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Heat treatment of liquid ultrafiltration concentrate influences the physical and functional properties of milk protein concentrate powders

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ABSTRACT

Liquid milk protein concentrate (MPC; 18.3 and 16.5%, w/w, total solids and protein, respectively) was heat treated at 80 °C (low-heat), 100 °C (medium-heat) and 120 °C (high-heat) for 30 s, or did not undergo heat treatment (control), prior to spray drying. Viscosity of the liquid MPC increased with increasing heat treatment temperature. Physical properties of MPC powders were influenced by heat treatment, with the size of powder particles generally increasing with increasing temperature. Heat treatment of ultrafiltration concentrate influenced the heat stability of MPC powders, with high-heat treated MPC having highest heat stability at pH 6.9 and 7.0 (140 °C). However, particle size distribution profiles demonstrated a decrease in powder dispersion with increased heat treatment temperature. This study demonstrated that heat treatment of ultrafiltration concentrate at temperatures ≥100 °C can present challenges with solubilising subsequent MPC powders.

1. Introduction

Milk protein concentrate (MPC) powder is used in nutritional food and beverage formulations as it contains a significant amount of high-quality protein. However, it is recognised as having sub-optimal dispersibility and solubility in water, generally attributed to non-covalent interactions between micellar caseins that are facilitated by the low concentration of lactose (Anema, Pinder, Hunter, & Hemar, 2006; Havea, 2006), and this phenomenon is accelerated by elevated storage temperatures of >37 °C (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010).

Ultrafiltration (UF) and diafiltration (DF) are used to create protein-enriched concentrates from skim milk, which are evaporated and spray dried to create MPC powder. Considering that liquid concentrate derived from membrane filtration is soluble (e.g., particle size distribution in the casein micelle range), but the product obtained following spray drying has significantly impaired rehydration performance, it seems important to explore the intermediate processing steps and what effect they could have on the physicochemical properties of such powder. One of these unit operations commonly performed is heat treatment, whereby the concentrate derived from membrane filtration is heated at a pre-determined temperature, for a defined time, prior to evaporation, likely to alter functional properties of the powder and therefore the ability to meet customer requirements. For example, in the case of skim milk powder, whey protein denaturation may be favourable at this stage to improve gelation or heat stability in the final product (Kelly & Fox, 2016).

Interestingly, despite heat treatment having a considerable role in dairy processing, the number of scientific studies reporting its effects on the functional properties of liquid and spray dried MPC is quite limited. Ho et al. (2018) investigated the influence of pH (6.2–7.2) and heat treatment (45, 55, 65 and 75 °C for 20 min) on the viscosity and heat stability of

liquid MPC (19.8%, w/w, total solids). Heat stability at 130 °C was highest at pH 6.7 (32.3 min), but significantly decreased to 12.5 min at pH 7 and 11.5 min at pH 7.2, while viscosity and particle size increased significantly after heat treatment at 75 °C (pH 6.7). Another study by Ho et al. (2019) investigated the effect of heat treatment (85, 100 and 120 °C for 15–200 s) on the viscosity of liquid MPC derived from UF. Viscosity increased significantly with increasing temperature and holding time, which would have implications for atomisation of such concentrates during industrial spray drying. Tari, Gaygadzhiev, Guri, and Wright (2021) heat-treated (85 °C for 5 min or 125 °C for 15 s) liquid MPC (18.5%, w/w, total solids; 13%, w/w, protein) at different pH values (6.5, 6.7 and 6.9) and reported that viscosity was significantly higher after heat treatment, while β-lactoglobulin denaturation was greater than that of α-lactalbumin. Crowley et al. (2014) measured the heat coagulation time of reconstituted (3.5%, w/w, protein) MPC powders ranging in protein content from 35–90% (w/w) and reported that calcium ion activity played a significant role in reducing heat stability. A further study by Crowley et al. (2015) evaluated the behaviour of MPC powders, reconstituted to 8.5% protein (w/w), during heating at 120 °C to simulate in-container sterilisation, and reported that MPC containing approximately 85% protein (w/w) was extremely unstable to heating between pH 6.3 and 7.1.

Lin, Kelly, O'Mahony, and Guinee (2018) performed low-heat (LH; 72 °C for 15 s) and medium-heat (MH; 85 °C for 30 s) treatment of skim milk prior to membrane filtration and spray drying and reported that MPC powder solubility was marginally higher for MH-MPC (96.8%) than LH-MPC (95.4%). Gazi and Huppertz (2015) produced MPC powder from LH (72 °C for 15 s) and MH (95 °C for 45 s) treated skim milk and reported no difference in initial solubility, despite higher whey protein denaturation (25 and 65% for α-lactalbumin and β-lactoglobulin, respectively) in the MH-MPC compared with LH-MPC. However, the solubility of the MH-MPC powder decreased more rapidly when stored at

elevated temperatures (>37 °C), compared with LH-MPC. Khalesi and FitzGerald (2021b) recently compared the physicochemical properties of commercial MPC powders, differing in the level of denatured whey proteins generated by heat treatment, and reported higher solubility and lower particle size values for the powder with higher whey protein denaturation when dispersed in water at 50 °C. Most studies investigating the relationship between thermal processing and solubility of subsequent MPC powders have only applied heat treatment to the skim milk before UF, despite heat treatment commonly being applied to the liquid UF concentrate. Carr (1999) applied a range of heat treatments (72–130 °C for 30–45 s) to skim milk prior to UF and investigated the solubility of MPC powders produced, with heat treatment generally leading to a decrease in powder solubility.

It is evident that the influence of thermal processing following membrane filtration, particularly high-heat (HH) treatment, on the functional properties of spray-dried MPC powders, has not been fully elucidated and further research is necessary. While the denaturation of native whey proteins is likely one factor that may reduce powder rehydration performance, the extent to which rehydration deteriorates (or whether it does significantly) as the intensity of heat treatment applied to liquid UF concentrate increases (i.e., LH versus HH treatment) is not well defined (Gazi & Huppertz, 2015; Khalesi & FitzGerald, 2021b; Lin et al., 2018). Therefore, the objective of this research study was to investigate the influence of heat treatment after membrane filtration (i.e., UF and DF) on the physical and functional properties of MPC powders.

2. Materials and methods

2.1. Heat treatment of liquid milk protein concentrate and powder manufacture

Liquid milk protein concentrate (MPC; 18.3 and 16.5%, w/w, total solids and protein, respectively) was obtained from a dairy ingredient manufacturer following UF and DF of pasteurised skim milk. Three heat treatments were applied to the liquid MPC (18 L) using a pilot-scale Microthemics UHT/HTST system (MicroThermics, Raleigh, NC, USA) in the Bio-functional Food Engineering facility in Teagasc Food Research Centre (Moorepark, Co. Cork, Ireland): (i) low-heat (LH) treatment of 80 °C for 30 s, (ii) medium-heat (MH) treatment of 100 °C for 30 s, and (iii) high-heat (HH) treatment of 120 °C for 30 s, while the starting material was considered the control as it did not undergo additional heat treatment (i.e., it was only pasteurised by the ingredient manufacturer). Powders were produced using an Anhydro single-stage spray dryer (SPX Flow Technology, Denmark), equipped with a two-fluid nozzle atomisation system and configured in a counter-current flow mode, while the air inlet and outlet temperatures were set at 180 and 85 °C, respectively. The free moisture and ash contents of the MPC powders were determined using a TGA701 thermogravimetric analyser (LECO Corporation, St Joseph, MI, USA) at 102 and 550 °C, respectively. The protein nitrogen values of the liquid MPC and powders were obtained using a LECO FP628 nitrogen analyser (LECO Corporation) and the protein content was determined by multiplying the nitrogen concentration by a nitrogen-to-protein conversion factor of 6.38.

