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Effects of milk heat treatment and solvent composition on physicochemical and selected functional characteristics of milk protein concentrate

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ABSTRACT

Milk protein concentrate (MPC) powders (~81% protein) were made from skim milk that was heat treated at 72°C for 15 s (LHMPC) or 85°C for 30 s (MHMPC). The MPC powder was manufactured by ultrafiltration and diafiltration of skim milk at 50°C followed by spray drying. The MPC dispersions (4.02% true protein) were prepared by reconstituting the LHMPC and MHMPC powders in distilled water ($LHMPC_w$ and $MHMPC_w$, respectively) or milk permeate ($LHMPC_p$ and $MHMPC_p$, respectively). Increasing milk heat treatment increased the level of whey protein denaturation (from ~5 to 47% of total whey protein) and reduced the concentrations of serum protein, serum calcium, and ionic calcium. These changes were paralleled by impaired rennet-induced coagulability of the $MHMPC_w$ and $MHMPC_p$ dispersions and a reduction in the pH of maximum heat stability of $MHMPC_p$ from pH 6.9 to 6.8. For both the LHMPC and MHMPC dispersions, the use of permeate instead of water enhanced ethanol stability at pH 6.6 to 7.0, impaired rennet gelation, and changed the heat coagulation time and pH profile from type A to type B. Increasing the severity of milk heat treatment during MPC manufacture and the use of permeate instead of water led to significant reductions in the viscosity of stirred yogurt prepared by starter-induced acidification of the MPC dispersions. The current study clearly highlights how the functionality of protein dispersions prepared by reconstitution of high-protein MPC powders may be modulated by the heat treatment of the skim milk during manufacture of the MPC and the composition of the solvent used for reconstitution.
Key words: milk protein concentrate, milk heat treatment, solvent composition, functionality

INTRODUCTION

Developments in membrane filtration of milk since the 1970s have led to the availability of a range of high-protein powders, including milk protein concentrates (**MPC**), micellar caseins, whey protein concentrates and isolates, and α-LA. Milk protein concentrates with high protein content (e.g., ≥80%) are prepared by concentration of milk protein (casein and whey protein) using UF and diafiltration (**DF**) of the resultant retentate to dilute out most of the milk serum and its solids components, including lactose, soluble salts, and nonprotein nitrogen (**NPN**). Huppertz and Gazi (2015) reported that the level of denaturation of β-LG in commercial MPC powders varies from approximately 20 to 80% of total, indicating that the milk heat treatment applied during MPC manufacture varies extensively.

Milk protein concentrates are used extensively in food manufacture and formulation, with applications including dairy-based beverages, yogurt, fresh cheese products, recombined milk cheeses, ice cream, coffee whitener, high-protein bars, and alcoholic dairy beverages. During food formulation, MPC is exposed to environments differing substantially in TS content, the types and levels of ingredients, the composition of the solvent phase (e.g., ionic strength, pH, sugar content), and processing conditions (e.g., heat, acidification, rennet gelation, addition of ethanol). Nevertheless, MPC must provide the requisite functionalities or combinations thereof, including emulsification, gelation, foaming, heat stability, or nutritive value (Patel and Patel, 2014; Ikeda, 2015). High-protein MPC powders are more functional than other ingredients, such as skim milk powder or whey protein concentrates, in many applications owing to the combined functionalities of both casein and whey protein, their neutral flavor (e.g., compared with sodium caseinate), and their low lactose content (<3%). Lactose is a nonfunctional ingredient (i.e., inert carbohydrate filler) in many formulations, and high levels increase formulation cost, the risk of crystal formation in products such as ice cream, and browning in products subjected to high-temperature

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conditions during manufacture (e.g., ultra-high-heat treated products) or food service (e.g., formulated foods that are baked or grilled).

Several recent studies have reported the effects of manufacturing conditions on the functionality of high-protein MPC, including heat treatment of the skim milk before UF (Crowley et al., 2015; Gazi and Huppertz, 2015), alteration of calcium (Ca) content by preacidification of the skim milk before UF or DF (Luo et al., 2016; Eshpari et al., 2017), lowering the temperature of the milk during UF (Luo et al., 2015), addition of NaCl (Mao et al., 2012) or calcium-chelating salts to the skim milk (Ramchandran et al., 2017) before UF or the retentate before DF (Bhaskar et al., 2001; Guinee et al., 2009), and high-pressure treatment of the skim milk before UF (Udabage et al., 2012). Increasing the severity of milk heat treatment from 72°C for 15 s to 95°C for 45 s led to denaturation of 65% of total β-LG and 25% of total α-LA (Gazi and Huppertz, 2015) but had little effect on the heat coagulation time (**HCT**) of aqueous dispersions of the MPC (8.5% protein) at 120°C in the pH range 6.3 to 7.1 (Crowley et al., 2015).

The effect of solvent quality on the functionality of dispersions prepared from high-protein MPC has also been investigated. Crowley et al. (2014) evaluated the effect of substituting water with simulated milk ultrafiltrate (**SMUF**) or SMUF with lactose (4.6%) and urea (30 mg/100 g) on the HCT of protein dispersions (3.5%) prepared from low-heat MPC with 80% protein (wt/wt). The HCT of a water-based dispersion of MPC with 80% protein (3.5% protein) at 140°C remained very low (<2 min) at pH 6.3 to 6.9 and then increased as the pH was further increased to 7.2. The use of SMUF or SMUF with lactose instead of water introduced a maximum HCT at pH 6.7 to 6.8. However, cold dialysis of the water-based dispersion against reconstituted skim milk resulted in a type A HCT versus pH profile with a maximum HCT at 6.9 and minimum HCT at pH 7.1. There are 2 types of HCT versus pH profiles for bovine milk: type A, which is the most common and is characterized by a maximum HCT at pH 6.6 to 6.7 and a minimum HCT at pH 6.8 to 7.0, and type B, for which HCT increases progressively with pH increases in the range of 6.2 to 7.2 (O'Connell and Fox, 2003). Eshpari et al. (2015, 2017) altered the solvent composition of protein dispersions (3.2% protein) prepared from standard- or reduced-Ca MPC with protein content ≥80% by overnight dialysis against skim milk at 4°C. The pH of the nondialyzed standard-Ca and reduced-Ca samples was 7.1 and 6.68, respectively, whereas that of the corresponding dialyzed standard-Ca and reduced-Ca samples was 6.65 and 6.65, respectively. Dialysis increased the concentrations of nonsedimentable protein and Ca of both the standard-Ca and reduced-Ca dispersions, the HCT of the reduced-Ca dispersion, and the storage modulus (**G′**) of the rennet-treated standard-Ca dispersion. Meletharayil et al. (2016) studied the effects of increasing lactose content (~0.3, 5.6, and 11.2%, wt/wt) on the glucono-δ-lactone-induced gelation of 4% protein dispersions prepared from low-heat MPC with 80% protein. Increasing lactose content from 0.3 to 11.2% coincided with increases in the pH at onset of gelation (from pH ~5.35 to 5.55) and G′ at pH 4.6 (from ~340 to 460 Pa) and a reduction in the level of expressible serum (whey) on centrifugation at 3,000 × *g* (from 67 to 36 g/100 g).

To our knowledge, there is no comprehensive study on the combined effects of milk heat treatment and solvent composition on the functionality of MPC dispersions. The objectives of the current study were to investigate the effects of milk heat treatment (72°C for 15 s or 85°C for 30 s) during the manufacture of MPC powder and the solvent (water or milk permeate) used for reconstitution of the MPC powder on the composition, physicochemical, and key functional characteristics of the resultant MPC protein dispersions (4% true protein). Commercially, water and milk permeate are commonly used solvents in formulated food products.

MATERIALS AND METHODS

Manufacture of Low- and Medium-Heat MPC

The MPC was produced in the Bio Functional Food Engineering pilot plant unit of Moorepark Technology Limited (Teagasc, Moorepark, Fermoy, Co. Cork). Milk was separated at 55°C (Westfalia model MM1254 separator, Westphalia, Germany). Skim milk (~800 L) was split into 2 portions (~400 L); one was used for the manufacture of low-heat MPC (**LHMPC**), and the other for the manufacture of medium-heat MPC (**MHMPC**). Milk was pasteurized at 72°C for 15 s using a plate heat exchanger (APV Pasilac SSP pilot plant, APV DK 8600, Silkeborg, Denmark) for LHMPC or at 85°C for 30 s using a pilot-scale tubular heat exchanger (MicroThermics, Raleigh, NC) for MHMPC.

