Title: Effect of sonication on the viscosity of reconstituted skim milk powder and milk protein concentrate as influenced by solids concentration, temperature and sonication.

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Abstract

Skim milk powder (SMP) and milk protein concentrates (MPCs) are manufactured by evaporation followed by spray drying and are widely used as functional and nutritional ingredients. This study investigated the effects of temperature (40 to 60 °C) and total solids content (TS) on the viscosity of reconstituted MPC (rMPC) (≥30 % TS) and SMP (rSMP) (≥46 % TS) in laboratory conditions. Additionally, the influence of sonication in batch (70 % amplitude) and flow through systems (90% amplitude) was studied in a laboratory setting. The viscosity increased for all treatments with an increase in TS and decreased with an increase in temperatures. Overall, sonication in both batch (30 s) and flow through systems (10.1, 20.2, and 30.20 s) resulted in significant decreases in viscosity for both rSMP and rMPC. An increase in

viscosity was observed after post- sonication circulation; however, the viscosity did not return to the pre-sonication values.

1. Introduction

Skim milk powder (SMP) is a dried form of pasteurized skim milk that contains ≤ 5% moisture and ≤ 1.5% milkfat (by weight) (Smith, 2008). SMP has a standardized milk protein content of 34% as opposed to nonfat dry milk (NFDM), which does not (Smith, 2008). Milk protein concentrates (MPCs) are high-quality protein products that have various roles in terms of functionality and nutrition. MPCs and SMPs are complete proteins that contain both casein and whey proteins as opposed to whey protein concentrates (WPC) or isolates (WPI). In comparison with SMP, MPCs are higher in protein (42-85%) and lower in lactose (4-46 % depending on protein content) and minerals (6-7%) (Agarwal, Beausire, Patel, & Patel, 2015; Patel & Patel, 2014) .

SMP is standardized to 34 % protein by using either the milk retentate or permeate from ultrafiltration (UF) of milk. SMP is manufactured using pasteurized skim milk that is concentrated using evaporation followed by spray drying (Smith, 2008). MPC's are generally produced using skim milk, which is concentrated by UF. UF results in segregation of caseins, whey proteins, micellar salts, and residual fat in the retentate, whereas lactose, soluble salts, and non-protein nitrogen are removed with the permeate (Bastian, Collinge, & Ernstrom, 1991). Diafiltration (DF) is commonly applied to remove residual lactose and soluble minerals and to obtain a product with a high protein content (Patel & Patel, 2014). MPC is produced by further concentration of this UF retentate using evaporation followed by spray drying.

MPCs provide a range of functionalities such as water binding, viscosity, gelling, foaming/whipping, emulsification, and heat stability and are used in many protein-fortified foods but primarily in meal replacements, nutritional beverages and bars (Agarwal et al., 2015; Patel & Patel, 2014). MPCs, due to their lower lactose content, can impart a clean dairy flavor with reduced Maillard browning. Apart from serving as an excellent substitute for milk, SMP can be used in infant formulas, nutritional products for children, and fortification of dairy products along with serving as a functional ingredient in bakery products, snacks, and chocolate confectionaries (Lagrange, Whitsett, & Burris, 2015).

Processing of both SMP and MPC involves evaporation and spray drying which are both high heat treatments. It would be economical to obtain a solution of higher % total solids (TS) prior to spray drying. However, it is difficult to do so because an increase in viscosity is seen with a high solids content (Fernández-Martín, 1972; O'Donnell & Butler, 2008). An increase in viscosity poses a problem in the dairy processing industry since it leads to reduced flow rates, high pressure drops, decreased turbulence (lower rate of heat transfer), and severe fouling in heating operations. The production of concentrated skim milk, which is used in the production of both SMP and MPC, is limited to approximately 50% TS since large increases in viscosity are observed at TS $\geq$ 45 % (Enríquez-Fernández, Camarillo-Rojas, & Vélez-Ruiz, 2013). Fluid milk with $\geq$ 45 % concentration is difficult to atomize due to increase in apparent viscosity that leads to large droplets being formed in the atomizer; thus, decreasing the thermal efficiency of the spray dryer (Enríquez-Fernández et al., 2013; Zisu, Schleyer, & Chandrapala, 2013). Additionally, the viscosity of concentrated skim milk increases with time in a process called "age thickening" which is a result of structural build via noncovalent interactions between casein micelles (Bienvenue, Jiménez-Flores, & Singh, 2003).

A reduction in viscosity of skim milk concentrate and reconstituted whey protein powders was seen upon application of high power, low frequency ultrasound (Zisu, Bhaskaracharya, Kentish, & Ashokkumar, 2010; Zisu et al., 2013; Ashokkumar, Zisu, Bhaskarcharya, Palmer, & Kentish, 2009; Yanjun et al., 2014). Ultrasound is sound waves that have a frequency of greater than 20 kHz and produced using a sonication power source (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). Acoustic cavitation is a phenomenon where passing of these sound waves through a liquid causes the formation of alternating high-pressure (compression) and low-pressure (rarefaction) cycles. During rarefaction, small vacuum bubbles or voids form which increase in size during every compression and rarefaction cycle. These bubbles reach a volume at which no additional energy can be absorbed during the compression cycle, leading to bubble collapse. As a result of cavitation and bubble collapse, very high temperatures (≈5,000 K) and pressures (≈2,000 atm) are reached locally (Zisu et al., 2013).

Different applications of ultrasound are being explored or implemented in food processing for emulsification, filtration, viscosity modification, improvement of whey protein heat stability, improvement of meat tenderness, and inactivation of spoilage microbes (Chandrapala et al., 2012; Chemat, Zill-E-Huma, & Khan, 2011; Knorr, Zenker, Heinz, & Lee, 2004). Sonication has caught the interest of the food industry due to its potential to alter the functionality of foods as well as improve the shelf life and quality (Chandrapala et al., 2012; Knorr et al., 2004). Very few studies have investigated the influence of sonication on the viscosity of concentrated milk.

