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The influence of temperature on filtration performance and fouling during cold microfiltration of skim milk

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ABSTRACT

Changes in the physicochemical properties and distribution of constituents in skim milk during microfiltration (MF) at low temperature influence filtration performance and product composition. In this study, the influence of processing temperature within the cold MF range (4, 8 and 12 °C) on filtration performance, fouling and partitioning of proteins was investigated. MF at 4 °C required the greatest energy input due to the significantly higher ($p < 0.05$) viscosity of feed and retentate streams, compared to processing at 8 and 12 °C. The greatest and lowest extents of reversible and irreversible fouling during MF were observed on filtration at 12 and 4 °C, respectively. Chemical analysis of the cleaning solutions post-processing demonstrated that protein was the major foulant; the lowest protein content in the recovered cleaning solutions (50 °C water and 55 °C alkali) was measured after MF at 4 °C. The concentration of β-casein, β-lactoglobulin and α-lactalbumin in the permeate all decreased throughout MF, due to fouling of the membrane. The greatest decrease in concentration of β-casein in the permeate during MF was observed at 12 °C (18.1%) followed by 8 °C (17.1%) and 4 °C (13.6%). The results of this study provide valuable information on processing efficiency (i.e., energy consumption and protein yield) and membrane fouling during the processing of skim milk in the cold MF range.

1. Introduction

Microfiltration (MF) is a technology used for the separation of whey proteins from casein micelles in skim milk, allowing for the generation of functional ingredients such as ideal whey (i.e., that produced using filtration) and micellar casein concentrate [34]. MF of skim milk can be performed using polymeric or ceramic membranes [6,14,28], with the choice of membrane material influencing the efficiency of separation [46]. The temperature at which MF is performed is an important consideration, having significant implications on membrane performance, extent of membrane fouling and composition of the process streams and final ingredients generated. For example, MF of skim milk at high processing temperatures (i.e., 50 °C) results in the generation of ideal whey, containing essentially no β-casein; conversely, MF of skim milk at low temperatures (i.e., 4 °C) results in the generation of ideal whey enriched in caseins, specifically β-casein (i.e., β-casein enriched whey) [14]. This ability to selectively modify protein profile, coupled with lower rates of fouling, has prompted increased research and commercial attention in low-temperature MF approaches for processing of milk [33,38,42].

Fouling remains one of the main challenges during membrane filtration of milk and, in addition to process performance-related challenges, membrane selectivity is also often adversely affected by fouling [5]. The extent of fouling is influenced by a variety of factors including the composition of the feed, pre-treatment of the product, membrane pore size, transmembrane pressure (TMP) and the temperature of filtration [4,45]. During filtration of dairy streams such as milk and whey, it is generally accepted that proteins are the major foulants, due to their interactions with each other and the membrane, with the former often exacerbated by calcium-mediated cross-linking [26,45]. Tan et al. [45] investigated the composition of internal and external foulants after the cold MF (i.e., 6 °C) of skim milk and reported elevated levels of whey proteins in the foulant material, suggesting a higher affinity of whey proteins, compared to caseins, for the membrane material.

Abbreviations: MF, microfiltration; UF, ultrafiltration; CF, concentration factor; VCF, volume concentration factor; NWP, normalised water permeability; TMP, transmembrane pressure; PHE, plate heat exchanger; CCP, colloidal calcium phosphate; β-lg, β-lactoglobulin; α-lac, α-lactalbumin.

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It has been shown that the temperature at which filtration is performed can significantly influence the extent of fouling [33,44,22]. Steinhauer et al. [44] studied the effect of temperature on membrane fouling during the filtration of whey and whey proteins (i.e., β-lactoglobulin; β-lg), reporting that filtration at 50 °C resulted in greater membrane fouling compared to at 10 °C. Méthot-Hains et al. [37] reported that, during the MF of skim milk, operating at 50 °C resulted in a more rapid rate of flux decline compared to at 10 °C; the authors also reported that higher membrane resistance and greater reversible fouling were measured at 50 °C, with similar results reported by Luo et al. [33]. However, although the higher degree of fouling experienced during filtration at high temperatures (i.e., 50 °C) can largely be attributed to proteins, the precipitation of colloidal calcium phosphate (CCP) on the surface or within the pores of the membrane during the filtration of dairy streams, could also contribute to fouling at the higher processing temperatures [21,41].

Membrane fouling during MF of dairy streams can impede the permeation of proteins through the membrane, thereby altering the partitioning dynamics and yield of individual proteins in the permeate [5,22]. Le Berre and Daufin [30] reported that, during cold filtration (i.e., 4 °C) of sodium caseinate suspensions, the permeability of β-casein was limited by the formation of a reversible fouling layer. Beckman and Barbano [5] studied the effect of concentration factor (CF) on serum protein removal during the MF of skim milk at 50 °C, and reported that fouling of the membrane hindered the permeation of whey proteins into the permeate at the different CFs (i.e., 1.50, 2.25 and 3.00×). The authors reported that the protein concentration in the permeate decreased by 0.022, 0.034 and 0.035% per hour at 1.50, 2.25 and 3.00× CF, respectively.

Although operating at low temperature (i.e., 4 °C) allows for the generation of novel ingredients (i.e., enriching permeate in a particular casein such as β-casein), changes in the physicochemical properties (i.e., increased viscosity) and distribution of milk constituents can alter membrane performance and composition of process streams. Therefore, the objective of this study was to investigate the impact of low temperatures at 4, 8 and 12 °C on the processing performance, protein partitioning, fouling and energy consumption during the MF of skim milk. The results of this study provide valuable information on processing efficiency (i.e., energy consumption and protein yield) and membrane fouling in the cold MF range, which can be applied in optimising filtration conditions for the generation of novel milk protein ingredients.

