Effect of adjusted pH prior to ultrafiltration of skim milk on membrane performance and physical functionality of milk protein concentrate

X. Luo, T. Vasiljevic, and L. Ramchandran¹

Advanced Food Systems Research Unit, College of Health and Biomedicine, Victoria University, Melbourne, Victoria 8001, Australia

ABSTRACT

Processing conditions during ultrafiltration of skim milk influence properties of the casein micelle and thereby the physical properties of milk protein concentrate (MPC). The aim of the study was to establish the effects of pH adjustment of skim milk feed to obtain MPC with desired emulsification properties. The ultrafiltration was conducted using commercially pasteurized skim milk with the pH adjusted to 6.7 (control), 6.3, 5.9, or 5.5 at 15°C until a volume concentration factor of 5 was reached. Effects of pH adjustment on selected physico-chemical properties (Ca content, particle size, ζ -potential) and functionalities (solubility, heat stability, emulsification capacity, and stability) of MPC were determined. Lowering the feed pH solubilized colloidal calcium phosphate that substantially contributed to modifying the properties of casein. This caused a reduction in the particle size while increasing the net negative charge. The structural modifications in proteins were manifested in the Fourier transform infrared spectra. Subsequent concentration did not induce any further protein structural changes. Such modifications to the casein micelles and colloidal calcium phosphate negatively affected the solubility and heat stability of the corresponding MPC powders. However, the emulsion activity index improved only until the pH of the feed was lowered to 5.9 and declined when pH was dropped to 5.5, followed with the loss of stability. Readjusting the pH of MPC powder dispersions to 6.7 restored their surface properties and thereby their functionality. Lowering the feed pH also negatively affected the membrane performance by clogging the membrane pores and lowering the flux, particularly at pH 5.5. Adjusting pH to 5.9 produced MPC with optimum emulsifying properties with minimal influence on membrane performance.

Key words: pH, ultrafiltration, milk protein concentrate, calcium

INTRODUCTION

Milk protein concentrate (MPC) is a product obtained by membrane processing and drying, containing all the CN and whey proteins present in skim milk, thereby delivering the value of the same CN to whey protein ratio as skim milk. This product provides a protein-rich nutritional source of milk solids, allowing textural modifications with enhanced emulsification capacity that has been used in cheese, processed cheese, and yogurt manufacturing and is commercially available in a range of protein levels (Ye, 2011). Physical functionality of MPC is an important characteristic governing applications of these protein concentrates in food products. Fundamental research on the functional properties of proteins and how these properties can be manipulated by processing are thus essential to improve MPC functionality. Emulsifying ability is one of the most important functionalities of MPC (Cameron et al., 1991). In an emulsion, a dispersion of one liquid in another, such as oil in water, a protein acts as a surface active component forming an interface between these 2 immiscible liquids. This reduces the interfacial energy and therefore increases the stability of the emulsion by creating a more thermodynamically stable system (Becher, 2001).

Several studies have shown that functionality of MPC including solubility and emulsification could be related to properties of the CN micelle (Schuck and Demeler, 1999; Schuck et al., 2002; Udabage et al., 2012). Structural changes of the CN micelle are primarily governed by mineral equilibrium in milk and a state of the colloidal calcium phosphate (CCP). Approximately two-thirds of the milk calcium is involved in complexation with phosphate, creating the CCP and stabilizing the CN micelle framework at the normal pH of milk (Walstra, 1990). The remaining portion is commonly termed soluble calcium and found in the serum phase of milk, thus creating equilibrium between the colloidal and soluble phase (Pierre and Brule, 1981). Any envi-

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¹Corresponding author: lata.ramchandran@vu.edu.au

ronmental change (temperature, pH, ionic strength) in milk would lead to a shift in the CN-mineral equilibrium, resulting in an increase or decrease in concentration of free calcium ions, dissolution of the CCP from the micelle and release of specific CN from the micelle (Griffin et al., 1988; Holt, 1992; Gaucheron, 2005), and consequently causing the change of the micelle size. Reduction of the particle size, calcium content, or both appears to be a plausible strategy in producing MPC with good emulsifying properties (Ye, 2011).

