Prevention of low-temperature gelation in milk protein concentrates by calcium-binding salts

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ABSTRACT

The objective of this study was to determine the effect of adding low concentrations of calcium-binding salts on the prevention of low-temperature gelation in milk protein concentrates (MPC). The MPC were created by a combination of ultrafiltration and diafiltration, standardized from 14 to 17% (wt/vol) protein content and mixed with one of 5 calcium-binding salts (sodium citrate, sodium hexametaphosphate, sodium polyphosphate, sodium pyrophosphate, and sodium monophosphate) adjusted to a pH of 6.75. The flow properties, apparent viscosity, and gel strength were determined for MPC containing a wide range of calcium-binding salt concentrations. Low-temperature gelation occurred in MPC with 16.0% and higher protein content. Low-temperature gelation at 16.0% protein content was prevented by the addition of any of the 5 salts tested at low concentrations (0.30 mM or less; sodium citrate, sodium hexametaphosphate, sodium polyphosphate, sodium pyrophosphate or sodium monophosphate), with sodium polyphosphate and sodium monophosphate being the most consistent in preventing low-temperature gels. All MPC samples exhibited shear-thinning behavior ($n = 0.52\text{--}0.72$), which increased (lower n values) as the protein content increased and decreased by addition of salt. At concentrations of salt above 1.00 mM, thermally irreversible gels were observed with relative strength dependent on the salt and protein content.

Key words: low-temperature gel, calcium-binding salt, emulsifying salt, milk protein concentrate

INTRODUCTION

Reductions in transportation cost by removing water from milk through the production of milk powders and milk protein concentrates (MPC) can have a large ef-

fect on industrial profit. However, the production of milk powder requires concentration and evaporation steps that are energy demanding and costly, and the use of a liquid MPC can provide cost reductions and better maintain the functionality of liquid milk (Corredig et al., 2019).

Kieferle et al. (2019) showed that for both liquid and reconstituted MPC, increasing the protein content resulted in higher viscosity at low temperature (10°C) and increased shear-thinning behavior. However, reconstituted MPC demonstrated consistently higher apparent viscosity and shear-thinning behavior at much lower concentrations than that of fresh MPC. Furthermore, protein-protein interactions in reconstituted MPC at ~12% protein content resulted in gel-like behavior and flow prevention, whereas fresh MPC remained liquid with up to 13% protein content. They also reported that predicting a precise viscosity based on protein content was not reliable, due to differences in composition and processing history.

The primary barrier preventing an extensive use of liquid MPC in the food industry is the formation of low-temperature gels, which makes the handling of MPC difficult for industrial applications. The phenomenon was first reported by Amelia and Barbano (2013), who studied the shelf life of micellar casein concentrate at low temperatures. Experiments by Lu et al. (2015, 2017) further explored the relationship between protein content and temperature in reconstituted micellar casein concentrates. In these studies, they found that a protein content of 17 to 18% at around 7°C resulted in a low-temperature gel (LT-gel).

Calcium-binding salts are known to affect the rheological properties of concentrated milk systems. Calcium-binding salts added above a critical concentration dissociate casein micelles (de Kort et al., 2011; Culler et al., 2017; McCarthy et al., 2017; Saricay et al., 2019) and promote gel formation in milk systems (Mizuno and Lucey 2005, 2007; Kaliappan and Lucey, 2011). Mizuno and Lucey (2005) found that tetrasodium pyrophosphate (0.1–2%) promoted gel formation in MPC adjusted to ~3% (wt/wt) protein content, pH 5.8 (com-

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mon processed cheese pH), and stored overnight. In a follow up study where tetrasodium pyrophosphate was added at 3.8 to 9.5 mM and the pH adjusted to 5.7 to 7.0, they found that weak gels formed at all concentrations when stored overnight at low temperature or when heated (25–45°C; Mizuno and Lucey, 2007). They also found that at a fixed salt concentration (7.6 mM) the turbidity decreased significantly as the pH decreased, indicating micellar dissociation.