The pasteurized skim milk was UF at 50°C (10 kDa; total membrane area: 27 m^2; ST28 3838 UF membrane; Synder Filtration, Vacaville, CA) to 21% TS. The resultant retentate was diluted with deionized water (50°C) at a retentate:water weight ratio of 1:1, diafiltered to 21% TS using UF at 50°C, and spray dried (Anhydro spray dryer, SPX Flow Technology Danmark A/S, Soeborg, Denmark) using nozzle atomization at inlet and outlet air temperatures of 180 and 85°C, respectively. The LHMPC and MHMPC powders (~4 kg of each type) were packed in silver aluminum bags and stored at 15°C until they were used for analysis. Both LHMPC

and MHMPC were each produced on 2 separate occasions (trials), and both powder types were produced from the same milk on each occasion.

Milk Permeate

During the preparation of MPC, a portion (10 L) of permeate obtained during UF of the pasteurized skim milk (72°C at 15 s) was collected. The sample was divided into 250-mL quantities, each of which was rapidly frozen in liquid nitrogen and then placed in a freezer at −20°C until required.

Preparation of Protein Dispersions from MPC Powders

Protein dispersions with 4.02% true protein (wt/wt) were prepared by dispersing the MPC powder in distilled water ($\mathbf{MPC_w}$) or milk permeate ($\mathbf{MPC_p}$) at 50°C while continually stirring at 500 rpm (IKA RT10 magnetic stirrer, IKA-Werke GmbH & Co. KG, Staufen, Baden-Württemberg, Germany) for approximately 2 h and holding and stirring overnight at 4°C to ensure protein hydration. Prior to analysis, the MPC dispersions were warmed to 40°C and held for 30 min to reverse cold aging and then cooled to 25°C for analysis.

To eliminate the effect of the difference in pH between the MPC_w (~7.0) and MPC_p (~6.65) on the serum composition, particle size, and zeta potential of the water- and permeate-based protein dispersions, the pH of the subsamples of the water-based dispersions was adjusted to 6.65 at room temperature. The protein dispersions prepared from the LHMPC powder in distilled water, water followed by pH adjustment to 6.65, or milk permeate are denoted as $\mathbf{LHMPC_w}$, $\mathbf{LHMPC_{w\text{-}pHa}}$, and $\mathbf{LHMPC_p}$, respectively; the corresponding dispersions prepared from MHMPC powder are denoted as $\mathbf{MHMPC_w}$, $\mathbf{MHMPC_{w\text{-}pHa}}$, and $\mathbf{MHMPC_p}$, respectively.

Solubility of Milk Protein Dispersions

The solubility of MPC dispersions after preparation and after overnight hydration at 4°C while stirring was determined by measuring the percentage of TS that remained nonsedimentable on centrifugation at 700 × *g* for 10 min at 24°C using the method described by Carr (1999). The TS were measured using the CEM SMART Trac II (CEM Corp., Charlotte, NC). Solubility is expressed as percentage solubility, defined as percentage TS in supernatant as a percentage of TS in the original dispersion.

The solubility was also determined indirectly by measuring the insolubility index using a modification of the International Dairy Federation standard method for dried milk and dried milk products (IDF, 1989). The modifications involved using a weight (~5 g) sufficient to give a true protein content of 4.02% (wt/wt) and permeate instead of water as a solvent for the preparation of the $LHMPC_p$ and $MHMPC_p$ dispersions. The dispersions (24°C) were centrifuged at 172 × *g* (Funke Gerber, type SuperVario-N; Funke-Dr.N.Gerber Labortechnik GmbH, Berlin, Germany), and the volume of sediment (mL) was measured visually.

Ultracentrifugation of Milk Protein Dispersions

Protein dispersions were ultracentrifuged at 100,000 × *g* at 25°C for 1 h to determine the proportions of sedimentable and nonsedimentable proteins and minerals. The resultant supernatant was filtered through glass wool to ensure removal of any residual fat. The sediment layer (pellet) was lyophilized at −46°C (FreeZone Freeze Dry Systems, Labconco, Kansas City, MO) under vacuum ($\leq 130 \times 10^{-3}$ mBar).

Compositional Analysis of Skim Milk, Milk Permeate, MPC Powder, Protein Dispersion, Supernatant, and UF Permeate

Skim milks and MPC powders were characterized for gross composition including fat and TS using the CEM SMART Trac II; lactose was assayed using a Megazyme lactose/galactose assay kit (Megazyme International Ireland, Bray, Co. Wicklow, Ireland); and total protein, noncasein N, NPN, Ca, and P were measured by International Dairy Federation standard methods, as described by Lin et al. (2016). The protein dispersion, supernatant obtained on ultracentrifugation, and UF permeate were analyzed for total protein, noncasein N, NPN, Ca, and P using International Dairy Federation standard methods and individual caseins by reverse-phase HPLC as described by Lin et al. (2016). Additionally, the protein dispersions were assayed for fat and TS using the CEM SMART Trac II and lactose by the Foss MilkoScan FT+ (Foss Electric A/S, Hillerød, Denmark). The concentration of ionic calcium ($[Ca^{2+}]$) was measured at room temperature using the sensION+ 9660C calcium combination ion selective electrode (Hach Lange, Barcelona, Spain), where calibration was performed using $CaCl_2$ solutions (0.0, 0.5, 2.5, 5.0, and 10.0 m*M*) as described by Lin et al. (2016).

Casein number, which refers to casein N as a percentage of total N, was calculated as follows:

Casein number = 100 – noncasein N (% of total N).

Native whey protein (WP), expressed as a percentage of total protein, was calculated by the formula

$$\text{WP} = 100 - \text{casein number} - \text{NPN},$$

where casein number is casein N as a percentage of total N, and NPN, expressed as a percentage of total N, are measured on the unheated (raw) skim milk. The percentage of whey protein denaturation (WPD) was calculated according the following relationship (Guinee et al., 1997):

$$\%\ \text{WPD} = \frac{100\left(\text{WP}_{\text{sm}} - \text{WP}_{\text{hm}}\right)}{\text{WP}_{\text{sm}}},$$

where WP_{sm} and WP_{hm} denote the levels of native whey protein in the unheated skim milk and pasteurized skim milks (72°C for 15 s or 85°C for 30 s), respectively. Similarly, the percentage of whey protein denaturation of the LHMPC and MHMPC dispersions was calculated.

Physicochemical Characteristics of Protein Dispersions

The mean particle size, expressed as z-average (nm), and zeta potential (mV) of the protein dispersions were determined using a Malvern Zetasizer Nanoseries Nano-ZS (Malvern Instruments Ltd., Malvern, UK), as described by Lin et al. (2016). Casein hydration was measured by lyophilization of the pellet obtained on ultracentrifugation (100,000 × *g* for 1 h at 25°C) and expressed as grams of water per gram of sedimented casein (Lin et al., 2016).

Rennet Gelation

Protein dispersions were adjusted to pH 6.55 at 21°C and equilibrated for 15 min. Chymosin (single strength Chy-Max Plus, 200 international milk clotting units/mL; Chr. Hansen, Hørsholm, Denmark) was diluted 20-fold in distilled water and added to give 1.03 international milk clotting units/g of protein. Gel formation was monitored by measuring the change in G′ over time using low-amplitude strain oscillation rheometry (CarriMed, type CSL^{2}500, TA Instruments, New Castle, DE). The following parameters were calculated from the resulting G′ versus time curves as described by Lin et al. (2016): rennet gelation time, maximum gel firming rate ($\mathbf{GFR_{max}}$), and storage modulus at 60 min ($\mathbf{G'_{60}}$).

HCT and Ethanol Stability

The pH of protein dispersions was adjusted to values in the range 6.2 to 7.2 at 21°C by incremental addition of 0.1 *N* HCl or NaOH. The HCT of pH-adjusted protein dispersions was measured in a thermostatically controlled oil bath (Hettich Elbanton Special Product, Hettich Benelux B. V., Geldermalsenat, the Netherlands) at 140°C, as described previously (Lin et al., 2016).

For the measurement of ethanol stability (**ES**), the protein dispersions were adjusted to pH values ranging from 6.2 to 7.0 at 21°C. The pH-adjusted samples were blended with aqueous ethanol solutions ranging in concentration from 30 to 98% (vol/vol) at a volume ratio of 1:2.4 (protein dispersion:ethanol solution), and the mixture was agitated for 30 s (Whirlimixer, Fisons, Holmes Chapel, UK). The ES was recorded as the minimum concentration of aqueous ethanol solution required to induce flocculation.

