Influence of hydrodynamic cavitation on the rheological properties and microstructure of formulated Greek-style yogurts

G. H. Meletharayil,^{*1} L. E. Metzger,^{*} and Hasmukh A. Patel^{*†2}

^{*}Midwest Dairy Foods Research Center, Department of Dairy Science, South Dakota State University, Brookings 57007

[†]Dairy Foods Research and Development, Land O'Lakes Inc., Arden Hills, MN 55126

ABSTRACT

With limited applications of acid whey generated during the manufacture of Greek yogurts, an alternate processing technology to sidestep the dewheying process was developed. Milk protein concentrate (MPC) and carbon dioxide-treated milk protein concentrate (TMPC) were used as sources of protein to fortify skim milk to 9% (wt/wt) protein for the manufacture of Greek-style yogurts (GSY). The GSY bases were inoculated and fermented with frozen direct vat set yogurt culture to a pH of 4.6. Owing to the difference in buffering of MPC and TMPC, GSY with TMPC and MPC exhibited different acidification kinetics, with GSY containing TMPC having a lower fermentation time. The GSY with TMPC had a titratable acidity of 1.45% lactic acid and was comparable to acidity of commercial Greek yogurt (CGY). Hydrodynamic cavitation at 4 different rotor speeds (0, 15, 30, and 60 Hz) as a postfermentation tool reduced the consistency coefficient (K) of GSY containing TMPC from 79.4 Pa·sⁿ at 0 Hz to 17.59 Pa·sⁿ at 60 Hz. Similarly for GSY containing MPC, K values decreased from 165.74 Pa·sⁿ at 0 Hz to 53.04 Pa·sⁿ at 60 Hz. The apparent viscosity (η_{100}) was 0.25 Pa·s for GSY containing TMPC and 0.66 Pa·s for GSY containing MPC at 60 Hz. The CGY had a η_{100} value of 0.74 Pa·s. Small amplitude rheological analysis performed on GSY indicated a loss of elastic modulus dependency on frequency caused by the breakdown of protein interactions with increasing cavitation rotor speeds. A steady decrease in hardness and adhesiveness values of GSY was observed with increasing cavitation intensities. Numbers of grains with a perimeter of >1 mm of cavitated GSY with TMPC and MPC were 35 and 13 grains/g of yogurt, respectively, and were lower than 293 grains/g observed in CGY.

The water-holding capacity of GSY was higher than that observed for a commercial strained Greek yogurt. The ability to scale up the process of hydrodynamic cavitation industrially, and the ease of controlling events of cavitation that can influence final textural properties of the product, make this technology promising for large-scale industrial application. Overall, the current set of experiments employed in the manufacture of GSY, which included the use of TMPC as a protein source in conjunction with hydrodynamic cavitation, could help achieve comparable titratable acidity values, rheological properties, and microstructure to that of a commercial strained Greek yogurt.

Key words: hydrodynamic cavitation, carbon dioxide, Greek yogurt, milk protein concentrate

INTRODUCTION

The per capita consumption of yogurt in the United States increased from 1.13 kg in 1980 to 6.76 kg in 2013 (USDA, 2014). The increased per capita consumption since 2010 is partly believed to be from the increasing popularity of Greek yogurt (GY). A widely used process for the manufacture of GY involves the concentrating of fermented milk solids by dewheying. The process of dewheying in a modern industrial setup employs centrifugal separators like the Quark centrifuge. The acid whey generated from the dewheying process has limited applications owing to its high mineral and lactic acid load. To sidestep the dewheying process, fortifying milk base with high protein milk ingredients is a choice available to GY manufacturers. The GY manufactured by the fortification route is known as Greek-style yogurt (GSY) in the industry. The alternative make process does offer technological challenges in terms of increased fermentation time and altered rheological and microstructural properties. In a study by Desai et al. (2013), sour taste was found to be the dominant sensory defect, whereas increased graininess, higher viscosity, and cohesiveness and decreased melt-away dominated the microstructural and rheological defects in GSY.

In the present study, a 2-pronged strategy was applied to address acidity, rheological, and microstructural de-

Received December 16, 2015.

Accepted May 23, 2016.

¹Current address: PepsiCo Global R&D, 100 Stevens Avenue, Valhalla, NY 10595.

