Manufacture of modified milk protein concentrate utilizing injection of carbon dioxide

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ABSTRACT

Dried milk protein concentrate is produced from skim milk using a combination of processes such as ultrafiltration (UF), evaporation or nanofiltration, and spray drying. It is well established that dried milk protein concentrate (MPC) that contains 80% (MPC80) and greater protein content (relative to dry matter) can lose solubility during storage as a result of protein–protein interactions and formation of insoluble complexes. Previous studies have shown that partial replacement of calcium with sodium improves MPC80 functionality and prevents the loss in solubility during storage. Those studies have used pH adjustment with the addition of acids, addition of monovalent salts, or ion exchange treatment of UF retentate. The objective of this study was to use carbon dioxide to produce MPC80 with improved functionality. In this study, reduced-calcium MPC80 (RCMPC) was produced from skim milk that was subjected to injection of 2,200 ppm of CO₂ before UF, along with additional CO₂ injection at a flow rate of 1.5 to 2 L/min during UF. A control MPC80 (CtrlMPC) was also produced from the same lot of skim milk without injection of CO₂. The above processes were replicated 3 times, using different lots of skim milk for each replication. All the UF retentates were spray dried using a pilot-scale dryer. Skim milk and UF retentates were tested for ζ -potential (net negative charge), particle size, and viscosity. All the MPC were stored at room (22 ± 1°C) and elevated (40°C) temperatures for 6 mo. Solubility was measured by dissolving the dried MPC in water at 22°C and at 10°C (cold solubility). Injection of CO₂ and the resultant solubilization of calcium phosphate had a significant effect on UF performance, resulting in 10 and 20% loss in initial and average flux, respectively. Processing of skim milk with injection of CO₂ also resulted in higher irreversible fouling resistances. Compared with control, the reduced-calcium MPC had 28 and 34% less ash

and calcium, respectively. Injection of CO₂ resulted in a significant decrease in ζ -potential and a significant increase in the size of the casein micelle. Moreover, RCMPC had a significantly higher solubility after storage at room temperature and at elevated temperature. This study demonstrates that MPC80 with a reduced calcium and mineral content can be produced with injection of CO₂ before and during UF of skim milk.

Key words: milk protein concentrate, carbon dioxide, ultrafiltration

INTRODUCTION

Ultrafiltration is a membrane separation technology that was introduced in dairy processing in the early 1970s. Ultrafiltration uses a semi-permeable membrane to fractionate components in milk based on their size (Huffman and Harper, 1999; Muthukumarappan and Marella, 2010). It has been extensively used by the dairy industry to produce a variety of dairy ingredients, including protein concentrates from cheese whey (Renner and Abd El-Salam, 1991; Marella, 2009) and milk (Jimenez-Flores and Kosikowski, 1986; Patel et al., 1991), fractionation of cheese whey proteins (Etzel and Chiu, 1997; Muller et al., 2003; Mehra and Kelley, 2004; Marella et al., 2011), and harvesting milk minerals from dairy co-product streams (Vyas and Tong, 2003; Mealy et al., 2013).

In production of milk protein concentrate with 80% protein (**MPC80**), skim milk is subjected to UF and diafiltration (**DF**). This process produces a liquid concentrate that is partially delactosed and high in protein. This liquid concentrate is further concentrated and spray dried to obtain MPC80 powder. During UF, water, lactose, NPN, and some soluble salts pass through the membrane and are collected in the permeate. Higher-molecular-weight constituents such as caseins, whey proteins, and some minerals are concentrated in the retentate stream (Jimenez-Flores and Kosikowski, 1986). The ratio of the volume of the milk to the volume of concentrate obtained during UF is known as volume reduction (**VR**). In a typical UF process, pasteurized skim milk is concentrated to a final volume of one-fifth to one-eighth of the volume of the original skim milk

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(VR of 5–8) to produce MPC that has 65 to 75% protein relative to total solids. For the production of MPC with higher protein content (protein content of 80% and above) relative to solids, DF is used in conjunction with the UF process. Diafiltration is the process of adding water to the product during UF and subsequent removal of it. Diafiltration removes additional lactose and soluble salts, thereby increasing the relative concentration of protein to total solids. As an example, in a typical manufacturing process, MPC with 80% protein relative to total solids is produced with a volume reduction of 5 with a DF level of 20 to 40% (based on volume of skim milk). This process produces a liquid MPC80 with 20 to 22% solids, and a protein:solids ratio of 80. The liquid concentrate obtained is typically further concentrated by nanofiltration before it is spray dried.

Several researchers have conducted extensive studies on the solubility of commercially available MPC powders and have reported poor solubility for MPC80 powders after extended storage (de Castro-Morel and Harper, 2002; Sikand et al., 2011; Udabage et al., 2012). Poor solubility of MPC80 can lead to nugget formation during cheese making (Bhaskar et al., 2007), bar hardening in high-protein bars (Imtiaz et al., 2012), and settling of insoluble material when used in drink mixes (personal communications with MPC80 customers). Previous research has demonstrated that processing and storage conditions affect physicochemical interactions among the proteins, salts, and sugars, and can affect the solubility of MPC80. The physicochemical interactions that have been shown to affect the functional properties of MPCs include casein-casein and casein-whey protein interactions (McKenna, 2000; Anema et al., 2006; Havea, 2006); hydrophobic association of casein micelles (Havea, 2006); formation of disulfide-linked β -LG and casein complexes (Kameswaran and Smith, 1999; Anema et al., 2006); sulfhydryl-disulfide interchange reactions, and hydrophobic interactions among the proteins (Mao et al., 2012); lactosylation, deamidation, and protein cross-linking (Le et al., 2012); and formation of a thin crust of fused casein micelles on the surface of powder particles (Fyfe et al., 2011). Several of these studies have shown a strong correlation between solubility of MPC80 and their Ca content (Bhaskar et al., 2007; Sikand et al., 2011, 2013; Ye, 2011) and it is theorized that Ca present in MPC may be promoting protein-protein interactions during processing as well as storage.