Model Stirred Skimmed Yogurt Preparation and Gel Formation

Protein dispersions (1 L) with 5.0% true protein in water with added α-lactose monohydrate powder or in milk permeate were prepared as described above; α-lactose monohydrate powder (>99.0% lactose; Arla Foods Ingredients, Sønderhøj, Denmark) was added to the water to give a total lactose level of 4.8% (wt/wt). The dispersions were heated to 85°C, held for 20 min while stirring continuously at 200 rpm (model RW16; IKA-Werke GmbH & Co.), cooled to 42°C in an ice-water bath, inoculated with direct-vat starter cultures YC380 and CH1 YoFlex (consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* at a weight ratio of 1:3; Chr. Hansen Ireland Ltd., Little Island, Co. Cork, Ireland), and incubated at 42°C (Heratherm Advance Protocol Microbiological Incubators, Thermo Scientific, Waltham, MA) until the pH reached 4.6. Subsamples (~20 mL) were withdrawn periodically during incubation, cooled to room temperature, and monitored for pH. The gelled dispersion was cooled to <8°C in ice water while stirring at 70 rpm (model RW16; IKA-Werke GmbH & Co.) and stored at 4°C for 36 h before analysis.

Immediately after starter culture inoculation, a well-mixed subsample (10 mL) of the dispersion was withdrawn and monitored for changes in loss modulus (**G″**), G′, and loss tangent (**tanδ**; tanδ = G″/G′) over a 9-h period at 42°C using low-amplitude strain oscillation rheometry as described for rennet gelation. Moisture

evaporation from the sample during measurement was prevented by placing a thin layer of tetradecane (Sigma-Aldrich, St. Louis, MO) on the surface and covering the sample with an evaporation blocker.

Water-Holding Capacity of Yogurt

Immediately after cooling to 8°C, 4 subsamples of each yogurt were poured into 50-mL stoppered centrifuge tubes, held at 4°C for 36 h, and centrifuged at 300 or 2,500 × *g* at 8°C for 30 min; the expressed serum was decanted and weighed. The water-holding capacity (**WHC**) was calculated as the total serum less the serum expressed on centrifugation per 100 g of yogurt.

Rheological Properties of Stirred Yogurt

Yogurt was stirred at 70 rpm for 1 min at room temperature (model RW16; IKA-Werke GmbH & Co.) to ensure homogeneity. A subsample (10 g) was placed in the measuring cell of a controlled-stress rheometer (Carri-Med, type CSL^{2}500, TA Instruments). The cell consisted of 2 coaxial cylinders, an outer cup (i.d. 27.5 mm), and an inner bob (diameter 25 mm). Following equilibration at 8°C for 5 min, the sample was subjected to a shear rate ($\dot{\gamma}$) sweep, whereby $\dot{\gamma}$ was increased from 10 to 120 s^{-1}. Shear stress ($\boldsymbol{\sigma}$; Pa) and viscosity (Pa·s) were measured as a function of $\dot{\gamma}$. The resultant $\dot{\gamma}$ versus σ data were fitted to the Herschel–Bulkley model using TA Rheology Advance Data Analysis software (version V5.7.0; TA Instruments):

$$\sigma = \sigma_o + K\dot{\gamma}^n,$$

where σ_o represents yield stress (Pa), K represents the consistency coefficient (Pa·s), and n represents the flow behavior index (Ramaswamy and Basak, 1991).

Statistical Analysis

The data were analyzed using a randomized complete block design, which incorporated the protein dispersions ($LHMPC_w$, $MHMPC_w$, $LHMPC_P$, and $MHMPC_P$) and 2 replicate blocks (samples prepared from the 2 separate trials of MPC made on different days). An ANOVA was carried out using the general linear model procedure of SAS 9.3 (SAS Institute, 2011), and the effects of heat treatment and reconstitution medium on each response variable were determined. Tukey's multiple comparison test was used for paired comparison of treatment means, and the level of significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Composition of MPC Powders and Milk Permeate

The compositions of heat-treated skim milk, MPC powders (LHMPC and MHMPC), and milk permeate (from low-heat skim milk) are shown in Table 1. Increasing the severity of heat treatment of the skim milk from 72 to 85°C resulted in an increase in the level of whey protein denaturation from 5.6 to 47% of total whey protein; otherwise, the gross composition of the low-heat and medium-heat skim milk samples was similar.

The composition of the LHMPC and HHMPC powders is similar to that reported for high-protein commercial (Patel and Patel, 2014) and experimental (Martin et al., 2010; Crowley et al., 2015) MPC powders. There was no significant difference between the levels of TS, total protein, lactose, fat, Ca, or P between the LHMPC and MHMPC powders.

Solubility of MPC Powders

The solubility of the MPC powders in water or permeate, following overnight holding at 4°C and heating of the dispersions at 40°C for 30 min to reverse cold aging, varied from 95.5 to 96.8% and was unaffected by heat treatment of the milk used in MPC manufacture or the solvent used for reconstitution of the MPC (Table 2). The solubility values are comparable with those (>95%) reported by Gazi and Huppertz (2015) for MPC powders with protein levels of 35 to 85% in water.

The insolubility index of MPC in water or permeate decreased from approximately 2.75 to 3.75 mL following dispersion to <0.18 mL of sediment (Table 2) after overnight holding at 4°C, indicating the beneficial effect of cold storage on protein hydration. This observation concurs with the findings of Ferrer et al. (2008), who found that overnight holding of protein dispersions from MPC powders with 56 to 90% protein was accompanied by a reduction in particle size where the dispersions were not subject to high shear (homogenization) during preparation.

Composition of Protein Dispersions

The composition of the MPC protein dispersions is shown in Table 2. Increasing the heat treatment of the skim milk before MPC manufacture did not significantly affect the gross composition or pH of the dispersions, as expected because of the similar compositions of the LHMPC and MHMPC powders. However, it

Table 1. Composition of unheated skim milk, heat-treated skim milk, milk protein concentrate (MPC) powders, and milk permeate

Item[1]	Effect of heat treatment on skim milk[2]			Effect of heat treatment on MPC powders[3]		Milk permeate
	Unheated	LHSM	MHSM	LHMPC	MHMPC	
TS (%, wt/wt)	89.9[a]	90.0[a]	90.3[a]	4.60[a]	4.56[a]	5.04
Protein (%, wt/wt)	4.23[a]	4.24[a]	4.23[a]	81.34[a]	81.54[a]	0.22
Casein (%, wt/wt)	3.47[a]	3.47[a]	3.47[a]	67.81[a]	67.82[a]	0.00
WP (%, wt/wt)	0.56[a]	0.56[a]	0.57[a]	12.97[a]	12.97[a]	0.01
WP denaturation (% of total WP)	0.0[c]	5.7[b]	46.9[a]	5.7[b]	46.9[a]	NA[4]
NPN expressed as protein (%, wt/wt)	0.23[a]	0.23[a]	0.23[a]	0.58[a]	0.54[a]	0.21
Lactose (%, wt/wt)	4.84[a]	4.86[a]	4.80[a]	2.66[b]	2.42[b]	4.79
Fat (%, wt/wt)	0.06[a]	0.05[a]	0.05[a]	1.40[a]	1.42[a]	0.00
pH	6.67[a]	6.66[a]	6.66[a]	—	—	6.58
Na (mg/100 g)	—	—	—	63[a]	75[a]	52
K (mg/100 g)	—	—	—	222[a]	224[a]	165
Ca (mg/100 g)	148[a]	147[a]	147[a]	2,409[a]	2,441[a]	28
P (mg/100 g)	100[a]	99[a]	99[a]	1,360[a]	1,348[a]	33
Mg (mg/100 g)	—	—	—	108[a]	109[a]	10

[a–c]Presented data are the mean values of duplicate trials; values in a row relating to the effect of heat treatment of skim milk with different superscripts differ significantly ($P < 0.05$). Values in a row relating to the effect of heat treatment on MPC powder with different superscripts differ significantly ($P < 0.05$).

[1]WP = whey protein.

[2]LHSM = low-heat-treated skim milk (72°C for 15 s); MHSM = medium-heat-treated skim milk (85°C for 30 s).

[3]LHMPC = MPC powder from LHSM; MHMPC = MPC powder from MHSM.

[4]Not applicable.

led to a reduction in the concentrations of Ca^{2+} and serum Ca ($P < 0.05$) in the permeate-based dispersion ($MHMPC_p$) and of serum Ca in the water-based dispersion ($MHMPC_w$). The reduction in serum Ca suggests precipitation of serum Ca and P as colloidal calcium phosphate (**CCP**).