²Corresponding author: HPatel@Landolakes.com

Table 1. Composition of CO₂-treated milk protein concentrate (TMPC) powder and milk protein concentrate powder (MPC)¹

Powder	TS (%, wt/wt)	Protein (%, wt/wt)	Ash (%, wt/wt)	Calcium (mg·g ⁻¹)
TMPC	96.59	71.92	4.84	12.8
MPC	95.37	67.88	6.73	19.2

¹Values are the means of data from triplicate analysis.

fects associated with the manufacture of GSY. Protein fortification by carbon dioxide (CO₂)-treated milk protein concentrate (**TMPC**) in GSY manufacture could potentially address higher acidity levels on account of its lower buffering when compared with control milk protein concentrate (**MPC**). The CO₂ treatment of milk before and during the process of UF and diafiltration employed in the manufacture of TMPC would partially demineralize milk, leading to the removal of micellar calcium phosphate (**MCP**) along with serum minerals. The use of hydrodynamic cavitation (**HC**) as a postfermentation tool was hypothesized to improve the rheological and microstructural properties of GSY. Hydrodynamic cavitation is a process having parallels to acoustic cavitation, with differences arising in the principle of cavity formation. Hydrodynamic cavitation uses velocity and pressure differentials generated by the flow of yogurt inside the cavitator toward the formation of cavities (Gogate and Bhosale, 2013). Pressure differential generates bubbles in low-pressure zones that collapse as they enter a high-pressure zone inside the cavitator. This collapse leads to the release of vast amounts of energy that could potentially influence protein interactions altering rheology and microstructure of GSY. Moreover, grains (that could potentially be a source of graininess) that are formed in GSY could serve as seed nuclei for the bubble to form, expand, and collapse, leading to a product with reduced grains that could lead to a product with superior mouthfeel (Earnshaw, 1998). To prove this hypothesis, the objective of this study was to determine the influence of TMPC as a protein source and hydrodynamic cavitation as a processing step on the chemical, rheological, and microstructural properties of GSY.

MATERIALS AND METHODS

MPC/TMPC Manufacture and Composition

The manufacturing procedure and composition (Table 1) of MPC and TMPC powders from pasteurized skim milk (72°C for 15 s) are described by Marella et al. (2015). Low heat nonfat dry milk was obtained from Associated Milk Producers (AMPI Inc., New Ulm, MN).

Preparation of Formulated Greek-Style Yogurts

Pasteurized skim milk was obtained from the Davis dairy plant of South Dakota State University. Nonfat dry milk and MPC or TMPC was added to skim milk at 50°C. The MPC or TMPC was added to contribute 6% (wt/wt) protein of the total 9% (wt/wt) protein in the GSY base formulation. The TS, protein, fat, lactose, and ash content of the GSY base along with the composition of a strained commercial GY purchased from a local grocery store is given in Table 2. The GSY base formulations mixed in a high-speed mixer were heated to 90°C for 10 min in a steam chest followed by cooling to 42°C. Frozen direct vat set yogurt culture (YC-X11) obtained from Chr. Hansen Inc. (Milwaukee, WI) was added at the rate of 0.02% (vol/vol). The pH of yogurt bases was measured every hour using a pH meter (CyberScan pH 110, Eutech Instruments, Singapore) to generate acidification profiles. Fermentation was arrested on reaching a pH of 4.6 by placing GSY in an ice bath followed by overnight storage in a cold room maintained at <5°C. All chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA) unless otherwise indicated.

Hydrodynamic Cavitation

An 8-inch APV hydrodynamic cavitator (SPX Flow Technology, Pastorsvej, Silkeborg, Denmark) was used for the HC of yogurts. The cavitator had specially designed rotors with indentations that influenced the flow trajectory of yogurts inside the cavitator. A total of 66 indentations were placed equidistant from each other on the 200-mm (8-inch) rotor. The speed of rotor that determines the extent of cavitation was controlled with a variable frequency drive attached to a 10-HP motor. The gap between the rotor and stator was 3 mm. The GSY was fed into the cavitator through a positive displacement pump. Four different rotor speeds of 0, 872, 1,745, and 3,490 rpm corresponding to frequencies of 0, 15, 30, and 60 Hz were used to generate cavitation during yogurt flow through the device. For cavitation at 0 Hz, yogurt was passed through the cavitator with an inactivated rotor. Cavitation was carried out at 8

Table 2. Compositional analysis (% wt/wt; means $\pm$ SE) of Greek yogurt base containing CO₂-treated milk protein concentrate (TMPC) and milk protein concentrate (MPC), in addition to the compositional analysis (means $\pm$ SE) of a commercial strained Greek yogurt

Item	TS	Protein	Fat	Lactose	Ash
Greek-style yogurt base with TMPC	16.72 $\pm$ 0.03	8.94 $\pm$ 0.05	0.54 $\pm$ 0.01	5.06 $\pm$ 0.03	1.11 $\pm$ 0.05
Greek-style yogurt base with MPC	17.37 $\pm$ 0.11	8.97 $\pm$ 0.03	0.37 $\pm$ 0.02	5.3 $\pm$ 0.03	1.27 $\pm$ 0.00
Commercial strained yogurt	14.96 $\pm$ 0.06	8.93 $\pm$ 0.08	0.25 $\pm$ 0.05	—	0.73 $\pm$ 0.00

to 10°C. Samples cavitated at each frequency were collected and stored below 5°C before analysis.