2.2. pH and colour of liquid milk protein concentrates

The pH of liquid MPC samples was measured at 25 °C using a SevenCompact pH meter S210 (Mettler Toledo, Greifensee, Switzerland). The colour of each MPC sample was measured using a Chroma Meter CR-400 (Konica Minolta Sensing Europe B.V., Nieuwegein, the Netherlands). The colour measurement was determined according to the

three colour coordinates: L*, a*, and b*. The value L* represents the sample luminosity or brightness, varying from black (0) to white (100); a* represents the colour varying from green (–) to red (+); b* represents the colour varying from blue (–) to yellow (+). The total colour difference (ΔE) was calculated using the formula reported by Kelleher et al. (2020).

2.3. Particle size of liquid milk protein concentrates

The particle size distribution of control and heat-treated liquid MPC was determined on the day of powder manufacture by dynamic light scattering using a Zetasizer nano (Malvern Instruments, Worcestershire, UK). MPC samples were diluted (1:20) in ultrapure water (25 °C) and placed in disposable cuvettes for analysis. The dispersant refractive index used was 1.33, the viscosity parameter was 0.89 cP, and the sample refractive and absorption indices were set at 1.45 and 0.001 (Power, Fenelon, O'Mahony, & McCarthy, 2020), respectively.

2.4. Viscosity of liquid milk protein concentrates

Viscosity of the control and heat-treated liquid MPC was measured the day following powder manufacture under cold (5 °C) and warm (40 °C) conditions using an AR-G2 controlled-stress rheometer (TA Instruments, Crawley, UK), equipped with a parallel plate geometry. Investigating the rheological behaviour of MPC at 5 °C is relevant for transport of liquid MPC, while analysis at 40 °C provides an insight into viscosity prior to evaporation. Samples were pre-sheared at a shear rate of 200 s^{-1} for 30 s, followed by a shear rate ramp from 0.1 to 300 s^{-1} over 5 min, with the temperature (5 or 40 °C) controlled using a Peltier system (± 0.1 °C). Analysis was performed in duplicate at both temperatures. The Herschel-

Bulkley model was fitted to the data using TA Data Analysis (TA Instruments, Crawley, UK) and the viscosity and rate index values were reported.

2.5. Calcium ion concentration

The concentration of ionic calcium in liquid MPC samples was determined the day following powder manufacture using a Sension+ MM340 benchtop meter equipped with a Sension+ 9660C calcium ion selective electrode (Hach Co., CO, USA). The ion selective calcium probe was calibrated with standard calcium solutions of 0.5, 1.0, 2.5 and 5.0 mM at 25 °C (Lin, Kelly, O'Mahony, & Guinee, 2016). A standard curve was obtained using the linear relationship between electrical output (mV) and the logarithm of ionic calcium concentration. Analysis was performed in duplicate.

2.6. Particle density, bulk density, interstitial and occluded air

Loose bulk density, tapped density (100 taps), particle density, interstitial air and occluded air were determined, as described by McSweeney, Maidannyk, Montgomery, O'Mahony, and McCarthy (2020). All measurements were recorded in duplicate.

2.7. Powder particle size distribution analysis

The particle size distributions of the MPC powders were determined using a Malvern Mastersizer (Mastersizer 3000; Malvern Instruments Ltd, Malvern, Worcestershire, UK) equipped with an Aero S dry powder dispersion unit. The refractive index and absorption index were set at 1.45 and 0.1, respectively. The air pressure was set at 2 bar and the

obscuration range was 0.1 to 6%. Measurements were recorded as the median particle diameter (D_{50}) and cumulative diameters (D_{10}) and (D_{90}), whereby 10, 50 and 90% of the sample volume is represented by particles smaller than the size indicated. The volume-weighted mean particle diameter ($D_{[4,3]}$) was also recorded.

2.8. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

MPC powders were reconstituted (1 h in 50 °C ultrapure water followed by magnetic stirring for 21 h at 4 °C) to 3.5% protein (w/w) and diluted (1:10) to give a concentration of 3.5 µg protein μL^{-1}. Samples for electrophoresis were prepared by combining the MPC solution with lithium dodecyl sulphate buffer and ultrapure water in Eppendorf tubes. A precast 12% bis-Tris Nu-PAGE gel was placed in an XCell Surelock Mini-Cell containing running buffer and antioxidant (Invitrogen, ThermoFischer Scientific, Dublin, Ireland), and 10 µL of each sample was added to the wells. Analysis was performed at a constant voltage of 200 V for 50 min (Buggy, McManus, Brodkorb, McCarthy, & Fenelon, 2017). Gels were then stained overnight using SimplyBlue Safe Stain (Thermo Fisher Scientific) and de-stained using ultrapure water.

2.9. Quantification of native whey proteins

MPC powder dispersions (3.5%, w/w, protein) for reverse-phase high performance liquid chromatography (RP-HPLC) analysis were prepared as described in Section 2.8. Sodium acetate buffer (0.1 M; pH 4.6) was added to MPC dispersions to give a final protein concentration of 0.25% (w/w) and these were centrifuged at 20,000 × *g* (4 °C) for 20 min to precipitate casein and non-native whey proteins. Prior to injection of the sample, the

supernatants were filtered through Captiva 0.2 μm filters (PES 25 mm; Agilent Technologies, Ireland). β-Lactoglobulin, α-lactalbumin and BSA standards (Sigma Aldrich, Ireland) were used for column calibration. RP-HPLC (1200 series; Agilent Technologies) was used to quantify native whey proteins, in unheated and heat-treated MPC samples, using a Waters 2487 dual wavelength absorbance detector at 214 nm. A silica-based C-18 RP-HPLC column (ZorBax 300SB-C18 5 μm, 4.6 × 150 mm; Agilent Technologies) was used for separation of native whey proteins using a gradient solvent program of 82% solvent A (99.9 % MilliQ water + 0.1% trifluoroacetic acid) and 18% solvent B (99.9% of acetonitrile + 0.1% trifluoroacetic acid). The column temperature was 40 °C and the eluent flow rate was 1 mL min^{-1} for 45 min. Data was processed using Waters Empower® software.

2.10. Heat coagulation time

The heat coagulation time of MPC dispersions (3.5%, w/w, protein) was determined over the pH range 6.7 to 7.2 at 140 °C, with the pH adjusted twice prior to analysis using 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. Glass tubes containing 3 mL of sample were immersed in a silicone oil bath and the time elapsed between placing samples in the oil bath and visible coagulation was recorded. Measurements were performed in duplicate.

