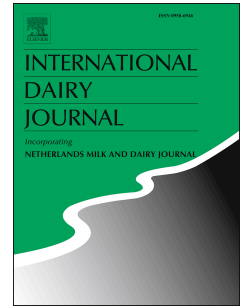


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Effect of pH and heat treatment on viscosity and heat coagulation properties of milk protein concentrate

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protein concentrate

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ABSTRACT

The effect of pH, adjusted using either hydrochloric acid (HCl), citric acid or sodium hydroxide, on calcium ion (Ca^{2+}) activity, and consequent changes in viscosity and heat coagulation time (HCT) of milk protein concentrate (MPC) was investigated. Reducing the pH of MPC dispersions resulted in a reduction in their viscosity, which subsequently increased during heat treatment. The maximum heat stability of MPC was observed at pH 6.7. Reducing the pH of MPC from 6.7 to 6.2 resulted in a significant ($P < 0.05$) increase in Ca^{2+} activity, and reduction in HCT. Such changes were more extensive using HCl compared with citric acid. Increasing the pH greater than 6.7 also led to a reduction in HCT but a decrease in Ca^{2+} activity. These results demonstrate the importance of pH adjustment, and choice of acidulant, on Ca^{2+} activity, viscosity, and HCT of MPC concentrates during processing.

Milk protein concentrates (MPC) are high quality protein ingredients obtained from skim milk (Martin, Williams, & Dunstan, 2010). MPC is produced by ultrafiltration (UF) of pasteurised skim milk, resulting in a retentate stream containing high levels of casein and whey proteins, which is typically dried to produce MPC powder ingredients, while the permeate stream, containing lactose, water and milk salts is removed (Bastian, Collinge, & Ernstrom, 1991; Green, Scott, Anderson, Griffin, & Griffin, 1984). The ratio of protein to total solids (TS) content is increased while the ratio of casein to whey proteins is maintained at a level similar to that in the original skim milk (Bastian et al., 1991; Green et al., 1984). The protein content of MPC can range from 35% (i.e., standardised skim milk powder) to ~85% (w/w) therefore MPC is considered to be a good source of protein with desirable nutritional, sensory and functional properties for a wide range of food applications (Banach, Clark, & Lamsal, 2014; Huffman & Harper, 1999) and is commonly used for protein fortification of cheese and yoghurt (Havea, 2006).

Following UF, liquid MPC concentrate is typically heat treated at high temperatures (~ 90–120 °C) depending on the required functionality of the ingredient. However, as these systems are concentrated in protein, heat-induced denaturation and aggregation of the whey protein fractions can result in high viscosity, lower total solids (TS) and possible gelation prior to spray drying (Murphy, Tobin, Roos, & Fenelon, 2013; Singh & Havea, 2003; Walstra & Jenness, 1984).

The TS content of liquid MPC after UF and heat treatment is typically increased by evaporation prior to spray drying (Bastian et al., 1991; Green et al., 1984); however, the maximum TS content of liquid MPC suitable for further processing is limited by its viscosity after evaporation. Increases in viscosity contribute to fouling during heat treatment and evaporation, resulting in increased droplet size during atomisation and affecting the rate of drying and final powder properties (Bienvenue, Jiménez-Flores, Singh, 2003; Fryer, 1989). Increased viscosity in evaporated, heat-treated MPC liquid concentrates is largely caused by the aggregation and interaction of denatured whey proteins on the casein micelle surface; however, there are several

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other factors that can affect the viscosity of MPC, such as protein content, TS, pH, presence of calcium chelators and buffering capacity (Anema, Lowe, Lee, & Klostermeyer, 2014; Anema, Lowe, & Li, 2004; Bienvenue et al., 2003; Langley & Temple, 1985; Singh, 2007). Adjusting the pH of skim milk concentrates has been shown to result in a change in the voluminosity of the casein micelles and the consequent viscosity (Karlsson, Ipsen, Schrader, & Ardö, 2005). Decreasing the pH of skim milk from 6.51 to 6.15 was shown to reduce viscosity; however, when the pH was reduced further (i.e., <6.15), viscosity increased again (Karlsson et al., 2005).

In addition to viscosity, the heat stability of MPC has been shown to be affected by TS and pH (Crowley et al., 2014; Dumpler & Kulozik, 2015; Sikand, Tong, & Walker, 2010). Dumpler and Kulozik (2015) examined the heat stability of skim milk concentrate and found that the heat coagulation time (HCT) decreased with increasing TS from 10–35% (w/w) across the pH range 6.3–7.3. It is well established that skim milk generally exhibits the typical Type A profile for HCT; whereby HCT decreases as pH is adjusted to greater or less than 6.7. Crowley et al. (2014) reported that rehydrated MPC powders at 3.5%, w/w, protein had reduced heat stability as the protein content of the powders increased from 35 to 90% (w/w, dry basis). The authors attributed this finding to a higher level of ionic calcium (Ca^{2+}) in dispersions prepared from powders with higher protein content. It is not only pH that can affect viscosity and HCT, but also the Ca^{2+} activity in the liquid MPC, which itself is very dependent on pH. Therefore, the type of acid used to reduce pH may influence equilibrium of calcium between the serum and micellar phases, subsequently influencing the physicochemical properties of MPC liquid concentrates and powders.