Titrateable Acidity

Titrateable acidity (TA) expressed as percentage lactic acid (LAC) per 100 g of GSY and GY was performed as per the procedure outlined by Bong and Moraru (2014). Approximately 10 g of yogurt was added to 10 mL of deionized water followed by the addition of 1 mL of phenolphthalein (0.5%, wt/vol), followed by titration with 0.1 *N* NaOH to the phenolphthalein end point:

$$\text{LAC \%} = (9 \times A \times N)/W,$$

where A is the volume in milliliters of the standard 0.1 *N* NaOH required for titration, *N* is the normality of the standard 0.1 *N* NaOH solution, and W is the weight of the sample expressed in grams.

Rheological Characterization

Steady rate sweeps and small oscillatory rheological measurements were performed on a stress-controlled rheometer (ATS Rheosystems, Bordentown, NJ). A plate and plate geometry (diameter 30 mm) with a gap setting of 2 mm were used for rheological testing. All rheological testing was carried out at 10°C.

Flow curves were generated by subjecting the GSY and commercial GY to shear rates between 1 and 100 s⁻¹. Delay and integration times were set to 10 s and the data obtained were fitted to the power law model.

$$\eta = \kappa \times \dot{\gamma}^{(n-1)},$$

where η is the viscosity (Pa·s), *n* is the flow behavior index, and κ is the consistency coefficient (Pa·s^{*n*}).

The frequency dependency on storage and loss modulus of yogurts was determined by first conducting an oscillatory stress sweep at a constant frequency of 0.15 Hz. The stress values at upper limits of the linear viscoelastic region were used to conduct frequency sweeps from 1 to 10 Hz.

Large Deformation Penetration Tests

Hardness, firmness, and adhesiveness of GSY and GY were measured using a TA-XT^{plus} Texture Analyzer (Texture Technologies Corp., South Hamilton, MA) equipped with a 5-kg load cell. Yogurts were transferred into cups (i.d. 54 mm and height 30 mm) and penetration tests were conducted using a 0.5-inch (1.27 cm) diameter cylindrical probe. A test speed of 0.5 mm·s⁻¹ and a posttest speed of 0.5 mm·s⁻¹ to a distance of 15 mm were applied. The maximum positive force attained during the penetration test was expressed as hardness, and the total positive area was expressed as firmness. Adhesiveness was expressed as the total negative area that was determined during the test.

Grains in Greek-Style Yogurts and Commercial Greek Yogurt

Grains in GSY and commercial GY was measured as per the method outlined by Remeuf et al. (2003) with modifications. A sample of approximately 0.1 g of yogurt was dispersed in 10 mL of water. Following gentle mixing, 500 μ L of this dispersion was poured into 10 wells of a 12-well cell culture cluster (Fisher Scientific, Pittsburgh, PA). The dispersions in all 10 wells were visualized under a stereomicroscope (SZX16, Olympus, Tokyo, Japan) with light projected from the bottom of the cell culture cluster. Grains appeared as dark objects on a white background, and representative images were captured. Images were analyzed as 8-bit images with ImageJ software (US National Institutes of Health, Bethesda, MD). Grains with perimeter >1 mm were enumerated and expressed as the number of grains in 1 g of yogurt.

Water-Holding Capacity

Water-holding capacity (WHC) of GSY and GY was performed according to the procedure of Meletharayil et al. (2015). The WHC of the yogurts was expressed as the ratio of the weight of pellet obtained after centrifugation to the weight of the yogurt and expressed in percentage.

Figure 1. Acidification profiles of Greek-style yogurt (9% wt/wt, protein) fermented with yogurt culture at 42°C containing either milk protein concentrate (○) or CO₂-treated milk protein concentrate (●). Error bars indicate SE.

Statistical Analysis

The ANOVA and significance were indicated by $P < 0.05$, using the statistical software SAS (version 9.3, SAS Institute Inc., Cary, NC). Three replicates of GSY were manufactured and each experiment was repeated at least 3 times.

RESULTS

Mineral and Acidification Profiles of Greek-Style Yogurts

The protein content of GSY bases was standardized to contain 9% (wt/wt) protein. Among GSY, yogurts containing MPC had the highest mineral content (Table 2) followed by yogurts containing TMPC. Strained commercial GY had the lowest mineral content among the 3. Compared with GSY with MPC as the protein source, a lower mineral content in GSY containing

TMPC can be attributed to a greater removal of coloidal and serum phase minerals during the process employed in the manufacture of TMPC (Marella et al., 2015). The GSY containing TMPC had significantly lower ($P < 0.05$) fermentation time to reach pH 4.6 as compared with GSY containing MPC (Figure 1). Lower acidification times to achieve pH of 4.6 in GSY containing TMPC can be correlated with a reduced mineral load leading to a reduction in buffering of the GSY base.