Evidently a simple UF followed by a diafiltration of skim milk, although efficient in removing soluble calcium, produced MPC with a poor solubility (Udabage et al., 2012) or emulsification (Ye, 2011). Modifications of the CN micelle appear a necessary step to achieve desired functionalities, and these can be brought about by several approaches that would modify properties and state of the CCP including temperature (Luo et al., 2015), pH (Dalglish and Law, 1989; Silva et al., 2013), or different chelators (Kaliappan and Lucey, 2011). Due to a multitude of effects, it is of a great importance to establish optimum processing conditions leading to MPC with required functionalities. Ultrafiltration temperature, for example, not only affected the CN micelle size but also the membrane performance with higher temperatures (50°C), resulting in a smaller particle size but an impaired flux and enhanced fouling than lower temperatures of 15 or 30°C (Luo et al., 2015). Due to a profound effect of pH on the properties of the CN micelle (Matia-Merino and Singh, 2007), it would be necessary to establish an optimum pH during UF processing that would result in efficient production of functional MPC. The CCP begins to dissociate when pH is decreased from that of normal milk (Van Hooydonk et al., 1986). Its dissociation can induce dissociation of individual CN (Holt et al., 1986) that can affect the structural integrity of CN micelle, which in turn may affect their functionality. The pH range between 5.5 and 6.7 appears critical for inducing fundamental changes in the CN micelle due to Ca^{2+} complexation with CN (Rabiller-Baudry et al., 2009).

Along with changes to CCP, structural changes to proteins are also likely to occur that may further bear relation to modifications in their functionality. Of the various techniques used to study protein conformation, Fourier transform infrared (**FTIR**) spectroscopy provides valuable information about protein folding, denaturation, protein-protein interaction, and subunit assembly, which in turn can aid to the understanding of protein functionality. The location of both amide I and II bands are sensitive to the secondary structural content of proteins (Carbonaro and Nucara, 2010). High sensitivity to small variations in molecular geom-

etry and H-bonding patterns make the amide I band especially useful for the analysis of secondary structural composition and structural changes in proteins (Kong and Yu, 2007). Characteristic secondary structural features such as β -sheet, β -turns, α -helix, and random coils can be identified by the characteristic peaks appearing at specific wavelengths. Furthermore, protein aggregation occurring as a consequence of intermolecular β -sheet formation with the involvement of hydrogen bonds results in the appearance of new bands (Lefevre and Subirade, 2001; Remondetto and Subirade, 2003; Eissa et al., 2006). Very few researchers have attempted to use FTIR to study proteins in MPC (Kher et al., 2007; Haque et al., 2010).

Overall, information on how modifications to feed conditions would influence the functionality of MPC, as well as processing performance of UF membranes is absent, although several predictions could be postulated as outlined above. Therefore, the aim of this study was to establish the effect of CCP solubilization by prior milk acidification on functionality of MPC and membrane performance. For this purpose, skim milk was acidified to pH 6.3, 5.9, or 5.5, ultrafiltered at constant temperature without diafiltration, and resulting powders assessed for selected functionalities.

MATERIALS AND METHODS

Sample Preparation

Skim milk, produced by a standard pasteurization process (72°C for 15 s) was obtained from a local supplier (Woolworths, Bella Vista, NSW, Australia). To prevent bacterial and fungal growth in the feed during the entire time required for the UF process (3–4 h), thimerosal (Sigma-Aldrich Pty Ltd., Castle Hill, NSW, Australia), a Hg-based preservative, was added at a concentration of 0.3 g/L.

As required by the experimental protocol, the samples were adjusted to pH 6.3, 5.9, or 5.5 at 15°C by the addition of an appropriate volume of 1 M HCl with constant stirring until a constant pH was established. The temperature of 15°C was selected based on our previous observations of appropriate membrane performance with improved emulsifying properties of the MPC produced (Luo et al., 2015). The pH of the control (6.7) and the other samples was finally adjusted as required by either 1 M HCl or 1 M NaOH. The total volume of all samples was kept constant by addition of an appropriate volume of Milli-Q water to prevent any dilution caused by adding different volumes of HCl (Silva et al., 2013).

Preparation of MPC by Ultrafiltration

All skim milk samples (feed) during UF were maintained at a constant temperature of 15°C using a water tank. Similar to our previous study (Luo et al., 2015), a SEPA CF membrane cell module (Sterlitech Corporation, Kent, WA) and a polyethersulfone membrane with a size of 190 × 140 mm and molecular mass cut-off of 20 kDa were used in all trials. The samples were pumped (T-VERTER, Hydrocell pump, Wanner Engineering Inc., Minneapolis, MN) through the membrane module at a pressure of 2 bars and frequency of 12.5 Hz during UF. The processing was stopped when volume concentration ratio (**VCF**) of 5 was achieved as inferred by measuring the volume of collected permeate. The retentate and permeate samples were collected when required and appropriately processed before frozen storage. For each experimental run, a new membrane sheet was used, which was wetted with Milli-Q water overnight before use. Before each UF run, the membrane module was flushed with Milli-Q water (15°C) for 10 min. The bulk of retentates at the end of UF runs was immediately frozen and freeze-dried in a freeze dryer (model FD300, Dynavac Eng. Pty. Ltd., Melbourne, Australia). Obtained powders were transferred into plastic containers and stored in a cool, dry room until required for analysis of functionality.