The objective of this study was to determine ionic conditions leading to the prevention of low-temperature gelation (**LT-gelation**) in MPC. It was hypothesized that adding relatively low concentrations of calcium-binding salt would partially dissociate the casein micelles, reduce micelle-to-micelle interaction, and prevent low-temperature gelation. This would result in altering the amount of native casein micelles. This hypothesis was based on the observations of Culler et al. (2017) and Saricay et al. (2019), who found that a specific concentration of calcium-binding salt (dependent on type) resulted in full micellar dissociation. This intermediate concentration would contain a proportion of altered or disassociated casein micelles preventing LT-gelation but retaining other functional properties. To test this hypothesis, we first determined the minimum protein content in liquid MPC where LT-gelation occurred, then tested 5 different salts for their ability to prevent LT-gelation. The calcium-binding salts used in this experiment were chosen as previous studies showed critical concentrations where full micellar dissociation was reported (Culler et al., 2017; Saricay et al., 2019). The objective of these experiments was to advance processing interventions that would increase the protein content of liquid MPC at an 80:20 casein:whey protein ratio, for cost effective applications in the food industry. Although the addition of calcium-binding salts may alter the native casein micelle structure, the resulting MPC would be suitable for dairy applications (high protein beverages or processed cheese) where casein micelles are expected to be altered.

MATERIALS AND METHODS

MPC Production

Milk protein concentrates with 17 to 19% total protein content (20–22% TS) were created by a combination of UF and diafiltration (**DF**). Pasteurized skim milk was obtained from the Penn State Berkey Creamery (University Park, PA) and stored at 4°C before use. Skim milk was placed in a 15-L beaker and equilibrated to 50°C (chosen to improve flow rate during filtration without denaturing whey proteins) for one hour using

a temperature-controlled water bath (Isotemp Heated Immersion Circulator, Thermo Fisher Scientific; Liu et al., 2014). Milk was concentrated using a XX42P0K80 Pellicon stainless steel UF holder (Millipore), equipped with a 10kDa Pellicon 3 filter (Millipore) and a peristaltic pump (620S, Watson Marlow) set at 100 rpm for a 2.3 L/min flow rate. The UF was conducted on 11.34 L of milk concentrated to 3.78 L (permeate was used to determine final volume; 7.56 L). The DF was performed by adding back 7.56 L of distilled water containing 5% of the protein-free permeate generated in the UF step to maintain a minimum ionic environment to prevent micellar dissociation (Culler et al., 2017; Saricay et al., 2019). The DF was performed 4 times with each step removing 7.56 L. The last DF step removed an additional 1.89 L of permeate to create 6-fold concentrated solution in a 1.89-L final volume. The liquid MPC were frozen until testing. Before testing, MPC were thawed in a temperature-controlled water bath (SHEL LAB Digital Water Bath, Sheldon Manufacturing) for one hour at 50°C; after thawing, the pH and ionic calcium content were measured at room temperature.

Calcium-Binding Salts

The calcium-binding salts used in this experiment were chosen because previous studies demonstrated their ability to disassociate casein micelles. The levels were chosen to be below the concentration where full micellar dissociation was reported (Culler et al., 2017; Saricay et al., 2019). To test the effect of calcium-binding salts on LT-gelation, sodium citrate (CAS 6132-04-3), sodium hexametaphosphate (CAS 10124-56-8), sodium polyphosphate (CAS 50813-16-6), sodium pyrophosphate (CAS 7758-16-9), and sodium monophosphate (CAS 7558-80-7) were added to MPC solutions with a final protein content of 14.2, 15.1, 16.0, or 17.0%. Concentrated salt solutions were produced at 1.0, 1.0, 1.0, 1.4, and 1.4 M for sodium citrate, sodium hexametaphosphate, sodium polyphosphate, sodium pyrophosphate, and sodium monophosphate, respectively. Each solution was adjusted to a pH of 6.75 ± 0.05 (using 2 N NaOH or HCl). Thirteen different concentrations of salt ranging from 0.01 to 100 mM were tested (Table 1). Salts were diluted with distilled water (calculated for either 14.2, 15.1, 16.0, or 17.0% protein content) before being mixed with MPC (held at 50°C for 1 h). Each sample was mixed (10 mL in 15-mL test tubes) by 10 s of vortexing followed by inversion (5 times) before being stored overnight at 4°C. After storage, the samples were placed upside-down for 30 min at 4°C before visual evaluation for liquid or gel behavior. Samples that sank to the bottom of the tube after being