2. Materials and methods

2.1. Materials

Low-heat skim milk powder was provided by a local Irish dairy company. Reconstituted skim milk was prepared by adding low-heat skim milk powder to ultrapure water over 2 h at 22 °C to attain a 3.20% (w/v) protein solution, at a native pH of 6.70. The skim milk was then stored at 4 °C for 48 h under gentle constant magnetic stirring to facilitate complete rehydration. Prior to microfiltration (MF), the reconstituted skim milk was filtered using filter paper (Whatman No. 113; pore size 30 μm). All chemicals, reagents and high-performance liquid chromatography (HPLC) standards, unless otherwise stated, were sourced from Sigma-Aldrich (Wicklow, Ireland) and were of analytical grade.

2.2. Microfiltration set-up

MF experiments were performed on a lab-scale, pressure-driven, tangential-flow filtration device (Pellicon 2 mini-holder; Merck Millipore, Tullagreen, Carrigtwohill, Ireland) as described by Crowley et al. [12], using a 1000 kDa molecular weight cut-off (equivalent to a 0.1 μm pore size MF membrane), V-screen, polyethersulfone membrane (Biomax, Merck Millipore) with 0.1 m^2 total membrane area. MF was performed at 4, 8 and 12 °C, at a cross flow velocity of 0.24 m/s, with a plate heat exchanger (PHE) recirculating the feed/retentate used to control the temperature throughout filtration. An equilibration time of 40 min was allowed at each of the specific temperatures prior to concentration to ensure stable conditions by returning both permeate and retentate lines to the feed vessel (2.5 L; i.e., full recirculation mode). During concentration, the retentate stream was recycled back to the feed vessel, while the permeate was continuously removed until a volume concentration factor (VCF) of 3 was achieved.

In order to assess changes in the partitioning of proteins during MF, permeate samples were taken at different stages throughout filtration, aliquoted in 2 mL Eppendorf tubes and frozen at −80 °C. Samples were collected as soon as permeate started to be produced (taken as VCF 1) and at a VCF of 2 and 3. The protein profile of these permeate samples were then analysed using reversed-phase high performance liquid chromatography (RP-HPLC) to determine the effect of fouling on the permeability of the membrane (outlined in Section 2.6).

2.3. Microfiltration processing performance

2.3.1. Transmembrane pressure and permeate flux

TMP (bar) and permeate flux were measured at 5 min intervals during MF. TMP was calculated according to the following equation:

$$TMP = \frac{P_i + P_o}{2} \quad (1)$$

where P_i and P_o are inlet and outlet pressure, respectively. Permeate flux ($L/m^2/h$) was recorded during processing by measuring the volume of permeate generated in a graduated cylinder over 30 s.

2.3.2. Energy consumption

Power meters (Energenie, Essex, UK) were used to measure the energy consumption (kWh) of both the feed/recirculation pump (i.e., mechanical energy) and the water bath (i.e., thermal energy) controlling the temperature of the PHE at each of the processing temperatures. For thermal energy calculations, it was assumed that the system had ideal thermal insulation and we the dissipation of thermal energy was considered negligible. The total energy at each of the processing temperatures was taken as the sum of the thermal and mechanical energy consumption required to reach a VCF of 3.

2.4. Determination of fouling and membrane cleaning

The extent of fouling, and the efficiency of cleaning, of the membrane was assessed by measuring normalised water permeability (NWP) after filtration, and after each of the cleaning solution cycles for comparison to reference values. The NWP ($L\ m^{-2}h^{-1}\ bar^{-1}$) was calculated as follows:

$$NWP = \frac{R \times F}{A \times TMP} \quad (2)$$

where R is permeate flow rate ($L\ h^{-1}$), F is the temperature correction factor based on water fluidity relative to 25 °C (–), A is the membrane area (m^2), and TMP is the transmembrane pressure (bar). Immediately after completion of filtration, 1 L of ultrapure water was flushed through the membrane to remove any remaining skim milk within the membrane and tubing. Following this, NWP was measured using 1.4 L of ultrapure water. This value was taken as the NWP of the fouled membrane after filtration and the water used to perform the NWP was collected and taken as the initial water clean. After this, 1 L of ultrapure water was recirculated through the membrane at 50 °C for 50 min (i.e., 50 °C water) with an applied TMP of 0.2 bar; this cleaning solution was collected and taken as the 50 °C water clean and a second NWP measurement was performed using 0.5 L of ultrapure water. Finally, 1 L of

0.4 N NaOH was recirculated through the membrane at 55 °C for 50 min (i.e., 55 °C alkali) with an applied TMP of 0.2 bar; this cleaning solution was collected and taken as the 55 °C alkali clean, and a final NWP measurement was performed on the cleaned membrane using 0.5 L of ultrapure water. Prior to each new trial, an NWP test was also performed on the clean membrane, to allow both the extent of fouling, and the efficiency with which each of the cleaning solutions restored NWP after filtration, to be calculated.

In addition to the cleaning procedures detailed above, the membrane was further cleaned on a separate day to ensure that the membrane was in the best condition for each subsequent trial. The cleaning steps applied were as follows; 2 L of 50 °C ultrapure water recirculated for 30 min, followed by 1 L of 55 °C 0.4 N NaOH recirculated for 50 min, 2 L of ultrapure water flushed to waste and 1 L of 3% (w/v) ethylenediamine tetra-acetic acid recirculated for 30 min at 25 °C. A TMP of 0.2 bar was applied for all cleaning steps. This ensured that the NWP for all processing trials was > 84% of that of the new membrane. For cleaning of the PHE, the membrane element of the unit was bypassed and a stepwise cleaning cycle was performed, consisting of ultrapure water (2 L), 1.0 N NaOH (1 L), ultrapure water (2 L), 1.0 N HNO_3 (1 L), followed by a final flush with ultrapure water (2 L).