Table 2. Compositional and physicochemical characteristics of milk protein dispersions[1]

Item[2]	Water-based protein dispersion		Water-based protein dispersion, pH adjusted to 6.65		Permeate-based protein dispersion	
	$LHMPC_w$	$MHMPC_w$	$LHMPC_{w\text{-}pHa}$	$MHMPC_{w\text{-}pHa}$	$LHMPC_p$	$MHMPC_p$
Protein dispersion						
TS (%, wt/wt)	4.9[a,B]	4.8[a,B]	—	—	10.3[a,A]	10.1[a,A]
Lactose (%, wt/wt)	0.1[a,B]	0.1[a,B]	—	—	4.8[a,A]	4.7[a,A]
Total protein (%, wt/wt)	4.06[a,B]	4.02[a,B]	—	—	4.26[a,A]	4.22[a,A]
Casein (%, wt/wt)	3.4[a,A]	3.4[a,A]	—	—	3.4[a,A]	3.4[a,A]
WP (%, wt/wt)	0.63[a,A]	0.61[a,A]	—	—	0.69[a,A]	0.66[a,A]
NPN (% of TN)	0.67[a,B]	0.62[a,B]	—	—	5.01[a,A]	5.01[a,A]
Total Ca (mg/100 g)	119[a,B]	121[a,B]	—	—	149[a,A]	147[a,A]
Ca (mg/g of casein)	35.4[a,B]	35.8[a,B]	—	—	44.4[a,A]	43.5[a,A]
Total P (mg/100 g)	68[a,B]	67[a,B]	—	—	103[a,A]	97[a,A]
Ionic Ca (m*M*)	4.01[a,C]	3.64[a,C]	7.77[a,A]	7.26[b,A]	6.48[a,B]	5.85[b,B]
pH	6.98[a,A]	6.99[a,A]	6.65[a,B]	6.65[a,B]	6.64[a,B]	6.65[a,B]
Solubility						
Solubility (%)	95.4[a,A]	96.8[a,A]	—	—	96.6[a,A]	96.5[a,A]
Sediment volume before hydration (mL)	2.50[a,A]	3.75[a,A]	—	—	2.75[a,A]	3.75[a,A]
Sediment volume after hydration (mL)	0.13[a,A]	0.06[a,A]	—	—	0.17[a,A]	0.12[a,A]
Casein hydration (g of water/g of casein)	3.22[a,A]	3.21[a,A]	3.25[a,A]	3.21[a,A]	3.26[a,A]	3.26[a,A]
Zeta potential (mV)	−28.0[a,A]	−28.0[a,A]	−24.2[a,B]	−23.1[a,B]	−19.9[a,C]	−20.3[a,C]
Particle size (nm)	198[b,A]	219[a,A]	190[b,B]	209[a,B]	158[b,C]	168[a,C]

[a,b]Presented data are the mean values of duplicate trials; values in a row relating to effect of milk heat treatment ($LHMPC_w$ and $MHMPC_w$, $LHMPC_{w\text{-}pHa}$ and $MHMPC_{w\text{-}pHa}$, and $LHMPC_p$ and $MHMPC_p$) with different lowercase superscripts differ significantly ($P < 0.05$).

[A–C]Values in a row relating to the effect of solvent ($LHMPC_w$, $LHMPC_{w\text{-}pHa}$, and $LHMPC_p$; $MHMPC_w$, $MHMPC_{w\text{-}pHa}$, and $MHMPC_p$) with different uppercase superscripts differ significantly ($P < 0.05$).

[1]Low-heat and medium-heat milk protein concentrate powders (LHMPC, MHMPC) manufactured from low-heat-treated (72°C for 15 s) or medium-heat-treated (85°C for 30 s) skim milk were dispersed in water ($LHMPC_w$, $MHMPC_w$) or milk permeate ($LHMPC_p$, $MHMPC_p$). Subsamples of $LHMPC_w$ and $MHMPC_w$ were pH adjusted to pH 6.65 and denoted as $LHMPC_{w\text{-}pHa}$ and $MHMPC_{w\text{-}pHa}$, respectively.

[2]WP = whey protein; TN = total nitrogen.

The use of permeate, rather than water, as solvent led to notable changes in gross composition, with the permeate-based dispersions ($LHMPC_p$ and $MHMPC_p$) having significantly higher concentrations of TS, lactose, total protein, NPN, Ca, P, [Ca^{2+}], serum Ca, and serum P than the corresponding water-based dispersions ($LHMPC_w$ and $MHMPC_w$; Tables 2 and 3). The higher concentrations of these compounds in the MPC_p dispersions are consistent with their presence in the permeate (Table 1). In contrast, the pH of the $LHMPC_p$ and $MHMPC_p$ dispersions (~6.65) was approximately 0.35 unit lower than that of the respective $LHMPC_w$ and $MHMPC_w$ dispersions, an effect most likely due to the lower pH of the permeate per se and the presence of salts in the permeate (e.g., NaCl, KCl, sodium phosphate, sodium citrate), which promote dissociation of carboxyl groups on AA residue side chains of the caseins and the resulting release of protons.

Subsamples of $LHMPC_w$ and $MHMPC_w$ were adjusted to a pH value (~6.65) similar to that of $LHMPC_p$ and $MHMPC_p$. The [Ca^{2+}] of the resulting pH-adjusted dispersions ($LHMPC_{w\text{-}pHa}$ and $MHMPC_{w\text{-}pHa}$) was significantly higher than that of $LHMPC_w$ and $MHMPC_w$ and that of $LHMPC_p$ and $MHMPC_p$. The decrease in pH coincided with an increase in the solubilization of micellar calcium as reflected by the increases in serum Ca and P.

Protein Profile of the Serum

Increasing the heat treatment of the milk from 72 to 85°C resulted in a lower concentration of protein in the serum phase of the $MHMPC_w$ and $MHMPC_p$ dispersions ($P < 0.05$), reflecting the heat-induced interaction of denatured whey proteins with the casein micelle (Singh and Creamer, 1991). Simultaneously, heat-induced dissociation of casein increased in the water-based dispersions, as evidenced by the higher concentration of nonsedimentable casein in the serum of $MHMPC_w$ compared with $LHMPC_w$ (Table 3). The increase in casein dissociation with intensity of heat treatment concurs with the findings of previous studies on reconstituted skim milk (Lin et al., 2018). Reducing the pH of the $MHMPC_w$ dispersion from 7.0 to 6.65 led to a reduction in concentration of protein in the serum, suggesting the reassociation of nonsedimentable casein or κ-casein–whey protein aggregates with the casein micelle. Previous studies have shown that heat-denatured whey proteins complex with dissociated κ-casein to form serum-soluble particles and aggregates; this occurs to a greater extent as the pH during heating is increased over the range of 6.5 to 7.5 (Ménard et al., 2005; Lin et al., 2018).

The substitution of water with permeate did not affect the total concentration of protein in the serum but resulted in a lower concentration of nonsedimentable casein and a higher concentration of NPN (Table 2, 3). The lower concentration of nonsedimentable casein on using permeate is analogous to that found on addition of $CaCl_2$ to sodium caseinate or MPC dispersions (Pitkowski et al., 2009; Sandra and Corredig, 2013), whereby calcium addition contributed to aggregation of the caseins.

For all dispersions ($LHMPC_w$, $LHMPC_p$, $MHMPC_w$, $MHMPC_p$), the levels of individual caseins in the serum, as a proportion of the total corresponding casein in milk, were highest for κ-casein and lowest for α_{S1}-casein (Figure 1). Increasing milk heat treatment led to

Table 3. Composition of serum obtained on ultracentrifugation of milk protein dispersions[1,2]

Item	Water-based protein dispersion		Water-based protein dispersion, pH adjusted to 6.65		Permeate-based protein dispersion	
	$LHMPC_w$	$MHMPC_w$	$LHMPC_{w\text{-}pHa}$	$MHMPC_{w\text{-}pHa}$	$LHMPC_p$	$MHMPC_p$
Protein (%, wt/wt)	$1.1^{a,A}$	$0.9^{b,A}$	$1.1^{a,A}$	$0.7^{b,C}$	$1.1^{a,A}$	$0.8^{b,B}$
Protein (%, milk protein)	$27.3^{a,A}$	$21.6^{b,A}$	$27.1^{a,A}$	$18.6^{b,C}$	$26.2^{a,A}$	$19.9^{b,B}$
Casein (%, wt/wt)	$0.4^{b,A}$	$0.5^{a,A}$	$0.4^{a,A}$	$0.4^{a,AB}$	$0.2^{a,B}$	$0.3^{a,B}$
Casein (% of milk casein)	$12.5^{a,A}$	$15.0^{a,A}$	$12.4^{a,A}$	$12.7^{a,AB}$	$6.7^{a,B}$	$8.3^{a,B}$
Ca (mg/100 g)	$16^{a,B}$	$11^{b,B}$	—	—	$39^{a,A}$	$35^{a,A}$
Ca (% of milk Ca)	$13.1^{a,B}$	$9.1^{b,B}$	—	—	$26.2^{a,A}$	$24.5^{a,A}$
P (mg/100 g)	$11^{a,B}$	$10^{a,B}$	—	—	$38^{a,A}$	$36^{a,A}$
P (% of milk P)	$16.5^{a,B}$	$15.5^{a,B}$	—	—	$36.7^{a,A}$	$37.2^{a,A}$

[a,b]Presented data are the mean values of duplicate trials; values in a row relating to the effect of milk heat treatment ($LHMPC_w$ and $MHMPC_w$, $LHMPC_{w\text{-}pHa}$ and $MHMPC_{w\text{-}pHa}$, and $LHMPC_p$ and $MHMPC_p$) with different lowercase superscripts differ significantly ($P < 0.05$).