The aim of this study is to investigate the effect of reducing pH using either a strong mineral acid (HCl) or a weak organic acid (citric acid) on the level of Ca^{2+} activity in liquid MPC obtained after UF of skim milk, the consequent effects on heat stability and changes in viscosity following heat treatment. Results from this study would enable a better understanding of the mechanisms responsible for, and allow control of viscosity development in MPC concentrates prior to spray drying that would be highly beneficial for improving process efficiency and product quality.

2. Materials and methods

2.1. Materials

Fresh liquid MPC retentate was obtained from a local dairy company and kept for maximum of 24 h at 4 °C prior to experimental procedures and analysis. The liquid MPC was manufactured by ultrafiltration (UF) and continuous diafiltration of pasteurised skim milk at <12 °C to 19.8% (w/w) TS (pH 6.7). No pH adjustment was carried out during the filtration process. The protein, fat, ash and lactose content of the MPC was 83.4, 1.07, 6.72 and 2.05% (w/w, dry basis), respectively. All other chemicals and reagents used in the study were of analytical grade and sourced from Sigma-Aldrich (Arklow, Co. Wicklow, Ireland).

2.2. Effect of heat treatment on the properties of milk protein concentrate

2.2.1. Heat treatment

Liquid MPC samples (40 mL) taken directly from the UF plant were transferred into 50 mL plastic vials (50 mL, 115 × 28 mm, polypropylene, Sarstedt, Co Wexford, Ireland), closed and heated in a thermostatically-controlled water bath at 45, 55, 65 and 75 °C for 20 min, followed by cooling to 25 °C using chilled water.

2.2.2. Rheological assessment of heat treated samples

The viscosity of liquid MPCs (19.8%, w/w, TS) after each heat treatment was measured using an AR-G2 controlled-stress rheometer (TA Instruments, Crawley, UK), equipped with a concentric cylinder geometry at a constant shear rate of 300 s⁻¹ for 5 min at 25 °C controlled by a Peltier apparatus (± 0.1 °C). Samples were visually free from foam/bubbles and measured within 1 h after cooling to 25 °C. All measurements were carried out in triplicate.

2.2.3. Polyacrylamide gel electrophoresis of heat treated samples

Protein profiles of liquid MPC samples after heat treatment were determined using pre-cast sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Novex Technologies, ThermoFischer Scientific) under reducing and non-reducing conditions using the method described by Buggy, McManus, Brodkorb, Carthy, and Fenelon (2017). After electrophoresis, the gels were stained overnight using 0.05% (w/w) Coomassie Brilliant Blue R-250 in 25% (v/v) isopropanol and 10% (v/v) acetic acid. After staining, the gels were de-stained using 10% (v/v) isopropanol and 10% (v/v) acetic acid solution until a clear background was achieved.

2.2.4. Particle size analysis of heat treated samples

The particle size distribution of MPC which was affected by heat-induced protein aggregation was determined by dynamic light scattering (DLS) using a laser-light diffraction unit (Mastersizer 3000, Malvern Instruments Ltd, Worcestershire, UK) equipped with a 300 RF (reverse Fourier) lens. Particle refractive and absorption indices for MPC were set at 1.38 and 0.001, respectively. Samples were diluted in deionized water to ~5% (w/w) and all measurements were recorded at ~7% laser obscuration at 20 °C. Size measurements were recorded as the median diameter ($D_{(50)}$), the cumulative diameters ($D_{(90)}$ and $D_{(10)}$) and the volume-weighted mean diameter ($D_{[4,3]}$), while size distribution profiles were obtained using polydisperse analysis. All measurements were carried out in triplicate.

2.3. Effect of pH and calcium ion activity on the heat stability of milk protein concentrate samples

2.3.1. pH adjustment of MPC samples

The pH of liquid MPC obtained after UF was adjusted to 6.2, 6.4, 6.6, 6.7, 7.0 and 7.2 by slow addition of 1 M citric acid, HCl and/or sodium hydroxide (NaOH). A standard pH meter

used to measure pH at 20 °C. The TS content of liquid MPC after pH adjustment was not significantly affected by dilution.

2.3.2. Calcium-ion activity analysis

Ionic calcium activity [Ca^{2+}] was calculated from a standard curve of 0, 5, 10, 15, 20 and 25 mM [Ca^{2+}] standards, prepared with CaCl_2 in a KCl and imidazole buffer (pH 6.7 and with ionic strength of 16, 32, 48, 64 or 80 mM). The standard curve was established from a linear relationship between $\log [\text{Ca}^{2+}]$ (mM) and the electrical output (mV) according to the Nernst equation (On-Nom, Grandison, & Lewis, 2010). Calibration slopes were between 27.9 and 29.7 mV (theoretical value = 29.6 mV). In addition, before each experiment, it was determined that a two-fold increase in [Ca^{2+}] increased electrical output by approximately 9 mV, in compliance with the Nernst equation (On-Nom et al., 2010). Samples and standards were measured at 20 °C after 30 s equilibration using a polymer membrane Ca-ion-selective electrode (Metrohm Ireland Ltd., Carlow, Ireland).

2.3.3. Viscosity measurements of pH-altered samples

The viscosity of liquid MPC samples (19.6 mL) was measured using the AR-G2 controlled-stress rheometer. Samples were pre-sheared at a shear rate of 200 s^{-1} for 0.5 min at 45 °C before viscosity was measured at a shear rate of 300 s^{-1} over 5 min. Subsequently, the temperature of the peltier system was ramped up to 75 °C at 5 °C min^{-1} , held for 5 min at 75 °C, before cooling to 45 °C at 5 °C min^{-1} , and held at this temperature for a further 5 min. Viscosity was measured at a constant shear rate of 300 s^{-1} . All measurements were carried out in triplicate and viscosities were recorded at 45 °C, a typical product temperature at the evaporation stage prior to spray drying.