This study investigated the effects of total solids (TS) and temperature on the viscosity of reconstituted milk protein concentrates (rMPC) and reconstituted skim milk powder (rSMP). Additionally, the influence of sonication on the viscosity of rMPC and rSMP at different TS and

92 temperatures was investigated using both batch and flow-through sonication systems in a
93 laboratory setting. Although there have been other studies that investigated the effects of
94 sonication in dairy systems (Zisu, et al., 2010; Zisu et al., 2013; Ashokkumar et al., 2009;
95 Yanjun et al., 2014) there are no published studies that investigated the effects of batch and
96 continuous sonication on reconstituted MPC and SMP at different solids (30-44% TS MPC, and
97 46-64% TS SMP) and at different temperatures (40 to 60 °C).
98 The temperatures and TSs used for this study were an attempt to mimic the conditions
99 used during the processing of milk concentrates. Since concentrated skim milk is evaporated at
100 temperatures between 50-70 °C, rMPC and rSMP were treated at 40, 50, and 60 °C to investigate
101 the influence of temperature on the viscosity (Singh, 2007). Also, MPC and SMP are evaporated
102 to obtain solids contents of about 30 and 50 % solids respectively, prior to spray drying (Agarwal
103 et al., 2015). Using this rationale, the TS used for this research was ≥30 % TS for rMPC and ≥ 46
104 % TS for rSMP.
105
106 **2. Materials and methods**
107 *2.1 Experimental design*
108 For the first part of the study, the effect of temperature and TS on the viscosity of rMPC
109 samples with TS of 30-44 % and rSMP samples with TS of 46-64 % was evaluated at 40, 50, and
110 60 ºC. For the second part of the study, the effect of temperature, batch sonication (versus no
111 sonication), and TS on the viscosity of rMPC samples with TS of 30-44 % and rSMP samples
112 with TS of 46-64 % were evaluated at 40, 50, and 60 ºC. For the third part of the study, the effect
113 of temperature, flow through sonication (versus no sonication), and TS on the viscosity of rMPC

114 samples with TS of 30-34 % and rSMP samples with TS of 50-54 % were evaluated at 60 ºC. All
115 experiments were replicated 3 times and analyzed in triplicate.
116
117 *2.2 Sample preparation*
118 MPC 70 (Darigold, Seattle, Washington, USA, low heat) evaporated to 32 % TS and low
119 heat SMP (Darigold, Seattle, Washington, USA; High Dessert Milk, Burley, Idaho, USA)
120 evaporated to 45 % TS before spray drying were used. Powders were stored at temperatures
121 between 20 and 25°C. The moisture content of the powders stated as 5.25 ± 0.10 for MPC and
122 4.37 ± 0.28 for SMP and this was confirmed using a Moisture Analyzer (Sartorius AG MA 150,
123 Göttingen, Germany). The moisture content of the powders was monitored over the time frame
124 of the experiments and no changes were observed. Thus, any changes in solubility as a result of
125 prior storage history are assumed to be consistent in all samples and did not contribute to
126 significant changes in viscosity.
127 Both rMPC and rSMP of known TS were made by weighing the solute (MPC or SMP)
128 with the addition of distilled water to make 400 ml solutions. Distilled water was heated to the
129 required temperature (40, 50, or 60 ºC) before being added to the solute. This mixture was
130 blended with a high shear blender (Ultra-Turrax with S25N-18G 10 dispersion tool) for 15 min
131 and kept in a water bath for 30 min at temperatures between 45 and 65 °C depending on the
132 experiment to be performed to maximize solubility. The TS content of samples was determined
133 using a Moisture Analyzer.
134 Overnight rehydration time was not possible in a laboratory setting at the % TS (30- 44 %
135 for MPC and 46- 64 % for SMP) we worked with because the samples would show age
136 thickening and would render viscosity measurements invalid. However, we do believe the steps

137 taken (mixing with a high-speed rotor blender and high water temperature) during sample
138 preparation were sufficient to achieve almost complete rehydration such that the powders were in
139 solution.

140 *2.3 Sonication treatment*

141 For batch sonication, a 30 ml sample of reconstituted concentrated milk was placed in a
142 double walled glass vessel (50 ml) at a constant temperature and sonicated at 70 % amplitude for
143 30 seconds using a 12.7 mm microtip and a Sonicator power source (QSonica Q500, Newtown,
144 CT, USA). A circulating water bath was used to maintain the appropriate sample temperature
145 (40, 50 or 60 °C). The viscosity of the samples was measured before and after sonication as
146 described below.

147 To simulate continuous operation, samples were reconstituted as described above and
148 pumped using a Masterflex 7529 pump (Cole-Palmer, Vernon Hills, IL, USA) at a flow rate of
149 1.8 L min^{-1} for a total of 60 min and 15 min for rMPC and rSMP, respectively before being
150 sonicated and a sample was collected at this time point. The sonication flow cell had a 261 mL
151 volume resulting in a 8.4 sec residence time (time sample was exposed to sonication) for the 1.8
152 L min^{-1} flow rate. For flow through sonication, the total volume of rMPC or rSMP used was 3 L
153 and the samples were recirculated through the system. Samples were collected for viscosity
154 measurements at 2, 4, and 6 min, which corresponded to total sonication residence times of 10.1,
155 20.2, and 30.2 s. For the continuous operation, rSMP and rMPC were sonicated (Heischler
156 UIP500hd, Ringwood, NJ, USA) at 90 % amplitude. Samples were recirculated through the flow
157 through system post sonication and samples were collected for viscosity measurements at 45 min
158 for rMPC and 30 min for rSMP. Schematics of the sonication systems is shown in Supplemental
159 Fig 1A. As shown in Figure 1A, two water baths were used. One water bath was to maintain the

sample temperature at 60°C. The stainless steel flow cell had a water jacket and was connected to the other water bath to maintain the temperature of the sample during sonication at 60°C.

The energy density (J/ml) for the samples sonicated in the batch and flow though system was calculated according to Chandrapala, Martin, Kentish, & Ashokkumar, (2014). The power readings ranged from 190-192 W in the flow through system. An average of 191 W was used and the calculated energy density at 10.0, 20.2, and 30.2 s of residence time was 0.64 J/ml, 1.28 J/ml, and 1.92 J/ml. The power readings for the batch sonication were an average of 63 W so the energy density for batch sonication was 63 J/ml with a 30 ml sample volume and 30 s sonication time.

2.4 Viscosity measurement

The apparent viscosity was measured for all samples using a viscometer (Fungilab-Expert series, Hauppauge, New York, USA) and a rheometer (AR-G2, TA Instruments, New Castle, DE) equipped with a concentric cylinder geometry. Viscometer spindles TL 5, 6, and 7 were used at the highest rpm's (10-200 rpm) attainable for that sample with type of spindle used to obtain a % torque between 20-100%. Measurements were taken at the three highest rpm's attainable and a mean of the viscosity values was calculated to be used for further analysis. For rheometer viscosity measurements, a steady state flow procedure was used to measure the viscosity as a function of shear rate (1×10^{-4}–300 s^{-1}) for both rMPC and rSMP and the mean of the viscosity at a steady state (highest shear rates) was recorded. Data from the viscometer were compared to that of the rheometer (for the solids and temperature experiments only). The viscosity measured was reported in Pa.s.