Table 1. Liquid (0.00) or gel behavior (0.25–0.75 indicates liquid and gel behavior was observed) of 14.2, 15.1, and 16.0% protein content milk protein concentrates with added sodium salts

Conc. mM	Citrate			Hexametaphosphate			Polyphosphate			Pyrophosphate			Monophosphate		
	14.2%	15.1%	16.0%	14.2%	15.1%	16.0%	14.2%	15.1%	16.0%	14.2%	15.1%	16.0%	14.2%	15.1%	16.0%
0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00
0.01	0.00	0.00	0.25	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.03	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00
0.05	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00
0.10	0.00	0.00	0.50	0.00	0.00	0.25	0.00	0.00	0.33	0.00	0.00	0.25	0.00	0.00	0.00
0.30	0.00	0.00	0.25	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00
0.50	0.00	0.00	0.50	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.33
1.00	0.00	0.00	0.75	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.33
3.00	0.50	0.50	1.00	1.00	1.00	1.00	0.00	0.00	0.33	0.00	0.00	1.00	0.50	0.50	0.67
5.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50	0.67	1.00	1.00	1.00	1.00	1.00	0.67
10.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
30.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
50.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
100.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Conc. = concentration.

held upside-down were categorized as liquid samples, whereas those that did not were categorized as gels.

MPC Composition Testing

Protein content was tested using the Dumas method (Nielsen, 2015) using a Leco FP-528 Nitrogen Analyzer (Leco Corp.). The nitrogen content was converted to total protein content using the conversion factor for milk protein (6.38). Samples were tested as powders; thus, a portion of the sample was freeze-dried (VirTis Genesis 35XL, SP Scientific) to determine total solids (wet basis). The protein content in liquid MPC was calculated by multiplying the total solids (freeze drying) with protein content of the dry powder (Nielsen, 2015).

Serum calcium was measured using a calcium probe (Perfect ION comb CA, Mettler-Toledo). Only the samples without added calcium-binding salts were tested. Samples were equilibrated to $22.0 \pm 1.0^\circ\text{C}$ for at least a half hour before testing. Ionic strength adjuster was added to all samples at a ratio of 200 μL per 100 mL of sample (as per the manufactures instructions). The pH was measured under the same conditions (SevenExcellence, Mettler-Toledo). The pH of samples that remained liquid did not change as a function of adding chelating salt, as the chelating salts were adjusted to a pH of 6.75, and this in combination with the strong buffering capacity of MPC (Liu et al., 2017) resulted in pH values that were the same as the control (no salt).

Rheology

Samples that showed liquid behavior were tested using a rheometer (Discovery HR-3, TA Instruments). A flow curve (shear rate vs. shear stress) was performed at 4°C from 1 to 100 s^{-1} and 100 to 1 s^{-1} using a plate and plate geometry (top plate, 25-mm diameter). A liquid sample (0.6 mL) was pipetted onto the center of the lower plate and the top plate was lowered to a gap of 1 mm. Samples were heated for 10 min at 30°C and inverted to ensure thorough mixing and then allowed to equilibrate at $22.0 \pm 1.0^\circ\text{C}$ for one hour before testing. The power law model for liquids (Steffe, 1993) was used to fit the data in the following equation:

$$\sigma = k\dot{\gamma}^n,$$

where σ is the shear stress (Pa), k is the consistency coefficient ($\text{Pa}\cdot\text{s}^n$), $\dot{\gamma}$ is the shear strain rate (s^{-1}), and n is the flow behavior index (dimensionless). Values were calculated from the apparent viscosity data collected at 1 to 100 s^{-1} and adjusted by numerical analysis, using Solver (Microsoft Corp.). The flow behavior index

and k values were adjusted to minimize the squared difference between the observed response and the predicted response. The resulting models had a correlation >0.999 .