To improve the functionality of MPC80, several researchers have developed new manufacturing processes that have focused on (1) application of physical treatments to the liquid concentrate before spray drying or to final dried MPC; or (2) process modifications that produce MPC with a modified mineral profile.

Banach et al. (2013) studied the effect of low- and high-shear extrusion and low-temperature toasting of MPC80 powders on the functional properties of the resultant modified MPC. Both of these treatments result in a decrease in solubility, hydrophobicity, and water-holding capacity. High-pressure homogenization (Augustin et al., 2012; Udabage et al., 2012), extrusion-porosification (Bouvier et al., 2013), microfluidization, and ultrasonication (Augustin et al., 2012) of liquid concentrates before spray drying have been reported to improve the solubility of MPC. Partial disintegration of casein micelle due to these high shear treatments and the resultant high level of nonmicellar casein is thought to cause the improved solubility (Udabage et al., 2012). Mao et al. (2012) and Sikand et al. (2013) developed a novel process to produce mineral modified MPC80 utilizing DF with different levels of monovalent salts added to the DF water. The mineral modified MPC80 produced from this process exhibited modified functionality with higher solubility compared with conventionally produced MPC80. Dybing et al. (2007) and Bhaskar et al. (2007) used a cation-exchange process to replace divalent ions, especially calcium, and reported improved functional properties for the resultant calcium-depleted MPC.

The modified functionality resulting from partial demineralization of MPC may be a result of protein disaggregation that occurs when calcium phosphate is removed from the casein micelle. A new approach that may be applicable for production of partially demineralized MPC is the injection of CO₂ before and during UF. Injection of CO₂ into milk has been extensively studied, and it is widely used to increase the shelf life of raw milk (King and Mabbitt, 1982; Law and Mabbitt, 1983; Werner and Hotchkiss, 2006) and pasteurized milk (Ma and Barbano, 2003; Hotchkiss et al., 2006). Previous research has demonstrated that CO₂ injection of cheese milk before rennet coagulation can be used to reduce the pH of milk and solubilize micellar calcium phosphate, which modifies the mineral profile of cheese produced from concentrated milk (Nelson et al., 2004). In a similar fashion, CO₂ injection could be used to reduce the pH of milk and solubilize micellar calcium and phosphate before and during UF to produce MPC with reduced calcium and mineral content. Additionally, when CO₂ is used as an acidulant, residual CO₂ can easily be removed by heating or applying vacuum, whereas other acidulants such as organic acids cannot easily be removed.

The overall objective of this research was to use injection of CO₂ before and during UF of skim milk and to determine the effect of CO₂ injection on the mineral content, casein particle size, ζ -potential, membrane performance, and solubility of MPC80.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

As described below, preliminary laboratory-scale UF experiments were conducted to select the correct VR and DF levels for production of MPC 80. Additionally, preliminary pilot-scale experiments were conducted where pasteurized skim milk was injected with carbon dioxide to determine various levels of dissolved CO₂. From these preliminary experiments, the required level of CO₂ injection before and during UF was selected for replicated analysis in a pilot scale study. In this study, 3 different lots of skim milk were injected with 2,200 ppm of dissolved CO₂ before UF, along with additional injections of CO₂ during UF. A portion of each lot of skim

milk was also used to produce MPC80 without CO₂ injection. The UF retentates were spray dried using a pilot-scale Niro dryer (Niro dryer model 1, Niro Inc., Columbia, MD) fitted with a centrifugal disc atomizer. All powders were evaluated for cold-water solubility, solubility after room temperature, and elevated temperature storage. A schematic of experimental design and the sample codes is shown in Figure 1.

Laboratory-Scale Experiments

Preliminary laboratory-scale experiments were conducted using a plate-and-frame unit (Marella et al., 2011). These experiments were conducted to select the required VR and level of DF needed to produce MPC80. In each test, 1,500 mL of pasteurized (63°C/30

Figure 1. Flow diagram of experimental design and sample codes (in parentheses). Both control (Ctrl) and reduced-calcium (RC) runs were repeated 3 times using different lots of skim milk. Skim = skim milk; UF = ultrafiltered milk; P = permeate; R = retentate; MPC80 = milk protein concentrate with 80% protein.

Figure 2. Schematic of pilot UF unit, which houses 2 spiral-wound elements (in 3838 format—965 mm long and 96.5 mm in diameter) in parallel.

min) skim milk was concentrated to VR of 4 to 8 in a continuous concentration mode. A polyethersulfone membrane (Parker process advanced filtration division, Parker Hannifin Corp., Oxnard, CA) with a molecular weight cut-off of 10 kDa in flat sheet configuration was used. The UF process was conducted at 15°C with a transmembrane pressure (TMP) of 207 kPa (30 psi). During these experiments, DF water from 0 to 50% (based on the feed volume) was added. Samples of skim milk and retentates were analyzed for TS and protein using standard methods of analysis (Hooi et al., 2004).