183 *2.5 Statistical analysis*

184 ANOVA and t-tests were performed to test for statistical significance (α=0.05) using SAS 9.4
185 and Excel statistics. Statistical significance of differences between viscosity measurements were
186 tested using t-tests. ANOVA was used to determine if solids and temperature have a combined
187 effect on the viscosity of rSMP and rMPC at the given temperature and TS parameters. For
188 ANOVA, the data obtained for both rMPC and rSMP was transformed to get a greater normal
189 distribution. rMPC was transformed using the logarithmic function and rSMP was transformed
190 using the square root function. ANOVA was performed using a complete block design for both
191 rSMP (46, 50, and 54 % TS) and rMPC (30, 32, 34, and 36 %TS) treated at 40, 50, and 60 °C.
192

193 **3. Results and discussion**

194 *3.1. Effect of solids and temperature*

195 Effect of solids and temperature on the viscosity of rMPC and rSMP can be seen in
196 Figure 1. The viscosity measurements with a viscometer when compared to that of rheometer
197 were not significantly different (data shown in Supplemental Fig A2), therefore viscometer
198 measurements are given. Since rMPC and rSMP are commonly evaporated at temperatures
199 between 50-70 °C to a TS of 30 and 50 %, respectively (Agarwal et al., 2015; Singh, 2007),
200 rMPC and rSMP were reconstituted at ≥ 30% and ≥ 46 % TS, respectively and treated at 40, 50,
201 and 60 °C. Overall, there was an increase in viscosity with an increase in solids content at each
202 temperature tested, for both rMPC and rSMP. For both rMPC and rSMP, the increase in viscosity
203 at 60 °C was linear initially and was exponential at ≥ 42 and ≥ 60 %TS, respectively. However,
204 the overall increase in viscosity was exponential in all other rSMP treatments while the viscosity
205 increase was linear at 40°C and exponential at 50°C in rMPC (with linear or exponential

206 regression $R^2 > 0.94$). For all TS, the 60 °C samples showed the lowest viscosity followed by 50
207 °C then 40 °C.

208 From ANOVA of rMPC and rSMP (Table 1), the effects of TS, temperature and their
209 interactions were statistically significant, indicating that both TS and temperature have a
210 combined effect on the viscosity of rMPC and rSMP. ANOVA determined the significant
211 variables with the largest effect for rMPC as temperature, followed by TS, and the interaction.
212 And for rSMP, the largest effect was TS followed by temperature, and the interaction.

213 When comparing Figures 1 A and 1 B, the Y axis of rSMP (Figure 1 B) is ten times
214 greater than that of rMPC (Figure 1 A). However, it should be noted that rMPCs are treated at
215 relatively lower TS as compared to rSMP in this study. At 50 °C, the viscosity of 44 % TS rMPC
216 was 0.6 Pa s, and that of a 46 % TS rSMP was 0.09 Pa s. Also, at 60 °C, the viscosity of 44 % TS
217 rMPC was 0.3 Pa s, and that of a 46 % TS rSMP was 0.07 Pa s. Hence, it can be said that at same
218 temperatures and approximately the same TS, rMPC has a higher viscosity as compared to
219 rSMP. This may be attributed to the higher protein content of rMPC. Moreover, rSMP thickened
220 with aging faster than rMPC.

221 With rMPC, a significant % increase in viscosity was observed at each TS (30, 32, 34,
222 and 36 %) at 40 °C and 50 °C as compared to 60 °C (Table 2). For rMPC, the greatest % increase
223 in viscosity (784.3%) was observed at 36% TS at 40 °C. For rMPC, the % increase at 40 °C as
224 compared to 60 °C was 304.2, 489.4, 513.9, and 784.3 % at 30, 32, 34, and 36 % TS,
225 respectively. rMPC at 50 °C showed a % increase of 228.9, 194.3, 197.2, and 215.9 %,
226 respectively at 30, 32, 34, and 36 % TS, as compared to 60 °C. The % increase of 36 % TS
227 rMPC at 40 °C, was approximately 3, 2, and 1.5 times higher when compared to 30, 32, and 34
228 % TS. At 50 °C, the increase in viscosity as compared to 60 °C was relatively proportional in

terms of TS. This implies that temperature had a greater effect than TS for the viscosity increases observed in rMPC within the ranges tested.

With rSMP, a significant % increase in viscosity was observed at each TS (46, 50, and 54 %) at 40 °C and 50 °C as compared to 60 °C (Table 2). For rSMP, the % increase in viscosity at 54 % TS at 40 °C and 50 °C was extreme (2446.2 and 1147.2 respectively) as compared to 60 °C. The % increase in viscosity at 46 % and 50 % TS at 40 °C and 50 °C compared to 60 °C was significant, but not as extreme, with values being 40. 5 and 24.5 %, respectively for 46 % TS, and 64.5 and 37.8 %, respectively for 50 % TS. At 40 °C, the % increase for 54 % TS rSMP was 61 and 38 times higher than at 46 and 50 % TS, respectively. Also at 50 °C the % increase for 54 % TS rSMP was 47 and 30 times higher than at 46 and 50 % TS, respectively. This implies that TS had a greater effect than temperature on the viscosity of rSMP within the ranges tested.

The increase in viscosity with increase in solids content and the decrease in viscosity with an increase in temperature seen with rMPC and rSMP was similar to the effect of temperature and solids content observed in skim milk by Fernández-Martin (1972) and in rMPC by O'Donnell and Butler (2008). However, for rSMP, temperatures ≤ 40 °C had a more dramatic effect on the viscosity as compared to temperatures greater than 40 °C, at ≤ 30 % TS (Fernández-Martin 1972). A similar trend was seen in this study with rSMP, where the increase in viscosity was exponential for all rSMP treatments while the viscosity increase was linear at 40 °C in rMPC.

For rMPC, the greatest % increase in viscosity (784.3 %) was observed at 36 % TS at 40 °C and that for rSMP was observed at 54 % TS at 40 °C. The viscosity of rSMP (0.14 Pa s) measured in this experiment was lower than the viscosity of a skim milk concentrate from an

evaporator (0.40 Pa s) measured by Zisu et al., (2013), when both had a 50 % TS concentration and treated at 50 °C.