2.3.4. Particle size measurements of pH-altered samples

The samples subjected to heat treatment using the rheometer (Section 2.2.4) were

2.2.4. All measurements were carried out in triplicate.

2.3.5. Heat stability of pH-altered samples

Heat stability of liquid MPC samples was determined as described essentially as described by Crowley et al. (2014), Davies and White (2009), and Dumpler and Kulozik (2015), with some minor modifications. The liquid MPC samples were adjusted to pH values ranging from pH 6.2 to 7.2. Samples (2.5 g) were added to glass test tubes (100 mm long, 13 mm internal diameter) and the tubes were sealed with silicone bungs, placed in a rocker and immersed in an oil bath containing heated mineral oil at a temperature of 130 °C. The heat coagulation time (HCT) was recorded as the time elapsed between immersing the sample in the oil bath and the onset of visible aggregation in the sample within the test tubes. All measurements were carried out in triplicate.

2.4. Statistical data analysis

Particle size data ($D_{(10)}$, $D_{(50)}$, $D_{(90)}$ and $D_{(4,3)}$), viscosity and HCT were analysed using one-way analysis of variance (ANOVA), with post hoc Tukey analysis using SPSS statistics software (SPSS V.18, IBM, New York, US).

3. Results and discussion

3.1. Effect of heat treatment on physicochemical properties of MPC

The effect of heat treatment temperature on the viscosity of MPC is shown in Fig. 1. Viscosity values of MPC dispersions showed a slight decrease with increasing heat treatment temperature from 25 (36.3 ± 4.7 mPa s) to 55 °C (27.7 ± 6.6 mPa.s) and a higher viscosity after heat treatment at 65 °C (30.1 ± 6.2 mPa s), although the effect was not significant ($P > 0.05$). However,

°C (Note: Rheological measurements were all carried out at 25 °C).

Protein profiles (SDS-PAGE) for liquid MPC samples analysed under reducing and non-reducing conditions before and after heat treatment are shown in Fig. 2. Under non-reducing conditions, the protein patterns of the samples heated at 45, 55 and 65 °C (Fig. 2A; lanes 2–4) were similar to that of the control sample. However, low band intensities corresponding to α -lac and β -lg, as well as changes to the band intensity of minor whey proteins were observed in the sample heated at 75 °C, indicating heat-induced denaturation and aggregation (Fig. 2A, lane 5), correlating with the higher viscosity of MPC heated at 75 °C, as shown in Fig. 1. Analysis of liquid MPC samples under reducing conditions showed similar intensities of casein and whey protein bands after each of the different heat treatments (Fig. 2B), demonstrating that the heat-induced protein-protein interactions were mediated mainly by disulphide bridging.

Particle size distribution profiles and $D_{(50)}$ and $D_{(4,3)}$ data showed a slight shift towards larger particles ($P > 0.05$) with increasing heat treatment temperature from 25 to 65 °C (Fig. 3A and B). However, the $D_{(50)}$ and $D_{(4,3)}$ values were significantly ($P < 0.05$) higher for MPC samples heated at 75 °C (Fig. 3B; Supplementary material Table S1).

3.2. Effect of pH on the calcium ion activity of liquid milk protein concentrate

Results for Ca^{2+} activity and viscosity of MPC as a function of pH are shown in Fig. 4. Ca^{2+} activity significantly ($P < 0.05$) decreased from 3.66 mM at pH 6.7 to 2.45 at pH 7.2 after addition of NaOH. Vaia, Smiddy, Kelly, and Huppertz (2006) showed calcium ion equilibrium between the casein micelle and serum phase to be strongly affected by pH, with increasing pH causing complexation of calcium with inorganic or organic phosphate. Conversely, Ca^{2+} activity of MPC dispersions increased with decreasing pH and was significantly influenced by the type of acid used (i.e., HCl or citric acid). The solubilisation of calcium phosphate as a direct effect of pH reduction is the likely cause of increased Ca^{2+} activity at pH values less than 6.7. Acidification of MPC

dispersions to pH 6.2 gave a Ca^{2+} activity of 4.91 and 10.5 mM when using citric acid or HCl,

respectively. Therefore, at pH 6.2, Ca^{2+} activity of the liquid MPC adjusted by citric acid and HCl were 1.74- and 2.87-fold that of the control sample (pH 6.7), respectively, with pH adjustment using HCl consistently resulting in a higher Ca^{2+} activity than that for citric acid across all acidic pH values studied (Fig. 4). The decrease in Ca^{2+} activity with increasing pH described in this study was also consistent with those shown in rehydrated MPC powders as reported by Crowley et al. (2014) (Supplementary material Fig. S1). Gaucheron (2005) explained how the addition of citric acid to MPC dispersions influences mineral equilibrium between the casein micelle and the serum phase, with an increase in levels of free citrate and calcium citrate in the serum phase and a concomitant decrease in Ca^{2+} activity.