Pilot-Scale Experiments

Pilot-scale experiments were conducted using the pilot UF unit shown in Figure 2. Two 10-kDa polyethersulfone membranes (Parker Hannifin Corp.) were used in parallel. Each membrane element was 97 mm in diameter and 965 mm in length with a 1.1-mm feed spacer and a total surface area of 5.7 m² per element. The inlet and outlet pressures to the membrane element were 345 and 207 kPa, respectively, resulting in a TMP of 276 kPa. All experiments were conducted at 15°C. Each experiment began with 189 kg of pasteurized skim milk. The feed was concentrated to a final

retentate of 37.85 kg, resulting in a VR of 5. To control the viscosity of the retentate and to remove soluble solutes, during the process, DF water (10% of the starting feed volume) was added at VR of 2, 2.5, 3, and 4, for a total DF of 40% of the feed volume.

Before UF, pasteurized skim milk was injected with CO₂ to obtain a dissolved CO₂ of 2,200 ppm in the skim milk. The CO₂ injection conditions are described below. After CO₂ injection, the skim milk was held for 1 h for equilibration. At the end of equilibration, the skim milk was warmed to 15°C and processed in the UF unit. At the end of the UF process, samples of permeate and retentate were collected for wet chemistry and measurement of dissolved CO₂. All of the retentate was collected for further drying to produce powder.

CO₂ Injection

A stainless steel sparger cup (12.7 × 9.5 × 25.4 mm) with 10-μm porosity was used to inject CO₂ into the skim milk. For injection of CO₂, the balance tank of the UF unit was filled with skim milk, membrane housings were isolated, and the feed pump was used to recirculate the skim milk through the piping of the UF unit (Figure 2). Backpressure of 138 kPa was applied dur-

ing recirculation. The CO₂ pressure was maintained at 527.1 kPa at the inlet to the sparger. The CO₂ flow rate was maintained at 14.1 L/min and the skim milk flow was at 57 to 68 L/min. During the sparging and the 1-h equilibration, the temperature of skim milk was maintained at 4 to 5°C using the inline heat exchanger in the UF unit. The initial pH of skim milk was 6.62, and after 30 min, the pH decreased to 5.71. At this point, CO₂ injection was stopped, and the milk was held for 60 min. Subsequently, the skim milk was warmed to 15°C and UF processing was started. During UF, the pH of the skim milk was continuously recorded and maintained at 5.8 with additional injection of CO₂ (at a flow rate of 1.5 to 2 L/min).

Measurement of Dissolved CO₂

Dissolved CO₂ was measured using an enzymatic assay. This assay used a CO₂ (liquid) reagent kit (Cat. No. C7502-120) and a test method (manual method test: CO₂ 405, Cat. No. C7502) supplied by Pointe Scientific Inc. (Canton, MI). The reagent kit and test method were originally intended for quantitative determination of CO₂ in blood serum, and the method is based on the principle that CO₂ (in the form of bicarbonate ions) reacts with phosphoenolpyruvate (PEP), in the presence of phosphoenolpyruvate carboxylase (PEPC) and forms oxaloacetate. Oxaloacetate oxidizes NADH in the presence of malate dehydrogenase, thereby decreasing the absorbance monitored between 405 and 415 nm. The decrease is proportional to the amount of CO₂ present in the sample:

This enzymatic method can be used for measuring CO₂ present as HCO₃⁻, CO₃²⁻, and dissolved gas. Dissolved CO₂ present in skim milk, permeate, or retentate samples was trapped as Na₂CO₃ using 4 M NaOH (Crow and Martley, 1991).

To measure the dissolved CO₂, approximately 0.5 g of sample (skim milk/permeate/retentate) was weighed into a 2-mL centrifuge vial that was previously flushed with N₂ gas. Then, 0.5 mL of boiled water and 60 µL of 4 M NaOH were added to the sample. After determining the final weight, the sample was mixed by vortexing followed by centrifugation of the vial at 17,091 × *g* at 20°C for 10 min. Any fat layer present was removed with a spatula. The UV-visible spectrophotometer was set at a wavelength of 405 nm and was zeroed with distilled water. A 1-mL sample of CO₂ reagent (PEP and PEPC) was added to a disposable cuvette flushed

with N₂ gas. The cuvette was then incubated at 37°C for 5 min. This incubation activates the enzyme present in the CO₂ reagent sample. At the end of incubation, 10 µL of the prepared sample was added to the cuvette and the contents were mixed by inverting 4 or 5 times. Absorbance was measured 30 s after adding the sample (A1); then, the cuvette was held in a water bath at 37°C for 6 min and a second reading (A2) was taken. Dissolved CO₂ (ppm) was calculated as follows:

$$\frac{(A1 - A2) \text{ Sample}}{(A1 - A2) \text{ Standard}} \times [\text{standard, ppm}] \times \text{dilution factor.}$$

Spray Drying

The UF retentates were spray dried using a single-stage pilot-scale dryer fitted with a Niro centrifugal disc atomizer (ASO 412E, Niro Inc., Columbia, MD). The inlet and outlet temperatures were maintained at 200 and 90°C, respectively. The powders were collected and packed in airtight containers and stored refrigerated (4°C) until further analysis was completed.