The gel strength was tested using a texture analyzer (TAX-T Plus, Stable Microsystems). Samples were tested at 4°C and kept in a chilled cooler before testing. The samples were tested in 15-mL centrifuge tubes and firmness was measured using a puncture test with a 6.35-mm-diameter (0.25-in) stainless steel ball probe. Each sample was punctured to a depth of 12 mm at a speed of 1 mm/s. The probe returned to the starting position at the same speed. The force during the process was collected and gel strength was measured as peak firmness force (grams; Mizuno and Lucey 2007).

Statistical Analysis

Each MPC at a given protein content was treated as a separate experiment. Experiments were analyzed as a completely randomized block design. Each batch of milk used to generate an MPC with all salts added was considered a block. One batch of MPC was used to make MPC-salt mixtures for all 5 salts and all 13 different concentrations at a single protein content. The MPC containing 14.2, 15.1, and 17.0% protein content were tested in duplicates, and the MPC containing 16.0% protein content was tested in triplicate. An additional replicate was run for sodium citrate, sodium hexametaphosphate, and sodium pyrophosphate. Analysis of variance was performed using SAS software (version 9.4, SAS Institute Inc.). Multiple mean comparison analysis was conducted using Tukey analysis ($P < 0.05$) after ANOVA showed significant treatment effects.

RESULTS AND DISCUSSION

The MPC samples had a pH ranging from 6.75 to 6.94 and the serum calcium in initial MPC (before salt addition) ranged from 323 to 448 mg/kg, 397 to 418 mg/kg, and 665 to 1,020 mg/kg, for the MPC containing 14.2, 15.2, and 16.0% protein content, respectively. Table 1 shows the liquid (white) and gel behavior (black) of all MPC and salt mixtures. Salt-free MPC at 14.2 and 15.1% protein content did not form LT-gels but formed salt-induced gels when salts were added at 3.0 mM or above for sodium citrate, sodium hexametaphosphate, sodium pyrophosphate, and sodium monophosphate, and 5.0 mM or above for sodium polyphosphate. At 16.0% protein content, LT-gels consistently formed in salt-free MPC and were prevented by the addition of calcium-binding salts. Each salt demonstrated the ability to prevent LT-gel formation at one or a range of

concentrations with different consistency. Only sodium polyphosphate and sodium monophosphate demonstrated consistent prevention of LT-gelation when used at 0.01 to 1.00 mM and 0.01 to 0.30 mM, respectively. For the other salts, LT-gels were consistently prevented at 0.03, 0.05, and 0.01 mM for sodium citrate, sodium hexametaphosphate, and sodium pyrophosphate, respectively. As Table 1 shows, other concentrations were able to prevent the formation of LT-gels but not consistently. This may be due to differences between batches of MPC because minor changes in pH were shown to affect viscosity and protein interactions in concentrated milk systems (Anema et al., 2007).

Table 2 shows the apparent viscosity at a shear rate of 100 s^{-1} for each MPC-salt mixture at 14.1, 15.2, and 16.0% protein content. The experimental variability among the different batches of MPC was greater than the differences due to type of salt or salt concentration. All samples were non-Newtonian and displayed shear-thinning behavior. There was no significant difference in apparent viscosity within the concentration of salt tested for each of the different salts, and the average apparent viscosity for each salt across the concentrations tested was reported. At 14.2% protein content, there was no difference in apparent viscosity between the control MPC and MPC containing the different salts, with values ranging from 160 to 201 mPa·s. At 15.1% protein content, MPC with added sodium citrate, sodium hexametaphosphate or sodium polyphosphate showed significantly lower apparent viscosity to an average of 209 mPa·s compared with the controls with no salt added, with an apparent viscosity of 294 mPa·s. Sodium pyrophosphate and sodium monophosphate were not significantly different from either the control or the MPC containing the other salts. At 16.0% protein content the control MPC samples consistency formed LT-gels whereas the MPC containing salt exhibited apparent viscosity ranging from 312 to 370 mPa·s. No differences in apparent viscosity were observed between any of the 5 salts but not all salts were equally consistent in preventing LT-gelation.