2.11. Particle size and solubility of milk protein concentrate dispersions

The particle size distribution of MPC dispersions was measured using a laser-light diffraction unit (Malvern Mastersizer 3000; Malvern Instruments Ltd, Worcestershire UK) equipped with a 300 RF lens, as described by McSweeney et al. (2020). Particle size measurements were recorded when the laser obscuration reached 3–4%. The solubility of

MPC powders was measured at 23 and 50 °C using a traditional solubility method, as described by McSweeney et al. (2020); powder solubility was given by the total solids content of the supernatant (obtained following centrifugation at 3000 × *g* for 10 min), expressed as a percentage of the total solids content of the initial dispersion. In addition, to investigate the rehydration behaviour of these powders under more industrially relevant conditions, powders were reconstituted for 1 h in ultrapure water at 50 °C using a 4-blade overhead stirrer operating at 500 rpm and then stirred magnetically (250 rpm) at 4 °C for 21 h. Finally, to investigate the relationship between heat treatment and storage, powders were also placed in sealed plastic containers and stored at 37 °C for 14 d, and the solubility after mixing (50 °C water for 30 s) was measured.

2.12. Statistical data analysis

Measurements were performed in triplicate unless otherwise stated, with results presented as mean ± standard deviation. Analysis of variance (one-way ANOVA; Tukey's HSD) was carried out using IBM SPSS (Version 28; Armonk, New York, NY, USA) statistical analysis package. The level of significance was set at $P < 0.05$.

3. Results and discussion

3.1. Physicochemical properties of liquid milk protein concentrates

The physicochemical properties of the liquid milk protein concentrate (MPC) samples are shown in Table 1. The pH did not change with heat treatment, with all MPC samples having a pH value of approximately 6.7. The z-average particle diameter increased slightly,

but not significantly with heating. Lin et al. (2018) reported z-average particle diameters of 190 and 209 nm for low-heat (72 °C for 15 s) and medium-heat (85 °C for 30 s) MPC powder dispersions that were pH adjusted to 6.65, while Tari et al. (2021) reported that MPC heat treated at pH 6.7 for (i) 85 °C for 5 min or (ii) 125 °C for 15 s did not significantly alter the particle size (volume-weighted mean particle diameters of 0.15 and 0.16 µm, respectively). Calcium ion concentration increased following LH and MH treatments, e.g., it increased from 3.23 for the control (C) to 3.79 and 4.08 mmol L^{-1} after LH and MH treatment, respectively, while there was no difference between MH and high-heat (HH) samples. This may have been caused by differences in viscosity, combined with the high total solids content of the samples, which subsequently influenced the measurements obtained by the probe. While Ho et al. (2018) reported that calcium ion activity of MPC increased following heat treatment at 75 °C for 5 min, it is widely reported that heat treatment decreases the concentration of ionic calcium in the serum phase of milk (Lewis, 2011). Future work involving mineral analysis of the colloidal and serum phases after ultracentrifugation would provide more clarity on this result. Changes in colour measurements were also observed post heat-treatment. Total colour difference, which takes each of the colour values into account, demonstrated that the colour increased as heat treatment temperature increased, e.g., 2.23 for LH-MPC and 4.65 for HH-MPC. Kelleher et al. (2020) reported a colour difference of 2.02 for a milk protein beverage containing a casein:whey ratio of 80.20 which had received a final heat treatment of 120 °C for 30 s, compared with an unheated control.

Analysis of viscosity demonstrated the significant effect of temperature on the rheological properties of liquid MPC (Fig. 1). When measurements were performed at 5 °C, C-MPC had the lowest viscosity of all samples, and this increased with heat treatment temperature. For example, the viscosity values from the Herschel-Bulkley model were 693 mPa s for C-MPC, compared with 4364 mPa s for HH-MPC (Table 2). This may be relevant

in relation to the transport of liquid protein concentrates for use in food and beverage formulations (Dunn, Barbano, & Drake, 2021). Viscosity was considerably lower when measured at 40 °C (Fig. 1; Table 2) but the same trend persisted (i.e., concentrate viscosity generally increased with heat treatment temperature). The rate index at 5 °C was highest for C-MPC (0.70) compared with the heat-treated samples (0.56–0.59), while it was similar for all samples when measured at 40 °C. Higher viscosity after heat treatment would likely limit the total solids content attainable during subsequent evaporation, thereby increasing energy costs during spray drying. Ho et al. (2019) also reported that viscosity of liquid MPC (19.8%, w/w, total solids) increased with increasing temperature of heat treatment (85, 100 and 120 °C), and this was likely caused by higher levels of whey protein denaturation and aggregation. Warncke, Kieferle, Nguyen, and Kulozik (2022) reported higher apparent viscosity for MPC following heat treatment for 30 min at 80 °C. Anema, Lowe, Lee, and Klostermeyer (2014) suggested that viscosity of skim milk concentrate increased after heat treatment, particularly at pH 6.5 and 6.7, due to the association of denatured whey proteins with the casein micelles, increasing their voluminosity. It is important to mention that viscosity analysis was performed in the current study after storing MPC samples at 4 °C overnight and not immediately after heat treatment, as it was reported by Tari et al. (2021) that viscosity of liquid MPC increased during storage.

3.2. Composition and physical properties of milk protein concentrate powders

The moisture contents of the MPC powders were 5.0, 5.6, 5.1 and 6.1% (w/w) for C-, LH-, MH- and HH-MPC, respectively, while the ash content was 7.8% for all four powders. Furthermore, the protein content was 87.1, 86.1, 86.9 and 85.9% (w/w) for C-, LH-, MH- and HH-MPC, respectively. The physical properties of the MPC powders are shown in Table 3.

316 Particle density was highest for C-MPC (1.17 g cm^{-3}), followed by LH- (1.09 g cm^{-3}), HH-
317 (1.03 g cm^{-3}) and MH-MPC (1.02 g cm^{-3}). However, loose bulk density was lower for C-
318 MPC (0.22 g cm^{-3}) than for heat-treated MPC powders (0.25–0.26 g cm^{-3}), while following
319 100 taps, bulk density increased by approximately 0.06 g cm^{-3} for all powders. Regarding the
320 air content of the powders, C-MPC had the highest interstitial air (272 mL 100 g^{-1}) but lowest
321 occluded air (16.9 mL 100 g^{-1}) values, while there were little differences between powders
322 produced from heat-treated concentrate, e.g., 221 mL 100 g^{-1} for LH-MPC compared with
323 216 mL 100 g^{-1} for HH-MPC. The slightly lower interstitial air content and higher bulk
324 density of heat-treated MPC powders compared with C-MPC may have been caused by the
325 higher viscosity of these concentrates, which resulted in less air being incorporated into the
326 feed.

327 Powder particle size increased significantly with increasing temperature of heat
328 treatment (Table 3). The volume-weighted mean particle diameter ($D_{[4,3]}$) increased from
329 27.4 µm for C-MPC to 43.9 and 71.4 µm for LH- and MH-MPC powders, respectively, while
330 there was no difference in the $D_{[4,3]}$ between MH- and HH-MPC powders. The increased size
331 of powder particles generated is most likely accounted for by the higher concentrate viscosity
332 after heat treatment (Fig. 1) as it would be more difficult for the nozzle to form small uniform
333 droplets. Rupp, Molitor, and Lucey (2018) reported that when the protein content of liquid
334 MPC was increased from 19 to 21 and 23% (w/w) using evaporation, there was a
335 corresponding increase in the viscosity of the concentrate and the $D_{[4,3]}$ values of the spray
336 dried powders (31, 37 and 50 µm, respectively). Similarly, Park, Stout, and Drake (2016)
337 reported a higher $D_{[4,3]}$ value for MPC powder produced from concentrate at 22% total solids
338 (46.8 µm) compared with concentrate at 12% total solids (34.2 µm).