Chemical Analysis

The skim milk, retentate, and permeate samples from MPC manufacture were collected and immediately cooled to 4°C. The TS, total fat, and ash of each sample was determined using standard wet chemistry procedures as described by Hooi et al. (2004). The pH of all the samples was measured in duplicate with a pH meter (pH meter 340, Corning Inc., Corning, NY) equipped with an Accumet gel-filled glass electrode with a spear tip (Fisher Scientific, Fair Lawn, NJ). Lactose was measured using an HPLC-based method, as described by Amamcharla and Metzger (2011) for milk except that a sample size of 1 g was used. Mineral analysis of the samples was done using inductively coupled plasma-optical emission spectroscopy.

Ultrafiltration Processing Data

The average flux and TMP were determined as follows: average flux is the permeate flow rate through the membrane and is expressed in liters per m² and hour (L/m²·h):

$$\text{Average flux} = \frac{\text{Permeate flow (L)}}{\text{Area of membrane (m}^2\text{)} \times \text{time (h)}}.$$

Transmembrane pressure is the driving force for the membrane separation and is determined by subtracting

the permeate pressure (P_p) from the average of the inlet (P_{in}) and outlet (P_{out}) pressures:

$$TMP = \frac{P_{in} + P_{out}}{2} - P_p.$$

Because permeate was collected at atmospheric pressure, its pressure was considered to be zero.

Resistances

In any membrane process, the mass flow through the membrane is expressed as flux, and it is a function of the driving force, resistance, and viscosity of the permeate. The mass flow is directly proportional to the driving force and indirectly proportional to the resistance (R) and viscosity (μ_p) of the permeate:

$$\text{Mass flow (flux)} = \frac{TMP}{R \times \mu_p}.$$

The clean water flux and flux during processing were used to calculate membrane resistance (MR) and resistance during processing (RDP) as follows:

$$MR = \frac{TMP}{\text{Clean water flux} \times \mu_p},$$

$$RDP = \frac{TMP}{\text{Process flux} \times \mu_p}.$$

The difference between RDP and MR is the overall fouling resistance, Rfo. A small Rfo indicates improved permeate rates through the membrane: $Rfo = RDP - MR$.

At the end of the process when the membrane was rinsed with water all the loosely adhered material on the membrane was removed. The water flux taken after rinsing is used to determine the resistance after processing and rinsing (RAPR):

$$RAPR = \frac{TMP}{\text{Final water flux} \times \mu_p}.$$

The difference between RAPR and MR is the irreversible fouling resistance, Rfi, where $Rfi = RAPR - MR$.

Viscosity

The viscosity of UF concentrates (17% solids) was measured at 20°C with a Viscoanalyzer (Reologica

Instruments AB, ATS Rheosystems, Rheometric Scientific Inc., Piscataway, NJ) using bob-and-cup geometry. The samples were conditioned at 20°C for 60 s before analysis. About 16 mL of each concentrate at 20°C was transferred into the cup of the rheometer, and the bob was lowered until the whole bob surface was covered. An equilibrium time of 25.0 s and a pre-shear rate of 10 s^{-1} for 20.0 s was applied. The viscosity of the solution was measured at a shear rate of $1.000\text{E}+0 - 2.50\text{E}+2$ per s with a delay time of $1.032\text{E}+0 - 2.000\text{E}+0$ s. The viscosity was reported at a shear rate of 100 s^{-1} .

Particle Size and ζ -Potential

The particle size of casein micelles in the starting skim milk and UF retentates was measured by dynamic light scattering using a Zetasizer Nano-ZS (with 633 nm He-Ne laser; Malvern Instruments Ltd., Malvern, UK). Skim milk and UF retentates were diluted 1:100 with corresponding UF permeates. Control skim milk (**Ctrl-Skim**) and control UF retentate (**CtrlUFR**) were diluted with control UF permeate (**CtrlUFP**) obtained from control runs. The diluted Ctrl samples had a pH of 6.82. Skim milk used for reduced-calcium (**RC**) skim and reduced-calcium UF retentate (**RCUFR**) were diluted with reduced calcium permeate (**RCUFP**) obtained from RC runs. The diluted RC samples had a pH of 6.02. Before measuring the ζ -potential (i.e., net negative charge), all diluted samples were filtered through 0.45- μm syringe filters. The electrophoretic mobilities, and hence the calculated ζ -potential of casein micelles, were measured at constant temperature of 25°C. The measurement interval was set to -50 to $+10$ mV.

Solubility in Water at Room Temperature

Five percent protein solutions (wt/wt) were made by dissolving milk protein concentrate powders in distilled water at $22 \pm 1^\circ\text{C}$. The solutions were stirred for 30 min using a magnetic stirrer and a stir plate (Fisher Scientific). The stirring speed was maintained at 300 rpm. After stirring, each solution was hydrated overnight at 4°C . After overnight hydration, the protein solution was again stirred for 30 min at 300 rpm. The pH of the protein solutions was adjusted to approximately pH 7.0 using 2.0 N NaOH, and then fine pH adjustment was made using 0.2 N NaOH. After 1 h of equilibration, the solubility method described by Havea (2006) was used with some modifications. Aliquots of each reconstituted MPC (50 mL) were centrifuged (CR4-12, Jouan Inc., Riviera Beach, FL) at $700 \times g$ for 10 min. The 5% protein solution (before centrifugation) and the supernatant (after centrifugation) were analyzed for TS. The TS was determined by forced draft oven (Fisher

Table 1. Protein levels in milk protein concentrates as a function of volume reduction (VR) and diafiltration (DF)¹

Run no.	VR	DF	TS	Protein	Protein:TS
1	4	20	16.72	11.75	70.3
2	4	30	16.77	12.14	72.4
3	5	0	20.73	13.34	64.4
3	7	0	23.07	16.48	71.4
3	8	0	27.48	20.58	74.9
4	5	30	18.56	14.55	78.4
5	5	40	17.84	14.40	80.7
6	6	50	14.26	11.95	83.8

¹All data were means of 2 runs.

