Short communication: Effects of nanofiltration and evaporation on the gel properties of milk protein concentrates with different preheat treatments

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ABSTRACT

This study aimed to evaluate the effects of different concentration methods (nanofiltration and evaporation) and heat treatments on the gel properties of milk protein concentrate (MPC). The MPC gels were produced using glucono- δ -lactone (GDL) as an acidifier with different preheat treatments (30 min at 80°C and 5 min at 92°C). We then evaluated the effect of preheat treatments on MPC gel properties, including storage modulus (G'), loss tangent ($\tan \delta$), firmness, whey separation, and microstructure. The results indicated that without preheating, evaporation (EP)-MPC had higher G' and firmness, and lower $\tan \delta$ and whey separation than nanofiltration (NF)-MPC. These results suggest that EP-MPC produced a better acid-induced gel than NF-MPC when no preheat treatments were performed. After preheating, however, except for a very small difference in the final G' (EP-MPC was higher), the 2 MPC did not differ significantly in firmness, final $\tan \delta$, or whey separation. Additionally, compared with the gel of unheated MPC, both preheat-treated gels (NF-MPC and EP-MPC) achieved increased G' and firmness and decreased $\tan \delta$ and whey separation. The preheat-treated MPC also displayed a more flexible-stranded network. These findings demonstrate that, given a suitable heating treatment, NF-MPC compares favorably with EP-MPC in achieving desired gel properties.

Key words: milk protein concentrate, nanofiltration, evaporation, preheat treatment

Short Communication

Milk protein concentrates (MPC), which contain 35 to 90% (wt/wt) protein, have been widely used in the production of cheese products, yogurt, nutritional

beverages, and other foods (Ye, 2011). Producing MPC using skim milk requires key unit operations including pasteurization, ultrafiltration, concentration of solids, and spray-drying in sequence (Martin et al., 2010). Multi-effect evaporation is the most widely used method to concentrate milk during the production of MPC; however, with this method, concentrated milk is inevitably exposed to a series of heating treatments, including preheating before evaporation and heating in the vessels of the evaporator. Considerable denaturation of milk proteins is known to occur (by measuring protein loss) during the evaporation process (Oldfield et al., 2005), which was substantiated in our previous study (Cao et al., 2015b) by measuring free sulfhydryl (SH) content. The denaturation of proteins impairs rehydration of milk proteins and could result in low solubility of the final powder. To achieve better solubility, our team tested a nanofiltration method in pilot-scale production of MPC to avoid the undesired effects of heating as seen in the conventional multi-effect evaporation, and we compared the solubility of the MPC produced by evaporation (EP-MPC) and by nanofiltration (NF-MPC). In our previous study (Cao et al., 2015b), the insolubility of MPC was measured according to the international standard ISO 8156:2005(E), which is widely used in the dairy industry. The NF-MPC had a much lower insolubility index of 0.32 mL compared with 0.9 mL for the EP-MPC (Cao et al., 2015b), and the NF-MPC maintained its advantage in solubility during a long-term storage experiment up to 24 wk (Cao et al., 2015a).

Nanofiltration significantly reduces the denaturation of milk proteins. However, suitable protein denaturation can enhance properties of milk protein gels such as gel strength. Heat treatment can induce denaturation and aggregation of β -LG with κ -CN on the periphery of casein micelles to facilitate the network formation of proteins, allowing the formation of stronger yogurt-like acid gels (Creamer et al., 2004; Van Vliet et al., 2004; Chevallier et al., 2016). In this sense, NF-MPC, with less heat-induced protein denaturation relative to EP-MPC, might form an acid-induced gel with lower gel

Received September 10, 2017.

Accepted February 4, 2018.

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strength. To date, however, no systematic study has offered deep insight into the gelation behavior and gel properties of NF-MPC and EP-MPC. Additionally, when used in the food industry, MPC will undergo additional heat treatments. For instance, in the production of yogurt using MPC as protein supplement, rehydrated MPC will be pasteurized before inoculation of lactic acid bacteria. The influence of these extra heating treatments on the gel properties of NF-MPC and EP-MPC remains unknown.