339

340 *3.3. Protein profile by electrophoresis*

341

342 The protein profile of MPC solutions under non-reducing conditions is displayed in
343 Fig. 2. Heat treatment did not appear to have a substantial effect on the α-caseins as the band
344 intensity was similar for all samples. However, the intensity of the κ-casein band was lower
345 for HH-MPC (lane 4), which suggests it dissociated from the casein micelle during heating
346 and formed aggregates with whey proteins in the serum phase. Dissociation of κ-casein from
347 the micelle has been reported by Sauer and Moraru (2012) during heating of micellar casein
348 concentrate at 110–150 °C. Anema and Li (2000) reported that dissociation of κ-casein in
349 reconstituted skim milk generally increased with increasing temperature from 60–120 °C.
350 SDS-PAGE protein profiles reported by Tari et al. (2021) and Ho et al. (2018) did not show
351 any differences in the intensity of the κ-casein bands between the control and MPC heated at
352 125 °C for 15 s and 75 °C for 20 min, respectively. Crowley et al. (2014) reported higher
353 levels of non-sedimentable κ-casein for an MPC85 powder reconstituted to 3.5% protein at
354 pH 6.5 and 6.7 when no heating was applied compared with 90 °C for 30 min, while this
355 trend was reversed at pH 7.1. Under non-reducing conditions in the current study, the
356 intensity of the β-lactoglobulin (β-lg) band decreased significantly with heat treatment,
357 particularly from LH to MH-MPC, with no band present for HH-MPC, while for α-
358 lactalbumin (α-la), faint bands were visible for all samples except HH-MPC. Ho et al. (2018)
359 reported lower band intensities for β-lg and α-la following heat treatment at 75 °C for 20 min.
360 Similarly, the SDS-PAGE gels produced by Tari et al. (2021) showed faint bands for these
361 two whey proteins when MPC was heat-treated at 125 °C for 15 s (pH 6.7 and 6.9) compared
362 with the control.

363

364 *3.4. Quantification of native whey proteins*

365

366 The quantity of native whey proteins in MPC and the extent of whey protein
367 denaturation induced by each heat treatment is presented in Table 4. Denaturation of both α-
368 la and β-lg increased with increasing heat treatment temperature in the range 80 to 120 °C,
369 and to a greater extent for β-lg. Vasbinder and de Kruif (2003) previously reported that β-lg
370 denatured more readily than α-la (70 and 40%, respectively) when skim milk was heated at
371 80 °C for 10 min at pH 6.7. In the current study, the quantity of α-la present was low overall
372 and decreased from 0.045 mg 100 mL^{-1} for C-MPC to 0.017 mg 100 mL^{-1} for HH-MPC,
373 corresponding to a 62% decrease in the concentration of native α-la (Table 4). This is
374 supported by the SDS-PAGE results in Fig. 2, whereby α-la was not visible for HH-MPC
375 under non-reducing conditions (lane 4). Similarly, extensive β-lg denaturation occurred
376 following HH treatment, with the concentration decreasing from 0.313 to 0.029 mg 100 mL^{-1},
377 and also correlates with the absence of a β-lg band on the gel in Fig. 2. Similarly, using RP-
378 HPLC, Gazi and Huppertz (2015) reported denaturation values of 25 and 65% for α-la and β-
379 lg following heat treatment of skim milk at 95 °C for 45 s prior to MPC manufacture.
380 However, Tari et al. (2021) reported considerably lower denaturation values of 3.02 and
381 33.4% for α-la and β-lg following heat treatment (125 °C for 15 s) of MPC (70%, w/w,
382 protein) at pH 6.7, when measured using ion exchange chromatography.
383 Heat treatment is often used to alter the quantity of native whey proteins in a resultant
384 powder as this can influence its industrial applications. Carr (1999) reported that increasing
385 the extent of whey protein denaturation by heat-treating skim milk prior to MPC manufacture
386 led to an increase in rennet coagulation time. However, denatured whey proteins play an
387 important role in the rheological properties of yogurt. For example, Lucey, Teo, Munro, and
388 Singh (1997) reported that gelation time of reconstituted skim milk powders decreased as
389 whey protein denaturation increased (up to 95 and 81% for α-la and β-lg, respectively) with
390 the intensity of heat treatment applied before spray drying. The association of denatured

whey proteins with casein micelles via disulphide bonding was identified as an important factor for increasing the stiffness of acid milk gels (Lucey, Tamehana, Singh, & Munro, 1998). Further research investigating the influence of native whey protein content in MPC powder on its functionality in different food systems (e.g., ability to form rennet and acid gels) would provide useful data for ingredient producers and end-users.

3.5. Heat stability

The stability of MPC dispersions to heating over the pH range 6.7 to 7.2 at 140 °C is shown in Fig 3, with heat stability generally increasing with increasing pH. This resembles the pH-heat coagulation time (HCT) profiles of type B milk and serum-protein free casein micelle dispersions described by Singh (2004). All MPC samples were unstable to heating at pH 6.7, with coagulation occurring after approximately 1 min. At pH 6.8, heat stability remained low (<2 min) for LH-, MH- and HH-MPC, but was slightly higher for C-MPC. Heat stability increased considerably to ~9 min at pH 6.9 for C-, LH- and MH-MPC, while HH-MPC did not coagulate until 3 min after this. This trend was also observed at pH 7, whereby HH-MPC remained stable for 2 min more than the other samples, however; a slight reduction in HCT was observed for HH-MPC at pH 7.1, but this increased again at pH 7.2. The calcium ion concentration in MPC solutions at pH 7.2 was 3.04, 2.95, 2.92 and 3.10 mmol L^{-1} for C-, LH-, MH- and HH-MPC, respectively, which suggests calcium ion concentration did not play a large role in the heat stability observed. Reduced dissociation of κ-casein has been identified as an important feature of higher heat stability in this pH range (Crowley et al., 2014), and higher electrostatic repulsion between proteins may be another contributing factor. The results suggest that denatured whey proteins in MPC can provide some improvements in heat stability at certain pH values (i.e., 6.9 and 7). Crowley et al.

(2014) reported that MPC powder (84%, w/w, protein) reconstituted to 3.5% protein (w/w) had extremely poor heat stability at or below pH 7 (HCT <1 min), while it increased to ~10 and 13 min at pH 7.1 and 7.2, respectively, likely due to decreased calcium ion activity. Crowley et al. (2015) reported that MPC powder produced from skim milk heated at 95 °C for 45 s showed higher heat stability at 120 °C in the pH range 6.8 to 7.1 than MPC produced from pasteurised skim milk, but it decreased at pH 7.2 and 7.3. Carr (1999) did not report a significant change in the heat stability (120 °C) of MPC powders produced from heat-treated skim milk up to whey protein denaturation levels of 86%, but that heat stability decreased significantly once denaturation reached 90%. Sunkesula, Kommineni, Meletharayil, Marella, and Metzger (2021) measured heat stability of reconstituted MPC (10% protein) at 140 °C and reported heat coagulation times of 13.02, 20.29 and 8.37 min at pH 6.7, 6.9 and 7.1, respectively. Khalesi and FitzGerald (2021a) reported HCT values of 2.2 and 2.7 min at 140 °C for MPC containing 16.6 and 6.0 g 100 g^{-1} of native whey proteins, respectively, with heat stability remaining higher for the powder containing less native whey at 110, 120 and 130 °C also. It is important to consider that heat stability results are difficult to compare across studies given the subjective nature of the test, particularly at specific pH values, but can provide useful information regarding trends in heat stability as pH changes.