In milk, at solids content of ≥ 40 %, the viscosity increases in a nonlinear manner with an increase in total solids content, which is similar to the exponential increase in viscosity at high solids seen in this study. In skim milk, an increase in solids content is accompanied by reduction in the volume fraction of water which in turn causes an increase in volume fraction of dispersed particles and the micelle-micelle interactions as the distance between the micelles becomes smaller (Bienvenue et al., 2003). Thus, the increase in viscosity seen with increase in solids content is due to increased intermolecular interactions between proteins. The decrease in viscosity with an increase in temperature has been attributed to a possible decrease in protein-protein interactions and an increase in protein-water interactions (Fernández-Martín, 1972; Herceg and Lelas, 2005).

During spray drying of milk powders, the temperature of the milk droplet does not exceed 70 °C and the powders are heated only for a few seconds, thus very minimal changes are observed in the behavior of milk components post spray drying when compared to the pre-drying concentrate (Singh, 2007). However, both evaporation and spray drying alter the soluble salt equilibrium of milk where a decrease in the solubility of calcium and phosphate is seen.

Previous research has shown that rehydration of milk powders is a function of dissolution (solubility) and mineral equilibration and is influenced by spray drying heat treatment, powder storage time and temperature (Anema, Pinder, Hunter, & Hemar, 2006), and rehydration temperature, times and shear (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2009; Chandrapala et al., 2014; Martin, Williams, Choong, Lee, & Dunstan 2008; Martin, Williams, & Dunstan, 2010). Low heat SMP is rapidly dissolved with just vigorous shaking at room

274 temperature for 20 s (Martin et al., 2008). This is not to state that a mineral equilibrium was
275 reached, but the sample is in solution. In contrast, MPC is known for having a low solubility.
276 The complete rehydration of milk powders is a result of two processes that occur simultaneously.
277 Dissolution of powder particles in the solvent and the transfer of water to the core of the powder
278 particles. Sikand, Tong, Roy, Rodriguez-Saona, & Murray (2011) found that the reason for low
279 solubility of high protein MPC's is due to decreased rate of water transfer to the core of the
280 protein particles. Mimouni et al. (2009) concluded that the rate limiting step in the compete
281 rehydration process of MPC 85 was the dissolution rate. They showed that there was a large
282 acceleration in rehydration of MPC85 with an increase in temperature from 24 to 35 ° C. In
283 addition Martin et al. (2010) showed that MPC 80 could be rapidly solubilized with vigorous
284 shaking followed by heating at 60 C for 5 min. Chandrapala et al. (2014) showed that a 10%
285 w/w solution of MPC 80 achieved dissolution at 90-95% using high shear for less than 10 min.
286 We used 15 min of high shear at temperatures greater than 40 ° C on the reconstitution of our
287 samples, therefore the rSMP and rMPC samples may not have been 100% soluble prior to
288 sonication so the decrease in viscosity may also be due to an increase in solubility as a result of
289 sonication as well as the disruption of protein aggregates.
290 *3.2. Effect of batch sonication*
291 Effect of sonication on the viscosity of rMPC and rSMP at 40 °C, 50 °C, and 60 °C in a
292 batch sonication system are displayed in Figures 2 and 3, respectively. Overall, there was a
293 decrease in viscosity after sonication for both rMPC and rSMP. An overall greater % decrease in
294 viscosity due to batch sonication was seen with an increase in % TS for rMPC. For rMPC, the %
295 decrease in viscosity as a result of batch sonication was greater at 50 °C, followed by 40 then

60°C. We were unable to determine the effects of sonication at %TS > 36 at 40 °C because the sample was too viscous.

In the case of rSMP, the highest values for % decrease in viscosity were seen at 54, 60 and 64 % TS at 60°C for batch sonication. We were unable to determine the effects of sonication at TS > 52 % at 40 and 50 °C as the samples were too viscous. Zisu et al., (2013) reported a 10% reduction in viscosity when skim milk concentrate was sonicated for a total of 1 min at 55 °C and at 50 % TS which is similar to the 22.1 % reduction seen in this study.

At 50 °C, the % decrease in viscosity of 44 % TS rMPC was 54.6 and that for a 46 % TS rSMP was 18.9. Also, at 60 °C, the % decrease in viscosity of 44 % TS rMPC was 44.3 and that for a 46 % TS rSMP was 19.2. Hence, it can be said that at same temperatures and approximately the same % TS, rMPC showed a higher reduction in viscosity as compared to rSMP in a batch sonication system. Samples were in solution prior to sonication, however, we do acknowledge that in a laboratory setting given our experimental parameters, 100 % solubility may not have been achieved. We believe the reduction in viscosity is majorly a result of breaking of protein aggregates due to sonication; however, an increase in solubility of reconstituted samples from sonication may have influenced the decrease in viscosity as well.

3.3 Effects of flow-through sonication

The effect of sonication on rMPC and rSMP at 60 °C in a flow-through recirculating sonication system is shown in Figure 4. Temperature and TS conditions were chosen to mimic the manufacturing conditions of SMP and MPC. For rSMP, % TS of ≥54 % in a continuous system required long heating times to form a continuous solution which resulted in age gelation of samples, therefore the highest TS used was 54. To achieve a steady state viscosity, rMPC was

run through the continuous system for 60 min. A steady state was determined by no change in viscosity. rSMP was run for a shorter time because an age thickening effect was observed when run for more than 15 min.

For rMPC, the decrease in viscosity with sonication is shown in Figure 4 A. When rMPC was run through the flow-through sonication system for 45 min after sonication, the decrease in viscosity was 33.2%, 17.2, and 10.3% for 30, 32, and 34% TS, respectively, as compared to pre-sonication. For rSMP, the decrease in viscosity with sonication is shown in Figure 4 B. When rSMP was run through the flow-through system for 30 min after sonication, the decrease in viscosity was 24.15, 4.0, and 11.5% for 50, 52, and 54% TS, respectively, as compared to pre-sonication.

Overall, there was an increase in viscosity with an increase in solids content and a decrease in viscosity with sonication for both rSMP and rMPC in the flow system, similar to the batch system. Sonication in a continuous flow-through system significantly decreased the viscosity of samples collected after sonication times of 10.1, 20.2, and 30.2 s as compared to the baseline prior to sonication (60 min for rMPC and 15 min for rSMP). For rMPC, the mean viscosity of the 34 % TS sample after 30.2 s residence time of sonication was lower than the mean viscosity of 30 % TS sample prior to sonication. Also, the mean viscosity at 34 % TS after 10.1 s of residence time of sonication was equivalent to that at 30 % TS prior to sonication. Therefore, if MPC is concentrated to 34 % TS via evaporation, only 10 s of sonication may be needed to obtain an equivalent viscosity as seen at 30 % TS. Furthermore, sonication of the 34 % TS rMPC for 30 s would yield a viscosity which was lower than that at 30 % TS pre-sonication values.

