Changes in the physical properties, solubility, and heat stability of milk protein concentrates prepared from partially acidified milk

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ABSTRACT

A limiting factor in using milk protein concentrates (MPC) as a high-quality protein source for different food applications is their poor reconstitutability. Solubilization of colloidal calcium phosphate (CCP) from casein micelles during membrane filtration (e.g., through acidification) may affect the structural organization of these protein particles and consequently the rehydration and functional properties of the resulting MPC powder. The main objective of this study was to investigate the effects of acidification of milk by glucono- δ -lactone (GDL) before ultrafiltration (UF) on the composition, physical properties, solubility, and thermal stability (after reconstitution) of MPC powders. The MPC samples were manufactured in duplicate, either by UF (65% protein, MPC65) or by UF followed by diafiltration (80% protein, MPC80), using pasteurized skim milk, at either the native milk pH ($\sim$ pH 6.6) or at pH 6.0 after addition of GDL, followed by spray drying. Samples of different treatments were reconstituted at 5% (wt/wt) protein to compare their solubility and thermal stability. Powders were tested in duplicate for basic composition, calcium content, reconstitutability, particle size, particle density, and microstructure. Acidification of milk did not have any significant effect on the proximate composition, particle size, particle density, or surface morphology of the MPC powders; however, the total calcium content of MPC80 decreased significantly with acidification (from 1.84 ± 0.03 to 1.59 ± 0.03 g/100 g of powder). Calcium-depleted MPC80 powders were also more soluble than the control powders. Diafiltered dispersions were significantly less heat stable (at 120°C) than UF samples when dissolved at 5% solids. The present work contributes to a better understanding of the differences in MPC commonly observed during processing.

Key words: milk protein concentrate, partial acidification, physical properties, solubility, heat stability

INTRODUCTION

Milk protein concentrate (MPC) powders are manufactured from skim milk by membrane filtration and spray drying. The major components of these shelf-stable food ingredients are protein, lactose, fat, and minerals. The proportion of these components varies depending on the extent of concentration and the type of membrane separation used. The proteins in MPC consist of caseins and whey proteins, present at the same ratio (4:1) as in milk.

Milk protein concentrate powders may present poor solubility (Mistry and Pulgar, 1996; McKenna, 2000; Anema et al., 2006; Havea, 2006; Baldwin and Truong, 2007; Mimouni et al., 2010; Sikand et al., 2011). Poor solubility has been attributed to the slow release of casein micelles from powder particles; it is shown that the slowly solubilizing material is almost entirely made up of caseins, whereas the whey proteins, minerals, and lactose are more soluble (Anema et al., 2006; Havea, 2006; Mimouni et al., 2010). It is widely believed that the processes involved in manufacture of MPC maintain the overall original state of casein micelles (Mulvihill and Ennis, 2003). The delicate ionic equilibrium between serum and micelles in milk (Holt et al., 1981) is shifted during membrane filtration. This change may cause a lasting effect on the casein micelles, affecting their rehydration and the functional properties of the resulting concentrates. It is known that certain processing steps can improve the rehydration properties of MPC powders; for example, heating the milk after pH adjustment before membrane filtration (Blazey et al., 2000), drying at lower temperatures (Schuck et al., 1994), adding salts before or after UF (Carr, 2002; Schuck et al., 1999, 2002; Mao et al., 2012; Sikand et al., 2013), adding sodium caseinate to the retentate before spray-drying (Schokker et al., 2011), adding polydextrose and whey proteins (Davenel et al., 1997), or partly depleting calcium by adding chelating agents, using cation exchange chromatography, or acidifying the milk (Bhaskar et al., 2001).

Little has been written about the effect of partial acidification of milk before UF on the composition, physical properties, and rehydration behavior of the re-

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sulting MPC powders. Investigating the effects of such manipulations on the thermal stability of the resulting powders after reconstitution is also of considerable interest, as these ingredients are often subjected to extensive heat treatments when incorporated into beverages. In such applications, the ability to withstand heating regimens as high as, for example, 121°C for 15 min, is very important.

In this study, we explored the effect of acidification to pH 6 by addition of glucono- δ -lactone (**GDL**), on the physical properties, reconstitutability, and thermal stability (after reconstitution). Milk pH was modified before ultrafiltration, which was then carried out with or without a subsequent diafiltration (**DF**) stage. This work attempted to use methods for reconstitution and observations commonly used in the industry to depict the properties of the powders under conditions relevant to processors.