3.6. Particle size distribution and solubility of milk protein concentrate dispersions

The particle size distribution profiles of MPC powders following reconstitution are shown in Fig. 4. When mixed in 23 °C ultrapure water for 30 s, each sample had a monomodal distribution in the size range 10 to 100 μm (Fig. 4A) and there was no significant difference between samples in relation to the D_{90} and $D_{[4,3]}$ values (Table 5). However, when the temperature of the water was increased to 50 °C, the volume of large particles (10–100

μm) was reduced for all powders, but only C- and LH-MPC had a new second peak in the size range 0.01 to 1 μm (Fig. 4B), suggesting that some of these powder particles dispersed. The overall size distribution was expanded, possibly due to powder particle swelling (Table 5), and there was a significant difference between C-MPC and samples that were heat-treated. For example, when the water temperature was 23 °C, the $D_{[4,3]}$ values were 35.3, 39.9 and 43 μm, but when the temperature was increased to 50 °C, the $D_{[4,3]}$ values were 24.8, 65.6 and 74 μm for C-, LH-, MH-MPC, respectively. To further elucidate the effect of heat treatment on MPC powder rehydration, overhead stirring was performed instead and for a longer duration, with the particle size distribution profiles for all samples shown in Fig. 4C. Under these conditions, all samples displayed a bimodal distribution. There was little difference in the dispersion of C- and LH-MPC, with the $D_{[4,3]}$ value being 11.2 and 8.95 μm, respectively. However, MH- and HH-MPC had significantly higher $D_{[4,3]}$ values of 51.5 and 55.5 μm, respectively. The dispersions prepared for 1 h were subsequently stirred magnetically overnight (4 °C) to investigate if the powders would eventually disperse and solubilise to the same extent, and the distribution is shown in Fig. 4D. The trend observed previously for C- and LH-MPC remained the same, with both distributions overlapping, but a greater difference was recorded between the MH- and HH-MPC, with $D_{[4,3]}$ values of 22.9 and 40.3 μm, respectively (Table 5).

The solubility values obtained following centrifugation of MPC dispersions, prepared in 23 and 50 °C water, generally followed the same trends as those observed in the particle size distribution data, as shown in Fig. 5. When measured at 23 °C, solubility was highest for C-MPC (87.8%), but decreased to 64.7, 45.9 and 48.8% for LH-, MH- and HH-MPC, respectively. When solubility was evaluated after reconstitution in 50 °C water, C-MPC remained the most soluble (95.6%), followed by LH- (92%), MH- (75.5%) and HH- (74.2%) MPC, demonstrating that heat treatment impaired the rehydration performance of MPC.

Furthermore, solubility measurements of MPC powders stored for 2 weeks at 37 °C demonstrated that heat treatment accelerated the deterioration in solubility. The solubility values were 93.6, 89.1, 61.1 and 33.2% for C-, LH-, MH- and HH-MPC, respectively. The greatest decrease in solubility was recorded for HH-MPC, as it was 74.2% initially, but only 33.2% after storage. These result agree with the study by Gazi and Huppertz (2015) which reported higher storage-induced solubility loss for MPC produced from skim milk heated at 95 °C for 45 s.

Carr (1999) applied several heat treatments to skim milk prior to membrane filtration and reported that MPC powder (83–86%, w/w, protein) solubility decreased with increasing temperature of heat treatment. Solubility, evaluated after stirring powders for 1 h in 50 °C water, was 95.5% for MPC produced from pasteurised skim milk, 81.2% when the heat treatment was 80 °C for 30 s, 81.1% for the skim milk processed at 100 °C for 30 s, and only 22.5% for the sample heat-treated at 120 °C for 45 s. This supports the results presented in the current study whereby powder solubility decreases with the intensity of heat treatment applied before spray drying. Furthermore, Carr (1999) reported that a higher homogenisation pressure (i.e., 200 bar instead of 150 bar) was generally required to promote powder solubilisation as the heat treatment temperature applied before spray drying increased. Gazi and Huppertz (2015) did not report a difference in solubility between MPC produced from pasteurised skim milk compared with an MPC powder derived from skim milk that was heat-treated at 95 °C for 45 s. Similarly, Lin et al. (2018) did not find a significant difference between MPC manufactured from pasteurised and medium-heat (85 °C for 30 s) treated skim milk; however, solubility was evaluated after stirring in 50 °C water for 2 h followed by overnight stirring, a point at which differences would be unlikely, as suggested by the particle size distribution data from overnight stirring for C- and LH-MPC in the current study (Table 5). Khalesi and FitzGerald (2021b) investigated the rehydration performance of MPC

powders (~85%, w/w, protein) containing different quantities of native whey proteins (16.6 and 6.0 g 100 g^{-1}) due to different heat treatment conditions (not disclosed). Particle size distribution was determined by stirring powders in 50 °C water for 1 h (5%, w/v, protein) and the D_{90} value reported for the MPC with more native whey was 3.13 µm, compared with 0.25 µm for the other powder. These values are considerably lower than those reported in the current study, which ranged from 32.7 to 128 µm, depending on the heat treatment applied. Particle size values within that range (i.e., 0.25–3.13 µm) are usually only attainable following intense high-shear treatment, such as the value reported by Pathania, Ho, Hogan, McCarthy, and Tobin (2018) of 0.4 µm when MPC was reconstituted using hydrodynamic cavitation. The D_{90} value of 39.6 µm for C-MPC is similar to the result of 59.4 µm reported by McCarthy, Kelly, Maher, and Fenelon (2014) for an MPC (81.4%, w/w, protein) prepared by overhead stirring in 50 °C water for 1 h. Khalesi and FitzGerald (2021b) also measured solubility of MPC powders after dispersing them for 30 min in water. Following a 4 h holding time, the average solubility was ~73 and 77% for the sample with more native whey protein, at 25 and 50 °C, and ~66 and 88% for the powder with less native whey protein at these temperatures, respectively. This seems contrary to the results presented in the current study which demonstrate that rehydration performance of MPC is impaired by the application of an intensive thermal process to the concentrate directly after membrane filtration and prior to spray drying, particularly upon reaching a temperature of 100 °C for 30 s. This is likely a result of the formation of whey protein and whey-casein aggregates which do not dissolve easily and remain even after overnight stirring, thereby further impairing MPC powder dissolution. Overall, the results suggest that heat treatment of ultrafiltration concentrate at ≥100 °C for 30 s can present processing challenges for users of MPC powder (i.e., will be more difficult to solubilise the powder). One approach for addressing this challenge may be the use of high-shear technologies to accelerate powder dissolution. It is important to

consider this as other powder applications may require high levels of denatured whey proteins, such as yogurt manufacture.

4. Conclusion

Heat treatment of liquid milk protein concentrate (MPC) after ultrafiltration can play an important role in modifying its functional properties and those of the resulting powders. Heating liquid MPC at 80–120 °C for 30 s increased viscosity. Physical properties of MPC powders were altered by heating of the concentrate, most notably an increase in bulk density and powder particle size. Protein profile analysis demonstrated a significant decrease in the proportion of native whey proteins as the temperature of heat treatment increased, which appeared to confer higher heat stability to MPC at pH 6.9 and 7. However, the creation of protein aggregates impaired the dispersion and solubilisation of MPC powders, with large particles remaining after extensive mixing. The results presented in this study will inform dairy ingredient researchers and manufacturers of the benefits and disadvantages of applying heat treatments to liquid milk protein concentrates prior to spray drying. Further studies involving the effect of both heat treatment and evaporation of MPC prior to spray drying on powder rehydration performance are required, and how this influences end-product functionality.

