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Influence of milk protein concentrates with modified calcium content on enteral dairy beverage formulations: Storage stability

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ABSTRACT

Control of calcium-mediated storage defects, such as age gelation and sedimentation, were evaluated in enteral high-protein dairy beverages during storage. To investigate the effects of reduced-calcium ingredients on storage stability, 2 batches each of milk protein concentrates (MPC) with 3 levels of calcium content were acquired [control, 20% calcium-reduced (MPC-20), and 30% calcium-reduced (MPC-30)]. Control and calcium-reduced MPC were used to formulate 8% (wt/wt) protein enteral dairy beverages. The formulation also consisted of other ingredients, such as gums, maltodextrin, potassium citrate, and sucrose. The pH-adjusted formulation was divided into 2 parts, one with 0.15% sodium hexametaphosphate (SHMP) and the other with 0% SHMP. The formulations were homogenized and retort sterilized at 121°C for 15 min. The retort-sterilized beverages were stored at room temperature for up to 90 d and particle size and apparent viscosity were measured on d 0, 7, 30, 60, and 90. Beverages formulated using control MPC with 0 and 0.15% SHMP exhibited sedimentation, causing a decrease in apparent viscosity by approximately 10 cP and clear phase separation by d 90. The MPC-20 beverages with 0% SHMP exhibited stable particle size and apparent viscosities during storage. In the presence of 0.15% SHMP, particle size increased rapidly by 40 nm on d 90, implying the start of progressive gelation. On the other hand, highest apparent viscosities leading to gelation were observed in MPC-30 beverages at both concentrations of SHMP studied. These results suggested that beverages formulated with MPC-20 and 0% SHMP would have better storage stability by maintaining lower apparent viscosities. Further reduction of calcium using MPC-30 resulted in rapid gelation of beverages during storage.

Key words: calcium-reduced MPC, high-protein dairy beverages, sodium hexametaphosphate

INTRODUCTION

Over the past decade, ready-to-drink, high-protein dairy-based enteral beverages have been in high demand because of their convenience and nutritional benefits. Enteral dairy beverages are defined as dairy beverages that are ingested orally to meet nutritional requirements (Smith and Garcia, 2011). Milk protein concentrates (**MPC**) are one of the preferred ingredients to formulate dairy beverages because they have the same casein-to-whey protein ratio as milk. By using MPC, protein content in dairy beverages can be increased; however, mineral-protein and protein-protein interactions during storage can result in defects such as sedimentation and age gelation (Datta and Deeth, 2001; Gazi and Huppertz, 2015).

Several research studies have reported various ways to improve storage stability of dairy beverages, either by using additives or by modifying the properties of ingredients used in the formulation. Cano-Ruiz and Richter (1998) delayed age gelation by adding cyclic polyphosphates such as sodium hexametaphosphate (**SHMP**) to the formulation before retort sterilization to dissociate casein micelles into submicelles. That study also reported that age gelation in retort-sterilized dairy beverages was more effectively controlled by cyclic polyphosphates than linear polyphosphates. As the amount of dissociated casein micelles increased, sedimentable caseins decreased during storage in dairy beverages (Harwalkar, 1982). Chen and O'Mahony (2016) observed the influence of adding glucose polymers to 8.5% protein beverages formulated using milk protein isolate and concluded that maltodextrin reduced sedimentation of proteins in beverages during accelerated physical stability testing. Petersen and Ward (2014) demonstrated that the sensory properties of beverages could be improved by modifying the MPC production process, in which they added transglutaminase enzyme to the milk during MPC production to produce liquid

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Table 1. Compositions of milk protein concentrate (MPC) powders used to formulate a high-protein dairy enteral beverage (mean ± SD, n = 2)

MPC	TS (%)	Protein (wt/wt) (%)	Calcium (%)	% Calcium reduction
Control	94.73 ± 0.18	80.77 ± 0.02	2.21 ± 0.04	—
20% Ca reduced	95.13 ± 0.89	80.77 ± 0.55	1.73 ± 0.02	21.7
30% Ca reduced	94.70 ± 0.17	80.02 ± 0.14	1.47 ± 0.02	33.5

MPC with a smaller particle size and lower apparent viscosity, resulting in a smooth-tasting beverage. It was also reported that increased heat treatment induced more casein micelle interactions with denatured whey proteins, delaying the dissociation of β-LG and κ-CN complexes during storage and thus delaying gelation (McMahon, 1996).