[A–C]Values in a row relating to the effect of solvent ($LHMPC_w$, $LHMPC_{w\text{-}pHa}$, and $LHMPC_p$; $MHMPC_w$, $MHMPC_{w\text{-}pHa}$, and $MHMPC_p$) with different uppercase superscripts differ significantly ($P < 0.05$).

[1]Serum phase of protein dispersions, obtained on ultracentrifugation at 100,000 × g at 25°C.

[2]Low-heat and medium-heat milk protein concentrate powders (LHMPC, MHMPC) manufactured from low-heat-treated (72°C for 15 s) or medium-heat-treated (85°C for 30 s) skim milk were dispersed in water ($LHMPC_w$, $MHMPC_w$) or milk permeate ($LHMPC_p$, $MHMPC_p$). Subsamples of $LHMPC_w$ and $MHMPC_w$ were pH adjusted to pH 6.65 and denoted as $LHMPC_{w\text{-}pHa}$ and $MHMPC_{w\text{-}pHa}$, respectively.

a greater increase in the degree of κ-casein dissociation in the water-based dispersions than in the permeate-based dispersions, as seen by comparing $LHMPC_w$ and $MHMPC_w$ and by comparing $LHMPC_p$ and $MHMPC_p$, respectively (Figure 1). The use of permeate instead of water significantly reduced the proportions of κ-, α_{S2}-, and β-caseins in the LHMPC dispersions ($P < 0.05$) and the proportions of κ-, β-, and α_{S1}-caseins in the MHMPC dispersions ($P < 0.05$; Figure 1). Reducing the pH of the $MHMPC_w$ dispersion from approximately 7.0 to 6.65 resulted in a higher $[Ca^{2+}]$, lower zeta potential (Table 2), a lower concentration of nonsedimentable casein (Table 3), and lower proportions of nonsedimentable κ-, β-, and α_{S1}-caseins in $MHMPC_{w\text{-}pHa}$ ($P < 0.05$). A similar trend was observed on reducing the pH of the $LHMPC_w$ dispersion, except that the change in concentration of nonsedimentable casein was not significant ($P > 0.05$; Tables 2 and 3).

Physicochemical Properties of Protein Dispersions

Increasing milk heat treatment coincided with an increase in particle size in both the MPC_w and MPC_p dispersions ($P < 0.05$) but did not affect casein hydration or zeta potential (Table 2). The increase in particle size is consistent with heat-induced denaturation of whey proteins and their interaction with κ-casein through thiol-disulphide interchange at the micelle surface (Singh et al., 1988; Corredig and Dalgleish, 1996; Anema et al., 2004).

The use of permeate in place of water significantly reduced particle size and zeta potential ($P < 0.05$), as seen by comparing $LHMPC_w$ and $LHMPC_p$ and comparing $MHMPC_w$ and $MHMPC_p$, respectively (Table 2). The lower particle size and zeta potential in the $LHMPC_p$ and $MHMPC_p$ dispersions is consistent with their lower pH, higher $[Ca^{2+}]$, and the higher concentration of other ionic species such as K^+, Na^+, and Mg^{2+} in the permeate (Table 1; Schmidt and Poll, 1986; Udabage et al., 2000; Philippe et al., 2003). Hence, when the pH of the $LHMPC_w$ and $MHMPC_w$ dispersions was reduced from approximately 7.0 to 6.65, the concentration of $[Ca^{2+}]$ increased and both zeta potential and particle size decreased (Table 2). The reduction in particle size on reducing the pH of the water-based dispersions from 7.0 to 6.65 concurs with the findings of Sinaga et al. (2017) and reflects the decrease in zeta potential.

Rennet Gelation of Protein Dispersions

Increasing the milk heat treatment during the manufacture of MPC led to a significant deterioration in rennet coagulability at pH 6.55, as evidenced by the inferior rennet coagulability (lower GFR_{max} and lower G'_{60}) of $MHMPC_w$ and $MHMPC_p$ compared with $LHMPC_w$ and $LHMPC_p$, respectively (Figure 2a and

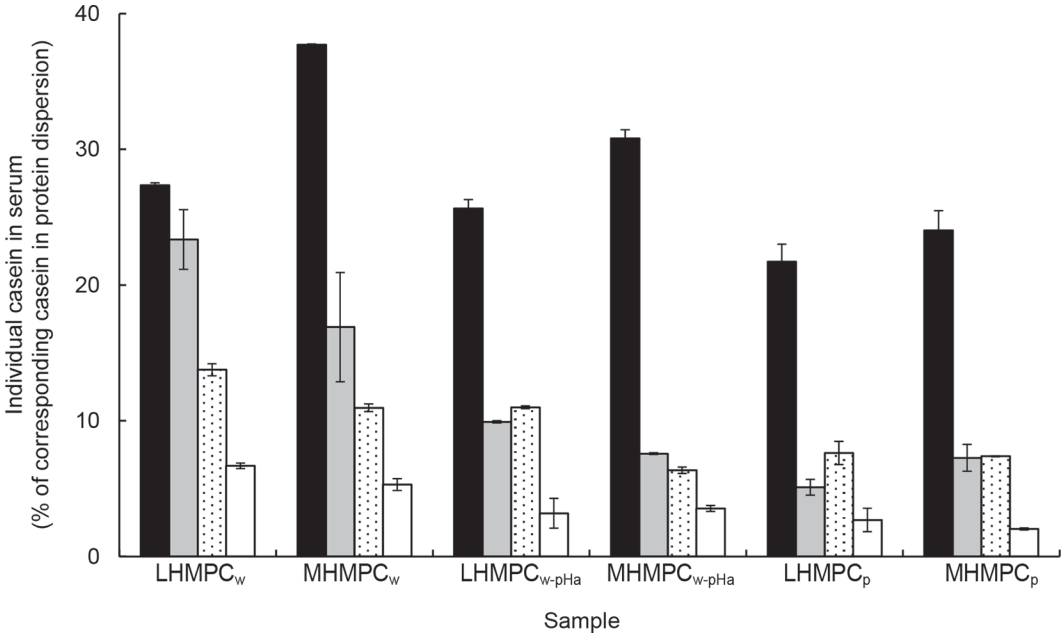

Figure 1. Proportions of individual caseins in the serum prepared by ultracentrifugation of the low-heat (LH) or medium-heat (MH) treated milk protein concentrate powders (MPC) reconstituted in water ($LHMPC_w$ and $MHMPC_w$), water followed by pH adjustment to 6.65 ($LHMPC_{w\text{-}pHa}$ and $MHMPC_{w\text{-}pHa}$), or milk permeate ($LHMPC_p$ and $MHMPC_p$). Black bars: κ-casein; gray bars: α_{S2}-casein; dotted bars: β-casein; white bars: α_{S1}-caein. Presented values are the mean values of duplicate trials; error bars represent standard deviations of the mean.

b). The adverse effect of high heat treatment on rennet gelation has been widely reported for milk (Guinee et al., 1997; Schreiber, 2001). Contributing factors include the reduction in the [Ca^{2+}] (Singh et al., 1988; Schreiber, 2001) and the presence of the denatured whey protein–κ-casein aggregates at the micelle surface or in the serum, which provide a steric hindrance to close approach and fusion of paracasein micelles (Ménard et al., 2005; Kethireddipalli et al., 2010; Lin et al., 2018).