This study aimed to clarify the effects of 2 concentration methods, nanofiltration and evaporation, and preheat treatments before gelation on the gelation behavior and final gel properties of MPC. We applied common preheat treatment conditions for yogurt production; that is, 85°C for 30 min and 92°C for 5 min (Lucey et al., 1998b; Ramasubramanian et al., 2012), and gel properties, such as rheological properties, firmness, whey separation, and microstructure, of both NF-MPC and EP-MPC were evaluated.

The NF-MPC and EP-MPC were supplied by Yinchuan Jinhe Dairy Industry Co. Ltd. (Ningxia, China). The manufacturing plant was equipped with spiral-wound ultrafiltration membranes with 10-kDa cutoff and spiral-wound nanofiltration membranes with 200-Da cutoff (Parker, Cleveland, OH). For the evaporation line, ultrafiltered milk was preheated to 75°C and fed into a 3-effect vacuum evaporator, with the first, second, and third evaporation temperatures set at 66, 60, and 56°C, respectively. The inlet and outlet temperatures of spray drying were set at 150–155°C and 85–90°C, respectively. The components of the 2 MPC (e.g., protein, fat, water, and minerals, including Ca, P, and Mg) were not significantly different but NF-MPC had significantly lower (<10%) content of Na and K than EP-MPC, and the whey protein nitrogen index (WPNI) of EP-MPC (5.68 ± 0.06 mg/g) was lower than that of NF-MPC (7.14 ± 0.07 mg/g) based on the supplier's data. All other chemicals and reagents such as glucono- δ -lactone (GDL), HCl, NaOH, and Rhodamine B were analytical grade and purchased from Beijing Chemical Reagent Company (Beijing, China).

The MPC solutions were prepared by dissolving 5 g of MPC powder in 100 g of demineralized water with stirring at 2,200 rpm for 1 h (Eurostar 20 digital, IKA, Staufen, Germany) at 60°C. All MPC solutions were kept at 4°C overnight after adding 0.01% (wt/wt) sodium azide. The pH of the MPC solutions was adjusted to 6.7 to 7.0 using 0.1 M HCl/0.1 M NaOH (Sandoval-Castilla et al., 2004).

The resulting MPC solutions were preheated in a water bath at 85°C for 30 min or at 92°C for 5 min with shaking (500 rpm) before cooling to ambient temperature (23°C). Solutions of unheated MPC were tested as

control samples. All MPC solutions were then blended with GDL at a concentration of 1.25% (wt/vol) to prevent possible microbial growth. Parts of the resulting solution (80 mL) were then poured into volumetric flasks to measure whey separation levels after incubation; the remaining solution was placed into beakers to measure rheological behavior and gel firmness of the acid MPC gel, respectively, after incubation. All the incubations were conducted at 30°C in an incubator (SPX-250B-Z, Shanghai Xunbo, China), and the pH of all the samples was recorded every 10 min.

The rheological behavior of the MPC gels were monitored by measuring loss tangent ($\tan \delta$) and storage modulus (G') values during incubation from 0 to 240 min, as described by Madadlou et al. (2010). Changes in $\tan \delta$ and G' during gelation of the MPC solutions were measured at a constant strain of 0.5%, a constant temperature of 30°C, and a frequency of 0.1 Hz on a rheometer (Physica MCR301, Anton Paar GmbH, Ostfildern, Germany). The $\tan \delta$ and G' were recorded every 5 min for approximately 3 h.

Measurement of gel firmness was performed on a TA.XTPlus (Stable Microsystems Ltd., Godalming, UK) using a 5-kg load cell when the pH of the MPC gel reached 4.6. The MPC gels in beakers were compressed using an acrylic cylinder probe (25 mm in diameter) with 30% strain at a speed of 1 mm/s (Sandoval-Castilla et al., 2004). Firmness (g) was defined as the peak force used in penetration.