3.3. Effect of pH on viscosity and particle size of liquid milk protein concentrate

Viscosity measurements performed before and after heat treatment (i.e., $75\text{ }^{\circ}\text{C} \times 5\text{ min}$) at pH values ranging from 6.2 to 7.2 are shown in Fig. 4. The viscosity of MPC prior to heat treatment significantly ($P < 0.05$) increased with increasing pH from 8.84 mPa s at pH 6.7 (i.e., control sample) to 14.7 and 38.7 mPa s at pH 7.0 and pH 7.2, respectively. Furthermore, viscosity decreased slightly to 5.24 and 6.30 mPa.s at pH 6.2 using HCl and citric acid, respectively. However, statistical analysis indicated no significant difference in viscosities among the samples in the pH range 6.7 to 6.2 (Supplementary material Table S2). A reduction in pH of MPC has been shown previously to result in a reduction in casein micelle voluminosity (Karlsson et al., 2015). In contrast, the viscosity of MPC post heat treatment ($75\text{ }^{\circ}\text{C} \times 5\text{ min}$) was slightly higher than that of non-heated MPC at pH 7.0 and pH 6.7 (18.9 and 15.0 mPa s, respectively; Fig. 4). For MPC samples adjusted to pH 6.4 using HCl (Ca^{2+} activity of 7.62 mM) the viscosity increased significantly ($P < 0.05$) from 5.99 to 97.7 mPa s on pH adjustment. This is compared with an increase in viscosity from 6.78 to 12.6 mPa s under the same extent of pH adjustment (Ca^{2+} activity of 4.92 mM) using citric acid (Fig. 4). At the lower pH value of 6.2, the use of HCl (Ca^{2+} activity

10.5 mM) led to gelation of MPC dispersions after heat treatment. However, adjusting the pH to 6.2 with citric acid (Ca^{2+} activity 6.36 mM) led to an increase in viscosity after heat treatment from 6.30 to 63.6 mPa s, but without any evidence of gelation having occurred. Anema et al. (2004) showed a similar effect with skim milk when adjusting the pH prior to heat treatment, reporting a higher level of whey protein association with the casein micelle during heat treatment at pH 6.5 compared with pH 7.1, leading to a significant increase in viscosity of the heated milk system at the lower pH. Vasbinder and de Kruif (2003) also showed that heat treatment at pH values less than 6.6 resulted in decreased levels of serum proteins and had down-stream implications on the properties of rennet and acid gels.

Although the increase in viscosity, driven by the denaturation/aggregation of whey proteins, was reported to be greater for milk samples heated at low pH (Anema et al., 2014), the influence of Ca^{2+} activity on the subsequent viscosity of heat treated MPC has not been previously studied. Heat treatment was found to considerably increase viscosity of liquid MPC at Ca^{2+} activities ≥ 6.36 mM (Fig. 4). Different acids used in pH adjustment resulted in differences in the levels of Ca^{2+} released to the serum phase and hence impacted on heat-induced viscosity differently. In fact, MPC at low pH, adjusted using HCl, had a greater Ca^{2+} activity compared with that adjusted by citric acid, and hence, was more susceptible to viscosity development during heat treatment (Fig. 4). Therefore, it was the Ca^{2+} activity of the MPC, which seemed to play the most significant role in the extent of viscosity increase during heat treatment. Also, it must be remembered that when relating Ca^{2+} activity to viscosity of MPC that the Ca^{2+} activity measured is influenced by the protein content of the system. Crowley et al. (2014) showed MPC powders rehydrated at 3.5%, w/w, protein to have a Ca^{2+} activity of 1.49 at pH 6.8, compared with the current study where MPC obtained directly from a commercial UF plant (16.5%, w/w, protein) had higher Ca^{2+} activity of 3.66 mM at pH 6.7.

Particle size data of MPC dispersions after heat treatment ($75\text{ }^{\circ}\text{C} \times 5\text{ min}$) are shown in Table 1. $D_{(50)}$ and $D_{[4,3]}$ of post heated samples at pH 7.0 and 7.2 were comparable with those of the control sample without heat treatment, while $D_{(50)}$ and $D_{[4,3]}$ of samples heated at pH less than 6.7

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were significantly ($P < 0.05$) larger than those of the control sample without heat treatment. Particle size analysis of MPC samples adjusted to pH 6.2 by HCl could not be measured after heat treatment due to gelation of the sample. However, large particles, presumably protein aggregates, were found for the post heat treated sample having an initial pH of 6.4 adjusted using HCl ($D_{[4,3]} = 48.8 \mu\text{m}$). $D_{(50)}$ and $D_{[4,3]}$ of post heated samples were observed to significantly ($P < 0.05$) increase in size with decreasing pH (Table 1).

3.4. Effect of pH and calcium-ion activity on heat coagulation time of MPC

The effect of pH on the HCT of MPC is shown in Fig. 5. Heat stability was observed to be at a maximum at pH 6.7 (HCT 32.3 min; Ca^{2+} activity 3.66 mM), while it significantly ($P < 0.05$) decreased at pH values greater than pH 6.7 (12.5 min at pH 7.0 and 11.5 min at pH 7.2). The reduction in HCT with increasing pH greater than 6.7 may be due to the dissociation of κ -casein from the casein micelle (Crowley et al., 2014). Therefore, even with a Ca^{2+} activity of 2.45 mM at pH 7.2, there was sufficient ionic calcium to cause heat-induced coagulation of the κ -casein depleted micelles (Crowley et al., 2014). At pH values less than 6.7 the HCT also decreased and was significantly ($P < 0.05$) lower when HCl was added as opposed to citric acid. The MPC at pH 6.4 adjusted by citric acid was relatively heat stable (HCT 22.5 min) while that adjusted with HCl had a significantly ($P < 0.05$) lower HCT of 2.47 min (Fig. 5). Lower HCT values were observed with higher Ca^{2+} activity for MPC samples adjusted using HCl (i.e., HCT 0 min; Ca^{2+} activity 10.5 mM at pH 6.2), compared with that adjusted to the same pH by citric acid (i.e., HCT 7.55 min; Ca^{2+} activity 6.36 mM at pH 6.2). According to Gao et al. (2010) the affinity of divalent ions (i.e., especially Ca^{2+}) to complex with citrate is much higher compared with monovalent ions which tend to remain in their free ionic form in simulated milk ultrafiltrate solutions.

