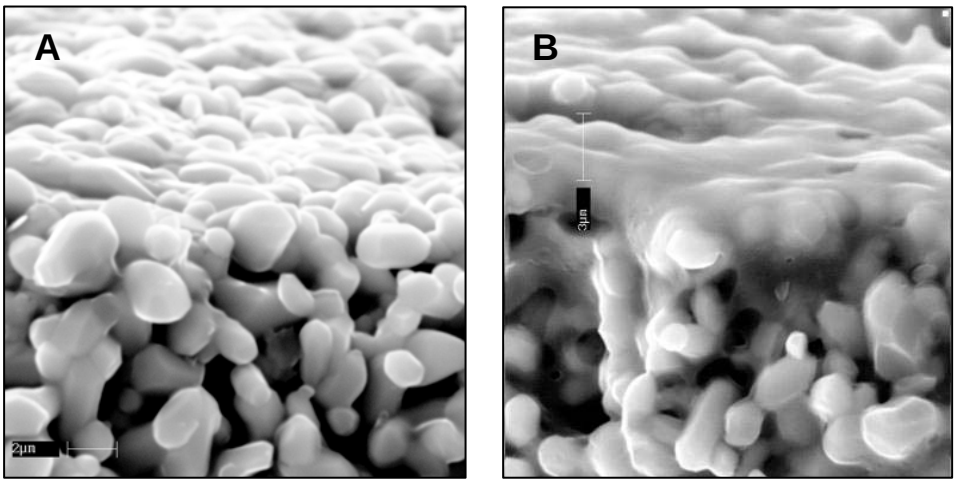

Figure 6: SEM micrographs of ceramic membranes
A – clean membrane; B – fouled membrane

The contribution of the fouling layer to the limited flow through the membrane was quantified using the cake filtration model, which has been proven to be an adequate descriptor of milk microfiltration (Guerra et al., 1997). Based on the cake filtration model, one can estimate the hydraulic resistance of the fouling layer using the formula:

$$J = \frac{\Delta P_{tm}}{\eta R_h}$$

where:

J = *flux (m/s)*

ΔP_{tm} = *transmembrane pressure (Pa)*

η = *viscosity (Pa × s)*

R_h = *hydraulic resistance* (m^{-1})

$R_h = R_m + R_f$

R_m = *hydraulic resistance of the membrane* (m^{-1})

R_f = *hydraulic resistance of the fouling layer* (m^{-1})

The hydraulic resistance of the membrane was estimated based on the flux data obtained during RO water runs, and it was determined to be 3×10^{11} m^{-1}. The hydraulic resistance of the fouling layer was estimated to be on the order of 10^{12} - 10^{13} m^{-1}, which clearly demonstrates that after the fouling layer is formed its resistance will actually control the flow through the membrane.

The SEM imaging allowed us to obtain visual proof of the effect of velocity on membrane fouling. When describing the process, one refers to a single value of the crossflow velocity, but this is in fact an average velocity for the flow channel. In reality, it is known that the velocity has a parabolic profile in the flow channel, meaning that its value varies from a maximum in the center of the flow channel to zero in the boundary layer, next to the membrane surface. When analyzing freeze fractured membrane sampled from the inner channels of the ceramic membrane and comparing them to freeze fractures from the outer channels, it was clearly observed that fouling was much more pronounced for the latter (Figure 7). The reason is that the membrane surface of the outer channels (Figure 7B) was exposed to a slower fluid flow as compared to the channels from the central zone of the membrane (Figure 7A). These micrographs thus represent a visual confirmation of the role of velocity on preventing the formation of a dense fouling layer.

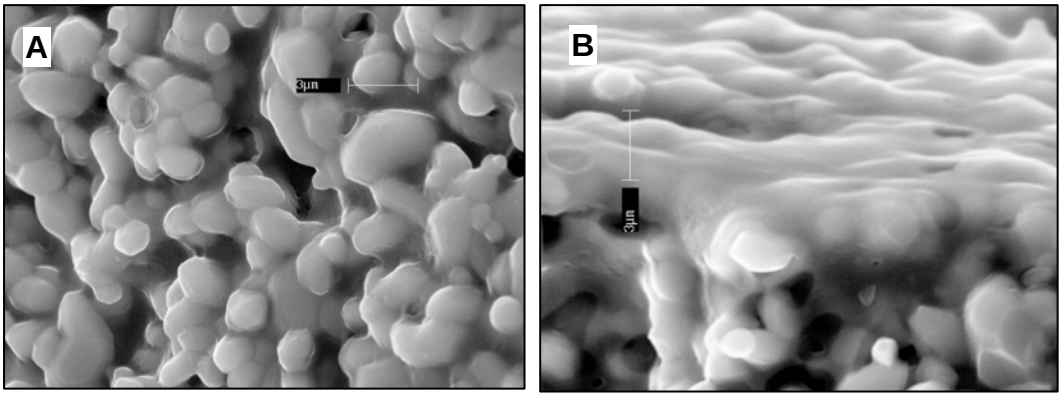

Figure 7: SEM micrographs of sections of ceramic membrane collected from: A – inner channel (high velocity); B – outer channel (low velocity)

At the same time, it becomes clear that unless the flow pattern in the outer channels is disrupted, there will always be a more pronounced retention of some components of the separated liquid on the surface of the membrane in the outer channels, regardless of the average crossflow velocity used for the microfiltration process. In case of milk CMF, this is one of the reasons why the protein composition of milk is slightly changed even under high crossflow velocity conditions (Table 1).