The use of permeate instead of water led to a notable deterioration in rennet coagulability, as shown by the longer rennet gelation time and lower GFR_{max} of $LHMPC_p$ relative to $LHMPC_w$ and the lower GFR_{max} and G'_{60} of $MHMPC_p$ relative to $MHMPC_w$ (Figure 2a and b; Table 4). An opposite trend might be expected considering the concomitant reductions in zeta potential and particle size. The negative effect of substituting water with permeate on rennet gelation is most likely

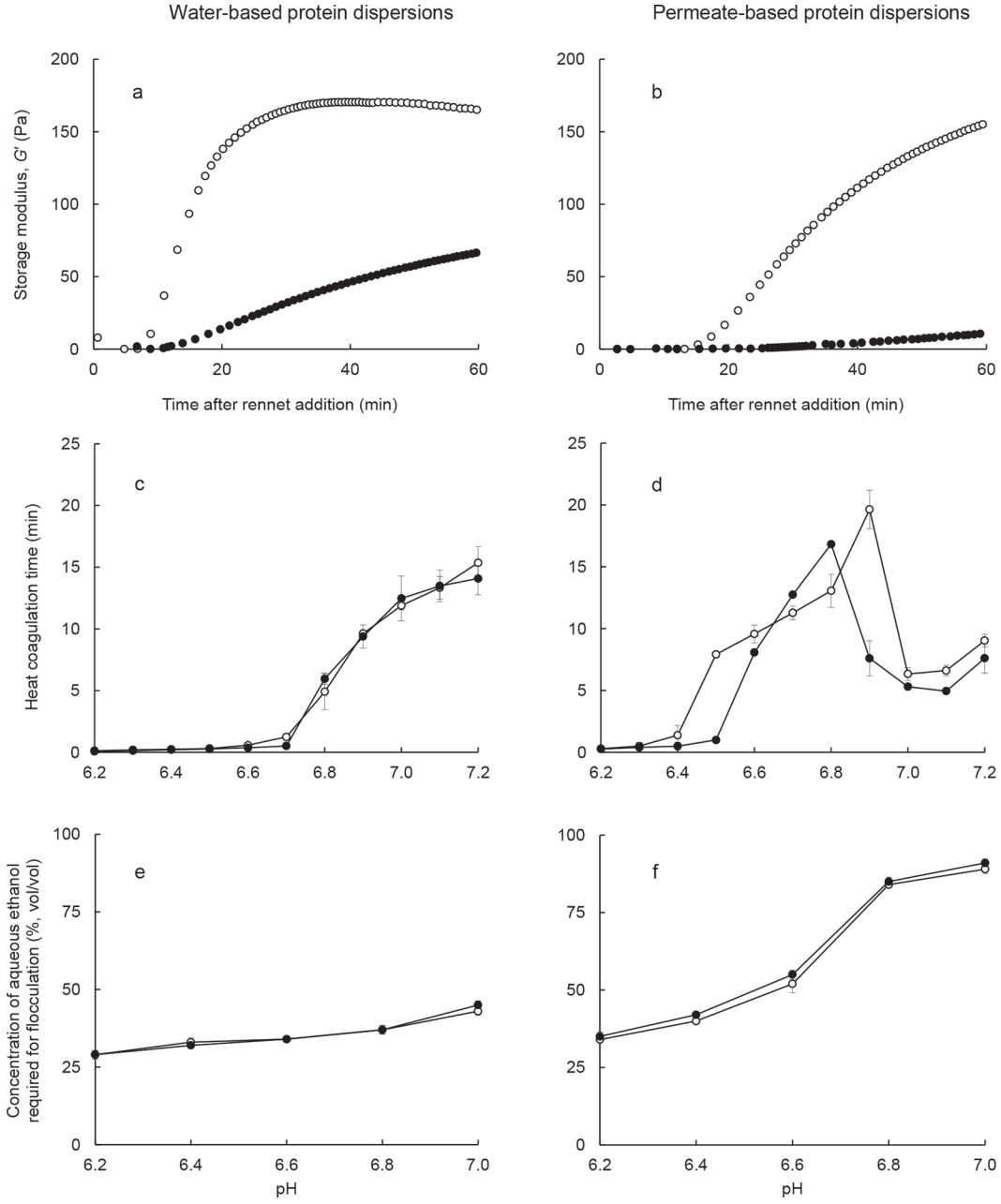

Figure 2. Effect of milk heat treatment (low heat: open symbols; medium heat: closed symbols) during the manufacture of milk protein concentrate powder on rennet gelation characteristics (a, b), heat coagulation time (c, d), and ethanol stability (e, f) of milk protein dispersions prepared by reconstituting the milk protein concentrate powder in water (a, c, e) or milk permeate (b, d, f). Presented data for heat coagulation time and ethanol stability are the mean values of duplicate trials; error bars represent the SD of the mean.

associated with the concomitant reduction in [Ca^{2+}], as seen by comparing $LHMPC_p$ with $LHMPC_{w\text{-}pHa}$ and by comparing $MHMPC_p$ with $MHMPC_{w\text{-}pHa}$ (Table 2), and the increase in concentration of soluble salts (e.g., NaCl, KCl, $MgCl_2$, Na citrate; Table 1). The higher concentrations of soluble salts and ionic strength of the serum phase of the MPC_p dispersions are likely to promote a salting-in effect of the casein in the MPC_p dispersions and thereby diminish casein–casein interaction by charge screening (Damodaran, 1997) and reduce the rennet coagulability relative to the MPC_w dispersions (Abou El Nour, 1998).

The strong rennet gelation behavior of the $LHMPC_w$ and $MHMPC_w$ contrasts with the observations of Martin et al. (2010), who reported the failure of an aqueous dispersion of an experimentally produced MPC in water (3.5% protein) to undergo rennet-induced gelation. The interstudy discrepancy may be related to differences in the concentrations of Ca, P, and [Ca^{2+}] and pH at rennet gelation. The use of cold UF-DF (10°C) by Martin et al. (2010), compared with warm UF–DF (50°C) in the current study, is likely to have depleted the concentration of [Ca^{2+}] and CCP in the MPC (Brule and Fauquant, 1981; Law and Leaver, 1998; Eshpari et al., 2015). Hence, Martin et al. (2010) found that the addition of $CaCl_2$ at concentrations of 2 to 3 mM to the aqueous-based MPC restored rennet-induced gelation. It is also likely that the non-pH-adjustment of the aqueous-based MPC dispersion (e.g., to pH 6.55–6.60) before rennet addition (Martin et al., 2010) would attenuate its rennet-induced coagulability (Nájera et al., 2003).

HCT of Protein Dispersion

Increasing the severity of milk heat treatment from 72°C for 15 s to 85°C for 30 s during MPC manufacture had no effect on the HCT of the water-based MPC dispersion as a function of pH in the range of 6.2 to 7.2 (Figure 2c). However, in the case of the permeate-based MPC dispersion (Figure 2d), it reduced the pH of maximum HCT from approximately 6.9 to 6.8 and the HCT at pH values 6.4, 6.5, and 6.9 ($P < 0.05$). The latter effect is typical of the trend reported for the effect of increasing heat treatment on the HCT versus pH profile of reconstituted milk powder (Carr, 1999; Lin et al., 2018).

The solvent system had a marked effect on the shape of HCT versus pH curves as seen by comparing the profiles of $LHMPC_w$ and $MHMPC_w$ with $LHMPC_p$ and

Table 4. Rennet-induced gelation and model stirred skimmed yogurt–making characteristics of milk protein dispersions[1]