Challenges to improving the storage stability of high-protein dairy beverages include sedimentation and age gelation. Sedimentation is a phenomenon that occurs due to aggregation of κ-CN–depleted casein micelles with formation of a protein layer at the bottom of a beverage container (Gaur et al., 2018). Sedimentation during early stages of storage is temporarily reversible upon mixing by resuspending the protein layer. However, increased concentration of proteins in the sedimentation layer during the later stages of storage results in a clear separation of a serum phase on the top and a protein sediment phase (Anema, 2017). The extent of sedimentation in beverages typically increases with higher storage temperature and longer storage periods (Cano-Ruiz and Richter, 1998; Malmgren et al., 2017).

Age gelation is another major storage defect in high-protein dairy beverages that concerns the dairy industry. Physical changes occurring during age gelation in dairy beverages has been explained in 4 stages by Datta and Deeth (2001). A short first stage of product thinning is followed by a longer second stage with a slight change in viscosity. The third stage can be identified by an increase in viscosity due to the gelation, and during the fourth stage the viscosity decreases due to breakdown of the gel, resulting in syneresis.

Casein micelle stability during storage is determined by factors such as ionic calcium, pH, composition of the beverage, and plasmin activity (Datta and Deeth, 2001; Anema, 2017). Partial demineralization of casein was proven to improve functional properties of MPC (Marella et al., 2015) as well as cause greater dissociation of casein micelles in skim milk (Xu et al., 2016). An earlier part of this study, determining the physiochemical properties of calcium-reduced MPC, proved that MPC-20 improved heat stability in 8% protein formulations without any chelating agent (Pandalaneni et al., 2018). The objective of the current study was to further investigate the influence of calcium-reduced MPC and the presence of a chelating agent on the storage stability of high-protein enteral dairy beverages.

MATERIALS AND METHODS

Experimental Design

Milk protein concentrate powders with 3 levels of calcium reduction, 0% (control), 20% (**MPC-20**), and 30% (**MPC-30**) were obtained from Idaho Milk Products Jerome, ID) in 2 batches, with the composition provided in Table 1. The SHMP was procured from Thermo Fisher Scientific (Waltham, MA). The MPC-20 is a commercially available calcium-reduced MPC known as IdaPlus 1085; MPC powders with desired calcium concentrations were produced by injecting carbon dioxide into pasteurized skim milk before and during the UF process, as described by Marella et al. (2015). The study was designed with 2 replications of MPC containing 3 calcium levels and 2 levels of SHMP (0% and 0.15%). Storage stability of the retort-sterilized enteral dairy beverages was evaluated by measuring particle size and apparent viscosity on 0, 7, 30, 60, and 90 d of storage, whereas color was measured only on d 0.

Preparation of High-Protein Enteral Dairy Beverages

Enteral dairy beverages were prepared using the formulation given in Table 2. Milk protein concentrates with varying calcium concentrations were reconstituted to 8% (wt/wt) protein solutions using two-thirds of the distilled water given in formulation (Table 2). Reconstituted solutions were prepared in a water bath maintained at 45°C and stirred using an overhead stirrer with a 4-blade propeller (Caframo, Georgian Bluffs, Ontario, Canada) to ensure complete solubilization. Reconstituted solutions were expected to have approximately 14.9% TS, 0.7% fat, and 7.9% protein. Other ingredients in the formulation [gums, corn maltodextrin, sugar (sucrose), potassium citrate, and canola oil] were mixed in the remaining one-thirds of water using a magnetic stirrer. The 2 solutions were mixed and the formulation was stirred for an additional 10 min. The pH of the formulations were adjusted to 7.0 using 0.5 *N* NaOH

Table 2. The formulation used to prepare enteral high-protein dairy beverages

Ingredient	Percent	Source
MPC85	9.76	Idaho Milk Products, Jerome, ID
Corn maltodextrin	2.90	Maltrin M100, GPC, Muscatine, IA
Sugar (sucrose)	1.21	C&H Sugar, Crockett, CA
Canola oil	0.68	Crisco, J.M. Smucker Co., Orrville, OH
Potassium citrate	0.12	Sigma Aldrich, St. Louis, MO
Cellulose gum	0.12	TICACEL-700 MCC, TIC Gums Inc., White Marsh, MD
Carrageenan	0.02	Ticaloid 100, TIC Gums Inc., White Marsh, MD
Cellulose gel	0.02	TICACEL-700 MCC, TIC Gums Inc., White Marsh, MD
Gellan gum	0.12	Ticagel Gellan DPB, TIC Gums Inc., White Marsh, MD
Water (distilled)	85.05	
Total	100.00	

only if pH was <7.0; no changes were made if pH was ≥7.0. The pH-adjusted formulations were divided into 2 equal parts, to which 0% SHMP and 0.15% SHMP were added. Subsequently, formulations were homogenized at 5,000 rpm for 30 s using a hand-held homogenizer (Polytron PT 2500 E, Luzern, Switzerland) and poured into 20-mL glass vials. Sealed vials were retort sterilized in an autoclave (Steris Amsco 3023, Sanford, FL) at 121°C for 15 min. Retort-sterilized vials were cooled and stored undisturbed at room temperature until they were opened on d 0, 7, 30, 60, and 90 of storage for further analysis.