Particle Size and Zeta Potential

Particle size and zeta potential of the feeds, retentates, and permeate samples were measured immediately after the collection of samples using a Malvern Zetasizer (model ZEN3600, Nano-ZS, Malvern Instruments Ltd., Malvern, UK) as previously described (Luo et al., 2015). The samples were diluted with Milli-Q water as required (1:100 for feed samples and 1:1,000 for MPC samples) to meet quality data requirement during measurement of particle size (Mellema et al., 2009). Thereafter, the samples were gently vortexed (10–15 s) and immediately introduced into the instrument. The measurements were carried out at room temperature (22°C).

Determination of Ca Content

The determination of the total Ca in feed and retentate samples followed the protocol described by St-Galais et al. (1992). The samples were first ashed at 550°C overnight and then the ash was solubilized in 0.1 M HCl. The soluble Ca was obtained by centrifugation of the feed and retentates at 33,000 × *g* for 60 min at 25°C (Williams et al., 2008) using Beckman LK 90 M ultracentrifuge with a Type 55.2 Ti rotor [Beckman

Instruments Australia (Pty) Ltd., Gladesville, NSW, Australia]. The obtained supernatant was ashed as described above. The Ca content in all samples was determined using an atomic absorption spectrophotometer (model AA-6300, Shimadzu Corporation, Kyoto, Japan) at 422.7 nm. The Ca concentration was inferred from a standard curve (1 to 10 ppm Ca).

ATR-FTIR

Changes in protein conformation were observed using an FTIR spectrofluorometer (Shimadzu IRAffinity-1, Shimadzu Corporation, Kyoto, Japan) equipped with IRsolution FTIR software (Shimadzu) as described previously (Luo et al., 2015). The samples were analyzed immediately after sampling and spectra were obtained in the absorbance mode using Vee-Max flat plate ZnSe ATR crystal (45 degrees; PIKE Technologies Inc., Fitchburg, WI). For each spectrum, an average of 80 scans was resolved at 4 cm⁻¹ in the range of 400 to 4,000 cm⁻¹ after atmospheric background subtraction. The interferograms thus obtained were ATR corrected, baseline adjusted to zero, smoothed (10 points), and deconvoluted with the aid of the software to recognize the corresponding peaks under the broad amide I region of 1,600 to 1,700 cm⁻¹.

Functionality of MPC

Physical functionality of MPC produced by the described protocol above was established within 2 wk after preparation. The powders were dispersed in Milli-Q water preheated to 50°C. These dispersions were further divided into 2 equal portions: the first, which was further assessed without prior pH adjustment, and the second, in which pH was readjusted to pH 6.7 using 1 M NaOH. Both portions were subsequently stored at 4°C overnight with the constant stirring to allow for full hydration of the powders for analysis. The following day pH of all dispersions was confirmed and adjusted if required, followed by final concentration adjustments resulting in 5% (wt/wt) MPC dispersions. The powders contained between 52 and 55% protein on a dry base.

The protein solubility was estimated using the method originally proposed by Morr et al. (1985) and modified as suggested by Dissanayake et al. (2012). In brief, after overnight storage exactly 10 mL of protein dispersions was centrifuged (model J2HS, Beckman, Fullerton, CA) at 12,000 × *g* for 25 min at 20°C. The supernatant was filtered through a 0.45-μm filter and its protein content was assessed by the Kjeldahl method. The solubility of the powders was expressed as a percentage of the protein remaining in the solution after centrifugation (Luo et al., 2015).

Heat stability of MPC dispersions was assessed using the solubility method upon heating at 140°C for a defined time (Dissanayake et al., 2012) as reported previously (Luo et al., 2015). After reaching the required time, the samples were quickly removed from the oil and submerged into an ice-water bath. The stability of heated dispersions was assessed by measuring the protein content of the supernatant upon centrifugation as described above.

The emulsions were prepared as reported previously (Luo et al., 2015) by mixing one part of canola oil with 3 parts of protein dispersions. After producing fine emulsions by a 2-step homogenization process, the samples were immediately cooled to 25°C. Emulsification activity index was determined by measuring turbidity of emulsions following the established procedure (Pearce and Kinsella, 1978) and modifications previously reported (Dissanayake et al., 2012; Luo et al., 2015). The emulsion stability index (**ESI**) was determined after storage at 4°C for 24 h (Dissanayake et al., 2012; Luo et al., 2015).