2.5. Composition and rheological properties of process streams

Retentate samples were collected at 12 min intervals during MF and the total solids were determined using standard International Dairy Federation methodology [25]. Total nitrogen content of each of the cleaning solutions and process streams (i.e., feed, retentate and permeate) was determined using the Kjeldahl method using a nitrogen-to-protein conversion factor of 6.38 for the calculation of crude protein [23]; ash content was determined after heating samples to 650 °C until a white ash was obtained [24].

The ionic calcium concentration of feed, permeate and retentate samples was measured using a Titrando 907 autotitrator with Tiamo v2.2 software equipped with a calcium (Ca)-ion-selective electrode (Metrohm Ireland Ltd, Co. Carlow, Ireland) according to the method reported by Crowley et al. [11], with minor modifications. The probe was calibrated at 25 °C using buffer solutions of known calcium-ion concentration (0.50, 1.00, 2.50, 5.00, 10.0 mM). Samples were equilibrated with the probe for 1 min prior to taking measurements.

Apparent viscosity of feed, permeate and retentate streams was measured at a shear rate of 1000 s^{-1} using a rotational viscometer (HAAKE RotoVisco 1, Thermo Fisher Scientific, MA, USA) with a cylindrical double gap cup and rotor geometry (DG43, Thermo Fisher Scientific, MA, USA). The temperature at which the viscosity of the samples was determined was the same as the processing temperature used to generate the samples (i.e., 4, 8 and 12 °C).

2.6. Protein profile of process streams

The protein profile of the feed, retentate and permeate (final bulk permeate and individual in-process permeate at VCF 1, 2 and 3) samples were measured using RP-HPLC (Agilent 1220 Infinity II LC, Santa Clara, CA 95051 United States) with a C18 column (3.6 μm × 250 mm × 4.6 mm, Aeris Widepore, Phenomenex, UK) as described by Bonfatti et al. [7], Crowley et al. [13] and Bot et al. [8], using solvents A (10.0% HPLC-grade acetonitrile, 89.9% ultrapure water and 0.10% TFA) and B (89.9% HPLC-grade acetonitrile, 10.0% ultrapure water and 0.10% TFA); the injected volume was 40 μL, and detection was carried out at 214 nm. Prior to analysis, samples were filtered through 0.45 μm filters (Minisart® RC25, Göttingen, Sartorius AG, Germany). The concentrations of individual proteins were determined by preparing standard curves of the respective proteins ($R^2 > 0.99$) and results are expressed as mg protein per mL of sample.

2.7. Statistical analysis

All experimental analyses were conducted in triplicate, with samples being produced from three independent trials for each processing temperature, unless otherwise stated. Results are expressed as mean ± standard deviation. Analysis of variance (one-way ANOVA; Tukey's HSD test) was performed using R i386 version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) on mean values. The level of significance was determined at $p < 0.05$ to determine whether statistical differences were present between the mean values. Linear regression analysis by least squares minimisation was performed using Microsoft Excel 2007. The goodness-of-fit was evaluated based on the correlation coefficient (R^2), with an $R^2 > 0.99$ indicating a strong correlation.

3. Results and discussion

3.1. Processing performance

The temperature at which filtration is performed influenced both the permeate flux and the rate of permeate flux decline (Fig. 1). Initial permeate flux values of 13.2, 12.0 and 11.0 L/m^2/h were measured at 12, 8 and 4 °C, respectively. Permeate flux decreased in a linear manner throughout processing, resulting in final flux values of 7.4, 7.0 and 6.2 L/m^2/h at 12, 8 and 4 °C, respectively. The rate of decline in flux, determined by calculating the slope of the flux-time profiles, was highest at 12 °C (−0.059), followed by 8 °C (−0.049) and 4 °C (−0.042).

From Fig. 1, it is evident that, at the beginning of MF, there are large differences in permeate flux at the different temperatures; however, after 45 min, differences in permeate flux are considerably less, with permeate flux reaching similar values towards the end of filtration. The higher flux recorded at 12 °C was expected, due to the significantly lower ($p < 0.05$) apparent viscosity (2.06 mPa.s) of the permeate at 12 °C than at 8 and 4 °C, with values of 2.21 and 2.48 mPa.s, respectively (Table 1). McCarthy et al. [35] reported higher initial flux values when MF of skim milk was carried out at 50 °C, compared to 8.9 °C; in agreement with the results from the present study, those authors also observed that flux decline was more rapid at the higher processing temperature, and attributed this to greater fouling. Similar results have been previously reported by Luo et al. [33] and Méthot-Hains et al. [37], who reported that ultrafiltration (UF) of skim milk at low processing temperatures (i.e., 10–15 °C) results in a lower extent of flux decline than at high processing temperatures (i.e., 50 °C).

TMP is a key processing parameter determining filtration process performance. Initial TMP values of 0.11, 0.12 and 0.14 bar were measured at 12, 8 and 4 °C, respectively. During MF at each of the processing temperatures, TMP increased gradually for the first 70 min of filtration and after this, the greatest increase in TMP was observed at 12 °C.