Whey separation levels were determined using a volumetric flask-based method as described by Lucey et al. (1998b). After blending GDL with each MPC solution, 80 mL of the resulting solution was poured into a 100-mL glass volumetric flask to fill it to the base of the neck. The flask was then placed in a water bath maintained at 30°C until the pH reached 4.6. The free whey was poured off and weighed. The whey separation level was defined as the weight percentage of the whey in the MPC gel.

The microstructure of the MPC gels was observed under a confocal scanning laser microscope configured with an air-cooled Ar/Kr laser for single-photon excitation (TCS SP5, Leica Lasertechnik GmbH, Heidelberg, Germany) according to the method described by Lucey et al. (1998c) with some modifications. Rhodamine B, used as fluorescent protein dye, was dissolved in demineralized water to a concentration of 0.05% (wt/vol), and several drops of the resulting dye solution were added to 10 mL of tested MPC solution. Before the addition of GDL, the MPC solutions were stirred. After stirring for approximately 2 min, 10 μ L of the mixture was transferred to a concave slide, which was covered with a cover slide and sealed. The gels were examined on the Leica TCS SP5 confocal microscope with a

100× oil immersion objective lens (numerical aperture = 1.4). Settings included an excitation wavelength of 543 nm and a reflect line of 617 nm. All experiments were performed in duplicate, and only representative micrographs were presented.

The MPC gels were prepared in triplicate, and each of the triplicate samples was measured to present mean values of the variables. Analysis of variance determined the significance of differences between results in IBM SPSS statistics software (version 16.0; IBM Corp., Armonk, NY). The difference was regarded as statistically significant when $P < 0.05$.

Theoretically, nanofiltration method not only reduces heat-induced protein denaturation in comparison to EP-MPC, but also causes variation in components. The WPNI of the NF-MPC (7.14 ± 0.07 mg/g) and EP-MPC (5.68 ± 0.06 mg/g) indicated less denaturation of proteins in NF-MPC, as expected. Component analysis showed reduced content (by less than 10%) of Na and K in NF-MPC (Cao et al., 2015a). The retention of minerals in NF-MPC used this study is supported by results from Suárez et al. (2006), who also found disparity in the retention of monovalent ions (Na^+ and K^+) and divalent ions (Ca^{2+} , Mg^{2+} , and P) after nanofiltration, and attributed the difference to variation in the ions' molecular weight and charge. In a preliminary experiment, NP-MPC was supplemented with NaCl and KCl such that levels of Na^+ and K^+ were the same as those in EP-MPC, and no significant change in strength or whey separation of acid-induced NP-MPC gel was observed, implying that the loss of Na^+ and K^+ is too small to exert influence. In the following section, we report the heat-induced influence on the gelation behavior of the 2 MPC without considering the component difference in minerals.

The change in G' as a function of time during acidification of NF-MPC and EP-MPC is shown in Figure 1A. The G' value was constant at zero during the first 50 min, followed by an increase over time. The preheating conditions (without preheating, 85°C for 30 min, and 92°C for 5 min) had a significant effect ($P < 0.05$) on the G' profiles of the 2 MPC, resulting in considerable differences both during the reaction and at the end of the test.

When the MPC samples prepared without preheating were tested, EP-MPC achieved significantly higher G' values compared with NF-MPC from ~150 min to the end of the test ($P < 0.05$), suggesting that EP-MPC has an advantage in gel elasticity compared with NF-MPC when prepared without additional heat treatment. After preheat treatments, the final G' values of both EP-MPC and NF-MPC were increased compared with their equivalent samples without preheating, demonstrating the efficacy of the preheat treatment

in improving MPC gel elasticity. One possible explanation is that the number and strength of bonds between casein micelles were increased by denatured whey proteins in the serum as well as those associated with casein micelles during the evaporation process, resulting in higher susceptibility to protein coagulation during acidification (Lucey, 2004). Consequently, stronger interparticle interactions can form, as indicated by the higher G' value of the prepared gel (Anema et al., 2004). After the same preheating conditions, the EP-MPC still achieved higher final G' values than NF-MPC. Considering that the protein in EP-MPC has