As expected, an increase in protein content resulted in increase in the apparent viscosity regardless of what salt was added. Karlsson et al. (2005) reported that as casein concentration increased beyond 12 to 13%, apparent viscosity increased exponentially. Assuming an 80:20 ratio of casein to whey protein, the casein content in the liquid controls was 11.36 and 12.8%, for the MPC containing 14.2 and 15.1% total protein content, respectively, well within the range of strong increase in apparent viscosity previously reported. It has also been shown that at lower temperatures the increase in apparent viscosity is even stronger (Nöbel et al., 2012, 2016).

Table 2. Mean apparent viscosity (mPa·s; shear rate = 100 s⁻¹ at 4°C) ± SD of liquid samples at 14.2, 15.1, and 16.0% protein content containing selected sodium-salts¹

Protein content, %	Control	Citrate	Hexametaphosphate	Polyphosphate	Pyrophosphate	Monophosphate
14.2	193 ± 4 ^{ab}	160 ± 33 ^{ab}	201 ± 56 ^a	166 ± 27 ^{ab}	161 ± 43 ^{ab}	149 ± 43 ^b
15.1	294 ± 30 ^a	210 ± 27 ^b	205 ± 26 ^b	212 ± 36 ^b	241 ± 30 ^{ab}	227 ± 30 ^{ab}
16.0	gel	312 ± 63 ^a	350 ± 70 ^a	370 ± 87 ^a	363 ± 47 ^a	357 ± 97 ^a

^{a,b}Different letters within each row indicate significant differences ($\alpha < 0.05$) using the Tukey test.

¹All liquid samples at a fixed protein content were pooled together for each type of salt.

The ability to prevent LT-gelation at 17.0% protein content was tested using sodium polyphosphate (0.01–1 mM) as this was the most consistent salt in preliminary tests. However, the 2 replicates tested failed to prevent LT-gelation.

Table 3 shows the consistency coefficient (k) and flow behavior index (n) calculated from the power law model for each salt type at each protein level tested. Because there was no significant difference due to salt concentrations within a salt tested, when liquid behavior was observed, the estimated rheological parameters describing the MPC were averaged across all salt concentrations within each of the salts tested. As reported in previous studies, MPC showed non-Newtonian shear-thinning behavior. All MPC became increasingly shear thinning as the protein content increased. Interestingly, all MPC recovered their original viscosity immediately after shearing (i.e., were time independent) indicating that the viscosity increase was probably due to protein-protein interactions that were disrupted by shear stress but reform when this stress was removed. Although all salts demonstrated shear-thinning behavior at 14.2 and 15.1% protein content, MPC containing 14.2% protein exhibited no significant difference in flow behavior index (average $n = 0.69$) and consistency coefficient (average $k = 0.79 \text{ Pa}\cdot\text{s}^n$) vs. control MPCs without salt. At 15.1% protein content, the MPC control showed significantly

higher consistency coefficient ($k = 1.81 \text{ Pa}\cdot\text{s}^n$) vs. MPC where sodium citrate, sodium hexametaphosphate or sodium polyphosphate were used to prevent LT-gelation (average $k = 0.96$). At 16.0% protein content were control MPC consistently formed LT-gels, MPC containing sodium polyphosphate exhibited a significantly lower flow behavior and higher consistency coefficient than sodium citrate, but no differences were found among the remaining salts.