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Table 1

Physicochemical properties of liquid control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate (MPC) samples prior to spray drying. [a]

MPC	pH	z-average diameter (nm)	Calcium ion concentration (mmol L^{-1})	L*	a*	b*	ΔE
C	6.68	183 ± 8.8[a]	3.23 ± 0.06[a]	75.7 ± 0.14[a]	–3.33 ± 0.02[a]	–0.77 ± 0.05[a]	–
LH	6.71	181 ± 5.8[a]	3.79 ± 0.05[b]	77.8 ± 0.13[b]	–3.61 ± 0.01[b]	–0.03 ± 0.05[b]	2.23 ± 0.02[a]
MH	6.71	189 ± 7.1[a]	4.08 ± 0.08[c]	79.2 ± 0.08[c]	–4.03 ± 0.02[c]	–0.28 ± 0.02[c]	3.61 ± 0.06[b]
HH	6.72	195 ± 4.6[a]	4.11 ± 0.02[c]	80.3 ± 0.02[d]	–4.11 ± 0.01[d]	–0.54 ± 0.01[d]	4.65 ± 0.12[c]

[a] Abbreviation: ΔE, total colour difference. Values within columns not sharing common superscript letters differ significantly ($P < 0.05$). All measurements were recorded at 25 °C.

Table 2

Viscosity and rate index values for liquid control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate (MPC) samples at 5 and 40 °C, derived from the Herschel-Bulkley model.

MPC	Viscosity (mPa s)		Rate index	
	5 °C	40 °C	5 °C	40 °C
C	693.3 ± 24.1	23.7 ± 5.6	0.70 ± 0.00	0.78 ± 0.04
LH	3033 ± 304	74.9 ± 36.1	0.59 ± 0.01	0.77 ± 0.09
MH	3467 ± 348	73.4 ± 22.1	0.59 ± 0.01	0.79 ± 0.06
HH	4364 ± 473	96.4 ± 27.1	0.56 ± 0.01	0.76 ± 0.05

Table 3

Physical properties of control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate (MPC) powders. [a]

MPC	Density parameters (g cm^{-3})			Volume parameters (mL 100 g^{-1})		$D_{[4,3]}$ (μm)
	p_p	p_b	p_t	V_{ia}	V_{oa}	
C	1.17 ± 0.00	0.22 ± 0.01	0.28 ± 0.00	272 ± 0.3	16.9 ± 0.1	27.4 ± 0.6[a]
LH	1.09 ± 0.00	0.26 ± 0.00	0.32 ± 0.00	221 ± 0.0	22.9 ± 0.9	43.9 ± 3.8[b]
MH	1.02 ± 0.00	0.26 ± 0.00	0.33 ± 0.00	205 ± 1.0	29.1 ± 1.1	71.4 ± 2.6[c]
HH	1.03 ± 0.00	0.25 ± 0.00	0.32 ± 0.00	216 ± 0.7	28.4 ± 0.3	73.4 ± 3.5[c]

[a] Abbreviations are: p_p, particle density; p_b, loose bulk density; p_t, tapped bulk density; V_{ia}, volume of interstitial air; V_{oa}, volume of occluded air; $D_{[4,3]}$, volume-weighted mean particle diameter. Values within column not sharing common superscript letters differ significantly ($P < 0.05$).

Table 4

Concentration (mg 100 mL^{-1}) and denaturation (%) of whey proteins in control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate (MPC) samples following heat treatment. [a]

MPC	α-Lactalbumin	β-Lactoglobulin
C	0.045 ± 0.001	0.313 ± 0.002
Denaturation	–	–
LH	0.039 ± 0.002	0.222 ± 0.004
Denaturation	14.4 ± 1.7	29.0 ± 1.4
MH	0.030 ± 0.004	0.098 ± 0.014
Denaturation	33.0 ± 9.5	68.6 ± 4.4
HH	0.017 ± 0.000	0.029 ± 0.001
Denaturation	62.2 ± 0.7	90.7 ± 0.4

[a] Denaturation is expressed as the percentage decrease in concentration of native whey proteins relative to the control sample.

Table 5

Particle size values for control (C), low heat (LH), medium heat (MH) and high heat (HH) milk protein concentrate (MPC) dispersions. [a]

Rehydration procedure	MPC	D_{90} (μm)	$D_{[4,3]}$ (μm)
23 °C for 30 s	C	55.0 ± 1.2^{a}	35.3 ± 0.6^{a}
	LH	65.3 ± 2.7^{b}	39.9 ± 1.7^{b}
	MH	71.4 ± 3.4^{c}	43.0 ± 1.7^{c}
	HH	66.6 ± 2.8^{bd}	41.2 ± 2.0^{bcd}
50 °C for 30 s	C	85.2 ± 3.6^{a}	24.8 ± 2.2^{a}
	LH	147 ± 8.2^{b}	65.6 ± 4.1^{b}
	MH	151 ± 15^{bc}	74.0 ± 6.4^{c}
	HH	171 ± 14^{d}	78.6 ± 4.4^{cd}
50 °C for 1 h	C	39.6 ± 4.5^{a}	11.2 ± 2.2^{a}
	LH	32.7 ± 5.3^{a}	8.95 ± 2.9^{a}
	MH	115 ± 12^{b}	51.1 ± 4.3^{b}
	HH	128 ± 18^{b}	51.5 ± 8.1^{b}
4 °C for 21 h	C	21.4 ± 3.5^{a}	4.90 ± 0.68^{a}
	LH	23.1 ± 5.2^{a}	6.61 ± 2.03^{a}
	MH	60.9 ± 7.9^{b}	22.9 ± 3.0^{b}
	HH	95.0 ± 9.3^{c}	40.3 ± 6.8^{c}

[a] Abbreviations are: D_{90}, particle size below which 90% of material volume exists; $D_{[4,3]}$, volume-weighted mean particle diameter. Dispersions were obtained after high-shear mixing at 23 °C and 50 °C for 30 s using a solubility index meter, overhead stirring (500 rpm) in 50 °C ultrapure water for 1 h and magnetic stirring (250 rpm) for 21 h (4 °C). The dispersions used for overnight magnetic stirring were those prepared by overhead stirring powders in 50 °C ultrapure water for 1 h. Values within columns, for each rehydration procedure, not sharing common superscript letters differ significantly ($P < 0.05$).

Figure legends

Fig. 1. Viscosity as a function of shear rate at (A) 5 °C for control (■), low-heat (◆), medium-heat (▲) and high-heat (●) milk protein concentrates and at (B) 40 °C for control (□), low-heat (◇), medium-heat (△) and high-heat (○) milk protein concentrates (18.3%, w/w, total solids).

Fig. 2. Sodium dodecylsulphate-polyacrylamide gel electrophoresis profiles of milk protein concentrate solutions under non-reducing conditions: control (lane 1), low-heat (lane 2), medium-heat (lane 3) and high-heat (lane 4).

Fig. 3. pH-heat coagulation time profiles at 140 °C for control (■), low-heat (◆), medium-heat (△) and high-heat (○) milk protein concentrate powders reconstituted to 3.5% (w/w) protein.