The work carried out in the current study has shown the relationship between calcium ion activity and HCT at representative solids content at which heat treatment at industrial scale most often occurs (i.e., directly after UF and prior to evaporation). Dumpler and Kulozik (2015) showed

maximum heat stability at pH 6.7 across all solids content but that HCT decreased with increasing solids content. However, the high ionic concentration of skim milk is not completely representative of MPC systems whereby much of the soluble minerals and ions have been removed during UF and diafiltration. The HCT of the MPC samples in the current study were typical of Type A profiles, with HCT increasing with concomitant increasing pH from 6.2 to 6.7 and decreasing thereafter up to pH 7.2 (Fig. 5).

4. Conclusion

This work has highlighted the challenges posed by thermal processing of liquid MPC concentrates at high total solids content (19.8%), whereby Ca^{2+} activity plays a significant role in viscosity and heat stability. Adjusting the pH of MPC to 6.4 using citric acid prior to heat treatment resulted in lower viscosity. Conversely, adjusting pH with HCl led to a release of Ca^{2+} from the colloidal to the serum phase, as represented by the high measured Ca^{2+} activity levels, and hence reduced HCT of the concentrate, and concentrate viscosity post heat treatment. Reducing the pH of MPC after ultrafiltration using citric acid could therefore allow for higher TS to be achieved during evaporation, as viscosity will be reduced while heat stability remains unaffected.

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Fig. 1. Apparent viscosity profile (shear rate 300 s^{-1} ; $25 \text{ }^\circ\text{C}$) of MPC (solids content of 19.8%, w/w) after heat treatment at 45, 55, 65 and $75 \text{ }^\circ\text{C} \times 20 \text{ min}$, respectively. Values are the means of triplicate data analysis. Viscosity values not sharing a common letter differ significantly ($P < 0.05$).

Fig. 2. SDS-PAGE electropherogram of MPC under (A) non-reducing and (B) reducing conditions. Lane 1 represents the control sample at $25 \text{ }^\circ\text{C}$ while lanes 2–5 indicate the samples with heat treatment at 45, 55, 65 and $75 \text{ }^\circ\text{C} \times 20 \text{ min}$, respectively.

Fig. 3. Particle size distribution profiles of MPC at $25 \text{ }^\circ\text{C}$ (unheated) (—) and heated at 45°C (----), 55°C (····), 65°C (— · —) and 75°C (— —) for 20 min (A) and size of particles in milk protein concentrate as function of heat treatment temperature (B): $D_{(50)}$ (\diamond) and $D_{[4,3]}$ (\blacksquare) are the median diameter and the volume weighted mean diameter; the values for the unheated control are given as (— —) and (— —) for $D_{(50)}$ and $D_{[4,3]}$ respectively. Values are the means of data from triplicate analysis. Values of $D_{(50)}$ and $D_{[4,3]}$ not sharing a common letter differ significantly ($P < 0.05$).

Fig. 4. Calcium ion activity and apparent viscosity as a function of pH in control MPC (\blacksquare) at pH 6.7 and MPC adjusted with hydrochloric acid (\square), citric acid (\blacksquare) or sodium hydroxide (\blacksquare). Bars represent calcium ion activity while symbols indicate the viscosity. Viscosity measurements were performed at a constant shear rate of 300 s^{-1} at $45 \text{ }^\circ\text{C}$ before (\circ) or after (\diamond) heating at $75 \text{ }^\circ\text{C}$ for 5 min.

Fig. 5. Heat coagulation time (HCT) as a function of pH in MPC adjusted with hydrochloric

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Table 1Particle size of MPC samples at different initial pH and heated at 75 °C for 5 min. ^a