3.2. Effect of processing temperature on energy consumption during filtration

The mechanical and thermal energy requirements during the MF of skim milk are strongly dependent on processing temperature (Table 1). At 4 °C, significantly higher ($p < 0.05$) mechanical energy requirements were measured compared to at 8 and 12 °C, with values of 27.6, 24.6 and 22.9 × 10^{-3} kWh, respectively. This can be attributed to the significantly higher ($p < 0.05$) viscosity of the skim milk feed and retentate at 4 °C; the viscosity of the final retentate generated at 4, 8 and 12 °C was 9.82, 8.58 and 7.11 mPa.s, respectively. The energy required to maintain the MF plant at 4 and 8 °C was also significantly higher ($p < 0.05$) than at 12 °C (Table 1). These results are in accordance with those of Méthot-Hains et al. [37], who reported that optimal conditions, in terms of energy consumption, were achieved when UF of skim milk was performed at a higher temperature (i.e., 50 °C), compared to a lower

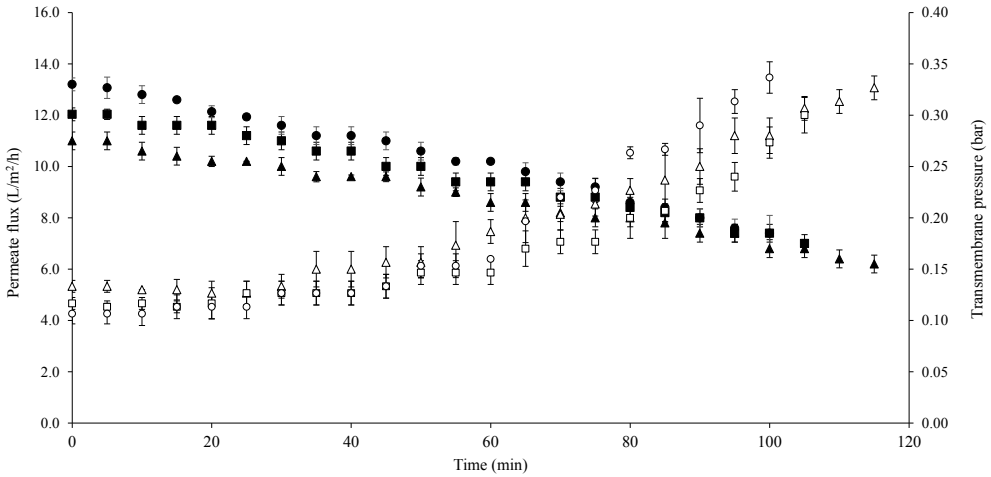


Fig. 1. Permeate flux (closed symbols) and transmembrane pressure (open symbols) as a function of time during the microfiltration of skim milk at 4, 8 and 12 °C: permeate flux at 4 °C (▲), 8 °C (■) and 12 °C (●); transmembrane pressure at 4 °C (△), 8 °C (□) and 12 °C (○). Filtration was continued until a volume concentration factor of 3 was achieved.

Table 1
Mechanical and thermal energy requirements to reach a volume concentration factor of 3 during the microfiltration of skim milk and the apparent viscosities of skim milk feed, permeate and retentate at 4, 8 and 12 °C.

Processing temperature (°C)	Energy consumption (10^{-3} kWh)			Viscosity (mPa.s)		
	Mechanical	Thermal	Total	Feed	Retentate	Permeate
4	27.6 ± 1.10^{c}	872 ± 21.0^{b}	900	3.69 ± 0.10^{c}	9.82 ± 0.32^{c}	2.48 ± 0.16^{b}
8	24.6 ± 0.60^{b}	846 ± 17.0^{b}	871	3.13 ± 0.09^{b}	8.58 ± 0.46^{b}	2.21 ± 0.21^{a}
12	22.9 ± 0.50^{a}	791 ± 30.0^{a}	814	2.70 ± 0.08^{a}	7.11 ± 0.48^{a}	2.06 ± 0.05^{a}

[a-c]Values followed by different superscript letters within a column are significantly different ($p < 0.05$).

temperature (i.e., 10 °C). In addition to higher process stream viscosity, the higher energy requirements at 4 °C may be partly attributed to the generation of heat through frictional forces (i.e., pumping), which would increase the energy required to maintain the lower temperature; longer processing times required to reach a VCF of 3 at 4 °C also contributed to higher energy requirements.

3.3. Influence of processing temperature on retentate and permeate composition

MF processing at 12 °C resulted in the greatest increase in total solids in retentate throughout filtration and the highest total solids in the retentate at the end of MF (14.1% w/w), compared to at 8 and 4 °C, with values of 13.9 and 13.8% (w/w), respectively. However, higher total solids were measured in permeate generated at 4 °C, with values of 6.56, 6.51 and 6.49% (w/w) for permeates generated at 4, 8 and 12 °C, respectively. MF of skim milk to a VCF of 3 resulted in significant ($p < 0.05$) increases in levels of protein in retentates, with the highest protein content in the retentate generated at 12 °C (7.80%) (Table 2). For permeates, a significantly higher ($p < 0.05$) protein content was measured at 4 °C, followed progressively by permeates generated at 8 and 12 °C. The higher degree of dissociation of β-casein into the serum phase of milk at lower temperatures [36], allowing for increased partitioning thereof into the permeate, is likely to have resulted in the higher protein content, in addition to the higher total solids measured in the permeate generated at 4 °C.

Ash content of retentates after MF was also significantly ($p < 0.05$) higher than that of the initial skim milk, due to the concentration of casein micelles and associated CCP (Table 2). As expected, a lower ash content was measured in permeates than retentates, while the ash content was significantly ($p < 0.05$) higher in the permeate generated at 4 °C, with values of 0.51, 0.47 and 0.46% for permeates generated at 4, 8 and 12 °C, respectively. A similar trend was observed for ionic calcium, with the highest concentration of ionic calcium measured in the permeate generated at 4 °C (3.07 mM). The solubilisation of CCP at low temperatures [17] has been shown to result in increased calcium concentration in permeate following the MF of skim milk [35].

Table 2
Ash, protein and ionic calcium concentrations in skim milk feed, retentate and permeate samples generated from the microfiltration of skim milk at 4, 8 and 12 °C.