Particle Size

The dynamic light scattering technique was used to measure particle size with a Zetasizer Nano ZSP (Malvern Instruments Ltd., Malvern, UK) using a method adapted from Silva et al. (2001). The equipment used a helium/neon laser at 633 nm wavelength and a backscattering angle of 173° at 10 mW. Retort-sterilized beverage vials were vortexed for 10 s to obtain a homogeneous mixture, diluted 50 times in calcium imidazole buffer, and then transferred into a disposable cuvette for analysis. Calcium imidazole buffer was prepared as described by Anema (1997). Average hydrodynamic diameter (z-average mean) was calculated by an autocorrelation function from the intensity scattering of particles measured at 25°C at 20-s intervals with 2 repeated measurements. Mean particle size of replicates are reported in the results and discussion.

Apparent Viscosity

The apparent viscosity of retort sterilized dairy beverages was measured using a controlled stress rheometer (ATS RheoSystems, Bordentown, NJ) in a bob and cup geometry at 20°C (Pandalaneni et al., 2018). A sample volume of 13 mL was used for measurement of apparent viscosity at a shear rate of 100 s^{-1} and compared statistically. Mean apparent viscosity of replicates are reported in the results and discussion.

Color

Color parameters of retort sterilized beverages were measured using a hand-held colorimeter (HunterLab, Miniscan XE, Reston, VA); L* is a measure of whiteness, a* is a measure of green-to-red on a negative to positive scale, respectively, and b* is a measure of blue-to-yellow on a negative to positive scale, respectively (Nasirpour et al., 2006). White and green standards were used to calibrate the colorimeter.

Statistical Analysis

The results obtained were statistically analyzed using SAS (version 9.1, SAS Institute Inc., Cary, NC). For analysis of variables on the same day of storage, split-plot analysis was used with calcium-reduced MPC as whole-plot factors and concentrations of SHMP as subplot factors. We used PROC GLIMMIX procedure for analysis and Tukey's test to determine any significant differences between treatment levels, which were declared significant when $P \leq 0.05$. For statistical analysis of variables during storage, repeated measures analysis was used. The PROC GLM function was used to identify significance between the main effects.

RESULTS AND DISCUSSION

When a high-protein milk system at neutral pH is heated above 80°C, whey proteins denature and form aggregates between themselves. However, association of denatured whey proteins with casein micelles is limited by the chaperone-like activity of κ-CN (Guyomarc'h et al., 2009). In addition to the denaturation and aggregation of whey proteins during heating, calcium phosphate will precipitate, leading to mineral imbalance. Consequently, calcium migrates from the colloidal

Table 3. Mean squares (MS) and probabilities (in parentheses) of changes in particle size and apparent viscosity in retort sterilized enteral high-protein dairy beverages during 90 d of storage

	Particle size (nm)		Apparent viscosity (cP)	
Factor[1]	df	MS	df	MS
Whole plot				
MPC	2	54,367.74 (<0.01)	2	50,417.84 (<0.01)
Rep (n = 2)	1	130.74 (0.32)	1	8.06 (0.71)
SHMP	1	2,837.72 (0.01)	1	7,221.11 (<0.01)
MPC × SHMP	2	26,403.41 (<0.01)	2	6,727.81 (<0.01)
Error	3	92.98	3	46.81
Subplot				
Time	4	1,770.31 (<0.01)	4	505.78 (<0.01)
Time × MPC	8	846.59 (<0.01)	8	1,368.50 (<0.01)
Time × SHMP	4	257.8 (0.07)	4	1,021.12 (<0.01)
Time × MPC × SHMP	8	1,264.43 (<0.01)	8	1,227.27 (<0.01)
Error(Time)	12	91.70	12	21.19

[1]SHMP = sodium hexametaphosphate; MPC = milk protein concentrate.

phase to the serum phase to reestablish the mineral equilibrium (Dalgleish and Law, 1989). Upon further heating, casein micelles destabilize and form aggregates due to lack of sufficient colloidal calcium phosphate. Pyne (1958) reported that chelating agents are added in lower concentration to stabilize the casein micelles and to increase heat stability. However, addition of chelating agents in higher concentrations will chelate calcium beyond the critical limit, resulting in casein micelle dissociation into nonmicellar caseins (Augustin and Clarke, 1990; Singh, 2004). The dissociated casein micelles and whey proteins form reversible aggregates under mild heat treatments. Increasing the severity of heat treatment will cause formation of irreversible aggregates of nonmicellar caseins and denatured whey proteins (Singh and Fox, 1987; Panouillé et al., 2004).