Figure 1. Effect of incubation time (A) on storage modulus (G' ; —) and loss tangent ($\tan \delta$; ---) and preheat treatments (B) on gelation time (bars) and gelation pH (stars) of evaporation (EP)-milk protein concentrate (MPC) and nanofiltration (NF)-MPC gels made by glucono- δ -lactone (GDL, 1.25% wt/vol)-induced acidification. The EP-MPC gels were prepared without preheat treatment (■), with preheat treatment at 85°C for 30 min (●), and with preheat treatment at 92°C for 5 min (▲), respectively; NF-MPC gels were prepared without preheat treatment (□), with preheat treatment at 85°C for 30 min (○), and with preheat treatment at 92°C for 5 min (△), respectively. In (B), EP-MPC (white bars) and NF-MPC (gray bars) prepared under different preheating conditions are presented. Error bars represent the SEM values (from triplicate experiments). Presented values are means from triplicate experiments.

undergone more heat treatments (in the evaporation stage) than NF-MPC, it seems that the lower final G' values of NF-MPC could be due to insufficient protein denaturation caused by less heating. For each MPC, the final G' values after heat treatments at 2 intensities (85°C for 30 min and 92°C for 5 min) did not differ. It is hard to determine the correlation between preheating intensities and G' values because just 2 heating treatments were tested in this study. It would be interesting to clarify the correlation in a future study by combining a series of heating treatments to determine the best heating conditions to attain optimized G' profiles.

The loss tangent is an index to monitor the viscoelastic behavior of a gel system during the gelation process, and a lower $\tan \delta$ value represents a higher elastic characteristic of the tested sample. In Figure 1A, all MPC samples had similar $\tan \delta$ curves; that is, the values of $\tan \delta$ increased to reach a maximum before gradually declining until the end of the test. The value of the maximum in the $\tan \delta$ curve ($\tan \delta_{\max}$) for MPC samples without heat treatment was higher than that of the samples with preheating ($P < 0.05$), and we detected no significant difference among the $\tan \delta_{\max}$ of preheated MPC samples ($P > 0.05$). A $\tan \delta$ curve with a significant maximum has been widely reported during the gelation of milk protein (Biliaderis et al., 1992; Roennegard and Dejmeek, 1993; Marle and Zoon, 1995). Lucey et al. (1998b) suggested that the appearance of $\tan \delta_{\max}$ is due to partial loosening of the gel network caused by solubilization of colloidal calcium phosphate. However, in the current study, the rapid increase of G' confirmed a persistent increase in the strength of the gel network. According to the definition of $\tan \delta$, mathematically, an increase of $\tan \delta$ indicates a faster increase of loss modulus (G'') than the increase of storage modulus (G') during the initial stage of gelation. The G'' is an indicator of viscosity behavior of material, and under our conditions, its increase was most likely due to the aggregation of casein proteins into short chains, which can increase the viscosity of the system, before further aggregation to form the 3-dimensional gel network. Except for the unheated NF-MPC sample, all samples had very similar $\tan \delta$ (~0.33) values at the end of the test, indicating similar viscoelastic characteristics. The effects of preheating on gelation time and gelation pH of NF-MPC and EP-MPC gels, which are defined as the time and pH when G' exceeds 1, are shown in Figure 1B. The EP-MPC gels had a shorter gelation time and a higher gelation pH than the NF-MPC gels ($P < 0.05$) without preheating. Gelation time was shortened and gelation pH was increased after preheating treatments. The decreased solubility of β -LG caused by heat-induced denaturation is a likely reason for the increase in gelation pH of

preheated MPC (Anema et al., 2004; Estévez et al., 2017). However, no obvious differences were observed between NF-MPC and EP-MPC in terms of gelation time or gelation pH after the same preheating conditions ($P > 0.05$; Figure 1B). Combining the results shown in Figure 1, the variation of $\tan \delta$ (Figure 1A) and pH (Figure 1B) during gelation depended on the preheating conditions. A faster decrease in pH reflects a rapid acidification process, during which it has been reported in previous studies from Anema (2009) and Liu et al. (2014) that a faster rearrangement of strands and micelle aggregation, and the removal of more colloidal calcium phosphate from the gel structure, happened and subsequently led to less elastic gels.