A similar effect was not seen for rSMP when looking at the viscosity changes between 50 and 54 % TS with sonication. It would take at least 30.2 s of sonication for the viscosity of 54% TS rSMP to be equivalent to the pre-sonication viscosity of the 52% TS rSMP. The differences in viscosity decrease for rSMP compared to rMPC may have been due to an immediate aging effect seen in the samples prior to the viscosity measurements. Depending on the flow-through sonication system, an increase in total sonication time to achieve a desired level of viscosity may be obtained by addition of multiple sonication flow cells in sequence in a processing facility. The sonication times used in flow (10.1 s) that resulted in a significant decrease in viscosity for rMPC are within a practical range.

With rMPC at 60 ° C, after 30 s of sonication, the % decrease in viscosity was greater for 30 and 32 % TS and lower for 34 % TS as compared to that seen in 30 s of batch sonication. Similarly, in the case of rSMP, the % decrease in viscosity after 30 s residence time in continuous sonication was greater for 46 and 50 % TS and lower for 54 % TS as compared to batch sonication.

In the flow-through system, a decrease in viscosity was seen after 10.1, 20.2, and 30.2 s of sonication respectively for both rMPC and rSMP as compared to pre-sonication observations for rSMP and rMPC, respectively (Figure 4). However, after 30 min (rSMP) and 45 min (rMPC) of post- sonication circulation through the continuous system, the viscosity increased but did not revert to the pre-sonication values.

Previous studies by Chandrapala et al., (2014), Yanjun et al., (2014), and Ashokkumar et al. (2009) have shown via particle size analysis of sonicated dairy systems that sonication breaks apart large aggregates leading to a decrease in particle size and a lower viscosity. Additionally, others (Martini et al., 2010) showed no change in whey protein sizes via SDS-PAGE after

sonication of a whey protein solution for 15 min at 60 C. Yanjun et al., (2014) did not observe protein degradation in MPC sonicated for up to 5 minutes via SDS-PAGE and Chandrapala et al., (2011) observed no changes in reverse-phase HPLC of whey samples sonicated for up to 60 min. These authors concluded that the physiochemical properties of casein micelles is unaffected by sonication and the viscosity reduction in dairy systems is primarily caused by the shear forces generated during acoustic cavitation, which disrupt noncovalent interactions (casein-casein and/or casein-whey protein interactions) forming aggregates (Zisu et al., 2010). After 30-45 min of recirculation post sonication, the increase in viscosity may be due to the ability of these non-covalent interactions to reform.

A similar effect of decrease in viscosity was observed by Zisu, Schleyer, and Chandrapala (2013), where high power low frequency ultrasound reduced the viscosity of skim milk concentrate in both batch and continuous processing. In their study, sonication could not prevent age thickening, however, sonication reduced the viscosity of the aged concentrate similar to that of the starting material. Aging of milk concentrates may be a result of either weakening of casein micelle interactions (Karlsson, Ipsen, Schrader, & Ardö, 2005) or flocculation of these micelles which may be due to loss of electrostatic repulsion during storage (Bienvenue et al., 2003).

4. Conclusion

From this study, it can be said that both TS and temperature significantly influence the viscosity of concentrated milk and can be used to modulate the viscosity of SMP and MPC concentrates. Overall, there was an increase in viscosity with an increase in solids content at each temperature tested, for both rSMP and rMPC. At the same temperatures and approximately the

same % TS, rMPC had a higher viscosity as compared to rSMP. This may be attributed to the higher protein content of rMPC. Moreover, temperature had a relatively greater effect on the viscosity for rMPC, while, for rSMP, TS had a greater effect on the viscosity

An overall greater % decrease in viscosity as a result of batch sonication was seen with an increase in TS for rMPC and rSMP. The % decrease in viscosity as a result of batch sonication ranged from 27.3- 54.6 % for rMPC and 18.7- 44.3 % for rSMP. Sonication in a flow through continuous operation significantly decreased the viscosity of samples collected after sonication times of 10.1, 20.2, and 30.2 s as compared to pre-sonication. An increase in viscosity was observed after post- sonication circulation; however, the viscosity did not return to the pre-sonication values.

We do acknowledge that the decrease in viscosity seen may be a result of increased solubility along with the disruption of protein aggregates due to sonication. Increased solubility of rMPC along with aging of rSMP may have led to the differences in decrease in viscosity of these two reconstituted concentrates. If MPC is concentrated to 34 % TS via evaporation, only 10 s of sonication may be needed to obtain an equivalent viscosity as seen at 30 % TS. Furthermore, sonication of the 34 % TS rMPC for 30 s yielded a viscosity, which was lower than that at 30 % TS pre-sonication values. For practical application of this research, this work needs to be repeated with fresh concentrates to determine whether the effect of sonication on the decrease in viscosity seen in this research is due to break down of aggregates or insolubility in the reconstituted samples or a combination of both. Moreover, the effect of sonication on transient aggregates formed during the process of concentration can also be studied.

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414

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496

Table 1. ANOVA for rMPC and rSMP samples when reconstituted at 30-36 % and 46-54% TS, respectively and treated at 40, 50, and 60 ° C.

Parameter	rMPC		rSMP	
	F statistic	P-value	F statistic	P-value
Total Solids	244.08	1.45×10^{-13}	1003.36	3.47×10^{-19}
Temperature	4679.22	3.58×10^{-21}	330.83	6.41×10^{-15}
Total Solids x Temperature	52.32	1.71×10^{-8}	315.13	2.15×10^{-16}

Table 2. Percent Increase in Viscosity of rMPC and rSMP at 40 and 50 °C as compared to 60 °C

%Total Solids	% Increase at 40°C	p-value	% Increase at 50°C	p-value
rMPC				
30	304.2	0.0003	228.9	7.07×10^{-6}
32	489.4	0.0020	194.3	0.0001
34	513.9	0.0005	197.2	8.80×10^{-8}
36	784.3	5.26×10^{-7}	215.8	1.23×10^{-7}
rSMP (%TS)				
46	40.1	0.0015	24.5	0.0012
50	64.5	0.0068	37.8	0.0006
54	2446.2	0.0023	1147.2	9.71×10^{-5}