Scientific) drying at 100°C for 4 h. The solubility was calculated as the TS of the supernatant, expressed as percentage of the TS of the 5% protein solution before centrifugation.

Solubility in Cold Water

Cold-water solubility was measured by dissolving MPC80 powders in cold tap water (at 10°C). To simulate the use of MPC80 in drink mixes, powders were dissolved in cold water by high-speed stirring (at 900 rpm) for 2 min. The initial mean pH of the 5% protein solutions was 6.33 and 6.96, respectively, for the RC and Ctrl treatments. Before measuring solubility, the pH of each 5% protein solution was adjusted close to pH 7.0 using 2.0 *N* NaOH and then fine pH adjustment were made using 0.2 *N* NaOH. After pH adjustment, the samples were allowed to equilibrate for 1 h, and solubility was measured as described previously for room temperature.

Statistical Analysis

All data were analyzed by MIXED procedure in SAS (SAS Institute Inc., Cary, NC) with a Type I error rate (α) of 0.05 to test for significant differences among the treatments.

RESULTS AND DISCUSSION

Effect of VR and DF Level on Protein:TS Ratio in MPC

Data obtained from laboratory-scale UF experiments conducted to determine the effect of VR and DF level on protein:TS ratio in MPC are shown in Table 1. Milk protein concentrate powders with a particular protein:TS ratio can be obtained by using a range of VR ratios and DF levels. In the present study, we selected a VR of 5 and DF level of 40% for production of MPC80.

Effect of CO₂ Injection on Ash and Calcium Contents of MPC

Average composition of UF retentates, permeates, and MPC powders manufactured in the study is shown in Table 2. Total solids, fat, protein, NPN, lactose, and protein:TS ratio did not differ ($P > 0.05$) for RCMPC and CtrlMPC. However, ash and Ca contents were significantly different ($P < 0.05$) for RCMPC and CtrlMPC powders. Similar results were observed for UF retentates and permeates. As seen from the ash and Ca contents of MPC powders, the ash content of RCMPC was 28% lower and Ca content was 33% lower as compared with the control. The reductions in ash and Ca contents were due to solubilization of calcium phosphate associated with casein micelles because of the reduced pH (Van Hooydonk et al., 1986; Dalgleish and Law, 1989; Law and Leaver, 1998), which passed into the permeate during the UF-DF process used in production of MPC. The mean dissolved CO₂ levels in skim milk, UF retentate, permeate, and 5% protein solutions of MPC powders are shown in Table 3. The skim milk used in the control MPC had 171 ppm of dissolved CO₂, whereas the CO₂-injected skim milk had 2,137 ppm of dissolved CO₂. The dissolved CO₂ in the RCUF retentate and permeate was 782 and 1,263 ppm, respectively, whereas the dissolved CO₂ in the CtrlUF retentate and permeate was 106 and 102 ppm, respectively. These dissolved CO₂ levels for RC and Ctrl treatments were different ($P < 0.05$) for the milk, retentate, and permeate. The pH values of the RC and Ctrl feeds, UF retentates, and UF permeates were also significantly different ($P < 0.05$) for treatment and control samples. The pH of the reconstituted 5% protein solutions of RCMPC and CtrlMPC were significantly different ($P < 0.05$), whereas the dissolved CO₂ levels were not different ($P > 0.05$). Because there was no difference in the level of dissolved CO₂, the lower pH of the RCMPC solution was probably a result of the lower mineral content of this sample. These results also

Table 2. Mean composition (%; n = 3; SD in parentheses) of UF retentates, permeates, and milk protein concentrate (MPC) powders obtained in the study

Sample ¹	TS	Fat	Protein	NPN	Ash	Lactose	Ca	Protein:TS
UF retentates								
RCUF	18.10 ^a (0.15)	0.48 ^a (0.04)	14.59 ^a (0.26)	0.12 ^a (0.02)	0.93 ^b (0.03)	1.88 ^a (0.19)	0.257 ^b (0.02)	80.6 ^a (0.75)
CtrlUF	17.83 ^a (0.31)	0.47 ^a (0.02)	14.20 ^a (0.30)	0.14 ^a (0.03)	1.30 ^a (0.04)	1.75 ^a (0.08)	0.387 ^a (0.03)	79.6 ^a (0.92)
UF permeates								
RCUF	4.34 ^a (0.19)	ND ²	0.14 ^a (0.01)	0.14 ^a (0.01)	0.43 ^a (0.02)	3.8 ^a (0.1)	0.057 ^a (0.01)	3.23 ^a (0.12)
CtrlUF	4.38 ^a (0.09)	ND	0.14 ^a (0.01)	0.14 ^a (0.01)	0.35 ^b (0.01)	3.84 ^a (0.05)	0.031 ^b (0.00)	3.27 ^a (0.13)
Powders								
RCMPC	95.92 ^a (0.59)	2.51 ^a (0.19)	77.51 ^a (0.07)	1.35 ^a (0.11)	4.96 ^b (0.05)	10.53 ^a (0.37)	1.319 ^b (0.04)	80.86 ^a (0.45)
CtrlMPC	95.86 ^a (0.58)	2.51 ^a (0.14)	76.98 ^a (0.48)	1.28 ^a (0.13)	6.94 ^a (0.10)	9.89 ^a (0.51)	2.00 ^a (0.05)	80.03 ^a (0.21)