Sample	Processing temperature (°C)	Ash (%)	Protein (% w/v)	Ionic calcium (mM)
Feed	n.a.	0.74 ± 0.02	3.18 ± 0.04	2.45 ± 0.09
Retentate	4	1.16 ± 0.01^{ab}	7.59 ± 0.08^{a}	2.52 ± 0.07^{a}
	8	1.18 ± 0.03^{b}	7.77 ± 0.13^{b}	2.48 ± 0.08^{a}
	12	1.14 ± 0.02^{a}	7.80 ± 0.17^{b}	2.52 ± 0.12^{a}
Permeate	4	0.51 ± 0.01^{b}	0.634 ± 0.006^{c}	3.07 ± 0.16^{b}
	8	0.47 ± 0.01^{a}	0.617 ± 0.007^{b}	2.93 ± 0.07^{b}
	12	0.46 ± 0.02^{a}	0.585 ± 0.007^{a}	2.77 ± 0.04^{a}

[a-c]Values followed by different superscript letters within a column for each analysis within a specific sample set (e.g., ash content for retentate) are significantly different ($p < 0.05$).
n.a. = not applicable.

3.4. Effect of processing temperature on membrane fouling and composition of cleaning solutions

The extent of fouling after MF of skim milk at 4, 8 and 12 °C was assessed by measuring the NWP after filtration and after each individual cleaning step, in order to obtain information on the extent of fouling and the nature of the foulant (Fig. 2). The greatest decrease in NWP after MF was at 12 °C, with NWP decreasing to 68.6% of the original NWP

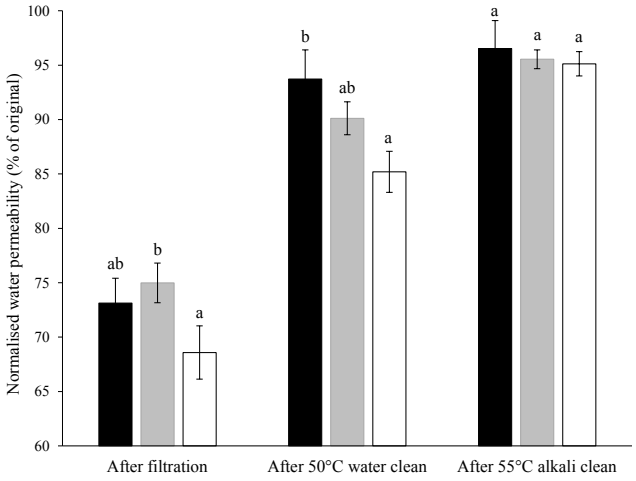


Fig. 2. Normalised water permeability, measured after filtration, after water clean at 50 °C and after alkali clean at 55 °C, as a percentage of the original normalised water permeability measured prior to filtration at 4 (■), 8 (▒) and 12 °C (□). [a,b] Mean values with different superscript letters within each individual cleaning step (e.g., after filtration) are significantly different ($p < 0.05$).

measured prior to filtration. Lower decreases in NWP were measured after MF at 8 and 4 °C, decreasing to 75.0 and 73.1% of the original NWP, respectively, indicating less fouling compared to that on MF at 12 °C. Barukčić et al. [4] measured water flux before and after MF of whey with different mean pore sizes (0.1, 0.5 and 0.8 μm), at 20 and 50 °C, and reported that fouling intensity was reduced at the lower processing temperature.

After determining the NWP of the fouled membrane, the membrane was cleaned using ultrapure water at 50 °C and the NWP was measured, yielding information on the loosely bound fouling material. Following cleaning with water at 50 °C, the NWP for the membrane increased at all selected processing temperatures, approaching that of the original NWP. The NWP of the membrane after MF at 4 °C (93.7% of the original NWP) was significantly higher ($p < 0.05$) than that of the membrane following MF at 12 °C (85.2% of the original NWP). The largest increase in NWP after the water clean at 50 °C was for the membrane following MF at 4 °C, with a 20.6% increase in NWP, while the smallest increase in NWP after the 50 °C clean was at 8 °C, followed by 12 °C, with NWP increasing by 15.1 and 16.6% at those temperatures, respectively. Therefore, these results indicated that MF at 4 °C resulted in the greatest amount of weakly attached foulant.

The NWP after the 55 °C alkali cleaning step was measured to provide information on the amount of strongly attached fouling material. Only a small increase in NWP was observed for the membrane following MF at 4 °C, compared to 8 and 12 °C with a 2.81, 5.42 and 9.92% increase in NWP, respectively; although more loosely bound fouling material was observed at 4 °C, lower amounts of strongly bound material were present. Cleaning of the membrane with water at 50 °C following MF at 4 °C restored NWP to values close to those prior to MF, suggesting that mild cleaning regimes may be adequate at 4 °C. The results indicate that more strongly bound fouling material was present following MF at 8 and 12 °C. Steinhauer et al. [44] studied the effect of temperature on membrane fouling during MF and UF of whey and individual whey proteins and the authors reported that the flux decrease during MF at temperatures ≤ 20 °C was due to the formation of a loosely packed fouling layer, whereas at higher temperatures (i.e., 40 °C) a more densely packed fouling layer was formed. The formation of a loosely packed fouling layer at 4 °C in the present study is supported by the effectiveness of water in the removal of foulant material.

The difference between the original and the final NWP after cleaning (data not shown) provided an index of the extent of irreversible fouling. Although the differences were not significant ($p > 0.05$), the lowest extent of irreversible fouling was measured at 4 °C, followed by 8 and 12 °C, with values of 3.47, 4.46 and 4.84%, respectively. As expected, irreversible fouling was considerably more minor than reversible fouling, as the former is often associated with operating at limiting flux (high TMP) conditions [43,22]. During MF, TMP remained low until the last 30–40 min of filtration and is therefore unlikely to have allowed sufficient time for extensive irreversible deposition (Fig. 1).