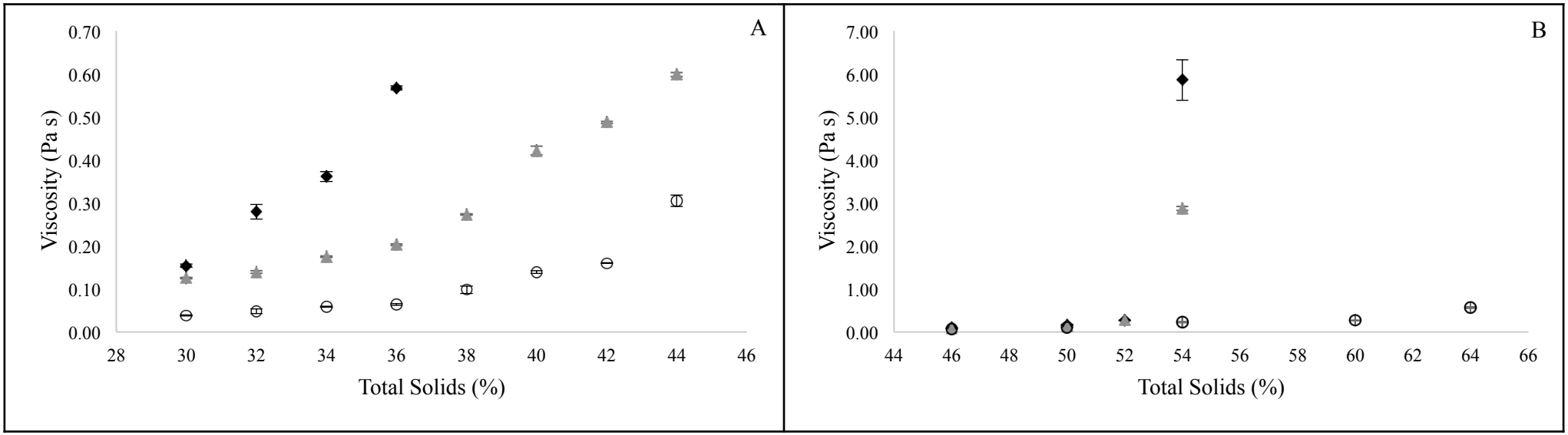

Figure 1. Viscosity of rMPC (A) and rSMP (B) at various solids content treated at ◆ 40 °C, ▲ 50 °C, and ○ 60 °C. Error bars indicate standard deviation.

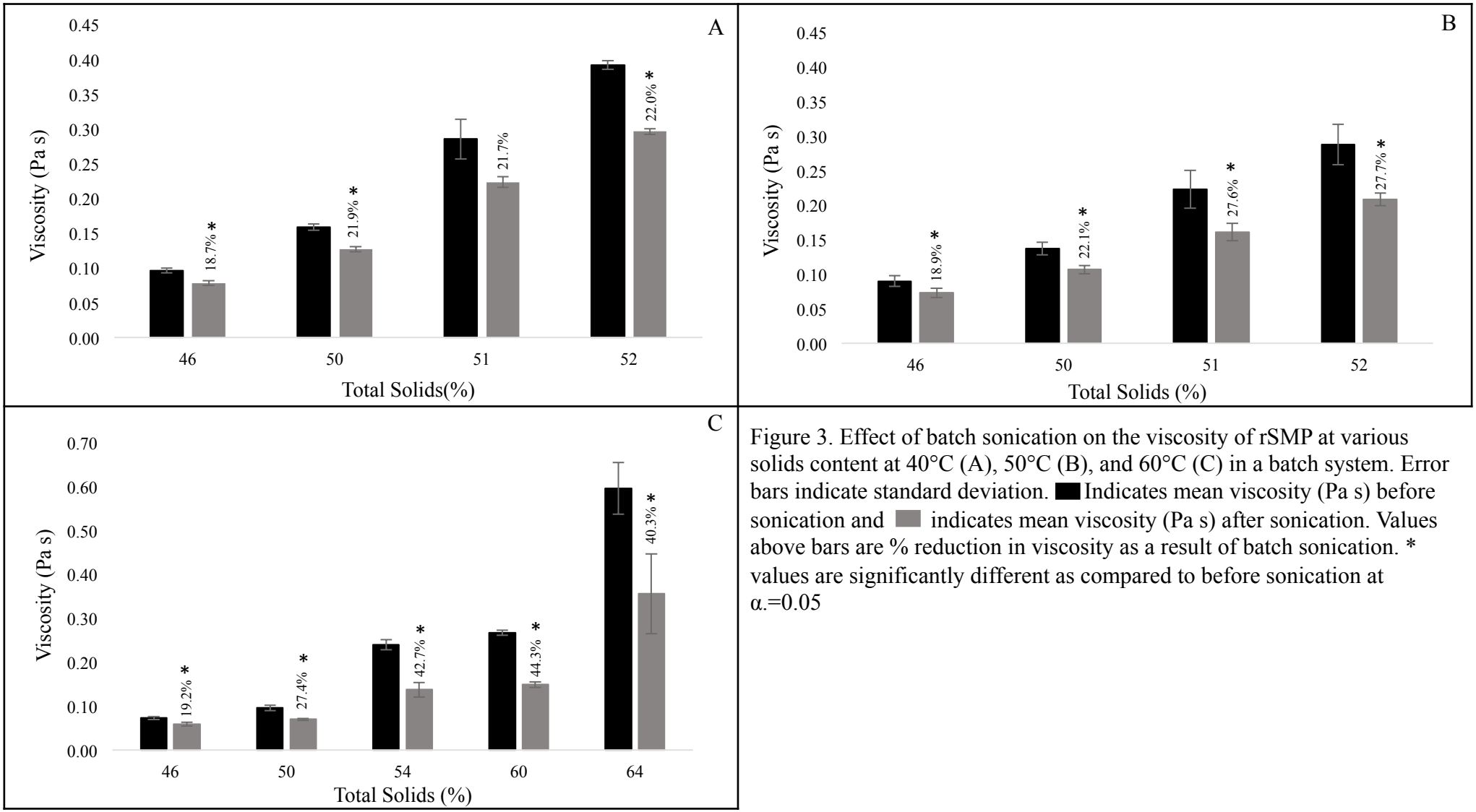

Figure 3. Effect of batch sonication on the viscosity of rSMP at various solids content at 40°C (A), 50°C (B), and 60°C (C) in a batch system. Error bars indicate standard deviation. ■ Indicates mean viscosity (Pa s) before sonication and ■ indicates mean viscosity (Pa s) after sonication. Values above bars are % reduction in viscosity as a result of batch sonication. * values are significantly different as compared to before sonication at $\alpha.=0.05$

