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ORIGINAL PAPER

Lower ultrafiltration temperature improves membrane performance and emulsifying properties of milk protein concentrates

X. Luo · L. Ramchandran · T. Vasiljevic

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Abstract Functionality of milk protein concentrates (MPC) can be influenced by process modifications that influence mineral partition and particle size. This work investigated the effect of processing temperature on functionality of MPC obtained by ultrafiltration (UF) of skim milk using polyethersulfone membrane. Skim milk was concentrated five times by UF operated at 15, 30 or 50 °C. Results indicated that the casein micelle size remained unchanged during UF at 15 and 30 °C, and decreased significantly from 92.7 to 83.6 nm at 50 °C, along with the reduction in net negative charge. Operating temperature also influenced the extent of calcium removal in permeate (24.9–16.6%) with minimal changes in protein structure. UF at high temperature (50 °C) reduced membrane performance due to fouling. Proteins and calcium were identified as the major foulants. Overall, operating UF at 15 °C gave better membrane performance as evidenced by better flux, less membrane fouling and shorter time required to reach a 5× concentration, as compared to that operated at 30 or 50 °C. Moreover, no changes in heat stability and solubility of the MPCs were observed while the emulsifying properties were better for MPC obtained at 15 °C.

Keywords Milk protein concentrate · Ultrafiltration · Calcium · Casein micelle · Emulsion · Membrane performance

1 Introduction

Milk protein concentrate (MPC), produced from skim milk by membrane concentration and drying, contains all the caseins and whey proteins present in milk with low concentrations of lactose and minerals. Such a MPC has been used as a functional ingredient in many food applications. For commercial and technological reasons, MPC

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is normally converted into powders (Faka et al. 2009) and has been used for a number of reasons in various dairy products that take up 25% of the dairy market. In recent years, a number of milk powders with high protein content (50–80%) has been developed using membrane technologies (Premaratne and Cousin 1991). Ultrafiltration (UF) or a combination of UF with diafiltration (DF) is widely used to separate and concentrate milk proteins (ST-Gelais et al. 1992). To be useful as functional ingredients, MPC powders should exhibit good functionality such as emulsification, high solubility and heat stability in food products. Conditions during production of MPC powder apparently affect their composition, most importantly mineral composition and protein concentration and structure, which in turn may influence their physical functionality (Sikand et al 2011). A recent work by Ye (2011) has, for example, demonstrated that emulsifying properties of MPC were influenced by particle size of casein micelles. The size of casein micelles was obviously affected by reduction of calcium content in the MPCs—the lower the calcium content in MPC the smaller the particle (droplets) size in emulsion leading to a better functionality.

Various processing conditions during concentration of milk proteins are known to affect their physical functionalities. For example, temperature of operation is an important parameter for skim milk concentration by a UF process. When milk proteins are subjected to thermal processing, depending on the heating conditions, whey proteins may undergo subtle structural changes or even reversible denaturation (Raikos 2010). Denatured whey proteins can interact with themselves and/or with κ-casein to form heat-induced protein aggregates (Donato et al 2007). Kinetics of protein denaturation and aggregation is affected by heating conditions and the environment, such as pH and ionic strength, which in turn affects the rate and extent of protein denaturation and extent of subsequent interactions of the whey proteins with the casein micelles, resulting in the change of a micelle size (Anema and Li 2003). UF at high temperatures above 70 °C (Lalande and Tissier 1985) favours membrane filtration by lowering liquid viscosity (Pace et al 1976; ST-Gelais et al. 1992) and increasing mass transport across the membrane (Marcelo and Rizvi 2008). However, a high temperature could accelerate membrane fouling caused by protein denaturation leading to protein interactions and gelling on membrane surfaces (Gautam 1994).

The effect of heat on protein denaturation and aggregation has been studied extensively and has been well reviewed (Considine et al 2007; Raikos 2010). However, the investigation of functional properties as affected by particle size and heat effect mainly focused on using reconstituted milk powders (Anema and Li 2003). The change of the casein micelle size induced by different heating conditions was reported by using centrifuged skim milk (Ono et al 1999). The production of MPC at 10 and 50 °C has been reported by Havea (2006) and Syrios et al (2011) but the effect on the properties of MPC has not been studied. Consequently, comprehensive information on the effect of temperature of UF processing on MPC functional performance is scarce.

This work investigated the effect of the processing temperature during a fivefold concentration of skim milk by UF on the functionality of the MPC and membrane performance. The results from this study may help establish a foundation for optimizing a process regime for the production of MPC with improved physical functionality.

2 Materials and methods

2.1 Preparation of milk concentrates

The milk used for this study was low-fat skim milk commercially pasteurized (72 °C/15 s) provided by a local supplier (Woolworths, Melbourne, Australia). The skim milk was heated and maintained at 15, 30 or 50 °C, respectively, using a double walled water tank. A SEPA CF membrane module and polyethersulfone (PES) membrane (190× 140 mm) with a molecular cut-off of 20 kDa were both purchased from Sterlitech Corporation (Kent, WA, USA). The set up was conditioned to the temperature of operation by running Milli-Q water at the same processing temperatures before running the milk sample. The skim milk was pumped through the membrane module at a pressure of 2 bar using a hydra-cell pump (T-VERTER 220V 1.5 kW 2HP, Wanner Engineering Inc., Minneapolis, MN, USA), and the concentrate was recycled into the feed container until the volume was reduced to one fifth of its original volume (volume concentration ratio=5). This was monitored by measuring the volumes of collected permeate during UF. The pH of all retentates was assessed at the end of the process and was in the range 6.4–6.5. Samples of permeate and retentate, collected at the start (0 h) and at the end of operation, were analysed immediately for particle size, zeta potential and conformational changes of proteins. A part of the concentrated samples was stored at −20 °C and used for calcium and protein analyses, and the remaining part of the frozen concentrates was freeze dried (Dynavac, Model FD300, Dynavac Eng. Pty. Ltd., Melbourne, Australia) and reconstituted for the functionality (e.g. emulsion) analysis. New membrane sheets were used for each run, which were wetted with Milli-Q water over night before use. The samples were frozen at −20 °C before freeze drying.