^{a,b}Values with the same superscript within UF retentates, UF permeates, and powders are not statistically different ($P > 0.05$).

¹RCUF and CtrlUF = reduced-calcium and control UF runs, respectively; RCMPC and CtrlMPC = reduced-calcium and control MPC runs, respectively.

²ND = not determined.

indicate that the dissolved CO₂ in the RCUF retentate was removed during the drying process.

Effect of CO₂ Injection on ζ -Potential and Particle Size of Casein Micelles

Data on ζ -potential of casein micelle in skim milk and UF retentates is presented in Table 4. We detected a significant difference ($P < 0.05$) in the ζ -potential of control skim milk (CtrlSkim) and CO₂ injected skim milk (RCSkim). Whereas the CtrlSkim had a ζ -potential of -11.34 mV, the RCSkim had a ζ -potential of -8.06 mV. Injection of CO₂ with the consequent reduction in pH and solubilization of calcium phosphate resulted in a decrease in the ζ -potential of the casein micelles. The ζ -potential is a measure of the net negative charge that casein micelles carry due to the presence of glycosylated residues of κ -casein. Cations present in the serum phase can interact with these negative charges, shielding the charges on the micelle (Baudry et al., 2005; Huppertz and Fox, 2006; Ahmad et al., 2008). The ζ -potentials of CtrlUFR and RCUFR were also ($P < 0.05$) different.

When compared with the feed (skim milk) used for the UF runs, the UF retentates had a significantly higher ζ -potential. The DF step used in the process of UF removes soluble minerals. This partial removal of soluble divalent calcium might have contributed to an increase in net negative charge on the casein micelle.

The data on particle size of casein micelles in skim milk and UF retentates are shown in Table 4. Significant ($P < 0.05$) differences were found in the average size of casein micelles in CtrlSkim, CtrlUFR, RCSkim, and RCUFR. In the present study, we detected an approximately 14% increase in the size of casein micelles as a result of the VR (5) and DF (40%) used during UF in the control (CtrlSkim vs. CtrlUFR). Injection of CO₂ resulted in swelling of the casein micelle and, for both RCSkim and RCUFR, a size increase of approximately 23% compared with the size of the casein micelles in skim milk. Previous research (Helen et al., 2010) also reported an increase in casein micelle diameter as a result of calcium removal. The mineral profile of the solution in which casein micelles are dispersed influences the organization of the micelle and its size (Ahmad et al.,

Table 3. Dissolved CO₂ levels and pH values (n = 3; SD in parentheses) in feed, UF retentate, UF permeate, and 5% protein solutions

Item	Feed		UF retentate		UF permeate		5% protein	
	CO ₂ , ppm	pH	CO ₂ , ppm	pH	CO ₂ , ppm	pH	CO ₂ , ppm	pH
RC run	2,137 ^a (59.09)	5.71 ^b (0.02)	782 ^a (76.05)	5.81 ^b (0.05)	1,263 ^a (126)	6.02 ^b (0.05)	132 ^a (21.55)	6.33 ^a (0.02)
Ctrl run	171 ^b (20.81)	6.75 ^a (0.06)	106 ^b (10.39)	6.84 ^a (0.06)	102 ^b (3.46)	6.82 ^a (0.03)	104 ^a (3.00)	6.96 ^b (0.05)

^{a,b}Values with same superscripts within a column are not statistically different ($P > 0.05$).

¹RC run and Ctrl run = reduced-calcium and control 80% milk protein concentrate runs, respectively.

Table 4. Mean ζ -potential (net negative charge) and size (nm) of casein micelles ($n = 3$; SD in parentheses) for skim milk and UF retentates¹

Item	CtrlSkim (pH = 6.82)	RCSkim (pH = 6.02)	CtrlUFR (pH = 6.82)	RCUFR (pH = 6.02)
ζ -Potential	-11.34 ^b (1.79)	-8.06 ^a (2.31)	-14.07 ^c (0.29)	-10.84 ^b (0.81)
Size	141.87 ^c (2.31)	173.37 ^a (1.4)	161.37 ^b (1.47)	174 ^a (3.24)

^{a-c}Values with the same superscripts within a row are not statistically different ($P > 0.05$).

¹CtrlSkim and RCSkim = pasteurized skim milks used in control and reduced-calcium runs, respectively; CtrlUFR and RCUFR are UF retentates obtained from control and reduced-calcium UF runs, respectively.

2008). The presence of higher levels of soluble calcium and phosphate ions in the serum phase of CO₂-injected skim milk (RCSkim) and RCUFR as a result of the lower pH may contribute to disaggregation and internal swelling of the casein micelles, thereby increasing their size. McKenna (2000) also reported an increase in the size of casein micelle as a result of concentration or DF and theorized that this was caused by solubilization of micellar calcium phosphate, which resulted in swelling of the micelle.