Gas surging technique. Small permeate fluxes increase the capital costs of membrane separation operations, since a larger membrane surface area is necessary to achieve the desired production capacity, and may also result in poor product quality due to the undesirable retention by the membrane of some chemical compounds that are critical for quality – i.e. proteins in case of milk. Uneven TMP along the membrane is one of the reasons for low efficiency of membrane separation processes. This does occur due to significant pressure drops along the membrane, on the retentate side. Techniques that use the uniform transmembrane pressure (UTP) are currently used by the Dairy Industry to address this problem, but typically such techniques require the partial recirculation of the permeate. For a process that yields a very small amount of permeate to start with, the usefulness of such a technique is limited. In this work, a different approach for counteracting the pressure drop and thus the uneven TMP was developed. This technique entailed a pressurized gas (CO_2) surge at the inlet of the membrane where the permeate pressure and thus the TMP is highest, in an attempt to destabilize the fouling layer, allowing for a sudden increase in flux.

The effect of gas surging on the fouling layer was evaluated by estimating the hydraulic resistance of the fouling layer during surging (Figure 8). It can be observed that R_f

was by several times lower during the CO_2 injection as compared to the periods when the CO_2 was turned off, though always still an order of magnitude higher than the hydraulic resistance of the clean membrane.

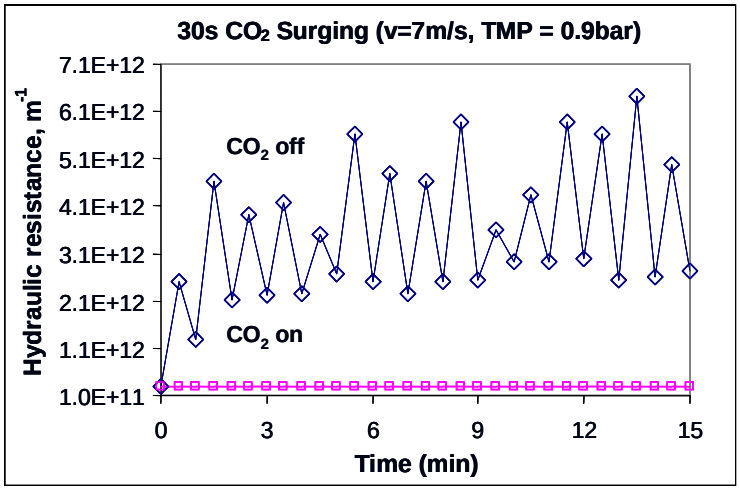

Figure 8: Effect of CO_2 surging on the hydraulic resistance during milk CMF experiments

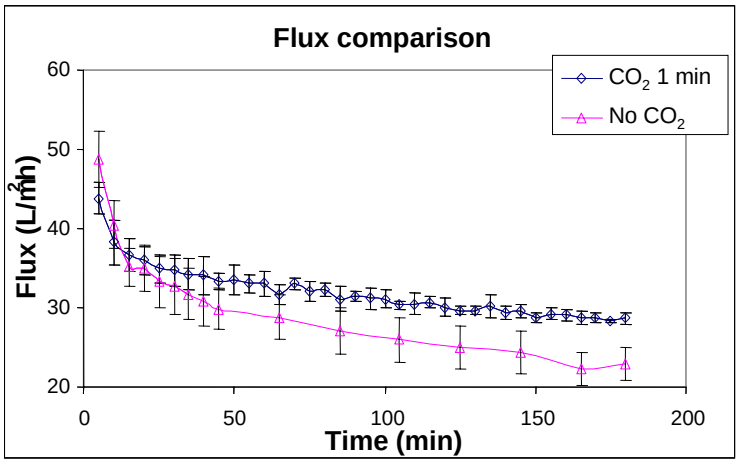

Figure 9: Comparison between the permeate flux obtained with and without CO_2 surging in milk CMF

Several combinations of pressure-time were tested and, following process optimization, a program consisting of CO_2 surges every minute for an average duration of 12 seconds at a pressure equal to that of the inlet pressure (1.38 bar) was selected. During the CO_2 surging runs, the permeate flux was significantly higher and more stable as compared to the fluxes obtained at the same process parameters (v = 7 m/s and TMP = 0.76 bar) but without gas surging. Both the overall larger flux and its steadiness are expected to increase the economical attractiveness of cold microfiltration of milk. In addition, the quality of the microfiltered milk is apparently not affected as a result of CO_2 surging, but this warrants further investigation.

Conclusions

This study demonstrates that cold microfiltration of milk can become a feasible method for microbial removal from raw skim milk, which could have significant benefits for the quality and shelf life of a wide variety of dairy product. Proper selection of the crossflow velocity and transmembrane pressure, coupled with the developed gas surging technique, can help maximize the yield of the process, while maintaining the chemical composition of the microfiltered milk relatively unchanged.

References

Saboya L. V., Maubois J.L. 2000. Current developments of microfiltration technology in the dairy industry. Lait 80:541–553

Guerra A., Jonsson G., Rasmussen A., Waagner Nielsen E. and Edelsten D. 1997. Low Cross-flow Velocity Microfiltration of Skin Milk for Removal of Bacterial Spores. Int. Dairy Journal 7: 849-861

Acknowledgements

The present study was funded by the New York State Milk Promotion Advisory Board.