2.2 Particle size and zeta potential measurement

Particle size and zeta potential of the skim milk, retentate and permeate samples were measured immediately after the collection of samples using Malvern Zetasizer (Model ZEN3600, Malvern Instruments Ltd, Worcestershire, UK). The samples with different concentrations were diluted with Milli-Q water as required (1:100 for 0 h milk samples and 1:1,000 for MPC samples) to meet quality data requirement during measurement (Mellema et al. 2009). Upon dilution, the samples were vortexed for 10–15 s and immediately introduced into the instrument. All measurements were carried out at room temperature (22 °C). The each measurement has taken less than 10 min to complete, which limited mineral equilibration between the casein micelles and aqueous environment, and thus maintained the integrity of the micelles, which is required for an accurate measurement (Beliciu and Moraru 2009). Duplicate measurements were conducted on each sample. The refractive index and viscosity of water used in calculations were 1.330 and 1.1442 mPa.s at 20 °C, respectively. The applied voltage was set at 40 V and the dielectric constant was 82.2. Hydrodynamic diameter (D_h) was measured by dynamic light scattering (DLS) in the same instrument under the same conditions using a Zetasizer software. Measurements were carried out at a scattering angle of 173 and wavelength of 633 nm. The average D_h was calculated using the Stokes–Einstein relation under assumption that particles had a spherical shape (Silva

et al 2013). The results are presented as a volume distribution. The experimental error on the average D_h was 5 nm.

2.3 Calcium content determination

The total calcium content of the permeate and retentate was determined using an atomic absorption spectrophotometer (AA-6300 SHIMADZU, Shimadzu Corporation, Kyoto, Japan) at 422.7 nm wavelength following the AOAC official method of analysis 991.25 (AOAC 2005) with some modifications in the sample preparation as suggested by ST-Gelais et al. (1992). The samples were prepared using 15% (*w*/*v*) trichloroacetic acid and 10% (*w*/*v*) lanthanum chloride (Sigma-Aldrich Pty Ltd, Castle Hill, NSW, Australia), and suitably diluted in order to comply with a range of standard concentrations using Milli-Q water. The calcium content was calculated and expressed as millimolar. The calcium retained on the membrane was also measured by analysing the calcium content of the membrane ash. Ash was obtained by taking two pieces of the membrane, one from the edge and the other from the centre of the exposed area (Delaunay et al 2006) and following the standard AOAC protocol (AOAC 2005). The calcium content was expressed in microgram per square centimetre (Hausmann et al 2013).

2.4 Analysis of proteins

The total protein nitrogen was measured using the Kjeldahl method as described by AOAC (2005). The protein conversion factor of 6.38 was applied. The protein profile of the feed and corresponding retentates obtained at different time points was also characterised using a high-sensitive protein 250 K Bioanalyzer 2100 (Agilent Technologies GmbH, Waldbronn, Germany). The samples were prepared and analysed under non-reducing conditions as per instructions of the manufacturer. The samples were introduced into an Agilent High Sensitivity Protein 250 kit, which contains a chip and reagents for protein labelling of proteins. The assay consists of two major analytical steps involving covalent labelling of proteins with a fluorescent dye and separation and detection of labelled proteins with on-chip-electrophoresis (Agilent). Additionally, commercial samples of whey protein isolate (WPI, BiPro, Davisco International Inc, Eden Prairie, MN, USA) and sodium caseinate (NaCN, NatraPro, MG Nutritionals, Murray Goulburn Co-operative Co. Ltd., Melbourne, Australia). The resolved peaks were compared with the external ladder protein standard, which was part of the kit.

2.5 ATR-FTIR

The change in protein conformation was observed using a Shimadzu IRAffinity-1, Fourier transform infrared (FTIR) spectrofluorometer (Shimadzu Corporation, Kyoto, Japan) equipped with IRsolution FTIR software (Shimadzu). The spectra of the prepared milk and corresponding retentates were obtained in the absorbance mode using Vee-Max flat plate ZnSe ATR crystal (45°; PIKE Technologies, Inc., WI, USA). For each spectrum, an average of 40 scans was recorded at 4 cm resolution in the range of 400–4,000 cm^{-1} after atmospheric background subtraction. The interferograms thus obtained were ATR corrected, baseline adjusted to zero and smoothed (10 points) with

the aid of the software in order to recognize the corresponding peaks under the broad Amide I region of 1,600–1,700 cm^{-1}. The peak areas obtained from peak tables of the manipulated spectra were used to identify and monitor changes in the FTIR spectra of the samples.