Fouling of the membrane after filtration has been shown to be predominately caused by interactions between proteins and the polymeric membrane material and the formation of protein-protein aggregates that can result in pore blocking [26]. In terms of fouling mechanisms attributed to different milk proteins, casein micelles/aggregates have been shown to cause pore blockage, while whey proteins cause internal fouling and adsorption to the surface of the membrane during the MF of skim milk [9]. Therefore, the composition (ash and protein levels) of the cleaning solutions were analysed to determine the influence of processing temperature on the composition of the fouling layer (Fig. 3a, b). Higher levels of protein and ash were measured in the initial clean (ultrapure water) following MF at 4 °C, although no significant ($p > 0.05$) differences between the selected processing temperatures were evident. The significantly higher ($p < 0.05$) viscosity of the feed and retentate at 4 °C is likely to have reduced turbulence at the membrane surface, thereby increasing concentration polarisation and reducing the efficiency of foulant removal from the surface of the membrane [20,1,27].

Protein and ash levels following the initial clean were significantly

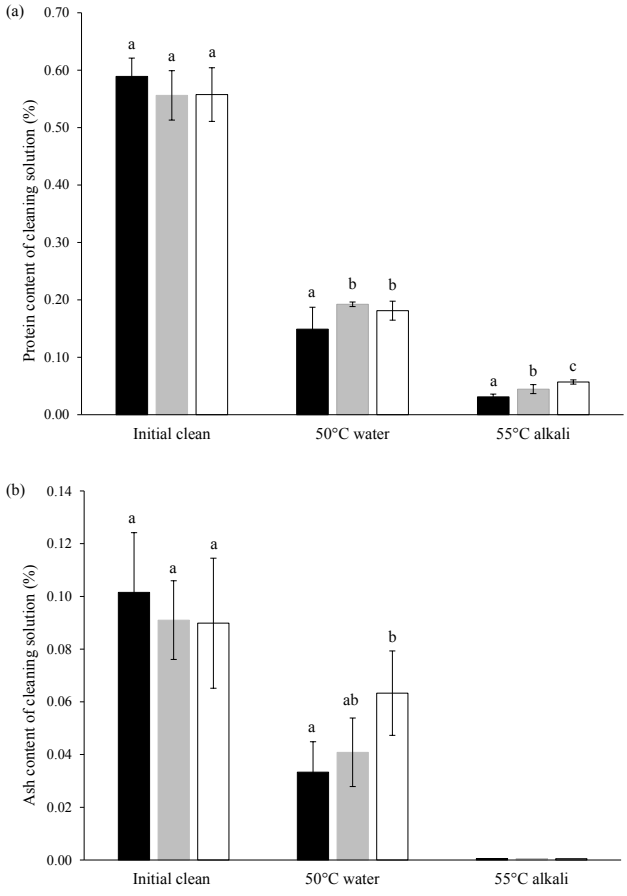


Fig. 3. Concentration of (a) protein and (b) ash in the initial water clean, water clean at 50 °C and alkali clean at 55 °C collected after the microfiltration of skim milk at 4 (■), 8 (▒) and 12 °C (□). Protein and ash values are related back to the total volume of cleaning solution used. [a,b,c] Mean values with different superscript letters within each individual cleaning step (e.g., after filtration) are significantly different ($p < 0.05$).

lower ($p < 0.05$) in the 50 °C water solution, with the lowest level of protein and ash following MF at 4 °C, with values of 0.15% and 0.03%, respectively. Similar results were observed in the 55 °C alkali solution, with significantly less ($p < 0.05$) protein removed from the membrane following MF at 4 °C, compared to that at 8 or 12 °C. Although proteins are the major foulants during filtration of dairy streams, minerals such as calcium phosphate can also contribute to fouling, affecting membrane performance [18]. The low levels of ash measured in the cleaning solutions in this study suggest that the contribution of minerals to membrane fouling were not as significant as that of proteins. However, the solubilisation of CCP at the low temperatures may have indirectly contributed to membrane fouling, as the solubilisation of CCP at low temperature results in an increase in the concentration of ionic calcium, which can facilitate the formation of protein aggregates and cause blocking of membrane pores. In addition, cleaning of membranes at high temperatures (i.e., 50–55 °C) may have resulted in some precipitation of CCP onto the membrane, preventing its removal [41].

3.5. Influence of temperature and volume concentration factor on protein partitioning

In order to understand how the partitioning of proteins changes throughout an MF run, as a function of temperature, permeate samples were collected at different VCFs (Table 3). α_s-Caseins were not detected in permeates, while the concentration of κ-casein did not change throughout MF (data not shown). Concentrations of β-casein, β-lg and α-lactalbumin (α-lac) in permeates all decreased throughout MF, with the greatest decrease measured for β-casein. The extent of decrease in concentration of β-casein in permeate from the beginning of MF (VCF 1) to the end (VCF 3) was in the order 18.1, 17.1 and 13.6% at 12, 8 and 4 °C, respectively. At low temperatures (i.e., 4 °C), β-casein partially dissociates from the micelle into the serum phase, with β-casein in the serum primarily existing in monomeric form [40,3]. As temperature increases, β-casein monomers begin to self-associate, yielding micelles of β-casein, with a larger radius than that of the monomeric form [3,32]. Paynes and Van Markwijk [40], reported that β-casein exists as a monomer at 4 °C but, as temperature increased to 8.5 °C, the degree of association increased to approximately 22. Similarly, subsequent research has shown that reducing temperature from 20 to 0 °C decreases the tendency to associate and hydrodynamic radius of β-casein micelles, with β-casein existing primarily in a monomeric state at 4 °C [31,16,39]. Therefore, it can be hypothesized that fouling during MF is likely to have affected the permeation of β-casein in polymeric state (i.e., at 8 and 12 °C) to a greater extent than that in the monomeric state (i.e., at 4 °C). In addition, the higher rate of decrease in β-casein concentration at 12 °C can be attributed to the greater extent of fouling (Fig. 2) at that temperature, reducing the permeability of the membrane.