Fig. 4. Particle size distribution profiles of control (■), LH (◆), MH (△) and HH (○) milk protein concentrate powders measured after reconstitution in ultrapure water for (A) 30 s at 23 °C, (B) 30 s at 50 °C (C) 1 h at 50 °C and (D) overnight stirring.

Fig. 5. Solubility values for control (C), low-heat (LH), medium-heat (MH) and high-heat (HH) milk protein concentrate dispersions after mixing for 30 s in 23 °C (dark grey bars) and 50 °C (light grey bars) ultrapure water, followed by centrifugation at 3000 × *g* for 10 min. Values within each temperature category not sharing a common superscript differ significantly ($P < 0.05$).

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

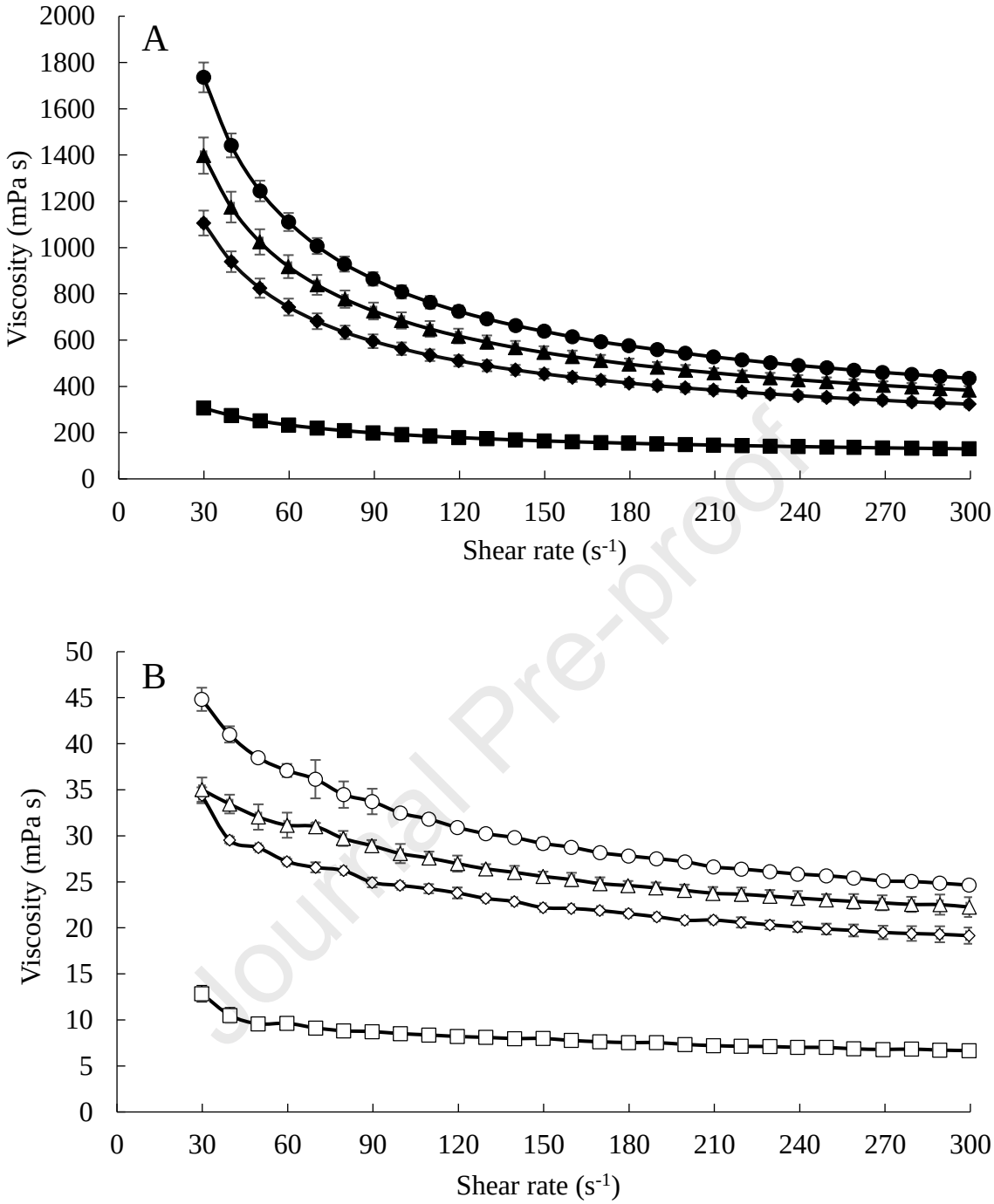


Figure 1

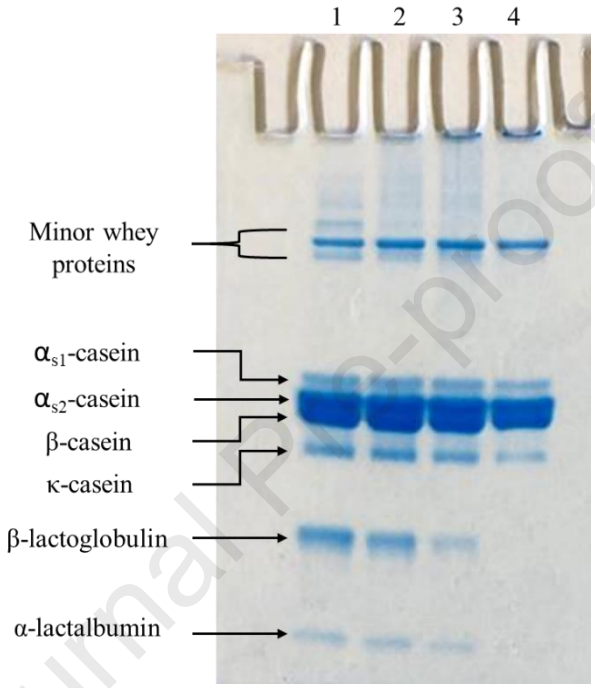
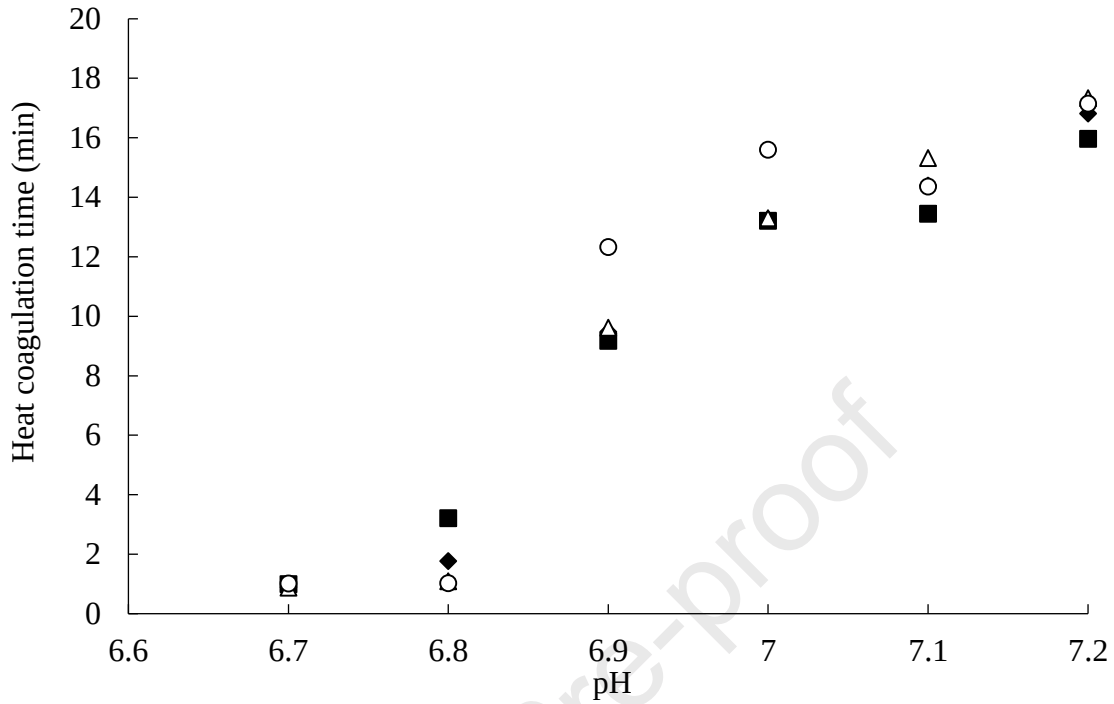