Effect of Beverage Formulation on Physicochemical Properties

We analyzed the effect of reduced calcium in MPC in the presence and absence of SHMP on particle size and apparent viscosity of retort sterilized beverages. The comparison of these properties was done between beverages immediately after retort sterilization.

Particle Size. The particle size was significantly ($P < 0.05$) influenced by the type of MPC, concentration of SHMP, and the interaction of MPC × SHMP in the beverages, as shown in whole plot in Table 3. In the absence of SHMP, no significant difference ($P > 0.05$) was observed between beverages formulated with control MPC (187 nm) and MPC-20 (202 nm); however, particle size significantly ($P < 0.05$) increased in beverages formulated with MPC-30 (302 nm), as shown in Figure 1. The increase in particle size in beverages formulated with MPC-30 can be attributed to reduced calcium association with casein micelles, which led to casein micelle dissociation and resulted in nonmicellar caseins. Consequently, when the formulation was heated, nonmicellar caseins and denatured whey proteins reaggregated to form approximately 300-nm particles.

In the presence of SHMP in the formulation, casein micelles dissociated to form heat-induced reaggregates of nonmicellar caseins and denatured whey proteins, causing an increase in the particle size in beverages (Panouillé et al., 2004). However, a significant increase ($P < 0.05$) in the particle size was only observed in beverages formulated with control MPC, of which particle size significantly increased ($P < 0.05$) from 187 (0% SHMP) to 274 nm (0.15% SHMP). In MPC-20 and MPC-30 beverages, further dissociation of casein micelles had no significant difference ($P > 0.05$) on the particle size. Significantly higher particle size was observed in beverages formulated with MPC-30, with 302 nm with 0% SHMP and 315 nm with 0.15% SHMP. As the calcium content was reduced, casein micelle dissociation occurred easily, even in the absence SHMP, resulting in the formation of larger heat-induced protein reaggregates.

Apparent Viscosity. The apparent viscosity was significantly ($P < 0.05$) influenced by the type of MPC, presence of SHMP, and the interaction of MPC × SHMP in the beverages as shown in whole-plot in Table 3. During retort processing, heat-induced protein reaggregates continued to grow in size and number until they formed a gel, leading to increased apparent viscosity (Panouillé et al., 2004). Beverages formulated with control MPC and MPC-20 had no significant ($P > 0.05$) difference in apparent viscosity, whereas beverages formulated with MPC-30 had significantly ($P < 0.05$) higher apparent viscosities, as shown in Figure 2. This significant increase was because of the easier dissocia-

tion of casein micelles due to lower calcium content in MPC-30 that increased heat-induced aggregates in the serum phase. The particle size data (Figure 1) obtained from beverages formulated with MPC-30 supported the apparent viscosity observation.

When 0.15% SHMP was added to the formulation, it interacted with available calcium ions in the serum phase, altering the mineral equilibrium. Consequently, colloidal calcium phosphate migrated into the serum phase and resulted in casein micelle dissociation, which facilitated heat-induced protein reaggregation. In the control formulation containing 0.15% SHMP, apparent viscosity significantly ($P < 0.05$) increased to 50.69 cP compared with 14.51 cP in control formulation with 0% SHMP. On the other hand, beverages formulated with MPC-30 produced the highest apparent viscosity compared with beverages formulated with control and MPC-20. The apparent viscosity of beverages formulated with MPC-30 significantly ($P < 0.05$) increased from 137.50 cP (0% SHMP) to 171.05 cP when 0.15% SHMP was added. As calcium percentage reduced in MPC, heat-induced reaggregates caused an increase in apparent viscosity (Figure 2) and particle size (Figure 1).