Titratable Acidity

Titratable acidity (TA) expressed as percentage lactic acid of GSY and GY are shown in Figure 2. The GSY containing TMPC had a TA of 1.45% (wt/wt) lactic acid compared with 1.95% (wt/wt) in GSY containing MPC. The TA values of GSY containing TMPC and that of the commercial GY were comparable. The reduced acidity in commercial GY and GSY containing TMPC can be attributed to a reduced buffering due to demineralization during dewheying and CO₂ application, respectively.

Rheological Characterization

The flow characterization properties of the GSY and commercial GY are given in Table 3. All yogurts exhibited a shear thinning behavior, a characteristic behavior of yogurts. The consistency coefficient values (κ) and flow behavior index value (n) of GSY was determined by fitting the flow data into a power law equation ($R^2 > 99\%$). The κ values of noncavitated GSY were higher than that of the commercial GY, which decreased as GSY were subjected to cavitation. The HC had a greater effect on the flow behavior of GSY containing TMPC with a larger decrease in κ and n values as com-

Table 3. Flow characterization (means $\pm$ SE) and grains of Greek-style yogurts (GSY) containing CO₂-treated milk protein concentrate (TMPC) and GSY with milk protein concentrate (MPC)¹

Sample	Flow index (n)	Consistency coefficient κ (Pa·S ⁿ)	Apparent viscosity (Pa·s, at 100 shear rate)	Grains/g of yogurt
GSY with TMPC				
0 Hz	0.07 $\pm$ 0 ^{ab}	79.4 $\pm$ 1.4 ^{bc}	0.49 $\pm$ 0.01 ^{de}	2,389 ^a
15 Hz	0.07 $\pm$ 0.04 ^{ab}	39.44 $\pm$ 3.85 ^{cde}	0.48 $\pm$ 0.04 ^{de}	833 ^b
30 Hz	0.06 $\pm$ 0 ^{ab}	28.74 $\pm$ 0.51 ^{de}	0.40 $\pm$ 0.01 ^{ef}	213 ^c
60 Hz	0.08 $\pm$ 0.02 ^b	17.59 $\pm$ 3.67 ^e	0.25 $\pm$ 0.03 ^f	35 ^d
GSY with MPC				
0 Hz	0.03 $\pm$ 0.01 ^b	165.74 $\pm$ 10.25 ^a	1.43 $\pm$ 0.01 ^a	2,436 ^a
15 Hz	0.07 $\pm$ 0.04 ^{ab}	85.42 $\pm$ 26.94 ^b	1.14 $\pm$ 0.13 ^b	400 ^c
30 Hz	0.07 $\pm$ 0.01 ^{ab}	63.7 $\pm$ 4.87 ^{bcd}	0.89 $\pm$ 0.09 ^c	250 ^c
60 Hz	0.02 $\pm$ 0.02 ^b	53.04 $\pm$ 1.64 ^{bcd}	0.66 $\pm$ 0.02 ^{de}	13 ^d
GY	0.16 $\pm$ 0.01 ^a	37.52 $\pm$ 2.55 ^{cde}	0.79 $\pm$ 0.08 ^{cd}	293 ^c

^{a-f} Means within the same column not sharing a common superscript are significantly different ($P < 0.05$).

¹ Also given is the flow characterization (means $\pm$ SE) of a commercial strained Greek yogurt (GY).

Figure 2. Titratable acidities of Greek-style yogurt (GSY; 9% wt/wt, protein) containing either milk protein concentrate (MPC) or CO₂-treated milk protein concentrate (TMPC) and a commercial strained Greek yogurt (CGY) at a pH of 4.5 ± 0.1 . Error bars indicate SE.

pared with GSY containing MPC. The GSY containing TMPC had lower apparent viscosity (η_{app}) values than GSY containing MPC across the entire range of shear rate applied (1 to 100 s^{-1}). However, the η_{app} at a value at 100 s^{-1} of commercial GY was higher than that of GSY containing MPC or TMPC. The n value of the commercial GY was 0.16 and was higher than n values of GSY containing MPC or TMPC, indicating a greater shear thinning of GSY subjected to cavitation. A similar n value of a strained commercial GY was reported by Bong and Moraru (2014).