Membrane Performance

Membrane performance was evaluated by measuring the permeate flux, followed by an examination of membrane surface and cross section using scanning electron microscopy after completion of UF as reported previously (Luo et al., 2015). The flux was measured every 30 min by continuously recording the weight of permeate, which also allowed for determination of weight reduction of the feed. The used membranes were rinsed with water, dried at room temperature, and stored at -20°C before the scanning electron microscopy examination. The scanning electron microscopy examination was carried out using a JCM 5000 bench top NeoS-copy scanning electron microscope (Swansey Internet Group, Manchester, UK). The scanning electron microscope was set at Vac-high PC-STD 15 KV during the sample imaging. To prepare samples for cross-section examination, the membrane was first wetted in Milli-Q water and then cut in liquid nitrogen. The membrane samples were mounted on double-sided carbon tape, dried at room temperature overnight, and coated with gold before examination.

Statistical Analysis

The experiments were designed as a randomized block design with pH as the main factor (4 levels) and replications as a block. The whole design was replicated 3 times with subsequent subsampling giving at least 6 independent observations ($n \geq 6$). One-way ANOVA was conducted to explore the influence of pH on the

functionality of MPC as the main effect, followed by Tukey honestly significant difference post hoc test to determine significant differences between the means at $P < 0.05$.

RESULTS AND DISCUSSION

Physicochemical Properties of Retentates

The Ca and phosphate distribution between serum and micellar (CN-bound) phases has great influence on the functional properties of dairy products. The CCP associated with CN micelles can be varied by various treatments such as heating and cooling (Holt, 1995; Luo et al., 2015), the addition of Ca-chelating agents (Udabage et al., 2000), or acidification (Lucey et al., 1996). Similar trend was obtained in the current study with Table 1 showing the total and calcium in the feed and retentates before and after UF, respectively. Indicatively, pH adjustment resulted in substantial solubilization of the CCP in the original feed. As expected, approximately one-third of the total Ca was soluble (Lewis, 2011) in the feed at native pH (control). The soluble portion increased to almost two-thirds of the total Ca with concomitant lowering of pH (Table 1). Whereas the total operating volume was reduced by 5 times, total Ca concentration increased by approximately 3.5 times, indicating that colloidal phase was concentrated. Even upon concentration, a fraction of Ca was in the soluble form, which may indicate reestablishment of the mineral equilibrium. At neutral pH, the soluble portion of the total Ca in the retentate was 15.5%. As the pH declined, this proportion increased from 17.9% at pH 6.3, to 20.9 at pH 5.9, and finally to 29.1% at pH 5.5. Li and Corredig (2014) have reported similar values at pH 5.5.

As the CCP was solubilized during acidification, the CN micelle structure did not appear to be substantially affected, which was reflected in the particle size distribution (Figure 1). The particle size distribution of the CN micelles shifted initially toward smaller sizes when pH of the feed was changed from 6.7 to 5.9 (Figure 1). No further change was observed when pH was adjusted to 5.5, being similar to particle size distribution at pH 5.9. At natural pH of skim milk, individual CN are associated in the form of micelles, the diameter of which varies depending on ionic conditions, but in general about 200 nm in diameter. The origin of stability of the micelles has been partly attributed to phosphocalcic bridge between phosphorylated lateral chains of CN structure and Ca (Law and Leaver, 1998). Upon acidification, proton addition causes association of H^+ with citrate, inorganic, and organic phosphates, inducing increase of ionic Ca concentration, and de-

crease in micellar hydration and zeta potential, leading to solubilization of micellar calcium phosphate (Law and Leaver, 1998). This also results in decline in CN micelle voluminosity (Vreeman et al., 1989) and its particle size (Silva et al., 2013). Another contributing factor to change in particle size could be dissolution of CN from the micelle, which has been found to be higher at lower pH values of ~ 5.5 even at 20°C (Dalglish and Law, 1988). It is not clear if UF process had an effect on the decrease of the particle size, although this was reported previously (Srilaorkul et al., 1991). Studies examining mineral equilibrium and effect on the CN micelle integrity in milk have been conducted in thermodynamically closed systems (a closed membrane processing loop), in which total mineral content remained constant. In our study, however, continuous removal of Ca by its permeation through the membrane would likely shift equilibrium between CCP and soluble Ca and thus induce further changes of the CN micelle, potentially leading to a reduction of micelle particle size. Furthermore, in the pH range between 5.2 and 5.9, the CN micelle undergoes many physico-chemical changes (Vreeman et al., 1989; Walstra, 1990), which, accompanied with a shift in the Ca equilibrium due to UF, would likely result in MPC with a smaller particle size (Srilaorkul et al., 1991).