| pH | pH adjustment | $D_{(10)}$ (μm) | $D_{(50)}$ (μm) | $D_{(90)}$ (μm) | $D_{[4,3]}$ (μm) |
|-----|------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| 6.7 | None; not heated | $(3.47 \pm 0.00) \times 10^{-2a}$ | $(1.26 \pm 0.00) \times 10^{-1a}$ | $(3.65 \pm 0.00) \times 10^{-1a}$ | $(1.68 \pm 0.00) \times 10^{-1a}$ |
| 6.7 | None | $(4.45 \pm 0.01) \times 10^{-2b}$ | $(1.46 \pm 0.00) \times 10^{-1b}$ | $(3.71 \pm 0.01) \times 10^{-1ab}$ | $(1.81 \pm 0.00) \times 10^{-1b}$ |
| 6.2 | Citric acid | $(5.48 \pm 0.47) \times 10^{-2c}$ | $(2.04 \pm 0.16) \times 10^{-1d}$ | $(6.36 \pm 0.33) \times 10^{-1c}$ | $(3.24 \pm 0.11) \times 10^{-1d}$ |
| 6.4 | Citric acid | $(5.48 \pm 0.42) \times 10^{-2c}$ | $(1.67 \pm 0.01) \times 10^{-1c}$ | $(4.00 \pm 0.05) \times 10^{-1b}$ | $(2.02 \pm 0.06) \times 10^{-1c}$ |
| 6.6 | Citric acid | $(4.64 \pm 0.01) \times 10^{-2b}$ | $(1.47 \pm 0.0) \times 10^{-1b}$ | $(3.68 \pm 0.0) \times 10^{-1ab}$ | $(1.81 \pm 0.00) \times 10^{-1b}$ |
| 6.2 | HCl | * | * | * | * |
| 6.4 | HCl | $(1.73 \pm 0.04) \times 10^{-1d}$ | $(1.28 \pm 0.08) \times 10^{1e}$ | $(1.60 \pm 0.04) \times 10^{2d}$ | $(4.88 \pm 0.16) \times 10^{-1e}$ |
| 6.6 | HCl | $(5.39 \pm 0.01) \times 10^{-2c}$ | $(1.63 \pm 0.00) \times 10^{-1bc}$ | $(3.85 \pm 0.00) \times 10^{-1ab}$ | $(1.95 \pm 0.00) \times 10^{-1c}$ |
| 7.0 | NaOH | $(3.35 \pm 0.01) \times 10^{-2a}$ | $(1.22 \pm 0.00) \times 10^{-1a}$ | $(3.57 \pm 0.00) \times 10^{-1a}$ | $(1.63 \pm 0.01) \times 10^{-1a}$ |
| 7.2 | NaOH | $(3.40 \pm 0.01) \times 10^{-2a}$ | $(1.25 \pm 0.00) \times 10^{-1a}$ | $(3.70 \pm 0.01) \times 10^{-1ab}$ | $(1.69 \pm 0.01) \times 10^{-1ab}$ |

^a The two samples at pH 6.7 are the controls with no pH adjustment, the first of these was also not heated to 75 °C for 5 min as an unheated control. $D_{(50)}$, $D_{(10)}$ and $D_{(90)}$ are the median diameter, the cumulative diameters whereby 50%, 10% and 90% of the volume is smaller than the size indicated, respectively. $D_{[4,3]}$ is the volume weighted mean diameter. Values presented are the means of data \pm standard deviations; values within a column not sharing a common superscript differ significantly ($P < 0.05$); a single asterisk indicates sample gelation.

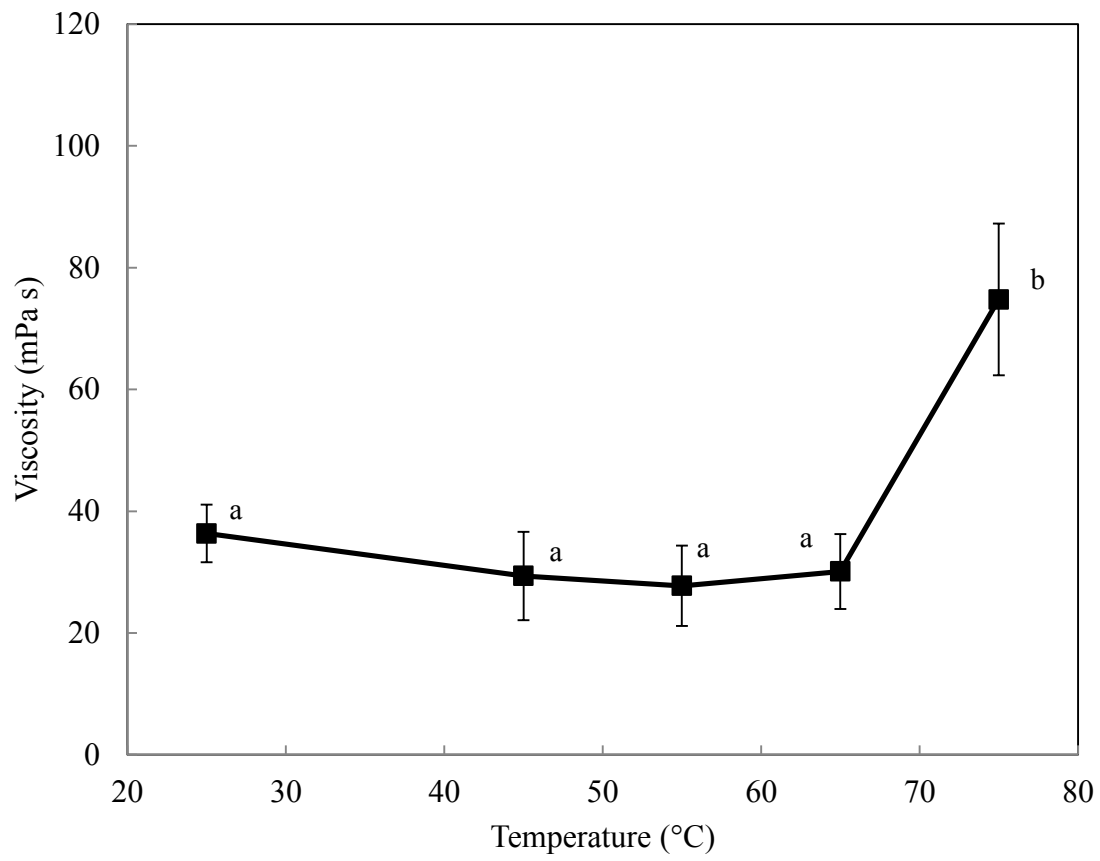


Figure 1.