2.6 Solubility of MPC

A 5% (*w*/*w*) dispersion was prepared from freeze dried MPC powders by mixing them with Milli-Q water followed by stirring for 2 h on a magnetic plate at room temperature (22 °C). The dispersion was left at 4 °C overnight to ensure complete hydration of the MPC powders. The powders contained between 52 and 55% protein on a dry base.

Protein solubility was estimated the following day by the method developed by Morr (1985) with some modifications. An aliquot of 10 mL of MPC dispersion was centrifuged (Centrifuge Model J2HS, Beckman, Fullerton, CA, USA) at 3,000×*g* for 25 min at 10 °C. The supernatants were assessed for their protein content by the Kjeldahl method. The solubility was estimated using the following equation:

$$\text{Solubility}, \% = \frac{\text{Protien content (supernatant)}}{\text{Total protein content in initial dispersion}} \times 100 \quad (1)$$

2.7 Heat stability of MPC

The heat stability of MPC was estimated as described by Dissanayake et al (2012). From the prepared MPC dispersions, approximately 2 mL of sample was sealed in narrow glass tubes (10 mm i.d.×120 mm) and placed in a rocking oil bath set at 140 °C for 10 s. After 10 s, the tubes were removed from the oil bath and cooled instantly in an ice bath. The stability of heated dispersions was determined by measuring the protein contents as described above for solubility method and expressed using the equation:

$$\text{Heat stability}, \% = \frac{\text{Protein content (supernatant of heated samples)}}{\text{Total protein content of original dispersion}} \times 100 \quad (2)$$

2.8 Emulsion properties

The emulsions were prepared by mixing one part of canola oil with three parts of MPC dispersion. A coarse emulsion premix was first prepared by homogenizing oil and MPC dispersion for 60 min using a laboratory mixer (R30, Fluco electric stirrer with a 4 blade mixing element, Fluco, Shanghai, China) and keeping the mixture in a water bath maintained at 50 °C for 20 min. Thereafter, the final emulsion was prepared by two stages of homogenization using an Ultraturrax (Pro Science PRO 250, Pro Scientific Inc., Oxford, CT, USA) at 10,000 rpm for the first stage (5 min) and at 15,000 rpm for the second stage (5 min), respectively. The emulsions thus formed were immediately cooled to 25 °C.

The emulsion activity index (EAI) was calculated using the method described by Pearce and Kinsella (1978) by measuring the turbidity and oil volume fraction of emulsion and calculated by:

$$EAI = \frac{2T}{\varphi C} \tag{3}$$

where T is turbidity; φ is oil volume fraction; C is the weight of protein per unit volume of aqueous phase before emulsion is formed.

The emulsion stability index (ESI) was analysed after the emulsion was stored at 4 °C for 24 h. Aliquots of 1 mL of the emulsion was diluted serially with 0.1 g.100 mL^{-1} sodium dodecyl sulphate (SDS) solution to give final dilution from 1/100 to 1/3,000 according to the concentration of samples. The absorbance of the diluted emulsion was determined using 1-cm path cuvette at wavelength of 500 nm using UV/Vis spectrophotometer. The turbidity of the emulsion was calculated by:

$$T = 2.303 \cdot \frac{A}{l} \tag{4}$$

where T is turbidity, A is absorbance at 500 nm and l is path length of cuvette.

The emulsion stability index was calculated using the equation:

$$ES = T \cdot \frac{\Delta t}{\Delta T} \tag{5}$$

where T is calculated turbidity value at 0 h; Δt is time interval (24 h) and ΔT is change in turbidity during the time period (24 h).

2.9 UF membrane performance

The performance of membrane was evaluated by measuring the permeate flux (every 30 min) and examining the membrane surface and its cross section using scanning electron microscope (SEM). The SEM examination was carried out using JCM 5000 bench top NeoScopy scanning electron microscopy (Swanzey Internet Group, Manchester, UK). The used membranes were rinsed with water, dried at room temperature and stored at −20 °C before examination. Cross-section samples of the dried membranes were prepared by wetting the membrane in Milli-Q water followed by cutting it in liquid nitrogen. The membrane samples were mounted on double sided carbon tape, dried at room temperature overnight and coated with gold before examination. The SEM was set at Vac-high PC-STD 15 KV during examination and imaging.

2.10 Statistical analysis

All experiments were at least replicated with three sub-samplings ($n \geq 6$). One-way analysis of variance (ANOVA) at 95% level of confidence was applied to analyse the differences caused by the main effect—temperature of ultrafiltration. Tukey Honestly Significant Difference (HSD) post hoc test was applied to differentiate significant differences between the means at $P < 0.05$. The statistical analysis was performed using a SAS software (SAS 1996).

3 Results and discussion

3.1 Changes in micelle size and zeta potential

The particle size distribution of skim milk and skim milk concentrates obtained by UF at different temperatures is shown in Fig. 1. Initially, the average particle size was not affected ($P \geq 0.05$) by processing temperature and ranged from 87.4±1.8 to 92.7± 3.8 nm for the milk equilibrated at 30 and 50 °C, respectively. Furthermore, the average particle size did not change noticeably ($P \geq 0.05$) after concentration at 15 or 30 °C. This is in agreement with the results of Martin et al (2010), who also reported no major change in micelle size during UF/DF at 10 °C. On the contrary, a significant decrease ($P < 0.05$) in particle size (from 92.7 to 83.6 nm) was observed (Fig. 1) when UF was carried out at 50 °C. This contradicts the observation by Green et al (1984), who reported that the size of casein micelles did not change during concentration of skim milk by UF at 50 °C. The observed particle size decreased at 50 °C may be contributed to plasmin-related dissociation of caseins. Plasmin is an endogenous protease present in milk and is relatively heat stable thus is not fully inactivated during commercial pasteurization. Furthermore, its activity optimum is close to physiological temperatures and increases in heat-treated milk (Kelly and McSweeney 2003). Since commercially pasteurized skim milk was used in this experimentation, it is possible that plasmin presents in the sample remained active and continued hydrolysing caseins at elevated temperatures during ultrafiltration.