Removal of minerals, in particular Ca, by UF affected zeta potential (Figure 2). As the feed was acidified and pH declined from 6.7 to 5.5, the net negative charge decreased from -32.8 to -27.6 mV. On the other hand, zeta potential of the corresponding retentates at the end of UF increased from -33.7 to -37.9 mV. This type of observation was expected because negatively charged sites of κ -CN would become neutralized by proton addition and solubilization of the CCP (Walstra, 1990). The exterior of the micelles mainly consists of hydrophilic negatively charged κ -CN, which provides both electrostatic and steric stabilization to the CN micelle. The negative charge is caused by dissociated carboxyl and ester phosphate group. When pH decreases, the CN-mineral equilibrium is shifted increasing the repulsion between the negatively charged AA in the CN

Figure 1. Particle size distribution of feed (solid line) and retentate (dotted line) at volume concentration factor of 5 obtained by UF of skim milk with adjusted pH at 6.7 (●), 6.3 (▲), 5.9 (■), or 5.5 (◆) at 15°C .

micelles, leading to a decrease in concentration of free Ca ions, dissolution of CCP from micelle, and release of specific CN from the micelle (Liu and Guo, 2008). Contribution of soluble Ca to charge screening at low pH appeared substantial as preferential removal of Ca from the micelle by CPP dissolution and by UF from the feed (Vasiljevic and Jelen, 1999) augmented negative surface potential of the particles (Figure 2) along with the neutralization effect of positively charged groups caused by lowering of pH. This could potentially contribute to the decrease in particle size due to greater electrostatic repulsion along with concomitant collapse of the κ -CN hairy layer (Walstra, 1990) as well as due to solubilization of CCP.

Structural Changes in Milk Proteins

Fourier transform infrared interferograms of the feed (Figure 3A) showed typical peaks associated with the secondary structure of proteins such as β -sheets (1,631–

Table 1. Total and soluble Ca (mean $\pm$ SD) in feed and retentates after $5\times$ volume concentration during UF of skim milk at 15°C with pH adjusted to 6.3, 5.9, or 5.5

pH	Total Ca (mM)		Soluble Ca (mM)	
	Feed	Retentate, VCF ¹ 5	Feed	Retentate, VCF 5
Control	31.1 ± 0.8	103.6 ± 0.2	12.4 ± 0.8	16.1 ± 0.3
6.3	32.2 ± 0.4	98.9 ± 1.2	15.3 ± 0.6	17.7 ± 0.4
5.9	31.4 ± 0.7	95.4 ± 0.7	17.9 ± 1.2	20.0 ± 0.2
5.5	32.7 ± 0.8	85.0 ± 0.4	25.2 ± 1.2	24.8 ± 0.1

¹VCF = volume concentration ratio.

Figure 2. Zeta-potential obtained at the beginning (—○—) and the end (---□---) of UF of skim milk at adjusted pH and constant processing temperature of 15°C. Error bars represent SD.

1,635 cm^{-1}), random coils (1,640–1,650 cm^{-1}), and α -helix (1,650, 1,657, or 1,658 cm^{-1}). Changing the pH of the feed before UF showed a shift in position along with increasing intensity of peak representing β -sheets. The peak appearing at 1,627 cm^{-1} in the control shifted to 1,629 at pH 6.3, 1,632 at pH 5.9, and finally to 1,634 at pH 5.5, suggesting the formation of disordered structures and intermolecular β -sheet associated aggregation as the pH of the feed was lowered. Similar changes were also exhibited by the peak representing random coil structure at 1,643 cm^{-1} . Moreover, appearance of well-defined peaks at 1,681 and 1,693 cm^{-1} , related to antiparallel intermolecular β -sheet aggregation and denatured compounds, respectively, indicated that lowering the pH of feed to 5.9 or 5.5 caused substantial denaturation and aggregation of proteins. The fact that whey proteins tend to form aggregates with CN at pH closer to their isoelectric point (pH $\sim$ 5.2; Nair et al., 2013) explains the appearance of such peaks (1,681 and 1,693 cm^{-1}) at pH 5.9 and 5.5 as well as the shift observed in peak representing β -sheets. Further, solubilization of CCP with decreasing pH in the feed could lead to dissociation of CN (McMahon and Oommen, 2013), which could further explain the increased possibility of protein interaction leading to formation of aggregates. This supports our earlier observation of increase in soluble Ca (to $>20\%$ of total Ca) when pH was lowered to 5.9 and 5.5, strengthening the possibility of CN dissociation due to solubilization of CCP.