Color. The L* (whiteness) was significantly ($P < 0.05$) influenced by the type of MPC but showed no significant ($P > 0.05$) effect by the concentration of SHMP. The a* (green to red) had no significant influence by both of the main effects (type of MPC and concentration of SHMP). On the other hand, b* (blue to yellow) had significant ($P < 0.05$) influence by type of MPC and concentration of SHMP. We found no significant ($P > 0.05$) interaction effect of MPC × SHMP ($P > 0.05$) in L*, a*, and b* of the beverages after retort sterilization. Irrespective of presence or absence of SHMP, we noted no significant ($P > 0.05$) difference in the L* and a* of beverages formulated with control MPC, MPC-20, and MPC-30, as shown in Table 4. However, the average b* values of MPC-20 (16.14) and MPC-30 (15.39) were higher compared with average b* of control (12.15). The increased b* in beverages formulated with MPC-20 and MPC-30 was assumed to be caused by exposed lysine residues (due to increased casein micelle dissociation), promoting interactions with lactose during retort processing and resulting in formation of browning compounds (Devi et al., 2015).

From these results, it can be summarized that the extent of casein micelle dissociation determined the size of heat-induced reaggregates. As more nonmicellar caseins are present in the formulation before retort sterilization, larger heat-induced reaggregates formed after retort sterilization. Dissociation of casein micelles, either by reduced calcium content or the addition of SHMP, also affected the b* color during retort sterilization.

Physicochemical Changes in High-Protein Beverages During Storage

Particle Size. The particle size of beverages throughout 90 d of storage was significantly ($P < 0.05$) influenced by the type of MPC, SHMP, time, and in-

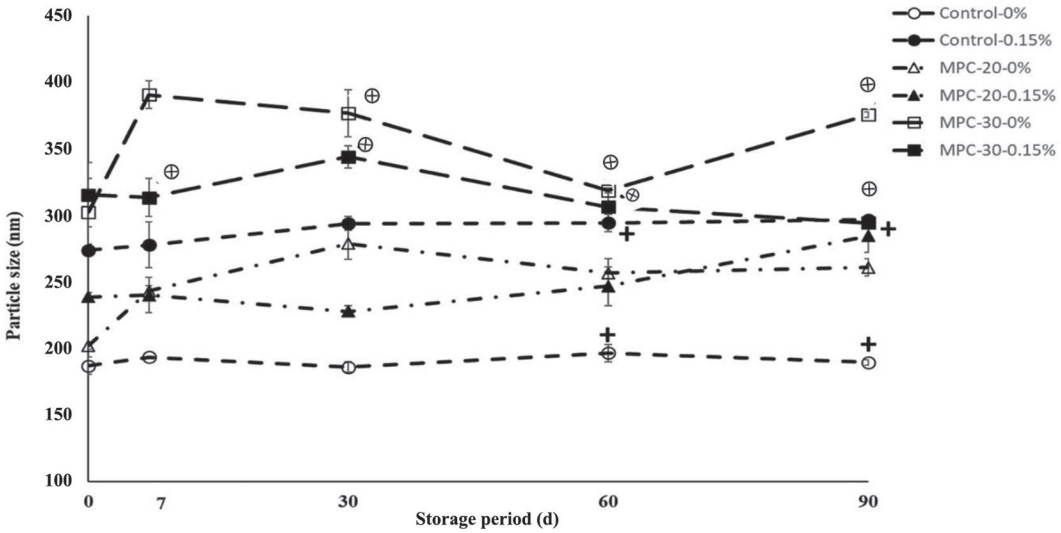

Figure 1. Particle size (nm) of retort-sterilized enteral dairy beverages during 90 d of storage at room temperature. Formulations were prepared using control [0% calcium-reduced milk protein concentrate (MPC)], MPC-20 (20% calcium-reduced MPC), and MPC-30 (30% calcium-reduced MPC) by adding 0 (open symbols) and 0.15% (closed symbols) sodium hexametaphosphate (SHMP). (+) indicates the visual observation of sedimentation and (⊕) indicates visual observation of age gelation during storage of enteral high-protein dairy beverages. Error bars indicate SD (n = 2).

Table 4. Color (L*, a*, and b*) values of the enteral dairy beverages measured after retort processing on d 0 (mean ± SD, n = 2)[1,2]

MPC	SHMP (%)	L*	a*	b*
Control	0	63.41 ± 1.06^{NS}	3.58 ± 0.01^{NS}	10.86 ± 0.72^{NS}
	0.15	65.36 ± 0.24^{NS}	3.95 ± 0.68^{NS}	13.44 ± 0.49^{NS}
20% Ca reduced	0	62.40 ± 0.02^{NS}	4.81 ± 0.61^{NS}	15.60 ± 0.66^{NS}
	0.15	62.56 ± 1.67^{NS}	4.87 ± 1.04^{NS}	16.69 ± 0.69^{NS}
30% Ca reduced	0	61.83 ± 0.97^{NS}	4.24 ± 0.00^{NS}	14.64 ± 0.37^{NS}
	0.15	62.61 ± 1.04^{NS}	5.12 ± 0.67^{NS}	16.15 ± 1.03^{NS}

[1]MPC = milk protein concentrate; SHMP = sodium hexametaphosphate; L* is the measure of whiteness on scale 0–100; a* is the measure green to red from negative to positive scale; b* is the measure of blue to yellow from negative to positive scale.