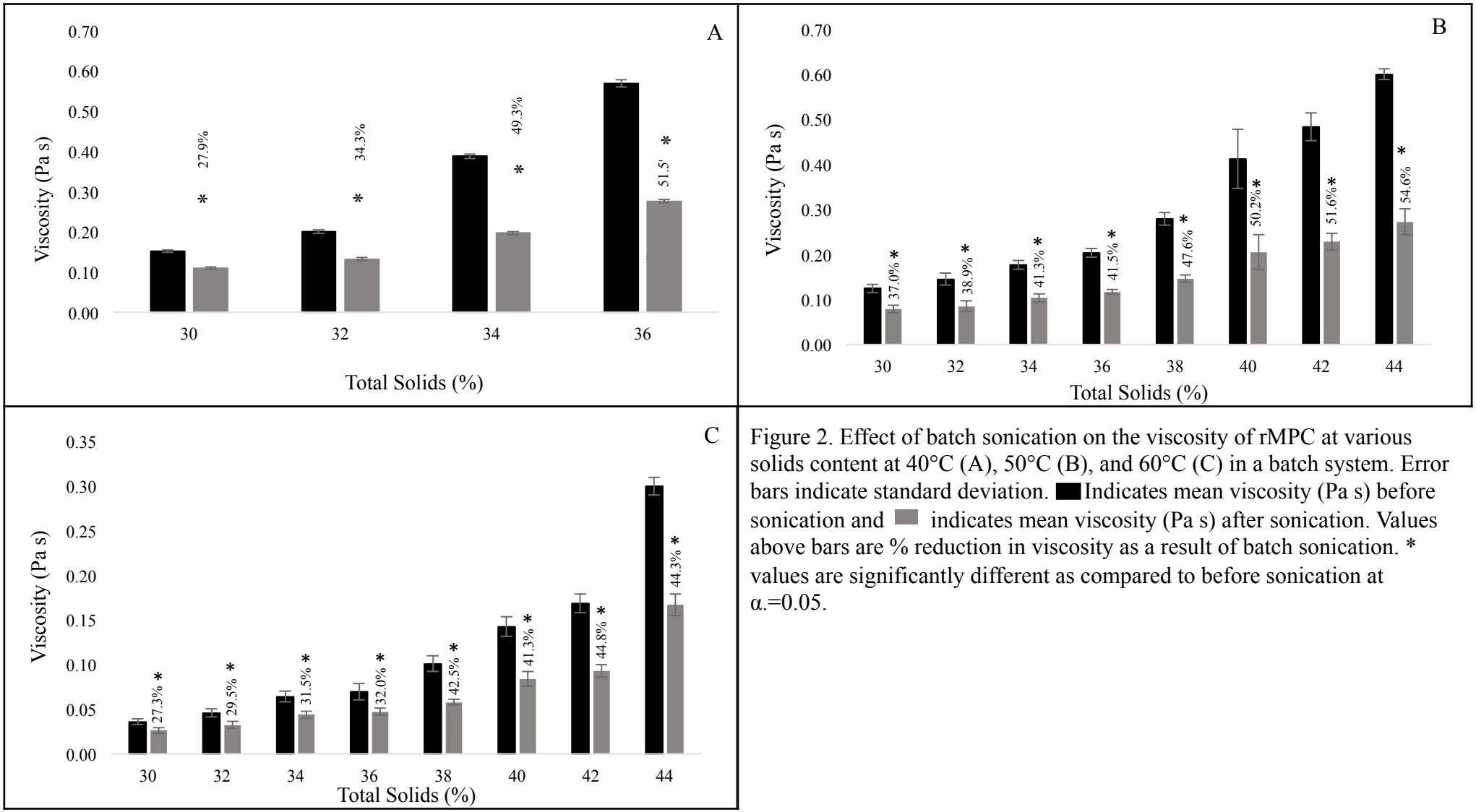

Figure 2. Effect of batch sonication on the viscosity of rMPC at various solids content at 40°C (A), 50°C (B), and 60°C (C) in a batch system. Error bars indicate standard deviation. ■ Indicates mean viscosity (Pa s) before sonication and ■ indicates mean viscosity (Pa s) after sonication. Values above bars are % reduction in viscosity as a result of batch sonication. * values are significantly different as compared to before sonication at $\alpha.=0.05$.

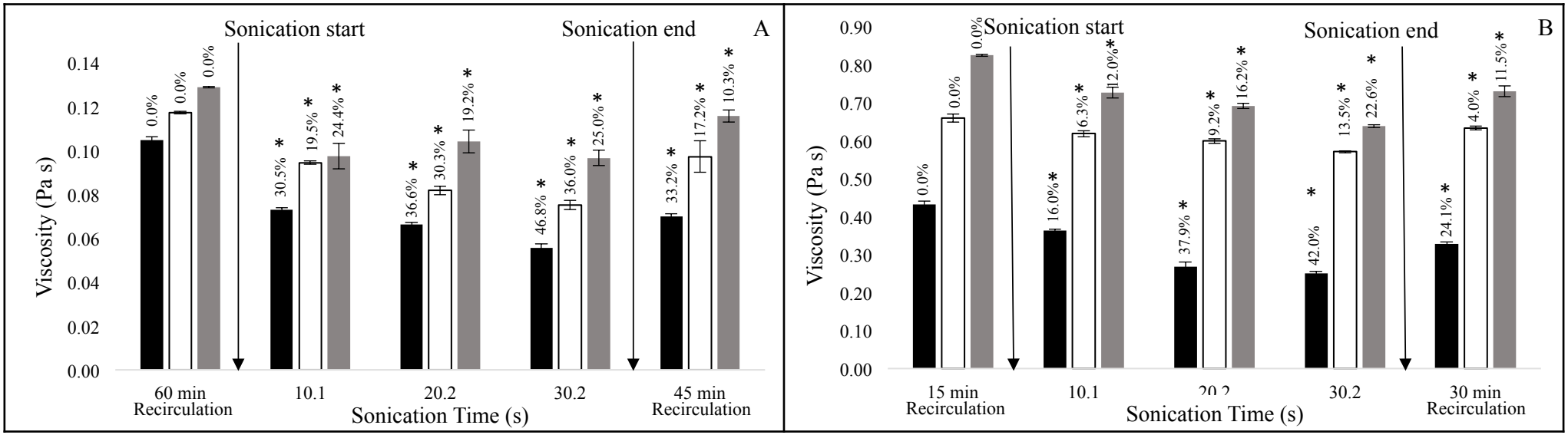

Figure 4. Effect of flow through sonication on the viscosity of rMPC (A) and rSMP (B) at various solids content at 60°C in a continuous system as compared to pre-sonication. Error bars indicate standard deviation. For rMPC (A), ■30 %TS, □32 %TS, and ■ 34 %TS. For rSMP, ■50 %TS, □52 %TS, and ■ 54 %TS. * values are significantly different as compared to before sonication at α.=0.05. On X axis, numbers indicate residence time in seconds. Values above bars are % reduction in viscosity as a result of flow through sonication.