The extent of decrease in the respective concentrations of β-lg and α-lac in the permeates during MF was lower than that observed for β-casein (Table 3). Unlike the trend observed for β-casein, the greatest percentage decrease in the concentration of α-lac was observed during MF at 4 °C, while MF at 12 °C resulted in a significantly ($p < 0.05$) lower decrease in the concentration of α-lac during MF. The largest decrease in β-lg during MF was observed at 8 °C (3.39%), while the lowest decrease was observed at 4 °C (2.30%). The reduced transmission of proteins as a result of altered membrane permeability due to fouling has been reported by numerous researchers [19,29]. It is well known that the whey proteins, in particular β-lg, are strongly responsible for membrane fouling during the filtration of skim milk and whey streams at low (i.e., <10 °C) [45] and high temperatures (45–55 °C) [38,22].

The differences in the transmission of β-casein and the whey proteins during MF at the different processing temperatures, may be attributed to the mechanism responsible for dissociation of β-casein. β-Casein is located primarily in the interior of the casein micelle and, at low temperature, β-casein first migrates to the surface of the casein micelle, from where it dissociates into the serum phase. At 4 °C, the rate of dissociation of β-casein into the serum phase is greater than at 8 and 12 °C, due to lower hydrophobic interactions [10,36]; therefore, β-casein physically removed in the permeate stream during MF is, at least partially, replenished by further dissociation from the micelle. As there is an equilibrium between micellar and serum β-casein [15], the greater rate of dissociation of β-casein from the micelle at 4 °C, as free β-casein permeates the membrane, may have resulted in the lower rate of decrease in β-casein during MF at 4 °C. On the other hand, for α-lac and β-lg, their serum phase concentrations are not in an equilibrium or dependent on the processing temperature; therefore, the differences observed for α-lac during MF are likely to be attributed to interactions with the membrane and fouling. α-Lac has the ability to bind Ca^{2+} [2], which could explain the influence of temperature on transmission of α-lac, as there was more calcium ions present at 4 °C, which may result in greater potential for calcium-mediated interactions between α-lac molecules and salt bridging between α-lac and the membrane.

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3.6. Influence of temperature on the protein profile of process streams

The profile and concentrations of individual proteins in the permeate and retentate streams were analysed to provide further insight into the

Table 3
Concentration of β-casein, α-lactalbumin and β-lactoglobulin in permeates collected at different stages throughout the microfiltration of skim milk at 4, 8 and 12 °C. Permeate was collected at a volume concentration factor (VCF) of 1, 2 and 3 and the percentage decrease in concentration of each protein, from the beginning to the end of microfiltration, was calculated.

Protein	Processing temperature (°C)	Protein content (mg/mL)			
		VCF 1	VCF 2	VCF 3	% decrease
β-casein	4	2.24 ± 0.06^{b}	2.09 ± 0.09^{ab}	1.93 ± 0.06^{a}	13.6 ± 0.52^{A}
	8	1.67 ± 0.02^{b}	1.50 ± 0.05^{a}	1.38 ± 0.06^{a}	17.1 ± 1.88^{B}
	12	1.05 ± 0.03^{c}	0.94 ± 0.01^{b}	0.87 ± 0.03^{a}	18.1 ± 2.73^{B}
α-lactalbumin	4	0.80 ± 0.005^{b}	0.79 ± 0.009^{b}	0.72 ± 0.003^{a}	9.96 ± 1.03^{B}
	8	0.82 ± 0.013^{c}	0.79 ± 0.007^{b}	0.77 ± 0.007^{a}	6.22 ± 2.24^{AB}
	12	0.84 ± 0.010^{b}	0.81 ± 0.008^{a}	0.81 ± 0.006^{a}	3.19 ± 1.39^{A}
β-lactoglobulin	4	2.16 ± 0.03^{a}	2.15 ± 0.06^{a}	2.11 ± 0.03^{a}	2.30 ± 0.68^{A}
	8	2.25 ± 0.04^{b}	2.17 ± 0.04^{a}	2.17 ± 0.04^{a}	3.39 ± 1.11^{A}
	12	2.35 ± 0.03^{b}	2.33 ± 0.03^{ab}	2.28 ± 0.02^{a}	2.61 ± 1.13^{A}

[a-c]Values followed by different superscript letters for the concentration of protein within a row are significantly different ($p < 0.05$).
[A-B]Values followed by different superscript letters for the percentage decrease in protein during filtration within a column for individual protein (i.e., β-casein) are significantly different ($p < 0.05$).
Values are means ± standard deviations from two independent trials at each processing temperature.