Effect of CO₂ Injection on UF

The effect of CO₂ injection on the UF process was assessed in terms of flux rates and fouling resistances. Data on initial (**Ji**) and average (**Javg**) flux are shown in Table 5. Both Ji and Javg were significantly lower ($P < 0.05$) for RC compared with control. Initial flux was

that in the first 2 min of the UF process. The initial flux was about 10% lower for RC, and Javg was about 20% lower compared with that of the control. The presence of higher levels of soluble minerals as a result of the lower pH from the injection of CO₂ resulted in significant differences in both Ji and Javg. The Javg obtained at various volume reductions during processing of skim milk into MPC80 with (RCUF) and without (CtrlUF) injection of CO₂ are shown in Figure 3. Injection of CO₂ into skim milk before and during UF led to about a 10% reduction in the flux compared with the control at the start of the UF process. The difference in flux increased to 20% by the time the VR reached 1.4 to 1.5. This difference remained at 20% for the rest of the process run. By the end of the process, RCUF run had an average flux of 9.07 L/m²·h with a 35% loss in flux, whereas the CtrlUF run had an average flux of 11.38 L/m²·h with a 27% loss in average flux.

Data on overall (Rfo) and irreversible (Rfi) fouling resistances are shown in Table 5. Both Rfo and Rfi were significantly higher ($P < 0.05$) for RCUF compared with the CtrlUF run. The Rfo as a function of VR is presented in Figure 4. For RCUF, the Rfo was about 44×10^{12} at the start, and it increased by about 80% by the end of the process. For CtrlUF process, the Rfo was about 39×10^{12} at the start, and it increased by about 58% by the end of the process. At the start of the UF process, Rfo for RCUF run was about 18% higher than that of the CtrlUF run, and by the time VR reached

Figure 3. Average flux rates (L/m²·h) obtained during processing of 189 L of skim milk into milk protein concentrate with 80% protein (MPC80) with injection of CO₂ (reduced-calcium UF, RCUF) and without injection of CO₂ (control UF, CtrlUF).

Table 5. Process parameters¹ ($n = 3$; SD in parentheses) for reduced-calcium (RCUF) and control (CtrlUF) UF runs

Treatment	Ji	Javg	Rfo	Rfi
RCUF	13.96 ^b (0.33)	8.71 ^b (0.63)	24.00 ^a (4.98)	88.62 ^a (9.81)
CtrlUF	15.67 ^a (0.12)	11.18 ^a (0.42)	12.57 ^b (3.49)	64.36 ^b (3.58)

^{a,b}Values with same superscripts within a column are not statistically different ($P > 0.05$).

¹Ji = initial flux (L/m²·h) for the first 2 min; Javg = average flux (L/m²·h) for the entire run; Rfo (dimensionless, $\times 10^{12}$) and Rfi (dimensionless, $\times 10^{12}$) = overall and irreversible resistance, respectively.

Figure 4. Overall fouling resistances, Rfo (dimensionless, $\times 10^{12}$), as a function of volume reduction. Volume reduction was calculated as a ratio of initial volume of the feed (skim milk) and the volume of the retentate at any particular interval of time. RCUF = reduced-calcium UF; CtrlUF = control UF.

1.4, the Rfo difference increased to approximately 35%. Subsequently, the difference remained at 35 to 40% for the rest of the UF process.

Overall fouling resistance is the sum of reversible and irreversible fouling of the membranes during processing of skim milk into MPC. It includes the effect of concentration polarization (mostly reversible) and fouling due to mineral deposit inside the pores of the UF membranes. The overall fouling resistance for RCUF run was 88.62×10^{12} , whereas for CtrlUF, overall fouling resistance was about 64.36×10^{12} . Injection of CO_2 before and during UF led to a 38% increase in the overall fouling resistance. In any membrane processing operation, immediately after the start of the process, the solutes present in the bulk of the fluid accumulate on the surface of the membrane, forming a thin layer of proteins, fat, and other macromolecules. This layer is known as the concentration polarization layer and it becomes the pseudofiltration layer (Marella et al., 2011). Formation of this layer commences after a very short filtration time; this layer is slightly compressible and it densifies as the process proceeds (Bryony et al., 2003). The macromolecules are held in this layer because of interactions among the proteins, minerals, and the membrane. Caseins are the main fouling material in membrane separation processes (Baudry et al., 2005). As seen from the particle size data presented in Table 4, the size of the casein micelle in RCUF was significantly greater than that of CtrlUF. Consequently, in RCUF, the pseudofiltration layer that formed on the membrane