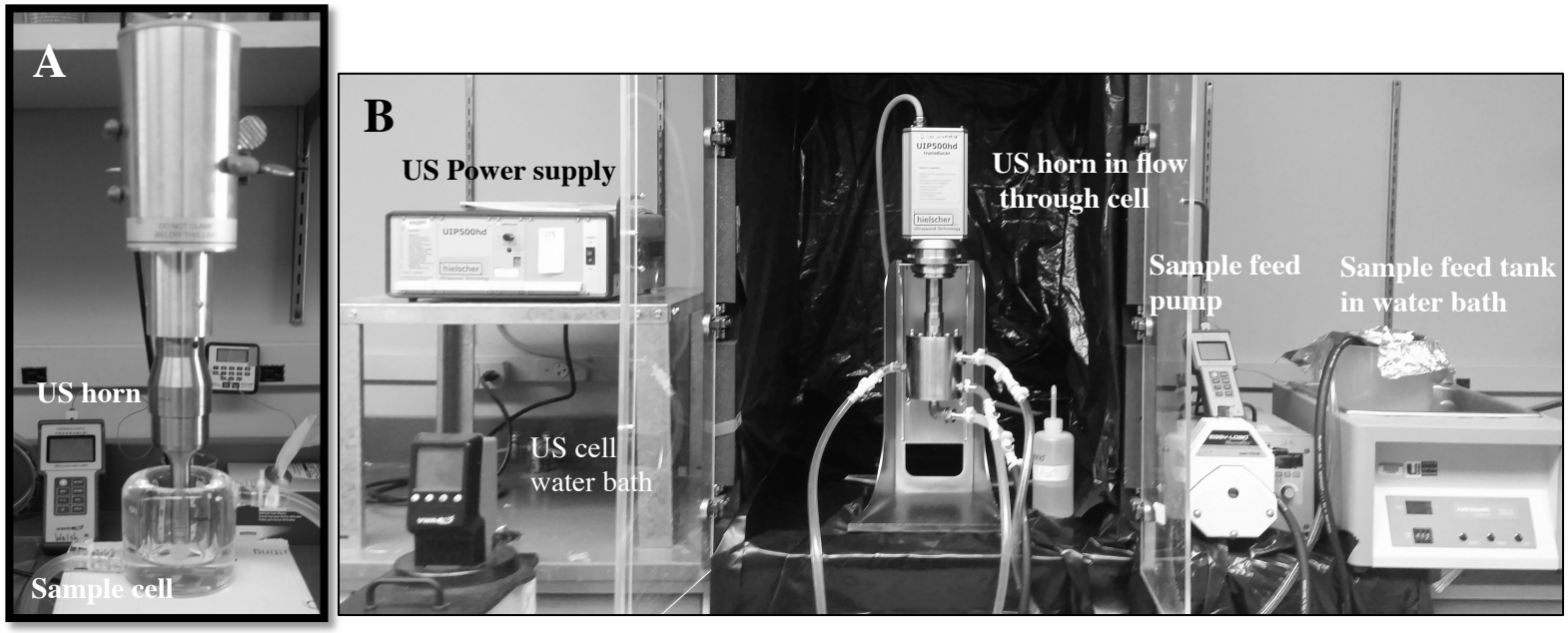

Figure A1. Schematics of the application of ultrasound (US) in batch (A) and in the flow through system (B).

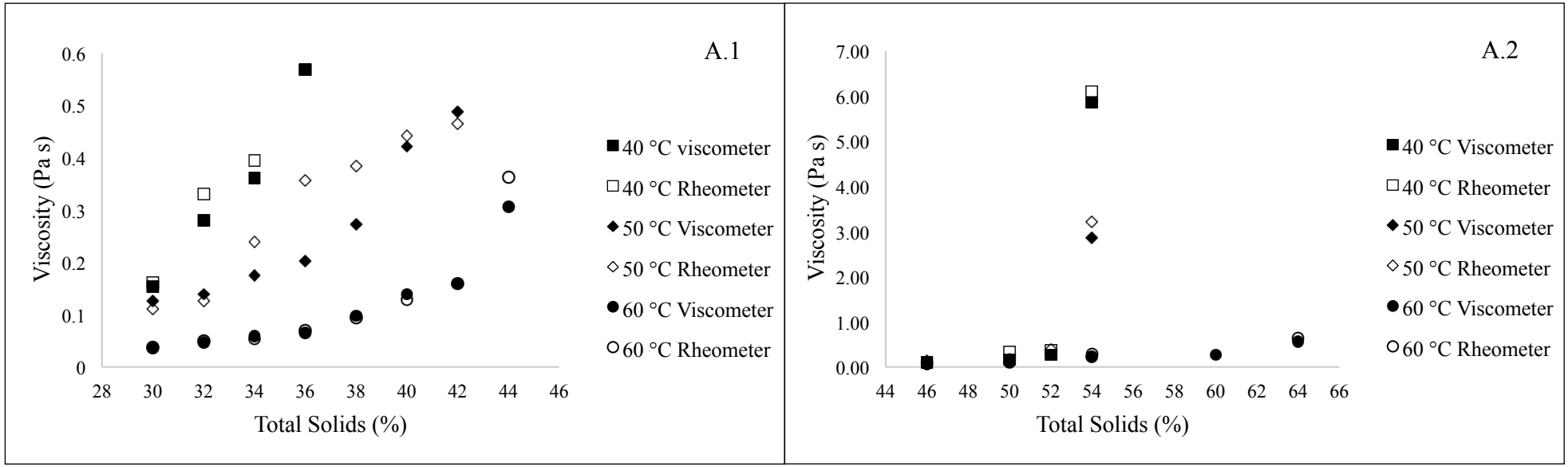

Figure A2. Viscosity of rMPC (1) and rSMP (2) at various solids content treated at 40 °C, 50 °C, and 60 °C comparing viscometer readings to that of rheometer.