MATERIALS AND METHODS

Materials

Pasteurized skim milk was obtained from Producer's Dairy Foods Inc. (Fresno, CA). Analytical-grade reagents were from Sigma-Aldrich Chemical Co. (St. Louis, MO). Glucono- δ -lactone was purchased from Roquette America Inc. (Geneva, IL). When added to an aqueous solution, GDL dissolves rapidly but hydrolyses progressively to gluconic acid, leading to a controlled decrease of pH, which suited the purposes of this research. Ultrapure water (Milli-Q Ultrapure Water Purification Systems, Billerica, MA) was used to prepare all the solutions.

Preparation of MPC Powders Acidified with GDL

Milk protein concentrate powders were manufactured in duplicate using pasteurized skim milk, either by UF to achieve an MPC with 65% protein (**MPC65**) or by UF followed by DF to achieve an MPC with 80% protein (**MPC80**). Controls were prepared at the native milk pH ($\sim$ pH 6.6) or GDL was added (at 3.25 g/L) to reach pH 6.0 before starting membrane filtration; GDL gradually decreases the pH of milk and is often used as a model for lactic acid fermentation in milk. The pH value of 6.0 was selected because little physical and chemical changes occur to the casein micelles at this pH (Alexander and Dalglish, 2004). By doing so, the effect of limited acidification could be studied. Figure 1 illustrates the various stages of MPC manufacture. The MPC65 and MPC80 powders were manufactured in the pilot plant of Dairy Products Technology Center at California Polytechnic State University (San Luis

Obispo). Pasteurized skim milk (300 kg) was ultrafiltered by using a model R12 cross-flow membrane recirculatory pilot plant unit (Niro Inc., Hudson, WI) equipped with dual 10-kDa-cut-off, spiral-wound polyethersulfone membranes (Snyder Filtration, Vacaville, CA). Ultrafiltration began at $5.8^\circ\text{C} \pm 1.5^\circ\text{C}$. During UF, the temperature was allowed to increase in such a way that by the end of the UF process, the temperature was $20 \pm 1.6^\circ\text{C}$. Milk was concentrated up to $5\times$ concentration, resulting in 60 kg of retentate. The UF retentate was then divided into 2 equal portions, 30 kg of which was spray-dried and the other 30 kg was diafiltered to achieve $6\times$ concentration using the same membrane pilot-plant unit, mentioned above. At the end of DF, 20 kg of retentate was collected and spray-dried. Both UF and DF retentates were spray-dried with a pilot Niro Filtermat Spray Dryer (Niro Inc.) to approximately 3.5% moisture, and the obtained MPC powders were immediately collected and sealed in airtight bags for further analysis. Inlet temperature was about 210°C and outlet temperature was 82°C . Four MPC powders were produced (Figure 1), 2 MPC65 and 2 MPC80. The control samples were named **UF-C** and **DF-C** and the GDL-treated samples were named **UF-G** and **DF-G**.

Composition

Powder samples were analyzed for proximate composition (AOAC International, 1995). Total protein was determined by Kjeldahl. Ash content was determined by ignition at 550°C in an electric muffle furnace (AOAC International, 1995; method 945.46; 33.2.10). Fat content was determined by the Mojonnier method (AOAC International, 1995; method 989.05; 33.2.26), and free moisture content by oven-drying method (AOAC International, 1995; method 990.20; 33.2.44). Lactose was determined by difference.

Total Ca content of the powders was determined by using Hewlett Packard 4500 ICP-MS (Agilent Technologies, Santa Clara, CA), according to EPA method 6020 A (US EPA, 2007).

Solubility Determination

The MPC powders were reconstituted in ultrapure water (5% wt/wt) for 3 h using a laboratory stage mixer (R010 Power, IKA Works, Wilmington, NC) at 960 rpm (speed setting #8) at $23^\circ\text{C} \pm 1.0^\circ\text{C}$. Aliquots (13 mL) of these samples were transferred to a series of 15-mL Falcon tubes and centrifuged at $700 \times g$ for 10 min at 23°C . The supernatant was separated from the pelleted material by withdrawing the supernatant into a pipette. Total solids (TS) contents of both bulk