should be more porous than that of CtrlUF. This porous layer should, in fact, permeate more material. However, the higher levels of soluble calcium phosphate present in RCUF run caused by the lower pH from CO_2 injection may adversely affect permeation by interacting with the components present in the porous layer. Also, the ζ -potential of casein micelle in CO_2 -injected skim milk and RCUF retentate was significantly greater than that of control skim milk and CtrlUF retentate (Table 4). When the negative charge (ζ -potential) on the casein micelles is higher, the electrostatic repulsion between the casein micelles increases. Compared with casein micelles present in CtrlUF retentate, casein micelles present in RCUF retentate have lower ζ -potential, reducing the electrostatic repulsion between the casein micelles. This reduction in ζ -potential may promote casein-casein interactions, contributing to increased fouling resistance. In contrast to Rfo, the irreversible fouling resistance, Rfi, is mainly due to deposition of minerals during skim milk UF. The RCUF run had Rfi of 24×10^{12} , which is about twice that of the CtrlUF runs. The higher levels of soluble calcium phosphate present in the skim milk due to injection of CO_2 before and during UF of skim milk led to an increased level of Rfi during the UF process. Calcium phosphate precipitates in the membrane pores, leading to increased irreversible fouling and the resultant decline of UF flux. Calcium phosphate is the predominant salt foulant in milk systems and it affects performance of the membrane process by decreasing the permeation rate. Higher levels of calcium and phosphate may also promote higher levels of membrane-mineral and protein-membrane interactions leading to higher levels of fouling resistances. Free ionic calcium also promotes protein-protein and protein-membrane interactions, leading to deposition of these materials on membrane surfaces (Rice et al., 2009). The effect of higher levels of minerals present in dairy process streams such as cottage cheese whey and acid whey has been studied and this loss of flux has been attributed to higher levels of calcium and phosphate in the process streams (Hayes et al., 1974; Hickey and Hill, 1980; Hiddink et al., 1981).

Effect of CO_2 Injection on Viscosity of UF Retentate

Viscosity of the UF liquid retentates (17% solids) was 1.99×10^{-3} and 23.6×10^{-3} Pa·s, respectively, for RCUF and CtrlUF. The RCUF had a lower ($P < 0.05$) viscosity compared with CtrlUF. We theorize that the higher viscosity of the CtrlUF was a result of the higher Ca content of the CtrlUF. We also believe that the lower viscosity of the RCUF could positively affect the performance of nanofiltration, which is com-

Figure 5. Solubility of milk protein concentrate (MPC) powders as a function of the storage period. RCMPC-RT and CtrlMPC-RT = reduced-calcium and control MPC powders stored at room temperature; RCMPC-40C and CtrlMPC-40C are reduced-calcium and control MPC powders stored at elevated temperature (40°C). Values with the same letters are not significantly different ($P > 0.05$).

monly used to concentrate UF retentate before spray drying.

Effect of CO₂ Injection on Solubility of MPC Stored at Room Temperature

Solubility is one of the most important functional properties of MPC80. Several studies have shown that MPC80 can lose solubility due to protein–protein and protein–mineral interactions during manufacture and storage. Several studies have reported wide variations in the solubility of commercially produced MPC (de Castro-Morel and Harper, 2002; Sikand et al., 2011). To evaluate the effect of storage period on solubility, the MPC produced in this study was stored at room temperature ($22 \pm 1^\circ\text{C}$) for 6 mo. The solubility during this storage period is shown in Figure 5. The control MPC retained its solubility for 30 d, after which a loss in solubility occurred during storage. By the end of the 180-d storage period, the CtrlMPC solubility was reduced by about 18%. In contrast, the RCMPC did not lose any solubility during storage: solubility at 180 d of storage was not ($P > 0.05$) different from that at d 0. It is theorized that storage of MPC results in protein-protein interactions that lead to the formation of insoluble complexes. The nature and extent of formation of these complexes have been studied by several researchers (McKenna, 2000; Havea, 2006; Singh, 2007). The MPC in this study differed in mineral and

calcium contents (Table 2). The differences in solubility between the control and RCMPC indicate that calcium and other minerals play a role in the loss of solubility in MPC during storage. Previous research also showed a strong correlation between solubility of MPC and their calcium content (Bhaskar et al., 2007; Sikand et al., 2011, 2013; Ye, 2011).

Effect of CO₂ Injection on Solubility of MPC Stored at 40°C

To accelerate the protein–protein interactions and calcium-mediated interactions among the proteins, RCMPC and CtrlMPC were stored at 40°C for 180 d; the results are shown in Figure 5. The RCMPC did not lose solubility ($P > 0.05$) for the first 90 d of storage. However, by 180 d at 40°C, a significant ($P < 0.05$) loss in solubility was observed. In contrast, the CtrlMPC lost ($P < 0.05$) solubility by 30 d, and solubility decreased more than 50% by d 90.

Cold Solubility

One of the important properties of any protein ingredient used in drink mixes is its solubility in cold water. To assess the solubility of MPC in cold water, tap water was used to disperse the MPC solutions. As explained in the Materials and Methods section, 5% protein solutions were made using high-speed stirring

for 2 min. The RCMPC had a ($P < 0.05$) higher cold-water solubility compared with CtrlMPC (92.15 and 73.63%, respectively). These results indicate that calcium and mineral contents also play an important role in cold solubility of MPC.

CONCLUSIONS

Carbon dioxide injection before and during UF of skim milk significantly affected ash and calcium contents of MPC80; ash content was reduced by 28% and Ca content by 34%. Injection of CO₂ resulted in a 2-fold reduction in the viscosity of the RCUF retentate compared with CtrlUF retentate. The reduction in viscosity may have a beneficial effect during further concentration of UF retentates before drying. However, we observed a ~20% decrease in flux of RCUF compared with the control, primarily due to solubilization of calcium phosphate. The RCMPC obtained from injection of CO₂ retained its solubility when stored at elevated temperature for about 90 d. Our results indicate that CO₂ injection can be used to produce MPC80 with reduced calcium and mineral contents and optimal functionality.

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