Figure 1 is a representative picture showing a gradual loss of whiteness as the salt concentration increased in the MPC, eventually resulting in a salt-induced gel. The kinetics of salt-induced gel formation were not studied because evaluations were made after overnight storage at 4°C. However, when salts were added to MPC at salt concentration $\geq 10 \text{ mM}$, a color change was immediately apparent in all salts, with the exception of sodium monophosphate. The white color of the MPC was lost and a pale-yellow color similar to that of sweet whey was observed. Furthermore, in MPC containing salt at concentrations of 50 to 100 mM, gel setting occurred within 30 s after salt addition. These gels were then allowed to sit overnight at 4°C before being tested for peak force using a texture analyzer, and the LT-gel formed with MCP at 16.0% protein content without salt was used as control. At all protein contents, the addition of $>10 \text{ mM}$ salt resulted in gels

Table 3. Mean ± SD for flow behavior index and consistency coefficient in liquid MPC containing selected sodium salts¹

Parameter and protein content	Sodium salt					
	Control	Citrate	Hexametaphosphate	Polyphosphate	Pyrophosphate	Monophosphate
Flow behavior index (n)						
14.2%	0.66 ± 0.01 ^a	0.70 ± 0.03 ^a	0.68 ± 0.03 ^a	0.71 ± 0.02 ^a	0.69 ± 0.03 ^a	0.72 ± 0.03 ^a
15.1%	0.61 ± 0.04 ^a	0.68 ± 0.02 ^a	0.69 ± 0.01 ^a	0.67 ± 0.01 ^a	0.65 ± 0.02 ^a	0.66 ± 0.01 ^a
16.0%	gel	0.57 ± 0.02 ^a	0.53 ± 0.03 ^{ab}	0.52 ± 0.03 ^b	0.53 ± 0.02 ^{ab}	0.54 ± 0.02 ^{ab}
Consistency coefficient (k)						
14.2%	0.94 ± 0.01 ^a	0.68 ± 0.28 ^a	0.93 ± 0.4 ^a	0.69 ± 0.25 ^a	0.70 ± 0.25 ^a	0.81 ± 0.07 ^a
15.1%	1.81 ± 0.47 ^a	0.97 ± 0.28 ^b	0.91 ± 0.26 ^b	1.01 ± 0.35 ^b	1.25 ± 0.28 ^{ab}	1.10 ± 0.22 ^b
16.0%	gel	2.42 ± 0.95 ^b	3.08 ± 1.08 ^{ab}	3.66 ± 1.75 ^a	3.16 ± 0.79 ^{ab}	3.26 ± 1.71 ^{ab}

^{a,b}Different letters within a row indicate significant differences ($\alpha = 0.05$) using the Tukey test.

¹All liquid samples at a fixed protein content were pooled together for each type of salt

Table 4. Peak force (g; mean $\pm$ SD) of milk protein concentrate (MPC) gel samples (16.0% protein content) containing selected sodium salts

Concentration, mM	Salt-free MPC	Sodium salt				
		Sodium citrate	Sodium hexametaphosphate	Sodium polyphosphate	Sodium pyrophosphate	Sodium monophosphate
0	25.3 $\pm$ 15.6					
3		6.8 $\pm$ 2.3 ^b	18.0 $\pm$ 3.7 ^b	4.2 $\pm$ 0 ^b	9.0 $\pm$ 1.4 ^b	8.4 $\pm$ 2.5 ^b
5		13.6 $\pm$ 4.1 ^b	59.8 $\pm$ 21.7 ^b	7.8 $\pm$ 2.6 ^b	12.2 $\pm$ 5.6 ^b	11.6 $\pm$ 1.8 ^b
10		44.4 $\pm$ 14 ^b	118.4 $\pm$ 17.8 ^a	25.1 $\pm$ 9.4 ^b	41.7 $\pm$ 11.5 ^b	26.5 $\pm$ 13.6 ^b
30		129.3 $\pm$ 6.9 ^a	170.8 $\pm$ 38.1 ^a	92.7 $\pm$ 14.3 ^{ab}	124 $\pm$ 8.1 ^a	62.8 $\pm$ 24.9 ^{ab}
50		131.8 $\pm$ 13.7 ^a	146.2 $\pm$ 17.2 ^a	136.7 $\pm$ 17.3 ^a	136.3 $\pm$ 18.7 ^a	97.4 $\pm$ 20.1 ^a
100		129 $\pm$ 28.6 ^a	110.5 $\pm$ 33.7 ^a	182.1 $\pm$ 55.6 ^a	87.1 $\pm$ 25.5 ^{ab}	107.2 $\pm$ 17.6 ^a