Zeta potential results (Fig. 2) showed that the proteins were negatively charged with no substantial change ($P > 0.05$) during concentration at 15 °C. However, at 30 and 50 °C, a decrease in the net negative charge in the retentates in comparison to those in their respective feeds was observed. This result is in agreement with the observations of Darling and Dickson (1979) that zeta potential of casein micelles increased with

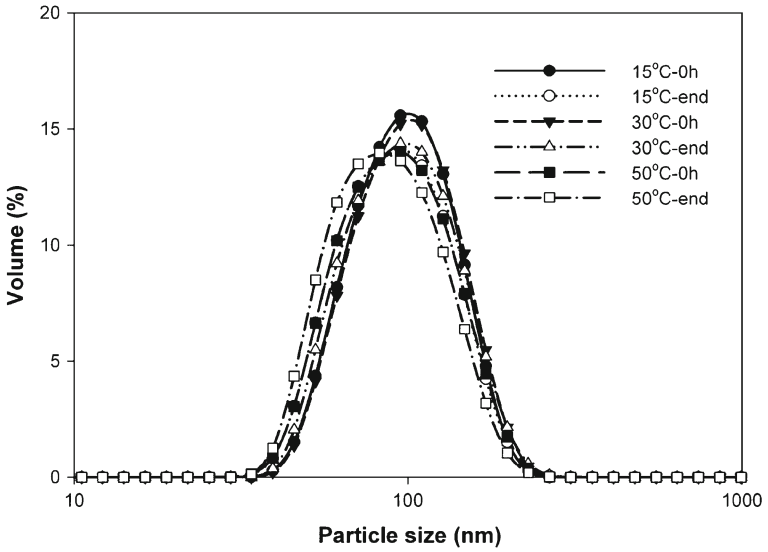

Fig. 1 The particle size distribution of retentates obtained by membrane ultrafiltration operated at 15, 30 or 50 °C, respectively

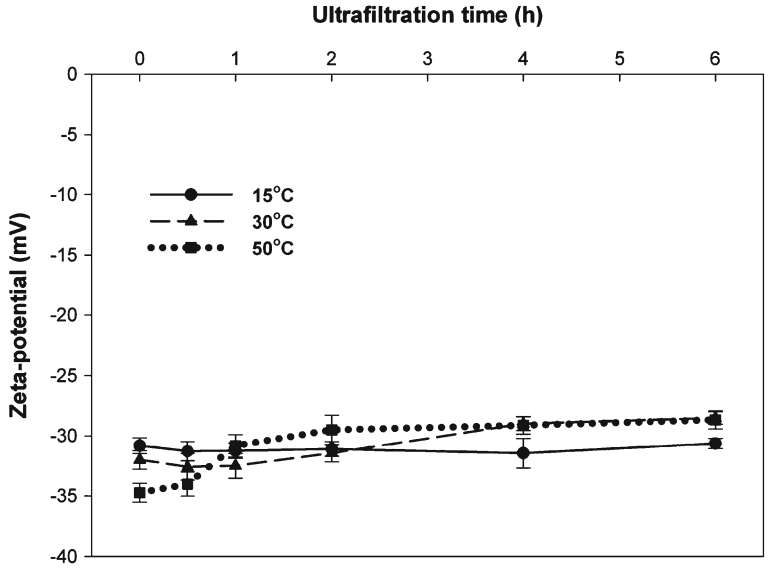

Fig. 2 Zeta potential as a function of concentration time of concentrated skim milk obtained by ultrafiltration at 15, 30 or 50 °C, respectively

increase in temperature from 10 to 50 °C. This reduction in net negative charge on casein micelles could also be contributed by possible modifications of surface charge as a consequence of hydrolysis by plasmin activity at temperatures closer to its activity optimum (Crudden et al 2005).

3.2 Changes in calcium content

The calcium content of retentate obtained during UF of skim milk operated at 15, 30 or 50 °C is shown in Fig. 3. The calcium content of the retentate increased with elevation of the operation temperature (from 15 to 50 °C) with corresponding decrease in the permeate. On an average, UF removed 24.9% of the calcium from skim milk into permeate at 15 °C compared to 20.2% at 30 °C and 16.6% at 50 °C, indicating that higher UF temperature coincides with less efficient calcium removal. Syrios et al (2011) also indicated that increased UF temperature resulted in reduced ionic calcium in the permeate when heating the skim milk to 50 °C prior to UF. Similar observations have been made by Chandrapala et al. (2010a, b), Lewis (2011), On-Nom et al (2010), Premaratne and Cousin (1991), Rose and Tessier (1959) and Vasiljevic and Jelen (1999).