	Water-based protein dispersion		Permeate-based protein dispersion	
Item	$LHMPC_w$	$MHMPC_w$	$LHMPC_p$	$MHMPC_p$
Rennet-induced gelation				
Rennet gelation time (min)	$6.9^{b,B}$	$9.9^{a,A}$	$13.9^{b,A}$	$18.9^{a,A}$
Maximum gel firming rate (Pa/min)	$233^{a,A}$	$46^{b,A}$	$93^{a,B}$	$14^{b,B}$
Storage modulus at 60 min (Pa)	$156^{a,A}$	$74^{b,A}$	$156^{a,A}$	$14^{b,B}$
Gelation during yogurt manufacture				
Denatured whey protein (% of total whey protein)	$79.7^{a,A}$	$82.3^{a,A}$	$76.3^{a,A}$	$81.7^{a,A}$
Gelation onset pH	$5.62^{a,A}$	$5.56^{a,A}$	$5.20^{a,B}$	$5.19^{a,B}$
Gelation onset time (min)	$182^{a,A}$	$187^{a,A}$	$149^{a,B}$	$150^{a,B}$
Storage modulus at pH 4.6 (Pa)	$470^{a,A}$	$316^{b,A}$	$171^{a,B}$	$108^{a,B}$
Time to reach pH 4.6 (min)	$450^{b,A}$	$470^{a,A}$	$205^{a,B}$	$210^{a,B}$
Yogurt properties				
Yield stress (Pa)	$3.4 \times 10^{-8a,A}$	$1.7 \times 10^{-7a,A}$	$4.9 \times 10^{-8a,A}$	$0.3^{a,A}$
Consistency coefficient (Pa·s^n)	$8.8^{a,A}$	$6.5^{a,A}$	$8.2^{a,A}$	$1.2^{b,B}$
Flow behavior index	$0.26^{a,A}$	$0.25^{a,A}$	$0.20^{a,A}$	$0.43^{a,A}$
Viscosity of sample at shear rate of 10 (1/s) (mPa·s)	$1{,}447^{a,A}$	$1{,}082^{b,A}$	$1{,}263^{a,B}$	$642^{b,B}$
Viscosity of sample at shear rate of 120 (1/s) (mPa·s)	$241^{a,A}$	$175^{b,A}$	$170^{a,B}$	$100^{b,B}$
WHC[2] (g of serum retained/100 g)				
300 × g	$66.8^{a,A}$	$66.7^{a,A}$	$66.7^{a,A}$	$65.2^{a,A}$
2,500 × g	$38^{a,A}$	$35^{a,A}$	$34^{a,A}$	$35^{a,A}$

[a,b]Presented data are the mean values of duplicate trials; values in a row relating to the effect of milk heat treatment during manufacture of milk protein concentrate ($LHMPC_w$ and $MHMPC_w$; $LHMPC_p$ and $MHMPC_p$) with different lowercase superscripts differ significantly ($P < 0.05$).

[A,B]Values in a row relating to the effect of the solvent used for dispersion of MPC ($LHMPC_w$ and $LHMPC_p$; $MHMPC_w$ and $MHMPC_p$) with different uppercase superscripts differ significantly ($P < 0.05$).

[1]Low-heat and medium-heat milk protein concentrate powders (LHMPC, MHMPC) manufactured from low-heat-treated (72°C for 15 s) or medium-heat-treated (85°C for 30 s) skim milk were dispersed in water ($LHMPC_w$, $MHMPC_w$) or milk permeate ($LHMPC_p$, $MHMPC_p$).

[2]Water-holding capacity (the quantity of serum retained by the yogurt following centrifugation at 300 or 2,500 × g).

$MHMPC_p$, respectively. Most notably, the replacement of water with permeate changed the HCT versus pH profile from a sigmoidal shape, where HCT remained essentially constant at pH values 6.2 to 6.7, and increased curvilinearly at a diminishing rate in the pH region of 6.8 to 7.2 (Figure 2c) to the more typical type A curve exhibiting a maximum HCT and minimum HCT (Figure 2d). The HCT versus pH profile of the water-based MPC dispersion (Figure 2c) is similar to that reported by Crowley et al. (2014). Le Ray et al. (1998) reported that the heat stability of an aqueous dispersion of phosphocasein (pH 6.67) at 95°C was significantly improved on substitution of water with milk permeate. Based on the analysis of the protein dispersions and their sera (Tables 2 and 3), the relatively low HCT of the water-based dispersions ($LHMPC_{w\text{-}pHa}$ and $MHMPC_{w\text{-}pHa}$) at pH 6.2 to 6.7 may be due to their relatively high $[Ca^{2+}]$ and degree of κ-casein dissociation (Figure 1), whereas the relatively high HCT at pH 6.8 to 7.2 may be associated with their higher zeta potential and lower $[Ca^{2+}]$ (Table 2). However, the presence of a greater range of salts (e.g., citrate, KCl) and higher concentrations of soluble salts (e.g., phosphate) in the permeate-based dispersions is also likely to alter the HCT versus pH profile from that in the water-based dispersions (Augustin and Clarke, 1990). It is noteworthy that Fox and Hearn (1978) found that type A milk was converted to type B milk on partial demineralization by dialysis against water.

Ethanol Stability of Protein Dispersion

The ES of MPC dispersions is shown in Figure 2e and f. Altering the heat treatment of the skim milk during MPC manufacture had little or no effect on the ES of the MPC_w or MPC_p dispersions over the pH range of 6.2 to 7.0. This trend contrasts with previous studies that reported a significant increase in ES of skim milk over the same pH range on high heat treatment of the milk (e.g., at 90°C for 30 min or 120°C for 2–30 min; Horne and Parker, 1981a; Lin et al., 2018) and attributed the increase to the heat-induced reduction in $[Ca^{2+}]$. The interstudy discrepancy may be related to the intensity of heat treatment and its effect on the change in $[Ca^{2+}]$ of the reconstituted MPC powder with pH.

Solvent composition had a major influence on ES, as shown by the markedly lower ES of the water-based dispersions ($LHMPC_w$ and $MHMPC_w$) compared with the permeate-based dispersions ($LHMPC_p$ and $MHMPC_p$), especially at pH 6.6 to 7.0. The detrimental effect of substituting permeate with water on the ES of MPC dispersions is indicative of a destabilization and aggregation of casein micelles (Horne, 2016), which is consistent with the associated increases in $[Ca^{2+}]$ and κ-casein dissociation (Table 2; Figure 1). It is noteworthy that the addition of NaCl to milk has been found to promote κ-casein dissociation (Tessier and Rose, 1964) and reduce ES in the pH region 6.6 to 7.0 (Horne and Parker, 1981b), which coincides with the region of the largest difference between the ES of the MPC_w and MPC_p dispersions (Figure 2e and f).

Model Stirred Skimmed Yogurt

Acidification and Gel Formation. The changes in pH and G′ during the acidification and gelation of MPC dispersions from trial 1 are shown in Figure 3; similar changes were observed in trial 2 (data not shown). Solvent composition had a major influence on the time to reach pH 4.6 (Table 4), the profile of the G′ versus time curve, gelation onset time (**GOT**), and G′ at pH 4.6 for both the LHMPC and MHMPC dispersions (Figure 3). The water-based dispersions ($LHMPC_w$ and $MHMPC_w$) required a significantly longer time (∼200 min) to reach the target pH (pH 4.6), which is consistent with their higher initial pH (∼7.0 vs. 6.65 for the permeate-based dispersions). Other factors contributing to the longer gelation time in the water-based dispersions may include the absence of minerals and vitamins that are normally present in milk serum and that are required for or stimulate the growth of starter bacteria (Hayek and Ibrahim, 2013).

Compared with the permeate-based dispersions ($LHMPC_p$ and $MHMPC_p$), the water-based dispersions had a longer GOT, a higher pH at gelation onset ($\mathbf{GOT_{pH}}$), and a higher G′ at pH 4.6 (Table 4; Figure 3) and were characterized by an inflection point (peak), which occurred at an advanced stage of gelation (∼70–100 min after the GOT; Figure 3a and b). This was also observed from plots of tanδ and G′ as functions of pH (Figure 3c–f). Hence, G′ increased following gelation onset at approximately pH 5.6 in the water-based dispersion, decreased abruptly to an extent dependent on the type of MPC ($LHMPC_w$ or $MHMPC_w$), and thereafter increased again as the pH decreased to 4.6. A similar though less pronounced peak was also observed by Meletharayil et al. (2015) during the glucono-δ-lactone-induced gelation of an aqueous dispersion of MPC with protein content ≥80% but not in dispersions from MPC powders with 50 to 70% or 85 to 90% protein. No such inflection point was observed for G′ versus time, G′ versus pH, or tanδ versus pH in the permeate-based dispersions, for which G′ and tanδ increased progressively from GOT at approximately pH 5.2.