Small Oscillatory Rheology Measurements

Frequency dependence of G' in GSY containing TMPC and MPC are shown in Figures 3 and 4, respectively. Yogurt gels are known to be frequency dependent with G' increasing with frequency (Ozer et al., 1997). The elastic component (G') in yogurts is influenced by the strength and number of nonrelaxing bonds, whereas the viscous component (G'') is influenced by relaxing bonds (Roefs et al., 1985). The correlation of cavitation intensity (with an increase in rotor speed) and G' in GSY was clearly evident (across the entire frequency range). At high rotor speeds (60 Hz), a breakdown in gel structure occurred, resulting in a decrease in the elastic moduli of GSY in the frequency range studied. The structural breakdown was greater in GSY containing TMPC. Frequency dependence of G' expressed as the slope, m of the elastic modulus, and frequency curve was ~ 0.16 in commercial GY and GSY before cavitation. Following cavitation of GSY containing MPC, the slope dropped to 0.06 at higher rotor speeds (30 and 60 Hz), indicating a structural breakdown. Moreover, in GSY containing TMPC, no frequency dependency

of G' (determined by the slope of the G' and frequency curve) was evident even at low rotor speeds (15 Hz). An abrupt decrease occurred in the G' values at higher frequency of GSY containing TMPC cavitating at maximum rotor speed, which suggests the occurrence of slip-page during the conduct of the experiment.

Penetration Tests

Hardness/firmness and adhesiveness values of the GSY containing TMPC and MPC are shown in Figures 5 and 6, respectively. The GSY not subjected to HC had higher hardness, firmness, and adhesiveness values when compared with commercial GY. However, with an increase in rotor speeds, a breakdown occurred in the structure of GSY. The structural breakdown of the GSY containing TMPC was greater when compared with GSY containing MPC. At maximum rotor speeds (60 Hz), GSY containing TMPC had lower hardness, firmness, and adhesiveness values compared with commercial GY (Figure 5). The GSY containing MPC had identical hardness, firmness, and adhesiveness values as strained commercial yogurts (Figure 6) at maximum rotor speeds (60 Hz).

Grains

The occurrence of grains in yogurts could possibly be linked to the denaturation of whey protein and its gelation during the manufacture of acid gels (Remeuf et al., 2003). Grains in the present study were defined as particles with a perimeter of $>1 \text{ mm}$. Commercial GY had 293 grains per gram, whereas GSY containing MPC and TMPC not subjected to cavitation had 2,389 and 2,436 grains per g, respectively, which were significantly higher ($P < 0.05$) than grains in commercial GY. The HC resulted in a steady decrease of the number of grains and was inversely related to rotor speeds employed in the experiment. The GSY containing MPC and TMPC cavitating at maximum rotor speeds (60 Hz) had 35 and 13 grains per g of yogurt, respectively. Figures 7 and 8 are representative images of grains of GSY with TMPC and MPC, respectively. Figure 9 is a representative image of grains in CGY. Images were captured under a stereomicroscope and processed using ImageJ software.

Water-Holding Capacities

The WHC of GSY containing TMPC was significantly higher ($P < 0.05$) to WHC of GSY containing MPC at the rotor speeds studied in this experiment (Figures 10 and 11). As a trend, cavitating GSY had a significantly higher ($P < 0.05$) WHC than commer-

Figure 3. Frequency sweep for Greek-style yogurt (9% wt/wt, protein) containing CO₂-treated milk protein concentrate at 10°C and in the linear viscoelastic region determined by stress sweep experiments. Open symbols are elastic modulus (G') values at 0 Hz (○), 15 Hz (□), 30 Hz (△), and 60 Hz (◇) of rotor speeds. Closed symbols are loss modulus (G'') values at ● (0 Hz), ■ (15 Hz), ▲ (30 Hz), and ◆ (60 Hz) of rotor speeds. Error bars indicate SE.

cial GY. The increase in WHC of yogurts subjected to cavitation suggests the incorporation of moisture as finely dispersed molecules into the protein matrix and is not expelled by centrifugal forces employed in this experiment. Other potential causes for a lower WHC of commercial GY as compared with a cavitated yogurt could include differences in starter culture strains and heat treatment history of milk used in its manufacture.