Concentrating the skim milk at any defined pH did not cause any major structural changes as was observed by the persistence of similar peaks in the corresponding retentates (Figure 3B). The peak intensities of some

typical peaks increased such as those appearing at 1,631 cm^{-1} (control, pH 6.3 and 5.9), as well as the peak at 1,634 cm^{-1} and those representing denatured components at pH 5.5, which could be a consequence of the concentration effect of UF. However, the appearance of well-defined peaks at 1,658 (control and pH 5.9 and 5.5) and 1,663 cm^{-1} (pH 6.3), indicative of α - and 3_{10} helical structures, respectively, suggest further randomness in the structural integrity of proteins as a consequence of UF or increased solubilization of CN as the pH was lowered.

Functional Properties of MPC

Milk proteins, due to their amphiphilic nature, play an important functional role in food. Among various

Figure 3. Fourier transform infrared interferograms depicting structural changes of proteins upon pH in feed (A) and the corresponding retentates (B) after UF at 15°C and volume concentration factor of 5. Pasteurized skim milk samples had pH adjusted to 6.3 (---), 5.9 (·····), and 5.5 (— · —) and included control (—) with pH of 6.76.

functional properties, solubility is of primary importance due to its effect on the other functionalities (Halling, 1981). Numerous studies pointed out that changes in operating conditions during membrane processing affect the composition and functionality of the MPC powders produced (Udabage et al., 2012). One of the limiting factors in widespread use of MPC powders is its poor solubility, which is further compromised during storage, mainly attributed to association of CN molecules by hydrophobic interactions (Havea, 2006) and migration of residual fat to the surface of the powder particle (Gaiani et al., 2009). Pasteurization of milk before MPC production has also been shown to induce a certain degree of protein aggregation mainly via disulfide bonds (Havea, 2006) that could consequently influence MPC solubility. Earlier, McKenna (2000) observed that large particle formed in MPC negatively affected its solubility. These particles consisted of CN micelles that somehow interacted and fused together. Figure 4 shows the comparison of solubility and heat stability of the reconstituted MPC powders obtained by freeze

drying of retentates concentrated from the skim milk adjusted to different pH. Solubility and heat stability of reconstituted powders (Figure 4) was established at adjusted and neutralized pH (6.7).

The solubility of MPC dispersions declined substantially ($P < 0.05$) from ~77 to 32% as pH of the feed was adjusted from 6.7 (control) to 5.5, respectively. However, this trend was reversed when pH of dispersions was neutralized back to 6.7. Solubility of MPC dispersions obtained at pH 5.9 and 5.5 was even greater ($P < 0.05$) than that of the control (87.9 ± 1.0 and 89.7 ± 0.7 , respectively). The heat stability followed a similar trend, declining from ~96% to ~8% at pH 6.7 and 5.5, respectively, as pH was lowered before UF. Neutralizing pH of the dispersions once again improved heat stability, which was comparable among the samples ranging between ~72 and 78% at pH 5.5 and 6.7, respectively (Figure 4). Among various factors influencing protein solubility, pH plays a central and important role. At pH above and below their pI, proteins have a net negative or positive charge, respectively, which contributes

Figure 4. Solubility, before (dotted) and after (dark gray) pH adjustment, and heat stability, before (cross-hatch) and after (diagonal lines) pH adjustment, of 5% (wt/wt) dispersions prepared from milk protein concentrate powders obtained by UF of pH adjusted skim milk at 15°C. Error bars represent SD.

to overall protein stability and consequently solubility. At pH closer to isoelectric point, CN micelle starts to change due to collapse of κ -CN hairy layer and solubilization of the CCP, resulting in compromised steric hindrance and lower electrostatic repulsions due to released Ca^{2+} (Table 1, Walstra, 1990). This, accompanied with a lower processing temperature, can result in dissolution of CN (Dalglish and Law, 1988), leading to a system with substantially different physical properties upon drying and redispersion. Modified surface properties likely resulted in poor dispersability and enhanced aggregation, which was manifested by poor solubility. Additionally, released β -CN might have been located on the surface of particles upon water removal, which further enhanced hydrophobic interactions upon reintroduction into solution driving particles toward aggregation and poor solubilization. These interactions, however, appeared minimized upon neutralization, which have likely recovered some of the initial surface properties.