During UF, about 10 mM soluble calcium (30% of total calcium), which is in the diffusible form, can pass through the membrane and accumulate in the permeate (Holt 1981). Removal of calcium during UF would result in some modifications in the salt system of milk such as solubilisation of colloidal calcium phosphate and migration of calcium from the micelles (Syrios et al 2011). These changes in the salt system could also have affected the structure of casein micelles in milk (ST-Gelais et al. 1992). It follows that a relatively larger proportion of calcium was removed through the permeate during UF at 15 °C as compared to 30 and 50 °C (Fig. 3). Further, small quantities

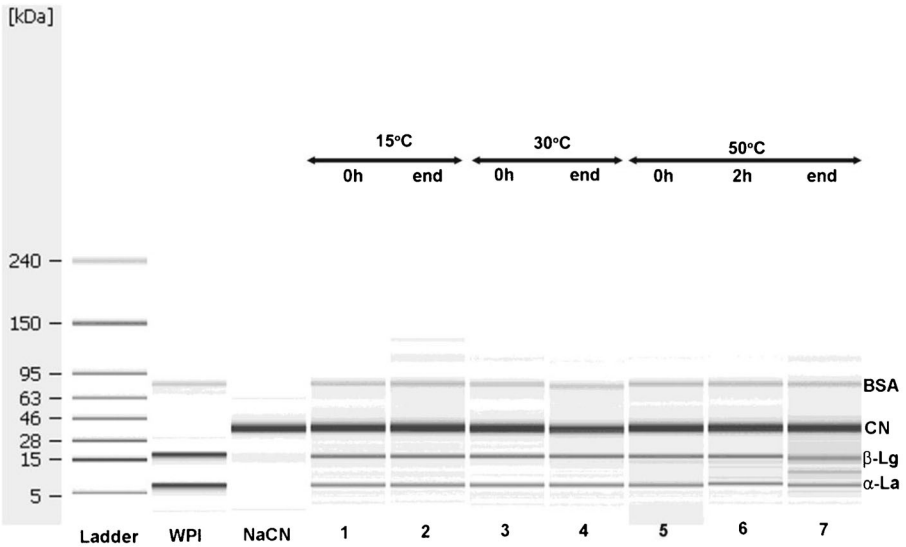

Fig. 3 Electrophoretic patterns under non-reducing conditions of feed and retentate samples obtained by ultrafiltration of skim milk operated at 15, 30 or 50 °C, respectively, at the beginning (0 h; feed), after 2 h and at the end of concentration. The ladder is composed from the external standard included in the protein kit (Agilent). The lanes WPI and NaCN were obtained by using commercial whey protein isolate and sodium caseinate, respectively, as external standards. The bands indicated present *BSA* bovine serum albumin, *CN* caseins, *β-Lg* β-lactoglobulin and *α-La* α-lactalbumin

of calcium were deposited on the membrane surface during UF (Table 1), the amount being similar at 15 and 30 °C but increasing ($P<0.05$) at 50 °C. It could be concluded that temperature of UF not only influenced the extent of removal of calcium into permeate, but also contributed to the extent of membrane fouling.

3.3 Changes in milk proteins

The change in protein content at the end of skim milk UF at different temperatures is shown in Table 2. As expected, the protein content of the concentrate increased as a consequence of UF. Further analysis of the protein distribution in the permeate as well as on the membrane revealed that a proportion of the total protein was retained on the membrane surface (Table 1) while negligible quantities of protein passed into the permeates (Table 2). These results indicated that proteins were likely the major foulants

Table 1 Protein and calcium content retained on the membrane surface during 5 volume concentration factor of skim milk by ultrafiltration (UF) conducted at 15, 30 or 50 °C

UF temperature	Protein ($mg.cm^{-2}$)	Ca ($\mu g.cm^{-2}$)
15 °C	1.34 ± 0.001^{a}	2.5 ± 0.01^{a}
30 °C	1.25 ± 0.001^{b}	2.0 ± 0.01^{a}
50 °C	2.18 ± 0.003^{c}	3.0 ± 0.01^{b}

All values presented are the mean±standard deviation ($n=6$)

Means in the same column with different letters are significantly different ($P<0.05$)

Table 2 Total protein (g.100 mL^{-1}) and calcium contents of retentates and permeates obtained during 5 volume concentration factor (VCF) of skim milk by ultrafiltration (UF) at 15, 30 or 50 °C

UF temperature	Retentate		Permeate	
	Total calcium (mM)	Total protein (g.100 mL^{-1})	Total calcium (mM)	Total protein (g.100 mL^{-1})
15 °C	99.7±1.4[a]	15.9±0.04[b]	6.90±0.99[c]	0.18±0.01[a]
30 °C	110.3±2.1[b]	15.4±0.02[a]	5.60±0.54[b]	0.22±0.01[b]
50 °C	111.7±1.5[b]	16.4±0.1[c]	4.60±0.32[a]	0.26±0.01[b]

All values presented are the mean±standard deviation (n=6)

Means in the same column with different letters are significantly different (P<0.05)

Total protein of skim milk feed was 3.3±0.11 g.100 mL^{-1}

Total calcium content of skim milk feed was 26.79±1.17 mM

during UF of skim milk, being significantly greater (P<0.05) at 50 °C than at 30 or 15 °C.