[2]Values were compared within column and with same superscript are not significantly different ($P > 0.05$); NS = no significant interaction effect between main effects.

teractions of MPC × SHMP, time × MPC, and time × MPC × SHMP. The time × SHMP interaction did not significantly ($P > 0.05$) influence particle size, as shown in Table 3. In the absence of SHMP, the particle size of beverages formulated with control MPC had no significant ($P > 0.05$) changes throughout 90 d of storage (Figure 1). However, in the presence of 0.15% SHMP in beverages with control MPC, particle size significantly ($P < 0.05$) increased from d 0 to 7, and thereafter had no significant change throughout 90 d of storage. The increased particle size from d 0 to 7 was attributed to heat-induced protein reaggregation. No noticeable changes were observed in the particle size of beverages formulated with control MPC (in both 0 and 0.15% SHMP) throughout 90 d of storage. However, a clear visual sedimentation and a clear phase separation was noted on d 60 and 90, respectively. Figure 1 shows the visual appearance of sedimentation as observed during storage.

In case of beverages formulated with MPC-20 with 0% SHMP, a significant ($P < 0.05$) and progressive increase in particle size was observed from d 0 to 30, and thereafter no significant increase was observed during the remaining storage period. The progressive increase

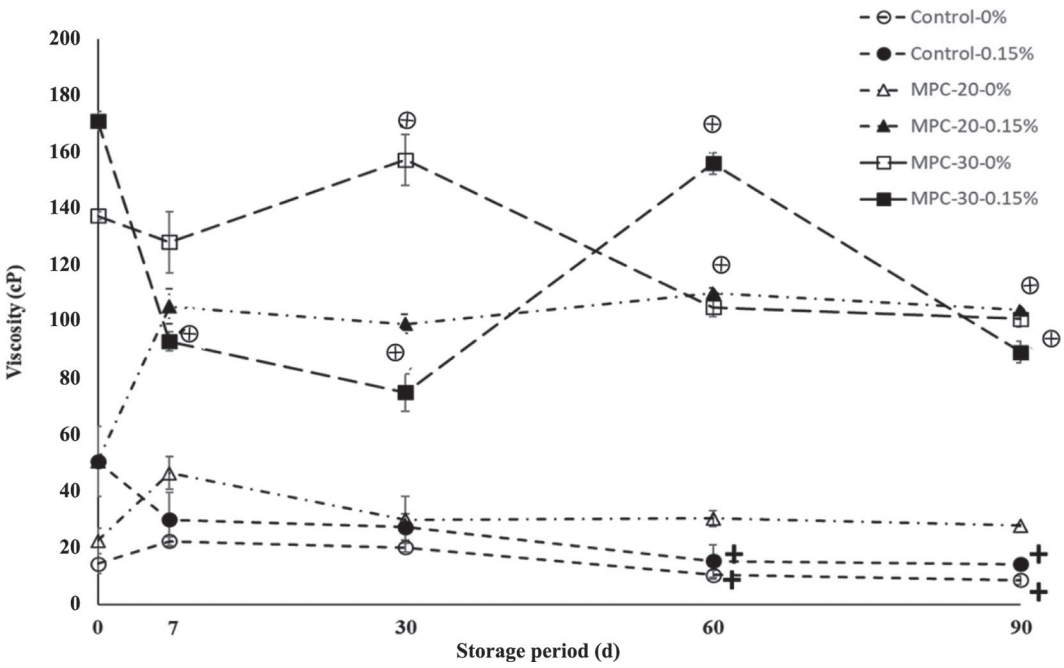

Figure 2. Apparent viscosity (cP) at 100 s^{-1} shear rate of retort-sterilized enteral dairy beverages during 90 d of storage at room temperature. Formulations were prepared using control [0% calcium-reduced milk protein concentrate (MPC)], MPC-20 (20% calcium-reduced MPC), and MPC-30 (30% calcium-reduced MPC) by adding 0 (open symbols) and 0.15% (closed symbols) sodium hexametaphosphate (SHMP). (+) indicates the visual observation of sedimentation and (⊕) indicates visual observation of age gelation during storage of enteral high-protein dairy beverages. Error bars indicate SD (n = 2).

in the particle size from d 0 to 30 was due to continued reaggregation of proteins. In case of beverages with MPC-20 and 0.15% SHMP, the particle size remained stable from d 0 to 60 and increased by approximately 40 nm from d 60 to 90 (284.78 nm). This significant ($P < 0.05$) increase in particle size was because of progressive gelation caused by network formation between heat-induced reaggregates (Panouillé et al., 2004; Tsioulpas et al., 2010).