Figure 2



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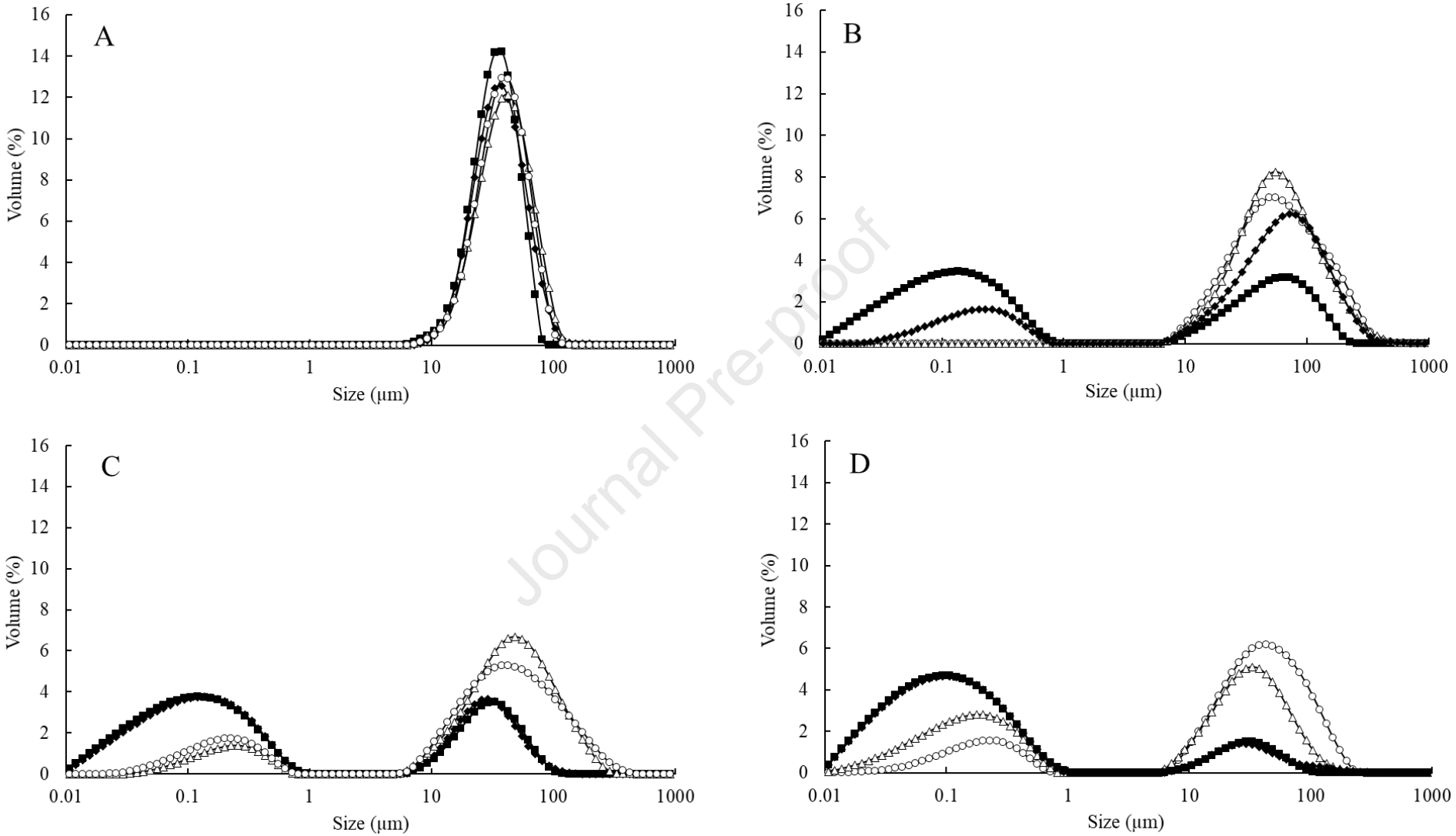

Figure 4

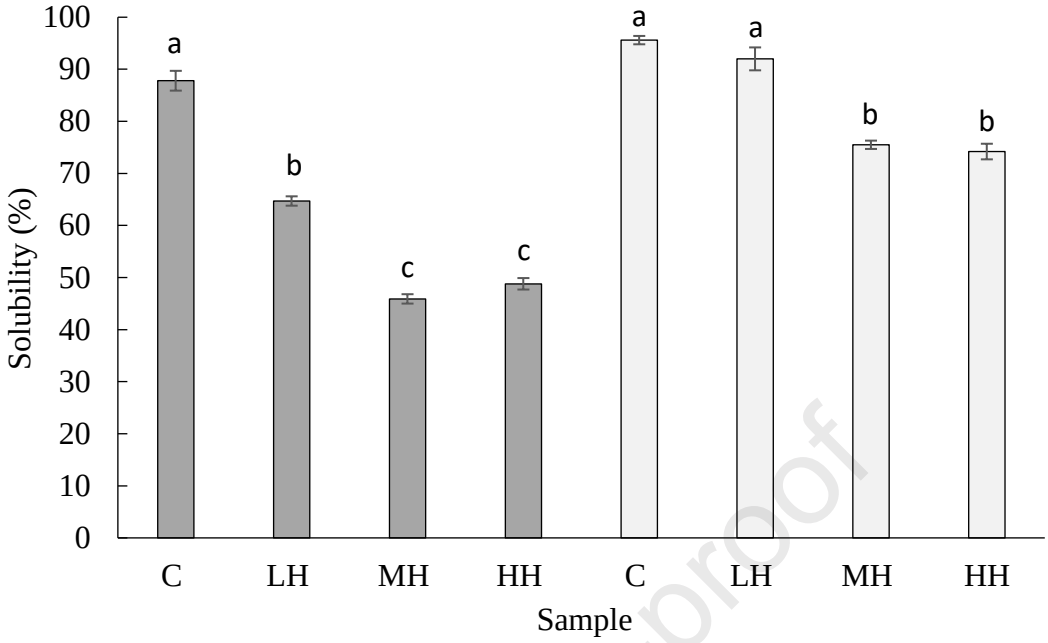

Figure 5

Credit author statement

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