Electrophoretic patterns obtained (Fig. 3) showed the protein profiles of the retentates obtained during UF (at time 0, 2 h and at the end of the operation) at 15, 30 and 50 °C, respectively. In the pattern obtained by the Bioanalyser, the bands representing α-casein, β-casein, κ-casein, β-lactoglobulin and α-lactalbumin are usually displayed across values from 12 to 46 kDa. Concentration of skim milk by UF resulted in the formation of some low molecular weight compounds (lanes 6 and 7), particularly when operated at 50 °C. Dalgleish and Law (1988, 1989) have indicated that small changes in colloidal calcium phosphate and other calcium phosphate nanoclusters can induce rearrangements within casein micelles that could result in solubilisation of some of the casein fractions from within the micelle. Further, the plasmin-assisted dissociation of caseins could also result in the release of low molecular weight fractions. Given that plasmin is associated with caseins (Dupont et al 2013), increase in protein concentration in the retentate could have resulted in casein hydrolysis and release of low molecular weight peptides particularly at 50 °C. Plasmin preferentially cleaves β-casein, while κ-casein basically remains intact, thus it still may provide stability to the casein micelle (Kelly and McSweeney 2003). Activity of plasmin has also been correlated to concentration of β-lactoglobulin (Aaltonen and Ollikainen 2011) which was also concentrated in the retentate. Thus, overall high-plasmin activity and plasmin-induced changes in zeta-potential (Fig. 2) might have promoted micellar disintegration (Crudden et al 2005), which also supports earlier observation related to reduced particle size of MPC obtained by UF at 50 °C.

Table 3 shows the major peak areas of interest obtained from interferograms of milk and corresponding MPCs obtained at the various temperatures of UF. Changing the temperature of operation from 15 to 50 °C resulted in a little change in the backbone structure of milk proteins (observed between 1,640 and 1,660 cm^{-1}) with β-sheet structures (1,635 cm^{-1}) predominating at 30 and 50 °C in all tested samples. Intensity of β sheets in MPCs decreased when UF was carried out at 15 °C, increasing slightly at 30 but then augmenting substantially at 50 °C. Such an enhancement of β-sheet intensity particularly at 50 °C is indicative of a structural change in proteins likely as

Table 3 Average peak areas at specific wave numbers obtained from peak tables of interferograms for skim milk at the start (0 h) of ultrafiltration and retentate obtained after 5 VCF by ultrafiltration at 15, 30 or 50 °C

All values presented are the means obtained from six interferograms

UF operation temperature	Sampling time	Wave number (1,635 cm^{-1})
15 °C	0 h	12.85
	End	12.23
30 °C	0 h	11.39
	End	11.76
50 °C	0 h	10.86
	End	12.20

a consequence of changes in surface properties such as zeta potential (Fig. 2) due to plasmin activity that could have resulted in a partial dissociation of the micelles and thereby reduced particle size.

3.4 MPC solubility and heat stability

Figure 4 shows the solubility (%) of freeze-dried MPC powders obtained at 15, 30 or 50 °C. As noted, operation temperature affected solubility of the MPCs slightly, with solubility of all samples below 80% and declining with increase of processing temperature. Our observation is similar to other reports which poor solubility of MPC powders attributed to their high-protein content (Fang et al 2011). Depletion of minerals and resulting dissociation of casein micelles are believed to contribute to the poor solubility of MPC powders (Sikand et al 2011). However, no such variations in solubility were

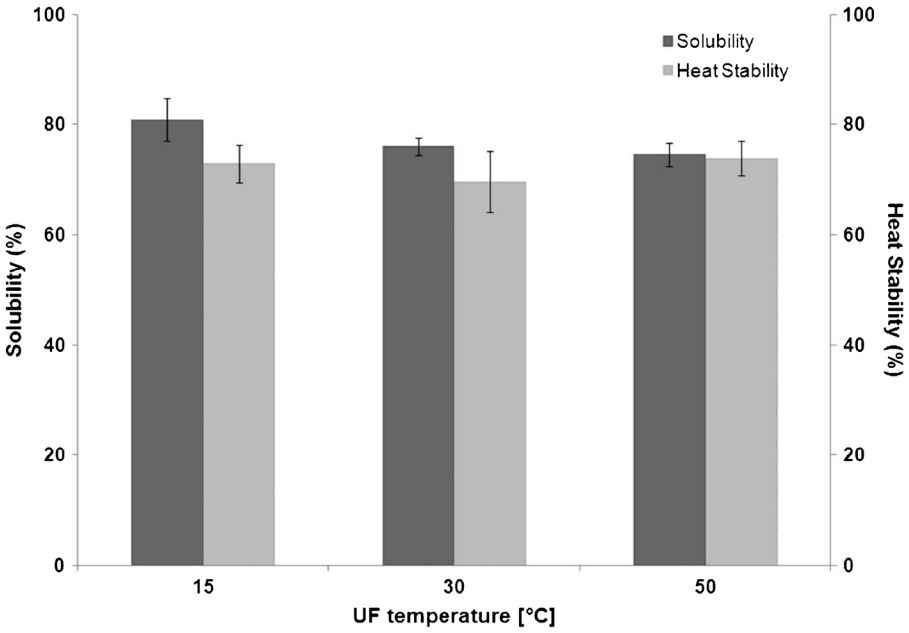

Fig. 4 Solubility and heat stability of MPCs obtained by ultrafiltration of skim milk at 15, 30 or 50 °C

observed in the current study (Fig. 5), probably because the MPC powders were freeze dried, while those reported so far used spray dried MPC powders.

Similar to solubility, only slight differences in heat stability among the powders were noted ($P \geq 0.05$, Fig. 5) across the tested processing temperatures. Although many factors are known to affect the heat stability of milk proteins, such as pH, proteins, salts and processing conditions (O'Connell and Fox 2003), there are no reports on the heat stability of MPC powders. Considering that there were no major conformational changes to proteins observed (Table 3), the similarity in heat stability can thus be expected.