Table 1. Composition (mean $\pm$ SD) of milk protein concentrate powders¹

Powder ²	Component ³ (g 100/g of powder)						pH
	Total protein	Fat	Lactose	Ash	Moisture	Total Ca	
UF-C	65.45 $\pm$ 0.5 ^a	2.38 $\pm$ 0.02 ^a	19.5 ^a	8.1 $\pm$ 0.2 ^a	3.5 $\pm$ 0.1 ^a	1.43 $\pm$ 0.03 ^a	6.95 $\pm$ 0.01 ^a
UF-G	65.13 $\pm$ 0.9 ^a	2.77 $\pm$ 0.10 ^{ab}	20.3 ^a	8.3 $\pm$ 0.2 ^a	3.6 $\pm$ 0.3 ^a	1.38 $\pm$ 0.07 ^a	6.52 $\pm$ 0.01 ^b
DF-C	80.52 $\pm$ 0.6 ^b	2.41 $\pm$ 0.01 ^b	5.2 ^b	7.8 $\pm$ 0.1 ^a	4.1 $\pm$ 0.3 ^a	1.84 $\pm$ 0.03 ^b	7.11 $\pm$ 0.02 ^c
DF-G	81.09 $\pm$ 1.0 ^b	2.52 $\pm$ 0.03 ^b	4.8 ^b	8.2 $\pm$ 0.3 ^a	3.5 $\pm$ 0.1 ^a	1.59 $\pm$ 0.03 ^c	6.60 $\pm$ 0.01 ^d

^{a-d}Letters indicate significant differences by Tukey's honestly significant difference procedure using $\alpha = 0.05$.

¹Values are the average of 2 experiments.

²UF-C = UF control; UF-G = UF and acidified with glucono- δ -lactone (GDL); DF-C = diafiltered control; DF-G = diafiltered and acidified with GDL.

³Lactose was measured by difference; values of pH were measured after reconstitution (see Materials and Methods).

samples were examined with a FEI Quanta 200 scanning electron microscope (FEI Company, Hillsboro, OR) operated at spot size of 4.5, accelerating voltage of 10 kV, and a working distance of 6 to 12 mm. Sample preparation and imaging were conducted in duplicate.

Heat Coagulation Time

The heat coagulation time (HCT) of the MPC solutions (5% wt/wt on solids) was determined following the method described by Davies and White (1959) with some modifications: a 3-mL aliquot of each solution was transferred to a heat-resistant screw-cap test tube (internal diameter 10 mm, length 100 mm). The test tubes were fixed to a metal platform and immersed in an oil bath maintained at 120°C. The HCT was recorded as the time elapsed between immersing the samples in the hot oil bath and the onset of visible clots. The HCT of the solutions was tested both before pH adjustment and after adjusting the pH to 6.95. The pH of sample UF-C (6.95) was used as a baseline to investigate the effect of pH on HCT in all samples.

Statistical Analysis

The sample treatments and chemical analyses in the present study were run in duplicate. Values were means of replicate determinations and the differences between the means of the treatments were compared by one-way ANOVA at a significance level of $P < 0.05$. The statistical analysis was conducted using the GLM command in Minitab (v.16.1, Minitab Inc., State College, PA). Differences between the treatments were tested using Tukey's honestly significant difference (HSD) intervals with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Composition

Table 1 summarizes the composition of the MPC powders. We observed no significant differences in

the ash and moisture contents across the powders ($P > 0.05$). The DF powders showed higher protein and lower lactose contents as a consequence of the diafiltration process ($P < 0.05$; Table 1). We found no differences in the compositional parameters with addition of GDL except for the total Ca content and pH after reconstitution.

As expected, the total Ca concentration was higher in DF powders than in UF powders because of the higher micellar casein content in MPC80 than in MPC65. The difference in the Ca content of control and GDL-treated powders was not significant for UF powders ($P > 0.05$); however, DF-G showed a significant decrease in Ca content compared with DF-C ($P < 0.05$). In addition, DF-G showed the lowest concentration of total Ca.

After reconstitution (5% wt/wt) in water, GDL-treated powders exhibited significantly lower pH values compared with their respective controls. Both UF and GDL significantly affected the pH of the powders after reconstitution (Table 1). Such differences in pH may play an important role in the processing properties of the powders; for example, in their thermal stability (Sauer and Moraru, 2012). Crowley et al. (2014) demonstrated that at 140°C, heat stability of MPC suspensions at pH < 6.8 decreased with increasing protein content. This was attributed to an increased Ca ion activity in the suspensions.