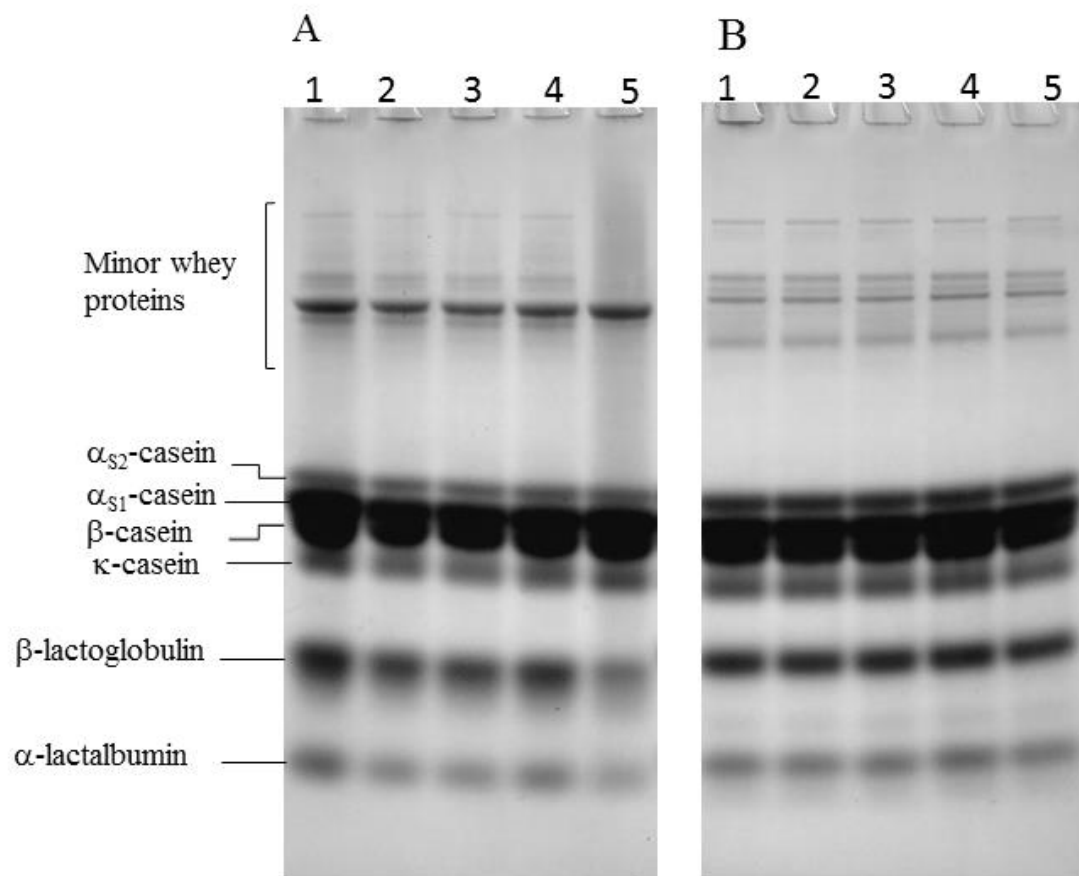
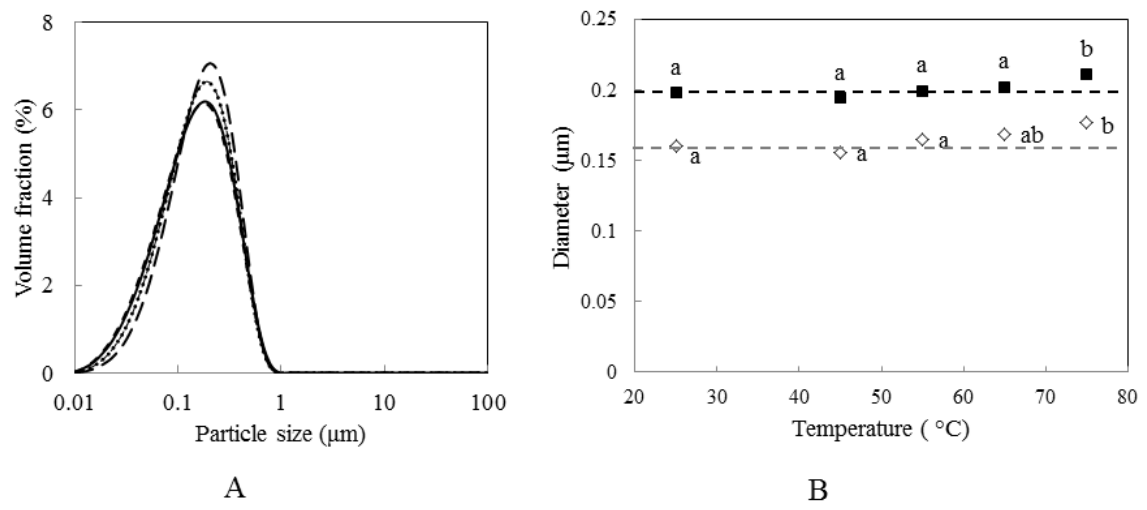


Figure 2.