DISCUSSION

Greek-style yogurts containing high-protein dairy ingredients manufactured by the application of membrane filtration processes to milk tend to differ in rheological properties as compared with GY manufactured from skim milk and subjected to the process of dewatering. Meletharayil et al. (2015) reported that high protein acid gels manufactured from reconstituted MPC powders of varying protein content showed variances in gelation times, gelation pH, and final G' values. This was mainly attributed to differences in protein interactions on heating between whey proteins and caseins owing to the altered ionic environment and Ca²⁺ ion activities of MPC dispersions. The MPC and TMPC powders

used for fortification of milk in the manufacture of GSY had significant differences in mineral profiles owing to differences in the method of manufacture. The process of CO₂ injection during the manufacturing of TMPC lowers the pH of milk before and during the process of ultrafiltration/diafiltration to 5.9. At this pH, a partial solubilization of phosphates and calcium (Dalglish and Law, 1989) partitions it to the permeate stream. The TMPC powder had a 28% reduction in mineral content and a 33% reduction in calcium content when compared with control MPC powder. The reduction in acidification times, as observed for GSY containing TMPC, can be correlated with lower mineral content of TMPC powders. Lower phosphates, citrate, and calcium in TMPC powders when compared with control MPC powders reduce the buffering of the GSY base. During the acidification of milk, a maximum in buffering capacity is observed around pH 5.1 and is reported to be the pH of maximum MCP solubilization (Lucey et al., 1993). Figure 1 shows a steady fall in pH from 5.1 to 4.6 over time for GSY containing TMPC, whereas a plateau was observed during the acidification of GSY containing MPC. This can be associated with decreased calcium levels in TMPC and removal of MCP during

Figure 4. Frequency sweep for Greek-style yogurt (9% wt/wt, protein) containing milk protein concentrate at 10°C and in the linear visco-elastic region determined by stress sweep experiments. Open symbols are elastic modulus (G') values at 0 Hz ($\circ$), 15 Hz ($\square$), 30 Hz ($\triangle$), and 60 Hz ($\diamond$) of rotor speeds. Closed symbols are loss modulus (G'') values at $\bullet$ (0 Hz), $\blacksquare$ (15 Hz), $\blacktriangle$ (30 Hz), and $\blacklozenge$ (60 Hz) of rotor speeds. Error bars indicate SE.

Figure 5. Hardness ($\square$), firmness ($\text{---}\bullet\text{---}$), and adhesiveness values of Greek-style yogurt (9% wt/wt, protein) containing CO₂-treated milk protein concentrate (TMPC) subjected to hydrodynamic cavitation at rotor speeds of 0, 15, 30, and 60 Hz. Also shown in the figure are the hardness, firmness, and adhesiveness values obtained on a commercial strained Greek yogurt. Error bars indicate SE.

the process of TMPC manufacture. The lower buffering observed in GSY containing TMPC was reflected in lower TA values. The TA expressed as percentage lactic acid using the phenolphthalein end point measures the buffering capacity of yogurts to pH $\sim$ 8.33 (Walstra et al., 2005). The lower amount of lactic acid produced to reach a pH of 4.6 coupled with lower amounts of phosphates and citrates in GSY containing TMPC yielded a product with a lower acidity when compared with GSY containing MPC. Commercial GY had acidity values similar to GSY containing TMPC on account of the removal of lactic acid and minerals during the process of dewheyng.

The collapse of cavities during the process of HC leads to the generation of shear forces. Cavitation intensity, which relates to the number of cavitation events occurring during the process of HC, can have a profound influence on the physical and chemical changes in a medium. Previous work by Badve et al. (2015) reported that in HC brought about by a stator-rotor assembly design (a similar design was used in our study), the cavitation intensity was directly proportional to rotor speeds. Increase in cavitation intensities resulted in physicochemical changes of GSY. The increase in WHC of GSY with an increase in rotor speeds related to the reported increase in the hydration of biopolymers in food subjected to HC (Mawson and Knoerzer, 2007).

Literature also cites altered protein conformation and structure when subjected to high-intensity ultrasonication leading to increased protein solubility and hydration (Moulton and Wang, 1982; Morel et al., 2000). The breakdown of gel structure was evident in steady shear rate sweeps of GSY. The consistency coefficient values κ and η_{app} values of GSY were lowest at maximum rotor speeds. GSY had greater shear thinning behavior as compared with commercial GY across the shear rates applied in the study indicating a lower tolerance to shear. A similar trend was reported by Bong and Moraru (2014) in GSY manufactured from milk fortified with micellar casein concentrates. A possible explanation could be the high gelation pH of dispersions containing MPC as protein sources. Meletharayil et al. (2015) reported an increase in gelation pH and decrease in gelation times when high protein acid gels were manufactured from reconstituted MPC powders. Gelation at a higher pH (>5.5) could exclude whey proteins from the acid gel network (isoelectric point, pI, $\sim$ 5.3) leading to a gel network of hydrophobically associated caseins that would shear on the application of low amounts of stress. A similar phenomenon may also be occurring in acid gels made from unheated milk that are reported to have a lower shear tolerance and elastic modulus owing to the exclusion of whey proteins in the formation of the acid gel structure (Anema et al.,

Figure 6. Hardness ($\square$), firmness ($\text{—}\bullet\text{—}$), and adhesiveness values of Greek-style yogurt (9% wt/wt, protein) containing milk protein concentrate (MPC) subjected to hydrodynamic cavitation at rotor speeds of 0, 15, 30, and 60 Hz. Also shown in the figure are the hardness, firmness, and adhesiveness values obtained on a commercial strained Greek yogurt. Error bars indicate SE.