3.5 Emulsifying properties of MPC

The emulsion activity index (EAI) and emulsion stability index (ESI) (Fig. 5) indicated that emulsion activity of MPC was slightly higher if obtained at 15 °C than at 50 °C. Emulsion stability also increased when operation temperature increased. The emulsion properties are known to be influenced by pH, Ca concentration, protein concentration and particle size (Hill 1996). The particle size in particular is understood to play an important role in the emulsifying capability of proteins (Ye 2011). The emulsifying properties and emulsion stability have been related to ionic strength and may be improved by a low ionic strength without affecting the emulsion capacity (Mena-Casanova and Totosaus 2011), as was also observed in our study. The lower the calcium content in MPC, the smaller the particle size in dispersion and droplet size in emulsion, leading to a finer and more stable emulsion. Changes in Ca^{2+} influence the extent of their binding with phosphoserine of caseins leading to an increase or decrease

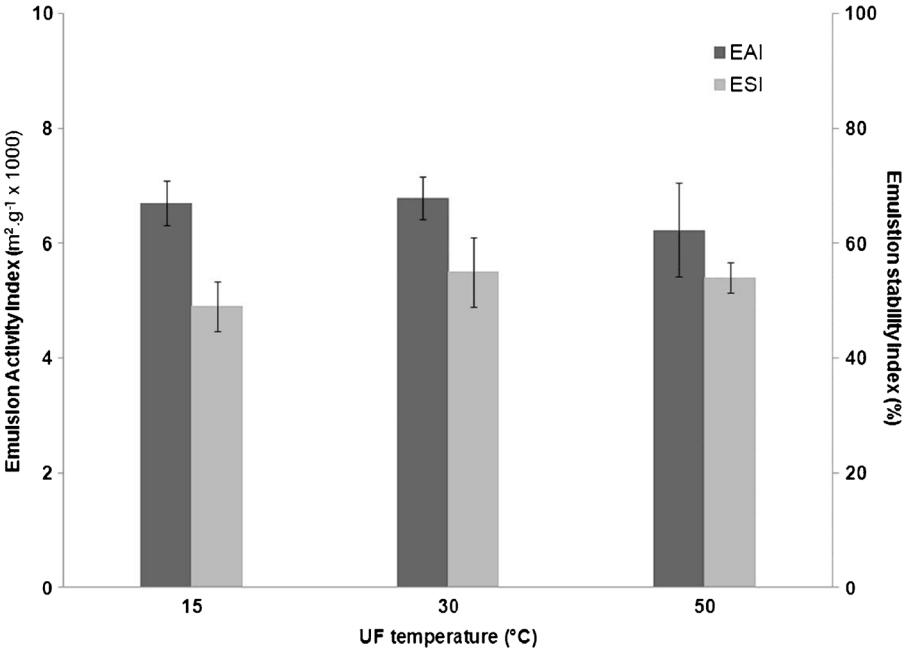

Fig. 5 The emulsion activity index (*EAI*) and emulsion stability index (*ESI*) of MPCs obtained by ultrafiltration of skim milk at 15, 30 or 50 °C

in electrostatic repulsion between the molecules, which in turn influenced the emulsion properties (Ye and Singh 2001). The results in our study showed that calcium migration during the UF can be affected by operation temperature (Fig. 3). Thus, Ca reduction could be achieved by concentrating proteins during the UF with minimal changes to protein conformation and thus without a major impact on their emulsifying properties.

3.6 Membrane performance during UF

The membrane performance was monitored by a flux measurement (Fig. 6) and SEM imaging presented in Fig. 7. In general, the flux declined steadily with the time during continuous UF. The rate of a flux decline was similar at 15 and 30 °C. However, at 50 °C, the rate of decline was rapid ($P<0.05$) during the initial 3 h of operation, after which it was steadied. Increased deposition of calcium and protein (Table 1) could have contributed to membrane fouling, leading to a decreased flux.

SEM images (Fig. 7) indicated that the active surface of membranes operated at 15 °C was cleaner as observed by the presence of some clear pores and lack of fouling in the cross sectional images. In contrast, membrane obtained after operation at 50 °C had some white particles sticking to the surface which could not be removed by washing, and the pores were blocked and deformed in some areas as shown in the cross section SEM images supporting the earlier finding that greater fouling occurred at higher temperatures. Analysis of the membranes (Table 1, Fig. 7) showed that more protein and calcium deposited on the membrane operated at 50 °C than that operated at 15 or 30 °C, with proteins being greater contributor than calcium at 50 °C. Thus, both proteins and calcium in milk contributed to the loss of membrane performance as a consequence of fouling, more so at 50 °C than at 15 °C.