Differences in the heat stability of milk have been attributed to variations in the composition of milk salts and presence or absence of the CCP (O'Connell and Fox, 2003). For example, Ghatak et al. (1989) reported that the heat stability of both bovine and buffalo milk is significantly correlated with soluble Ca content, whereas Pouliot and Boulet (1995) correlated the heat stability of concentrated milk with the ratio of colloidal Ca to colloidal phosphate. Furthermore, mode of milk protein concentration has a marked effect on heat stability. Concentrates prepared by UF were more stable than those produced by conventional evaporation, which was attributed to a reduction in the concentration of lactose and soluble Ca (Sweetsur and Muir, 1980). Lowering pH beyond 6.0 before UF resulted in concentrates with compromised heat stability (Muir and Sweetsur, 1984), once again indicating substantial changes of surface properties invariably leading to low solubility and heat stability. Apparently some of these physicochemical changes may be reversed by pH readjustment upon redispersion, thus improving solubility and heat stability. Similarly, Ezech and Lewis (2011) observed improved heat stability of skim milk when the pH was readjusted to 6.47 from 5.45, which they attributed to restoration of CCP.

Modified particle properties may ultimately lead to improved or compromised interfacial functionality. The emulsion activity index (EAI) and ESI of reconstituted MPC powders obtained by pH adjustment are presented in Figure 5. Lowering pH to 5.9 improved ($P < 0.05$) EAI; however, it consequently declined ($P < 0.05$) at pH 5.5. This trend was conversely reversed upon neutralization with EAI at pH 5.5 being similar

($P > 0.05$) to that at pH 5.9 and greater than EAI at pH 6.7 or 6.3 (Figure 5). The emulsion stability as indicated by ESI on the contrary was greater ($P < 0.05$) at the control pH and steadily declined ($P < 0.05$) as pH was lowered in the feed. Interestingly neutralization completely reversed this trend, with the most stable emulsion obtained from MPC obtained at pH 5.9, being almost unchanged during the time of experimentation.

Emulsion activity index is a measure of the capability of a protein to stabilize the interface between 2 immiscible phases, such as oil and water (Dickinson, 1992; Dalglish, 2006). Stability of an emulsion depends on the balance of several forces including attractive, repulsive, steric, and depletion forces (Dickinson, 1997). The structure of an emulsion can change in several ways including creaming, flocculation, or coalescence, or their combinations (Ferragut and Chiralt, 1996; Dickinson and Golding, 1997). Thus emulsion stabilization may be provided by hindering these processes and the manipulating forces driving them. For an emulsion to remain kinetically stable, repulsive forces need to be greater than attractive forces. From Figure 5, it is obvious that MPC powders had different emulsification properties likely governed by surface properties of constituting particles. Dispersions prepared by MPC without pH adjustment apparently had compromised solubility that consequently affected redistribution of protein particles on the interface and thus emulsifying capacity. Upon neutralization, many amphiphilic properties were restored and probably improved because MPC obtained at pH 5.9 and 5.5 had greater EAI and ESI than those obtained by UF at 6.7 or 6.3 (Figure 5). Such an improvement could partially be attributed to reduced particle size, although it does not appear as a governing factor at a lower pH because MPC obtained at 5.5 and 5.9 had similar particle size distributions (Figure 1). Loss of steric hindrance and hydrophilicity by collapsing κ -CN (O'Connell and Fox, 2003) might have rendered resulting CN micelles more hydrophobic and prone to associations, and thus poor solubility. This, however, upon pH neutralization this might have been counteracted by increased electrostatic repulsion due to lack of soluble Ca in solution, and thus improved solubility. However, rather looser structure due to depletion of the CCP at low pH and likely reorganization of individual CN (Anema and Klostermeyer, 1997; Vasbinder and de Kruif, 2003) resulted in smaller particles with enhanced emulsifying properties. Although it is not evident from the current study, dissolution of CN during UF at low temperature is expected; however, it is difficult to predict its fate during further processing and whether it plays any role in improved emulsification. A recent study has found that β -CN is

Figure 5. Emulsion activity index, before (dotted) and after (dark gray) pH adjustment, and emulsion stability index, before (cross-hatch) and after (diagonal lines) pH adjustment, of emulsions prepared from milk protein concentrate powders obtained by UF of pH adjusted skim milk at 15°C. Error bars represent SD.

highly hydrophobic and prone to self-associations, creating different micellar forms and modifying behavior of other proteins present (Liyanaarachchi et al., 2015).