Figure 7. Representative stereomicroscopic images of grains in Greek-style yogurts (9% wt/wt, protein) containing CO₂-treated milk protein concentrate and subjected to cavitation at rotor speeds of 0, 15, 30, and 60 Hz. Grains appear as dark objects and are further analyzed using ImageJ software (US National Institutes of Health, Bethesda, MD).

2005). On the contrary, GY from skim milk (employing the dewheyng step) have a gelation pH of ~ 5.1 and would include in the gel network caseins cross-linked by whey proteins leading to a gel with greater amounts shear tolerance (Lucey et al., 1997).

The GSY containing TMPC had lower κ values as compared with the GSY containing MPC. A decrease in MCP in GSY containing TMPC could influence the extent of casein crosslinking during acid gelation leading to a lower number and strength of bonds that could influence κ values. A similar correlation was observed

for the frequency dependence of G' values with GSY yogurts containing TMPC having a lower G' when cavitated at higher rotor speeds than GSY containing MPC (Figures 3 and 4). Commercial GY showed a frequency dependence of G' similar to those of noncavitated GSY (slope of G' and frequency curve of ~ 0.16 for commercial GY and noncavitated GSY). With increasing rotor speeds, an increase in the cavitation intensity may lead to the breakdown of nonrelaxing bonds that contribute to G' in GSY. Weaker intermicellar casein crosslinking in GSY containing TMPC owing to a lower

MCP content may have resulted in a greater decline in G' when subjected to the forces of cavitation. A similar correlation was seen in the penetration tests where GSY containing TMPC had a lower hardness, firmness, and cohesiveness values than GSY with MPC across the rotor speeds applied in the experiment (Figures 5 and 6). On HC, acid gels have decreased ability to resist compressive and shear forces due to the breakdown of the bonds. A decrease in adhesiveness values of GSY with increasing cavitation intensity can be correlated with improved mouthfeel during its consumption and is

generally not associated with GSY fortified with milk protein powders (Desai et al., 2013).

Subjecting GSY to HC decreased the number of grains thought to be formed as a result of local stresses developed during the gelation process (Lucey et al., 1997). These grains could serve as nuclei for the cavitation bubble to form, grow, and explode, thus releasing shear forces resulting in their breakdown (Figures 7 and 8). Overall, in the current set of experiments employed for the manufacture of GSY, the use of TMPC as a protein source in conjunction with HC could help achieve

Figure 8. Representative stereomicroscopic images of grains in Greek-style yogurts (9% wt/wt, protein) containing milk protein concentrate and subjected to cavitation at rotor speeds of 0, 15, 30, and 60 Hz. Grains appear as dark objects and are further analyzed using ImageJ software (US National Institutes of Health, Bethesda, MD).

Figure 9. Representative stereomicroscopic image of grains in commercial Greek yogurt (9% wt/wt, protein). Grains appear as dark objects and are further analyzed using ImageJ software (US National Institutes of Health, Bethesda, MD).

comparable TA values, rheological properties, and microstructure to that of a commercial strained GY.

CONCLUSIONS

The use of CO₂-treated MPC in conjunction with hydrodynamic cavitation can be used to control the increased viscosity, firmness, and acidity that is encountered in the manufacture of GSY containing added milk protein ingredients. An increase in cavitation intensities brought about by an increase in rotor speed led to a decrease in the frequency dependence of G' , indicating a breakdown of protein interactions. A decrease in the buffering capacities of the GSY base achieved by the removal of minerals using a combination of CO₂ treatment in tandem with UF and diafiltration led to comparable acidity values of GSY and GY at pH 4.6. Identical hardness, firmness, and adhesiveness values were observed between a commercial GY and GSY containing TMPC subjected to cavitation. Similar microstructure was also observed between commercial GY and GSY subjected to cavitation. The ability to scale up the process of hydrodynamic cavitation industrially

Figure 10. Water-holding capacities (WHC) of Greek-style yogurt (9% wt/wt, protein) containing CO₂ treated milk protein concentrate subjected to hydrodynamic cavitation at rotor speeds of 0, 15, 30, and 60 Hz. Also shown in the figure is the WHC of a commercial strained Greek yogurt. Error bars indicate SE.

and the ease of controlling the event of cavitation that can influence final textural properties of the product make this technology promising for large-scale industrial applications. The process used in this study could be a solution to the vexing problem of acid whey disposal faced by the dairy companies manufacturing GY.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of the Midwest Dairy Foods Research Center (Brookings, SD), the Agricultural Utilization Research Institute (St. Paul, MN), and the Agricultural Experiment Station (South Dakota State University; HATCH3ADP12-328100).