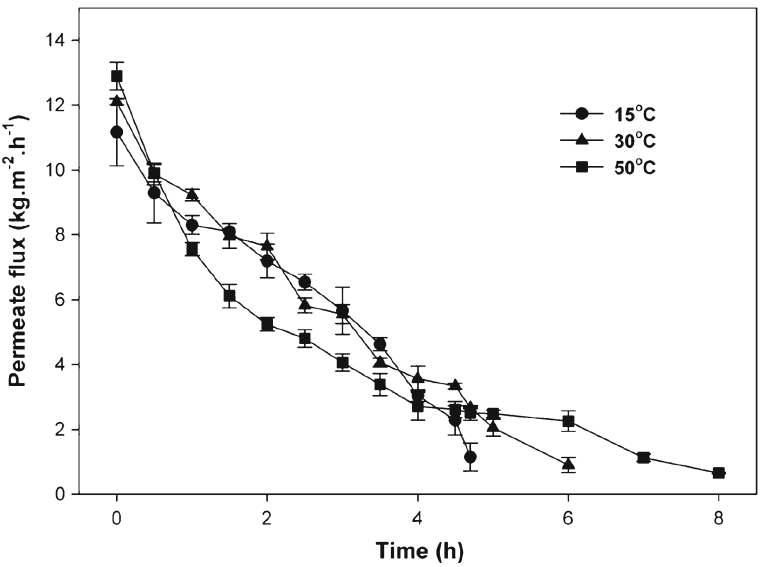

Fig. 6 The permeate flux during ultrafiltration of skim milk at 15, 30 or 50 °C, respectively

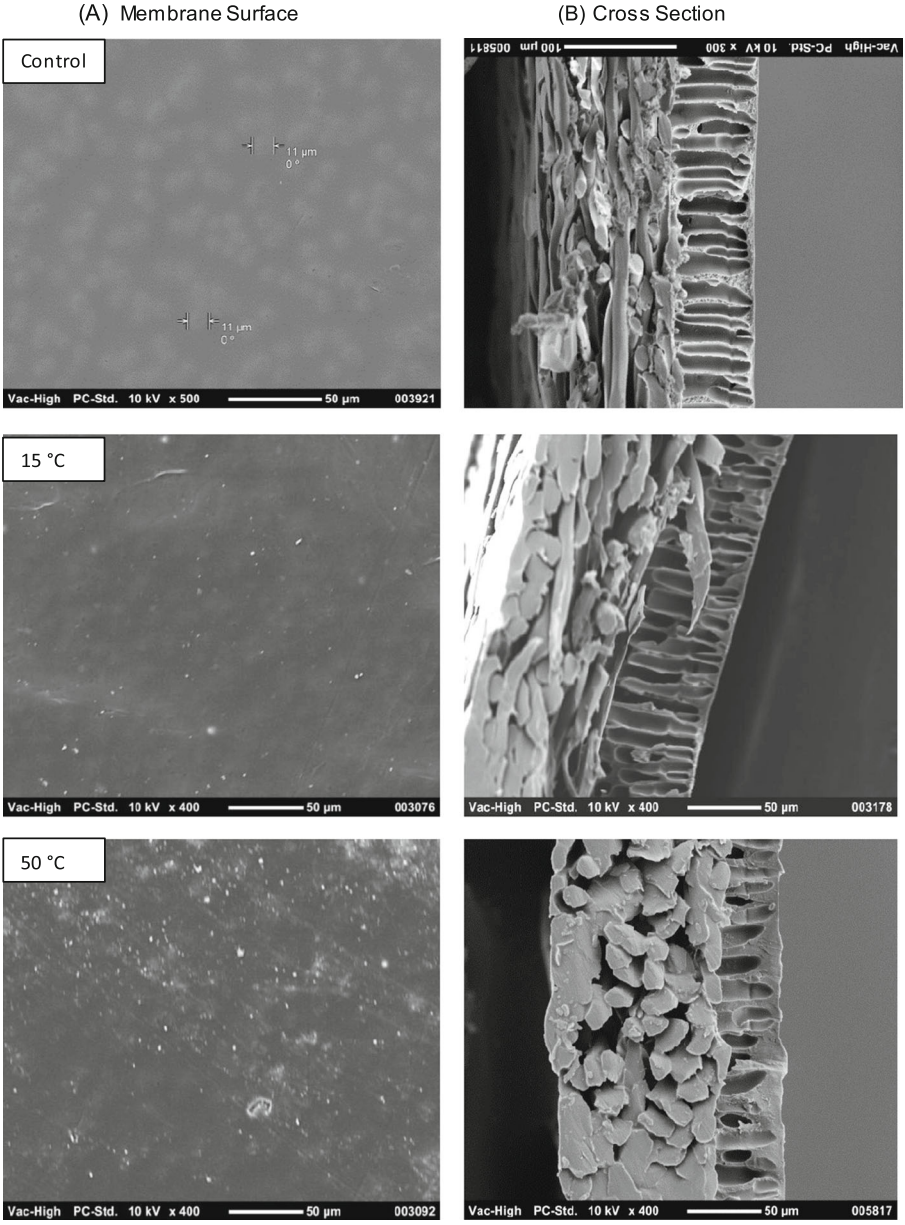

Fig. 7 The SEM images of membrane surface and cross section of new membrane (**a**), after skim milk ultrafiltration at 15 °C (**b**) and at 50 °C (**c**)

4 Conclusions

Careful selection of operation parameters can assist in producing MPC with a tailored functionality. Operation temperature is one such parameter that was found to have a significant effect on the average particle size of the MPC obtained. Overall, lower

temperature of operation (15 °C) produced MPC with a comparable small particle size, better emulsion functionality and less membrane fouling without much alteration in protein conformation, solubility and heat stability, in comparison to those produced at higher temperature (50 °C) of UF. This could be due to a greater removal of calcium in the permeate and likely with some involvement of plasmin activity. Further investigation is thus warranted to establish how additional manipulations of operating conditions, including feed modifications via pH adjustments, could govern the mineral balance in milk and consequently the functionality of the MPC produced by ultrafiltration.

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