In beverages formulated with MPC-30 and 0% SHMP, an increase in particle size by approximately 90 nm was observed from d 0 to 7, followed by a steady decrease until d 60. This increase of particle size on d 7 of storage can be attributed to progressive gel formation because of the network between heat-induced protein reaggregates (Panouillé et al., 2004). The gradual decrease in particle size followed by age gelation in the MPC-30 beverage was attributed to gel breakage and syneresis. When 0.15% SHMP was added to the formulation, the particle size significantly ($P < 0.05$) increased on d 30, followed by a gradual decrease by d 60 with no further change until d 90. From visual observation, it was evident that gel breakage with syneresis had already occurred by d 7 in MPC-30 beverage with 0.15% SHMP.

The results so far imply that reducing calcium in the beverage formulation led to nonmicellar caseins, which reaggregated with denatured whey proteins and among themselves to increase particle size during retort sterilization. However, when particle size continued to increase in number and size during storage, rapid gelation occurred due to the network formed between heat-induced protein reaggregates, as evident in MPC-30 beverages (Panouillé et al., 2004).

Apparent Viscosity. The apparent viscosity was significantly ($P < 0.05$) influenced by the type of MPC, presence of SHMP, time, and interactions of MPC × SHMP, time × MPC, time × SHMP, and time × MPC × SHMP, as shown in Table 3. In the case of beverages formulated with control MPC in the absence of SHMP, apparent viscosity (as compared with d 0) demonstrated no significant ($P > 0.05$) difference until d 30, followed by a significant decrease by d 60. This significant decrease of approximately 10 cP from d 30 to 60 can be attributed to sedimentation. However, distinct phase separation sediment protein layers at the bottom and top aqueous layers were not observed until d 90. In the beverage formulated with control MPC and with 0% SHMP, a reversible sedimentation was observed by d 7 and showed no significant ($P > 0.05$) effect on apparent viscosity. However, when 0.15% SHMP was added to the control formulations, a significant ($P < 0.05$) decrease in apparent viscosity was observed by d 7 in contrast to beverages with 0% SHMP. This can be attributed to casein micelle dissociation, resulting in heat-induced protein reaggregates with casein micelles in the presence of SHMP. From a previously published study by Pandalaneni et al. (2018), it was evident that casein micelles were not completely dissociated in control formulation as compared with calcium-reduced MPC when 0.15% SHMP was added. Hence, it can be assumed that association of heat-induced reaggregates with casein micelles can occur during retort sterilization. During storage, these heat-induced reaggregates dissociate from the casein micelles, resulting in prominent reversible sedimentation by d 7 (Datta and Deeth, 2001; Panouillé et al., 2004). During storage, a significant ($P < 0.05$) decrease in apparent viscosity by d 60 and 90 was also observed in beverages formulated with control MPC with 0.15% SHMP, which was attributed to sedimentation and phase separation. However, the particle size (Figure 1) in beverages formulated with control MPC with and without SHMP did not exhibit any significant ($P > 0.05$) changes during sedimentation or phase separation. Disruption of protein networks occurred during sample preparation for particle size analysis, which could be the reason for no change in particle size.

In case of beverages formulated with MPC-20 and with 0% SHMP, the apparent viscosity significantly ($P < 0.05$) increased from d 0 to 7 of storage and decreased by d 30, whereas we found no significant ($P > 0.05$) difference in the apparent viscosity until d 90. An increase in apparent viscosity on d 7 was attributed to the increased size of heat-induced protein reaggregates, facilitated by easier dissociation of casein micelles in MPC-20. These heat-induced reaggregates associate with casein micelles during retort sterilization and dissociate during storage, leading to reversible sedimentation by d 30 (Datta and Deeth, 2001; Panouillé et al., 2004). The absence of an increase or decrease in the apparent viscosity during storage confirmed that gelation or sedimentation had not occurred yet in MPC-20 beverages with 0% SHMP. On the other hand, when 0.15% SHMP was added, the apparent viscosity in MPC-20 beverages significantly ($P < 0.05$) increased by d 7, and thereafter no significant changes were observed. The significant increase in apparent viscosity by d 7 can be attributed to the increased size of heat-induced protein reaggregates, but the absence of any further decrease in apparent viscosity suggests that no reversible sedimentation occurred. When 0.15% SHMP was added to the beverages formulated with MPC-20, it was hypothesized that the nonmicellar caseins formed heat-induced protein reaggregates during retort sterilization and caused an increase in particle size and the apparent viscosity by forming a network. When the apparent viscosities of MPC-20 beverages