Figure 11. Water-holding capacities (WHC) of Greek-style yogurt (9% wt/wt, protein) containing milk protein concentrate subjected to hydrodynamic cavitation at rotor speeds of 0, 15, 30, and 60 Hz. Also shown in the figure is the WHC of a commercial strained Greek yogurt. Error bars indicate SE.

REFERENCES

- Anema, S. G., S. Lauber, S. K. Lee, T. Henle, and H. Klostermeyer. 2005. Rheological properties of acid gels prepared from pressure- and transglutaminase-treated skim milk. *Food Hydrocoll.* 19:879–887.
- Badve, M. P., T. Alpar, A. B. Pandit, P. R. Gogate, and L. Csoka. 2015. Modeling the shear rate and pressure drop in a hydrodynamic cavitation reactor with experimental validation based on KI decomposition studies. *Ultrason. Sonochem.* 22:272–277.
- Bong, D. D., and C. I. Moraru. 2014. Use of micellar casein concentrate for Greek-style yogurt manufacturing. Effect on processing and product properties. *J. Dairy Sci.* 97:1259–1269.
- Dagleish, D. G., and A. J. R. Law. 1989. pH-induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. *J. Dairy Res.* 56:727–735.
- Desai, N. T., L. Shepard, and M. A. Drake. 2013. Sensory properties and drivers of liking for Greek yogurts. *J. Dairy Sci.* 96:7454–7466.
- Earnshaw, R. G. 1998. Ultrasound: A new opportunity for food preservation. Pages 183–192 in *Ultrasound in Food Processing*, M. J. W. Povey and T. J. Mason, ed. Blackie Academic & Professional, London, UK.
- Gogate, P. R., and G. S. Bhosale. 2013. Comparison of effectiveness of acoustic and hydrodynamic cavitation in combined treatment schemes for degradation of dye wastewaters. *Chemical Engineering and Processing: Process Intensification* 71:59–69.
- Lucey, J. A., B. Hauth, C. Gorry, and P. F. Fox. 1993. The acid-base buffering properties of milk. *Milchwissenschaft* 48:268–272.
- Lucey, J. A., C. T. Teo, P. A. Munro, and H. Singh. 1997. Rheological properties at small (dynamic) and large (yield) deformations of acid gels made from heated milk. *J. Dairy Res.* 64:591–600.
- Marella, C., P. Salunke, A. C. Biswas, A. Kommineni, and L. E. Metzger. 2015. Manufacture of modified milk protein concentrate utilizing injection of carbon dioxide. *J. Dairy Sci.* 98:3577–3589.
- Mawson, R., and K. Knoerzer. 2007. A brief history of the application of ultrasonics in food processing. 19th ICA Congress, Madrid, 2007. Accessed Aug. 18, 2015. http://www.sea-acustica.es/WEB_ICA_07/fchrs/papers/ult-09-004.pdf.
- Meletharayil, G. H., H. A. Patel, and T. Huppertz. 2015. Rheological properties and microstructure of high protein acid gels prepared from reconstituted milk protein concentrate powders of different protein content. *Int. Dairy J.* 47:64–71.
- Morel, M. H., P. Dehlon, J. C. Autran, J. P. Leygue, and C. Bar-L'Helgouach. 2000. Effects of temperature, sonication time, and power settings on size distribution and extractability of total wheat flour proteins as determined by size-exclusion high-performance liquid chromatography. *Cereal Chem.* 77:685–691.
- Moulton, K. J., and L. C. Wang. 1982. A pilot-plant study of continuous ultrasonic extraction of soybean protein. *J. Food Sci.* 47:1127–1129.
- Ozer, B. H., R. K. Robinson, A. S. Grandison, and A. E. Bell. 1997. Comparison of techniques for measuring the rheological properties of Labneh (concentrated yogurt). *Int. Dairy J.* 50:129–133.
- Remeuf, F., S. Mohammed, I. Sodini, and J. P. Tissier. 2003. Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt. *Int. Dairy J.* 13:773–782.
- Roefs, S. P. F. M., P. Walstra, D. G. Dagleish, and D. S. Horne. 1985. Preliminary note on the change in casein micelles caused by acidification (skim milk). *Neth. Milk Dairy J.* 39:119–122.
- USDA. 2014. Dairy products: Per capita consumption, United States (Annual). Accessed Aug. 18, 2015. <http://www.ers.usda.gov/data-products/dairy-data.aspx>.
- Walstra, P., J. T. Wouters, and T. J. Geurts. 2005. *Dairy Science and Technology*. CRC Press, Boca Raton, FL.