Membrane Performance

Achieving a balance between process parameters to obtain MPC of required functionality and membrane performance is of paramount importance. The membrane performance was initially assessed by flux determination and further elaborated on by the scanning electron microscopy characterization. As expected, the flux declined steadily over time during UF irrespective of adjusted pH (Figure 6). Indicatively, the flux was similar ($P > 0.05$) for all UF runs at pH 5.9 and above. The flux deteriorated substantially ($P < 0.05$) as pH was adjusted to 5.5. In principle, it took approximately 3.5 h to achieve VCF of 5, whereas this time was extended at pH 5.5 to 4 h. Apparently, pH adjustment in the feed before UF induced significant changes in surface properties of the CN micelle that resulted in enhanced surface activity and subsequent

greater fouling at lower pH. This membrane fouling was also observed by scanning electron microscopy (Figure 7). The cross section of the membrane operated at pH 6.7 appeared clean as indicated by the presence of some clear pores on the surface and rigid supporting wall. As pH was lowered to 5.5, the extent of membrane fouling became substantial, with pores and supporting wall being almost completely blocked at pH 5.5 (Figure 7). The reduction in size of CN micelle (Figure 1) as well as change to its surface properties (Figure 2) at pH 5.5 could have promoted its adherence to membranes leading to extensive fouling. Overall, the slight flux decline appeared to follow the generation of the fouling layer and increased deposition of solids; flux is therefore controlled by diffusion resistance in this layer. However, the extent of fouling observed in Figure 7 was not in line with the observed differences in flux. Although the fouling layer at pH 5.5 appeared thicker, the flux was fractionally lower, especially in the initial stages. This indicated that the flux at this pH was hindered immediately, whereas further flux declined at a similar rate as that at other pH levels.

Figure 6. Permeate flux during UF of skim milk with adjusted pH at 6.7 (●), 6.3 (▲), 5.9 (■), or 5.5 (◆) at 15°C to a volume concentration factor of 5. Error bars represent SD.

Membrane fouling in the dairy industry mainly arises from the composition and properties of the treated fluid apart from the effects of operation parameters (e.g., pressure, temperature, and flow rate). Membranes employed in UF of milk are usually hydrophilic, which minimizes attraction between membrane surface and present proteins. Therefore, creation of a fouling layer is initiated by soluble salts, mainly Ca and phosphates (Hausmann et al., 2013). Salt deposition may be accelerated at a lower pH due to greater salt solubilization from the CN micelle. Patel and Reuter (1985) have also attributed flux impairment during UF processing at lowered pH to increased concentration of soluble Ca. Upon layer creation, these salts start interacting with the CN micelles, in which the modified structure allows for greater attraction and consequently greater deposition and fouling. However, although the fouling layer appeared thicker at low pH, the rate of flux decline among all runs was similar, which may be indicative of a highly porous layer (Hausmann et al., 2013). Additionally, the low pH is always accompanied by an

Figure 7. Scanning electron microscopy cross sections of membranes at the end of UF of skim milk with adjusted pH at 6.7, 6.3, 5.9, or 5.5 at 15°C and a volume concentration factor of 5.

increase in the viscosity of milk, which may further hinder the flux. Viscosity of skim milk can increase by 2.6 times for a VCF between 2 and 5 (Dinkov et al., 2008), which further explains the reduction in flux observed in all samples. All these factors have been implicated in fouling of membranes and questionable process feasibility (Muir and Sweetsur, 1984).

CONCLUSIONS

The pH of the skim milk feed had a significant effect on UF operation and the functionality of the final MPC powder. Lowering feed pH from 6.7 to 5.5 substantially reduced the size of CN micelle and their zeta potential while enhancing emulsion capability and emulsion stability of the MPC. However, it had a negative effect on the flux due to increased fouling. This was mainly attributed to the removal of Ca from the CN micelles and consequent reduction in micelle size during feed acidification and as well as during UF processing of the feed with lowered pH. Possible structural modifications of CN particles due to depletion of CCP at low pH and likely reorganization of individual CN resulted in smaller particles with enhanced emulsifying properties. The solubility and heat stability of MPC declined with lowering of feed pH, however, restoring the pH of the MPC dispersions to normal (pH 6.7) before analysis improved their functionality possibly due to the restoration of their surface properties. The lowering of the pH, however, reduced the membrane performance with increased membrane fouling resulting in longer times to achieve similar concentration levels. Overall, the most optimum pH was found to be 5.9 for UF at 15°C to obtain MPC with improved emulsifying properties without compromising membrane performance.

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