**Figure 3.**

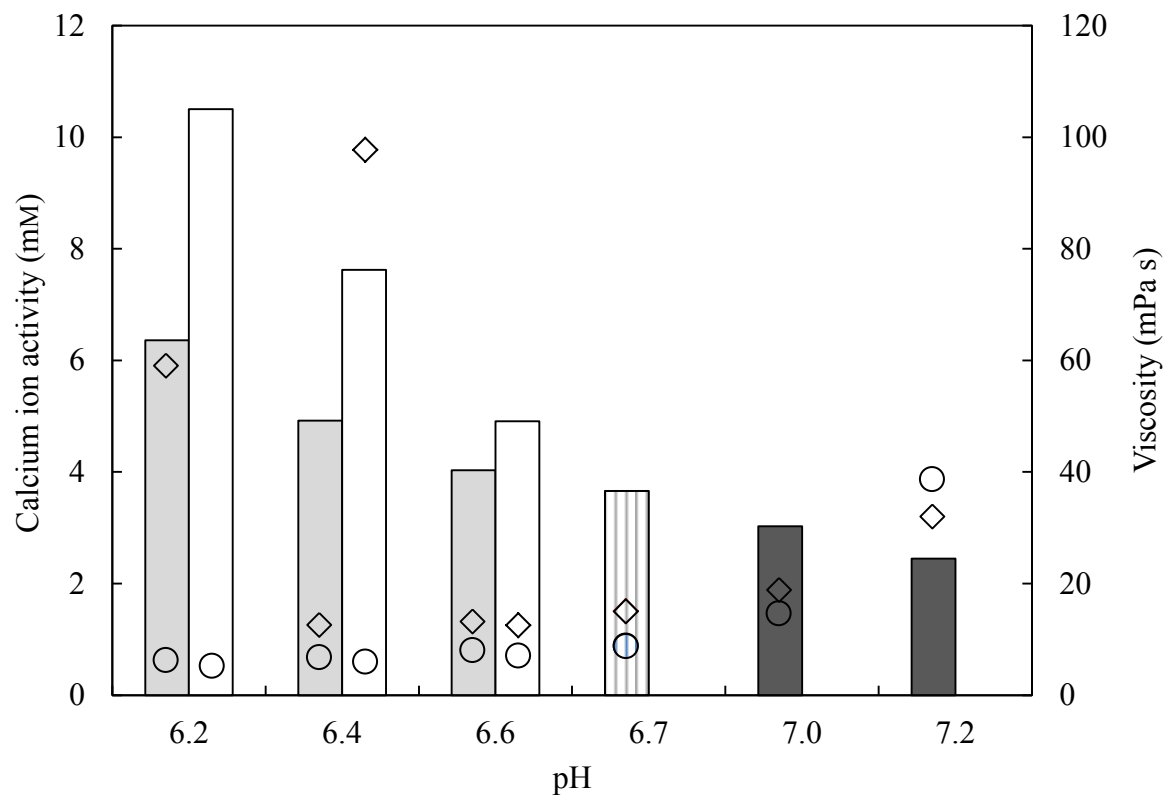


Figure 4.

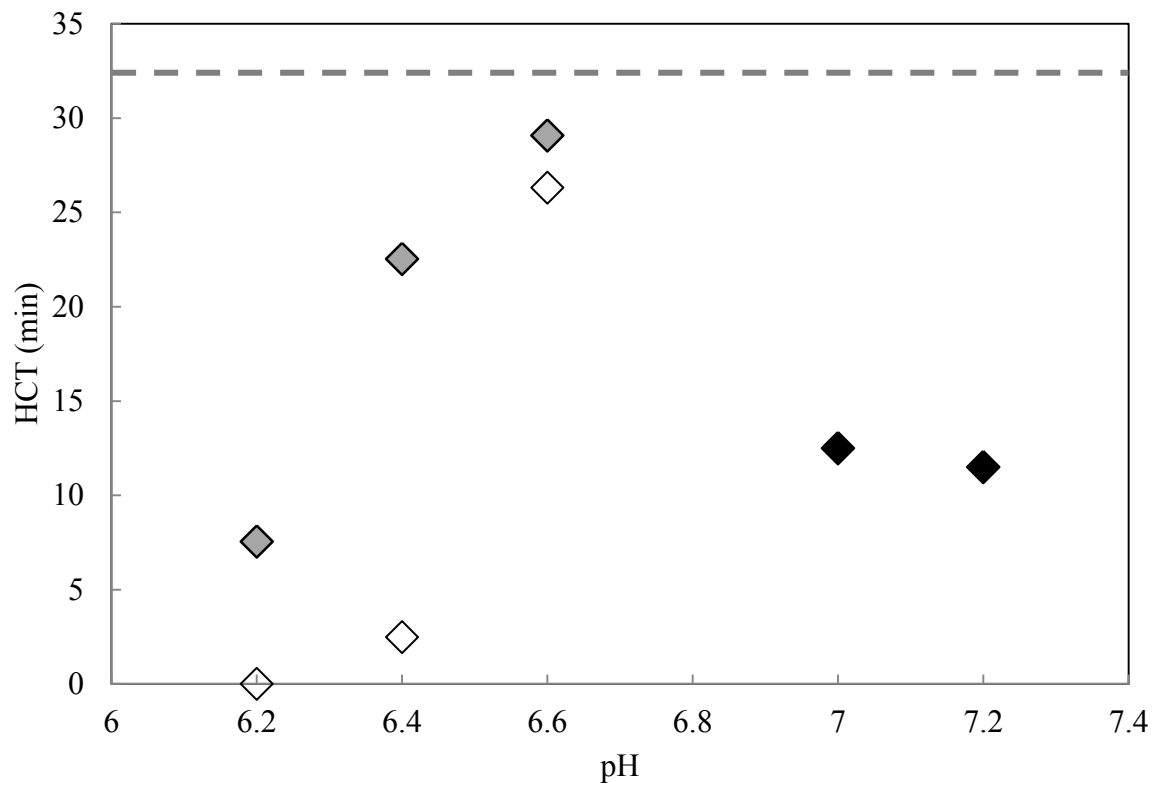


Figure 5.