Preheat treatments at 85°C for 30 min and 92°C for 5 min before gelation resulted in identical values of firm-

Figure 2. (A) Firmness and (B) whey separation degree of evaporation (EP)-milk protein concentrate (EP-MPC; white bars) and nano-filtration (NF)-MPC (gray bars) made by glucono- δ -lactone (GDL, 1.25% wt/vol)-induced acidification under various preheat conditions. Presented values are means ($\pm$ SD) from triplicate experiments.

ness ($P > 0.05$) for NF-MPC and EP-MPC gels, which were significantly higher than that of the unheated samples (Figure 2A). The result was in line with the finding for G' ; that is, the G' values were increased by preheating. The increased firmness of MPC gels was caused by the aggregation of denatured whey proteins during gel formation (Lucey et al., 1997b). Without preheating, the degree of whey separation of EP-MPC was significantly lower than that of NF-MPC ($P < 0.05$). Application of the preheat treatment, however, not only decreased whey separation but also eliminated the disparities in whey separation of the 2 MPC ($P > 0.05$; Figure 2B). Before and during gelation, excessive rearrangement of particles in the gel network is responsible for whey separation (Lucey et al., 1997a). This suggests that the higher the heat treatment, the less rearrangement of the network occurs, making gels stable and less susceptible to spontaneous whey separation.

Confocal laser scanning micrographs of acid-induced NF-MPC and EP-MPC gels are shown in Figure 3. Compared with NF-MPC, EP-MPC gels prepared without preheating appeared to be more homogeneous, with a fine meshed, branched, and interlinked microstructure (Figure 3A). After preheating, however, the microstructures of the 2 gels were less distinct. One plausible explanation is that branched and interlinked types of strands between clusters were caused by the preheat treatment of MPC, whereas unheated MPC may have coarser networks with larger pores, thinner strands, and less branching or tortuous strands. Structural rearrangements of preheated MPC gels result in crack formations and a rough surface due to the presence of gel strands and stronger fusion of casein particles (Peng et al., 2010; Meletharayil et al., 2015). Also, denatured whey proteins on the surface of casein micelles hinder the approach of other casein particles and lessen the formation of casein-dense micelle clus-

Figure 3. Confocal scanning laser micrographs of milk protein concentrate (MPC) gels made by glucono- δ -lactone (GDL, 1.25% wt/vol)-induced acidification under the following conditions: evaporation (EP)-MPC and nanofiltration (NF)-MPC gels were made without preheat treatment (A, a), with preheat treatment at 85°C for 30 min (B, b), and at 92°C for 5 min (C, c), respectively. The protein matrix is light (red) and pores are dark. Scale bars = 25 μ m. Color version available online.

ters; therefore, visible cracks and a rough surface can be seen (Lucey et al., 1997b,c).

According to the above findings, the gel properties of MPC produced by nanofiltration did not differ significantly from those produced by evaporation after the same preheat treatment, which may be due to the extent of denaturation in the whey proteins in the 2 MPC samples after preheat treatment. Even without preheat treatment, NF-MPC and EP-MPC showed few differences. Therefore, nanofiltration is an alternative approach to concentrate MPC because it compares favorably with evaporation in improving the final gel qualities of dairy products. Based on results from our previous study on MPC, NF-MPC exhibits higher solubility and similar gelation properties compared with EP-MPC. Future research will focus on the emulsification properties of NF-MPC and EP-MPC to gain a more complete profile of the MPC.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China Youth Fund Project (grant no. 31401517; Beijing, China), National Natural Science Foundation of China (grant no. 31471689; Beijing, China), the earmarked fund for Modern Agro-industry Technology Research System (CARS-36; Beijing, China), and Special Fund for Agro-scientific Research in the Public Interest (201303085; Beijing, China).

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