on d 7 at 2 levels of SHMP were compared, presence of 0.15% SHMP increased the apparent viscosity by approximately 6 times higher than the beverages with 0% SHMP, confirming rapid growth in the network between heat-induced protein reaggregates (Panouillé et al., 2004; Tsioulpas et al., 2010).

In case of beverages formulated with MPC-30 with and without SHMP, significantly ($P < 0.05$) higher apparent viscosities than control and MPC-20 were observed throughout the storage. A 30% reduction in calcium in MPC led to increased casein micelle dissociation, consequently forming heat-induced reaggregates between nonmicellar caseins and denatured whey proteins. Increased aggregate size and number enabled easier network formation, resulting in rapid gelation (Tsioulpas et al., 2010). Increased apparent viscosity from d 0 to 30 of MPC-30 beverages with 0% SHMP, followed by a decrease with no further changes, was attributed to age gelation, followed by gel breakage and syneresis. However, in beverages formulated with MPC-30 with 0.15% SHMP, the apparent viscosity rapidly decreased by approximately 80 cP from d 0 to 7. This rapid significant ($P < 0.05$) decrease can be attributed to gel breakage and syneresis by d 7, which was evident from the visual observation as well. Because the gel matrix was disrupted to obtain a homogeneous sample for particle size analysis, these changes were not exhibited in the particle size data (Figure 1). Beverages formulated with MPC-30 and 0.15% SHMP exhibited another significant ($P < 0.05$) rapid increase in apparent viscosity by d 60, which was assumed to be due to stronger gel formation by interlinking of protein reaggregates in the supernatant.

It is noteworthy that the apparent viscosities of beverages formulated with MPC-20 and with 0% SHMP were closer in range of apparent viscosities of control beverages. On the other hand, apparent viscosities of beverages formulated with MPC-20 with 0.15% SHMP were higher and closer to apparent viscosities of beverages formulated with MPC-30. Distinct differences in the apparent viscosity of beverages formulated with MPC-20, with and without SHMP, mark the importance of the amount of calcium to be chelated to obtain beverages with the preferred apparent viscosity. Though heat-induced reaggregates were formed in beverages formulated with MPC-20, the amount of calcium reduction was able to keep the reaggregates stable in the beverages. Results from our study demonstrate that the amount of calcium affects the rate at which gelation occurs. Gelation was distinct and occurred rapidly in beverages formulated with MPC-30 compared with MPC-20 beverages. Tsioulpas et al. (2010) also reported coagulation occurred with in-can sterilized skim milk when 0.2% SHMP was added. Those authors explained that the coagulation was due to an increase in casein micelle size as the concentration of SHMP increased, supporting the observations made in the current study. The particle size and apparent viscosity results of our study were in agreement with Anema (2017), who also observed an increase in particle size due to aggregation of casein micelles before the onset of gelation in UHT milk samples.

Summarizing the observations from our study and previously published studies, it is evident that use of 20% calcium-reduced MPC in enteral high-protein dairy beverages improves retort stability and storage stability without the addition of chelating agents. Because of the different heat treatments employed, along with the various compositions of dairy beverages in the literature, the apparent viscosities and particle sizes cannot be compared directly to explain the exact phenomenon of sedimentation and age gelation during storage. To develop a high-protein dairy beverage with improved storage stability, further studies are required to understand the role of nonprotein components.

CONCLUSIONS

Throughout 90 d of storage, retort-sterilized beverages formulated using control MPC exhibited no gelation, but visual sedimentation by end of the 90-d storage was distinct. On the other hand, MPC-20 beverages without SHMP had consistently lower apparent viscosity throughout storage with no visual sedimentation or gelation; however, when 0.15% SHMP was added, apparent viscosities significantly increased. Larger particle size and higher apparent viscosities leading to rapid gelation were found in beverages with MPC-30 with and without SHMP. These results suggest that using 30% calcium-reduced MPC in beverage formulations could lead to rapid gelation, whereas formulating beverages with 20% calcium-reduced MPC improved storage stability with and without SHMP. This study presents an option to improve storage stability of enteral high-protein dairy beverages formulated with 20% calcium-reduced MPC and without chelating additives such as SHMP.

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