Characterization of MPC Powders

Figure 2 summarizes the effect of concentration and GDL on the cold solubility of the powders. The MPC65 powders were significantly more soluble than the MPC80 powders. It is important to note that this solubility determination method (described earlier), conventionally used in the field, is based on total solids, and comparisons can be made only based on protein concentration. No significant differences in the solubility of UF-G powder were found compared with UF-C;

Figure 2. Cold solubility of powders after dispersion at 23°C for 3 h at 5% solids (wt/wt). UF-C = UF control; UF-G = UF and acidified with glucono- δ -lactone (GDL); DF-C = diafiltered control; DF-G = diafiltered and acidified with GDL. Letters indicate significant differences by Tukey's honestly significant difference procedure using $\alpha = 0.05$. Error bars represent standard deviations.

however, the solubility of DF-G was significantly higher ($P < 0.05$) than that of the corresponding control (DF-C; Figure 2). We concluded that dispersion of casein micelles at 23°C may be improved by acidification (addition of GDL) before UF in high-protein MPC.

These results were consistent with previous reports demonstrating that MPC is affected by the presence of Ca (Carr, 2002) or serum components at drying (Gaiani et al., 2005). Davenel et al. (1997) proposed that reducing the number of calcium phosphate bridges between the casein micelles, which would normally be produced during the drying of milk products, would improve the solubility of MPC powders. Gaiani et al. (2005) also reported that adding milk ultrafiltrate to native phosphocaseinates before spray-drying favored rapid rehydration. This improvement was attributed to the hygroscopic nature of the components present in the ultrafiltrate. Sikand et al. (2013) also reported that addition of salt (NaCl or KCl) during the diafiltration stage of MPC80 manufacturing improves solubility of the resulting powder. Mao et al. (2012) indicated that the addition of NaCl during diafiltration can modify the strength of hydrophobic interactions and disulfide interchange reactions and thereby affect protein aggregation and the solubility of MPC powders.

The low content of serum material in the high-protein DF powders (MPC80) was one of the causes for their inferior dispersability compared with the UF powders (MPC65). The extended loss of serum components during diafiltration allowed for additional proximity of casein micelles and facilitated the casein-casein interactions, during diafiltration and the subsequent drying. McKenna (2000) found that formation of relatively large (~100 μ m) particles in MPC is attributed to protein-protein interactions resulting from the fusion of

casein micelles. It was previously reported that when water is removed and the volume fraction of casein micelles is increased, the decreased distance between the casein micelles may result in the partial collapse of the κ -casein layer (DeKruif, 1998). As a consequence, the rate of aggregation of casein micelles in a concentrated system would increase because of an increase in the probability of effective collisions (DeKruif, 1998).

The addition of GDL did not affect particle density of any of the powders studied ($P > 0.05$; Figure 3). It is important to note that DF increased the particle density of MPC powders significantly ($P < 0.05$) due to the higher protein concentration in the retentate. Particle density influences other physical properties of the powder such as bulk density. It is important that powders have a high bulk density to reduce their volume, especially when they are to be shipped over long distances. A powder with high bulk density requires less packaging material. Other aspects of packaging such as selection of machinery for handling, requirement of packaging materials, and the container volume are also decided based on the density of a powder (Barbosa-Canovas and Juliano, 2005).

Figure 4 illustrates the surface morphology of the MPC powders as analyzed by scanning electron microscopy. The UF and DF treatments as well as the addition of GDL before filtration did not seem to affect powder size distribution or granule morphology. Kim et al. (2003) found that shape and morphology of a powder particle depends on the type of raw material, degree of heat treatment, and other compositional and processing parameters. The observations of the current experiment indicate that the changes found in the rehydration of the MPC powders cannot be attributed to modifications of particle shape or surface morphology.

Figure 3. Particle density of powders. UF-C = UF control; UF-G = UF and acidified with glucono- δ -lactone (GDL); DF-C = diafiltered control; DF-G = diafiltered and acidified with GDL. Letters indicate significant differences by Tukey's honestly significant difference procedure using $\alpha = 0.05$. Error bars represent standard deviations.