^{a,b}Different letters within each column indicate significant differences ($\alpha = 0.05$) using the Tukey test.

that were firmer than the LT-gels in MPC controls and were thermally irreversible even when heated to 50°C for 1 h. As expected, the gel strength increased as the protein content increased. Furthermore, salt-induced gels formed at the same salt concentrations for each salt regardless of the protein content tested. Table 4 shows the average firmness of MPC containing 16.0% protein content and all salt concentrations. Although the strength of the salt-induced MPC gels varied among salts, all salts exhibited a similar concentration dependent MPC gel strength behavior. At low levels of salt (3–5 mM), most salt-induced MPC LT-gels exhibited weaker structure than control MPC gels (salt-free). At salt concentrations of 30 mM and above the MPC gel strength increased significantly.

We hypothesize that salt-induced gels were likely the result of micellar dissociation followed by reaggre-

gation of the newly released casein monomers. All 5 salts tested were previously observed to cause micellar dissociation and resulted in a decrease in turbidity of skim milk as the concentration of salt increased (Culler et al., 2017; Saricay et al., 2019). However, previous studies reporting micellar dissociation were conducted in diluted milk or micellar casein systems (0.22 to 2.2% casein content), where full micellar disassociation was expected, resulting in near-translucent systems. Full micellar dissociation was not likely in MPC with 16% protein content due to crowding effects, resulting in gels that were turbid and not translucent.

Studies on the effect of calcium-binding salts on re-constituted MPC powder by Mizuno and Lucey (2005, 2007) failed to observe salt gels with sodium citrate, sodium pyrophosphate, or sodium hexametaphosphate, despite testing them at concentrations up to 100 mM,

Figure 1. Representative photograph of 16.0% protein content milk protein concentrate with 0 to 100 mM sodium hexametaphosphate (left to right).

probably due to the low protein content (1.7% wt/vol casein) and the pH (5.8) limiting the calcium-binding ability of salts (Pearce, 1980; Goldberg et al., 2002; Rahimi Yazdi and Corredig, 2012; Nielsen, 2015). In a similar study, McCarthy et al. (2017) failed to obtain salt-induced gels working with reconstituted MPC powder containing 6.5% (wt/vol) casein protein, up to 50 mM sodium citrate and 25 mM sodium hexametaphosphate, at a pH of 6.8. Contrary to previous studies reporting the lack of gelation in low protein content systems (i.e., below 7%) with relatively high salt concentration (e.g., 100 mM), our results indicate that MPC with protein content $\geq 14\%$ will form strong gels in response to high salt concentration. These salt-induced gels exhibited up to 7 times the strength of the corresponding salt-free MPC controls.

CONCLUSIONS

Low-temperature gelation occurred in MPC containing 16.0% (wt/vol) protein content and above and was prevented by addition of calcium-binding salt with varied consistency. Sodium polyphosphate and sodium monophosphate demonstrated a much more robust ability to prevent LT-gelation compared with sodium citrate, sodium hexametaphosphate, and sodium polyphosphate. For all salts, concentrations ≥ 3.00 mM in MPC with 14.2, 15.1, and 16.0% protein content formed gels that were not thermally reversible and were firmer than that of the LT-gel control. Future studies should explore the exact mechanism for how these calcium-binding salts interact with casein micelles and prevent LT-gelation. In doing so we believe further increases in protein content may be possible. The exact nature and rheological properties of these salt-induced gels should also be studied, as these gels appear to have unique and different shear and concentration dependent properties. These findings are significant because they allow for the dairy industry to increase the minimum protein content from 15 to 16% without worrying about the formation of a LT-gel. This may reduce the dependence of powders, which generally have a higher cost (protein content dry basis per kg) and different functional properties.

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