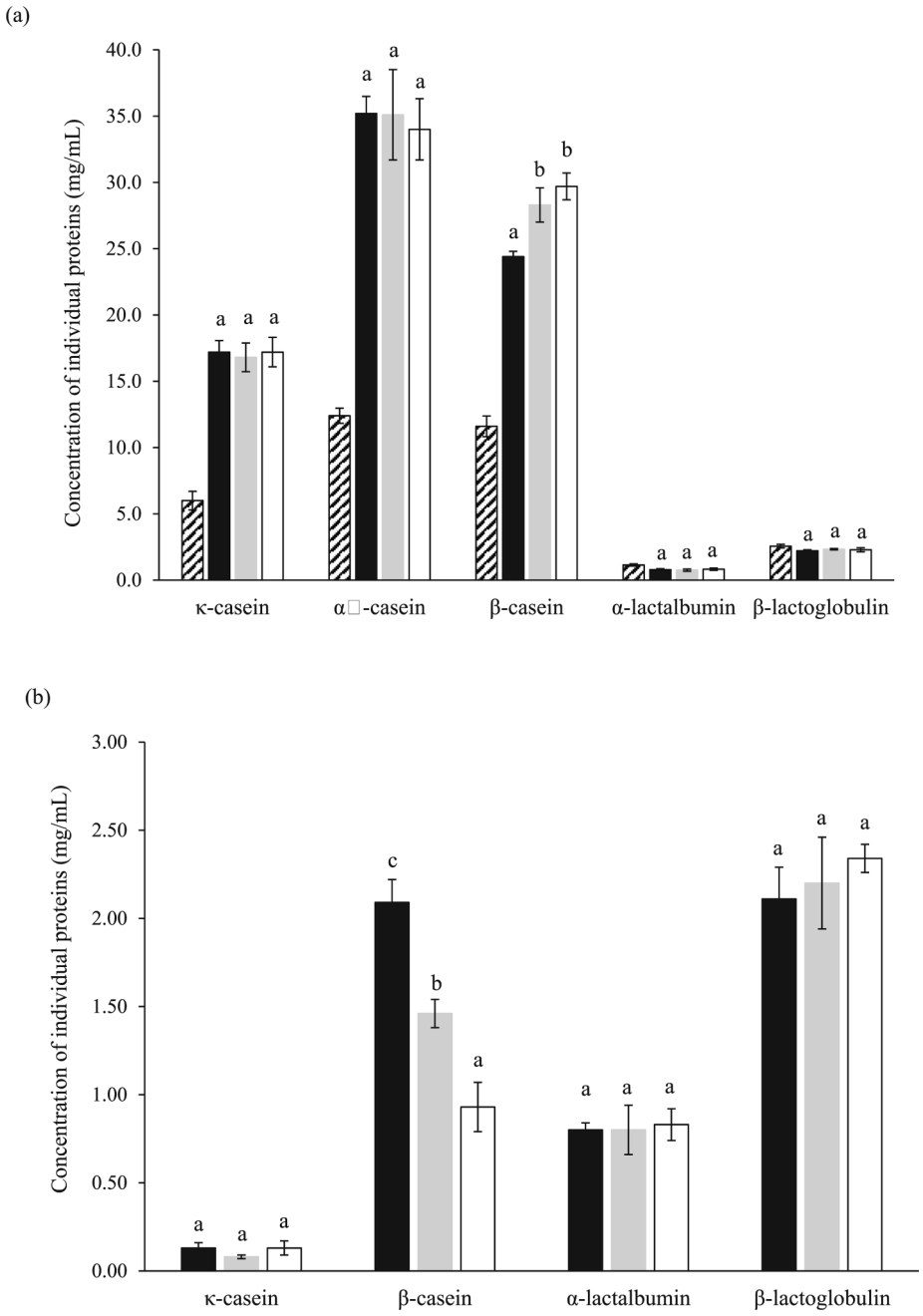


Fig. 4. Concentrations of individual proteins in (a) feed (skim milk: ▨) and retentate, and (b) permeate after microfiltration of skim milk at 4 (■), 8 (▩) and 12 °C (□) as measured using reversed phase-high performance liquid chromatography. [a,b,c] Mean values with different superscript letters within each individual protein are significantly different ($p < 0.05$). Different scale values were used between figure (a) and (b).

influence of processing temperature on individual protein partitioning (Fig. 4). As expected, the concentrations of all individual caseins were higher in the retentates than in the feed. No significant ($p > 0.05$) differences were observed in the concentrations of κ- and α_s-casein, in addition to α-lac and β-lg, between retentates produced at the different processing temperatures; however, significant differences were observed for β-casein. The concentration of β-casein was significantly lower ($p < 0.05$) in retentate produced at 4 °C compared to retentates produced at 8 and 12 °C, with values of 24.4, 28.3 and 29.7 mg/mL, respectively. This was expected as at low temperatures, hydrophobic interaction strength decreases, facilitating the dissociation of β-casein from the micelle into the serum phase of milk [36], where it can subsequently be transmitted through the membrane, into the permeate. As expected from the concentration of β-casein in the retentate, a significantly ($p < 0.05$) higher concentration of β-casein was measured in permeate following MF at 4 °C (2.09 mg/mL), followed by 8 °C (1.46 mg/mL) and 12 °C (0.93 mg/mL) (Fig. 4b). The β-casein content of the permeate generated at 4 °C represented 17.7% of the β-casein content of the original skim milk feed. No significant ($p > 0.05$) differences were observed between the concentrations of α-lac and β-lg in permeates generated at different temperatures, although the highest concentration of both was in permeate generated at 12 °C.

4. Conclusion

The specific temperature, in the range 4–12 °C, at which cold microfiltration of skim milk is performed significantly impacts membrane performance, fouling and protein partitioning. Performing microfiltration at 4 °C resulted in lower initial fluxes, compared to 8 or 12 °C, but ultimately a slower decline in permeate flux during processing was achieved. Filtration at 4 and 8 °C resulted in the lowest extent of reversible and irreversible fouling, although this processing temperature was associated with greatest mechanical and thermal energy requirements. Microfiltration at 4 °C resulted in permeate with the highest concentration of β-casein and the lowest decrease in the concentration of β-casein in permeate throughout microfiltration. The results of this study provide new information on the influence of temperature on membrane performance, fouling and protein partitioning during the cold MF of skim milk, and demonstrate that performing microfiltration at lower temperatures may enable production of next-generation dairy streams with novel protein fractions, due to enhanced membrane performance, altered protein partitioning and improved yields of β-casein.

CRediT authorship contribution statement

Thomas C. France: Conceptualization, Methodology, Investigation, Writing - original draft, Software. **Francesca Bot:** Methodology, Writing - review & editing, Software. **Alan L. Kelly:** Supervision, Writing - review & editing. **Shane V. Crowley:** Supervision, Writing - review & editing. **James A. O'Mahony:** Funding acquisition, Conceptualization, Supervision, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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