Particle Size of Reconstituted MPC Powders

After cold reconstitution for 3 h under stirring (see Materials and Methods), the particle size distribution of the powders was analyzed using light scattering, as shown in Figure 5. We detected no statistically significant differences in the particle size distribution of the reconstituted MPC powders. All dispersions showed a monomodal distribution of sizes, with a large peak appearing between 10 and 100 μm diameter. These results indicated that although the apparent solubility index of MPC80 powders was high (Figure 2), and with some differences between DF-C and DF-G, rehydration at 23°C under stirring for 3 h was not sufficient to regain the original size distribution of casein micelles. It is important to note that in light scattering, large particles contribute greatly to the signal. The average diameter of casein micelles is about 200 nm (De Kruif, 1998;

Holt, et al., 2003) and a higher temperature is needed for efficient recovery of the original casein micelles' size and thus for full rehydration of MPC powders. These results confirm previous reports that high temperature and a high shear of the dispersing liquid are needed for full solubilization of MPC powders (Ferrer et al., 2008; Mimouni et al., 2009).

Thermal Stability of Reconstituted MPC Powders

Table 2 summarizes the HCT for the dispersions after reconstitution at 5% solids. Powder UF-C showed high heat stability, with 107 min before any visible signs of coagulation. However, sample DF-C showed a very low heat stability that could not be recovered even after adjustment to the pH of the UF control (6.9). Regardless of acidification, DF dispersions were less stable compared with UF dispersion upon heating at 120°C.

Figure 4. Scanning electron microscopy images of (A; scale bar = 500 μm) UF control; (B; scale bar = 400 μm) UF and acidified with glucono- δ -lactone (GDL); (C; scale bar = 400 μm) diafiltered control; (D; scale bar = 400 μm) diafiltered and acidified with GDL.

Figure 5. Particle size distribution (% of volume) of reconstituted (5% wt/wt solids in water at 23°C) powders. UFC = UF control; UFG = UF and acidified with glucono- δ -lactone (GDL); DFC = diafiltered control; DFG = diafiltered and acidified with GDL.

Within treatment, acidification decreased the HCT of both UF and DF samples (Table 2).

When compared at the same pH value, both GDL-treated samples showed HCT similar to their controls, indicating that pH is a major factor in imparting heat stability of milk. It is indeed known that a rapid decrease in milk coagulation time occurs with decreasing pH (Fox and Nash, 1979). The DF treatment also strongly affected the heat stability of milk, suggesting that serum composition has a major effect on heat stability. However, it is important to point out that the differences in Ca content between DF-C and DF-G did not cause a significant difference in the coagulation time.

CONCLUSIONS

In the present study, we investigated the effect of partial acidification of milk before membrane filtration on the composition, solubility, and thermal stability of the final powder. Although acidification of milk to pH

6 did not significantly affect the proximate composition of the MPC powders resulting from each UF or DF stage, it increased cold solubility compared with controls. This suggested that partial acidification reduced (at least partly) the protein-protein interactions during drying that may contribute to decreased solubility of high-protein MPC powders. This study also clearly demonstrated that solubilization of powders at 23°C for 3 h under stirring does not fully recover the particle size distribution of the casein micelles, regardless of the treatment. Increased protein content of MPC powders had a strong effect on the heat stability of MPC dispersions, and DF dispersions were significantly less stable than UF dispersions. The preacidification of milk to pH 6 also significantly affected the thermal stability of the dispersions. Acidified MPC samples (both UF and DF) exhibited lower thermal stability compared with their controls; however, adjusting the pH to 6.9 resulted in increased thermal stability within treatments (UF/DF). This effect was more profound in the UF samples. After pH adjustment, UF-G showed higher thermal stability, reaching values similar to those for the UF-C. The results of this work, which was carried out under conditions close to industrial practices, should bring a better understanding of the changes observed during processing of different MPC powders. The possible effect of partial acidification on other processing properties of MPC (e.g., rennet gelation) may also need investigating, as these powders are often used in cheese making. The mechanisms involved at the molecular level that lead to such functional changes are yet to be understood.

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Table 2. Heat coagulation time (mean $\pm$ SD; $n = 3$) of reconstituted powders (5% wt/wt solids) at 120°C at original pH (before) and after adjusting pH to 6.95 (as a baseline pH for comparison; after)

Sample ¹	Heat coagulation time (min)	
	Before	After
UF-C	107 $\pm$ 1.0	107 $\pm$ 1.0
UF-G	0.5 $\pm$ 0.0	107 $\pm$ 1.0
DF-C	2.4 $\pm$ 0.5	2.0 $\pm$ 0.5
DF-G	0.2 $\pm$ 0.0	2.0 $\pm$ 0.5

¹UF-C = UF control; UF-G = UF and acidified with glucono- δ -lactone (GDL); DF-C = diafiltered control; DF-G = diafiltered and acidified with GDL.

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