The presence of an inflection point in the G′ versus time, G′ versus pH, or tanδ versus pH curves of the water-based MPC dispersions would appear to be a

more extreme form of the shoulder feature observed during acid-induced gelation of high-heat-treated milk (Lucey, 2016). The shoulder has been ascribed to the competitive effects of 2 physicochemical changes with opposite effects of G′: a pH-induced aggregation of denatured β-LG (complexed with the κ-casein at the micelle surface), which enhances micelle aggregation, and solubilization of CCP, which promotes hydration and disaggregation of casein (Meletharayil et al., 2015; Lucey, 2016). The occurrence of the inflection point in the G′ versus time or pH profiles of the water-based dispersions and its absence in the corresponding permeate-based dispersions cannot be attributed to differences in whey protein denaturation, which was similar (∼76–82% of total) for both (Table 4). Instead, it may reflect differences between the dispersions with respect to parameters that influence the degree and type of structural rearrangements within the gel—for example, proportions of serum Ca, P (Table 3), and CCP (Anema, 2009) and rate of pH reduction. Differences between the water- and permeate-based dispersions with respect to the course of tanδ with pH from GOT supports a greater potential for bonds and strands within the gel from the water-based dispersions

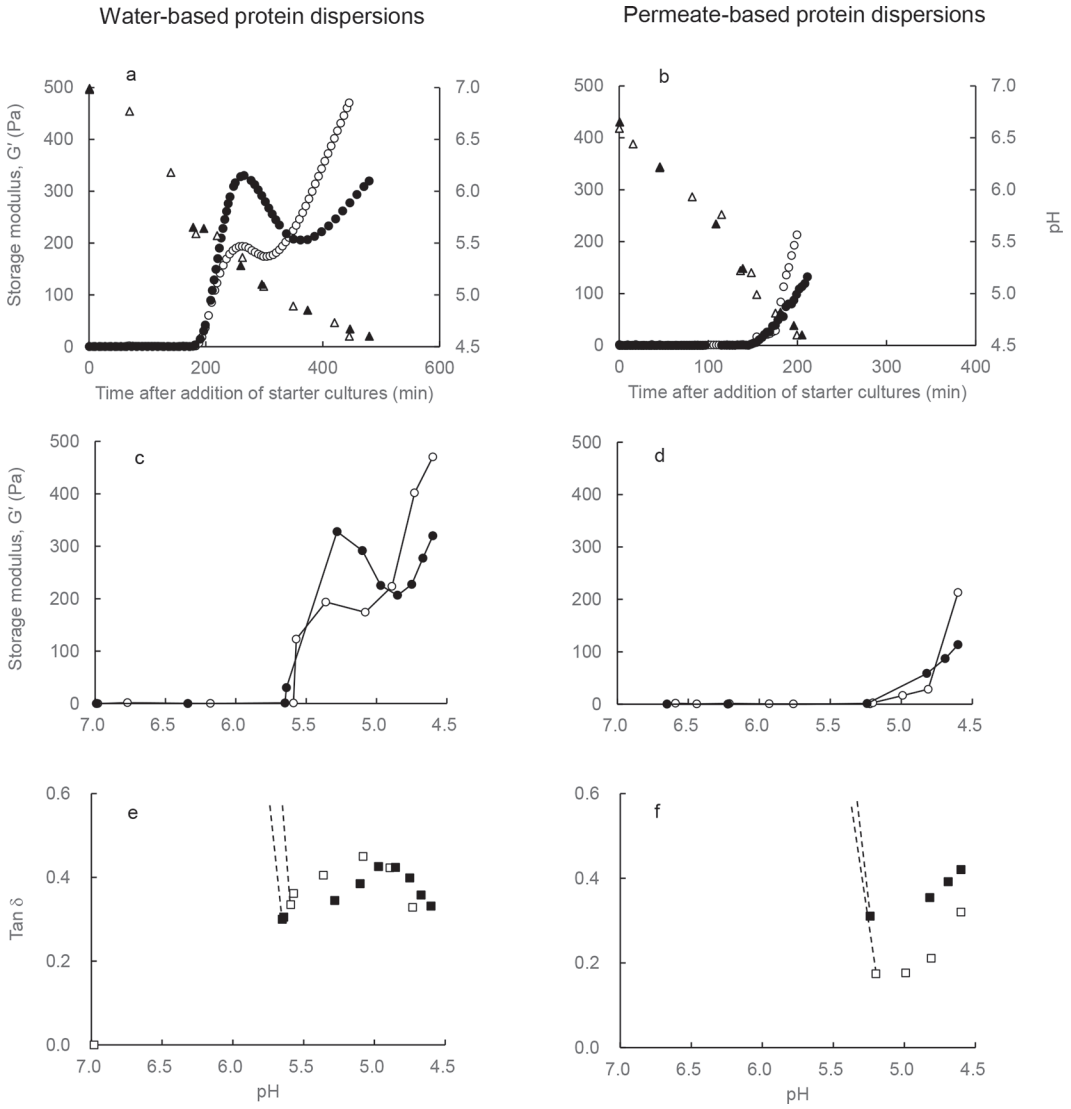

Figure 3. Effect of milk heat treatment (low heat: open symbols; medium heat, closed symbols) during the manufacture of milk protein concentrate powder on the gelation characteristics of model stirred skimmed yogurt from protein dispersions (5% protein) prepared by reconstituting milk protein concentrate powder in water (a, c, e) or milk permeate (b, d, f): changes in pH (Δ, ▲) and storage modulus (○, ●) as functions of time (a, b) and storage modulus (c, d) and tan δ (e, f) as functions of pH. Broken lines indicate that tan δ increased to values >1.0.

to relax and thereby facilitate more rearrangement of the gel and higher ultimate gel strength (i.e., G′; Lucey, 2016).

The relatively high GOT_{pH} for the water-based dispersions (~5.6 versus 5.2 for the permeate-based dispersions) may reflect their higher pH (~7.0 compared with ~6.65 for the permeate-based dispersion) of the milk at the heat treatment applied during yogurt manufacture (Table 2). The higher pH of the water-based dispersion during heat treatment is conducive to greater dissociation of κ-casein and the formation of serum-soluble complexes of κ-casein and denatured whey proteins (Vasbinder and de Kruif, 2003; Ménard et al., 2005; Lin et al., 2018). Similarly, previous studies have reported a marked increase in the GOT_{pH} of glucono-δ-lactone-induced gels prepared from reconstituted skim milk powder when the pH of the skim milk at heat treatment was increased from pH 6.2 to 6.5 to pH 6.9 to 7.1 before cooling, fermentation, and gelation (Vasbinder and de Kruif, 2003; Anema et al., 2004; Lakemond and van Vliet, 2008). The higher GOT_{pH} of the water-based dispersions probably reflects their higher pH (~7.0 compared with ~6.65 for the permeate-based dispersions; Table 2) and its influence on the partitioning of κ-casein (Figure 1) and denatured whey protein between the serum and the micelle. The proportion of denatured whey protein associated with the casein micelle decreases as the pH of milk at heating is increased from 6.6 to pH 6.9 to 7.5 (Vasbinder and de Kruif, 2003; Ménard et al., 2005; Lin et al., 2018). Anema et al. (2004) hypothesized that the serum-soluble denatured whey protein–κ-casein aggregates in milk heated at high pH may gel separately from the casein micelles. As the isoelectric pH of the serum-soluble aggregates (pH ~5.3) is higher than that of the casein micelles (pH ~4.6), the pH at which gelation occurs shifts to higher pH as the pH of the milk at heating increases. Alternatively, Lakemond and van Vliet (2008) suggested that the higher GOT_{pH} of acid gels from milk heat treated at higher pH values in the range of 6.2 to 6.9 was associated with several changes that affect particle aggregation and rearrangements processes before and just after gelation, including the structure of the casein micelle surface, extent of thiol interactions, and the size of heat-induced complexes. κ-Casein dissociation led to a smoother micelle surface (i.e., more devoid of protruding κ-casein) and less steric hindrance to the close approach of, and earlier bonding between, the casein micelles during acidification.

Rheological Properties. The shear rate versus shear stress data for all yogurts fitted to the Herschel–Bulkley model ($R > 0.99$). All yogurts exhibited a yield stress and shear thinning behavior (data not shown), reflecting the presence of an internal casein–whey protein network, which was disrupted during shearing. However, the yield stress values were low ($<1.7 \times 10^{-7}$ to 0.3 Pa, Table 4) and did not differ between yogurts ($P > 0.05$). Overall, the $LHMPC_w$ dispersion had the highest viscosity during shearing and the $MHMPC_p$ had the lowest.

Increasing the severity of milk heat treatment before MPC manufacture had no effect on the value of consistency coefficient but led to a significant reduction in viscosity over the entire shear rate range for both the water- and permeate-based dispersions (Table 4). This trend and the similar levels of denatured whey protein in all yogurt milks (Table 4) suggests that partial predenaturation of whey protein during MPC manufacture (e.g., ~48% in the MHMPC powder) reduces the viscosity of model stirred skimmed yogurt.

The use of permeate instead of water had effects similar to those obtained on increasing the severity of milk heat treatment during MPC manufacture except that it led to a reduction in the K value for the $MHMPC_p$ yogurt (Table 4). The effect of solvent may relate to differences in the rate of different physicochemical changes occurring during gel formation—namely, solubilization of CCP and reduction in the charge of proteins—and their effect on network formation and rearrangement before the end of yogurt manufacture (Lucey, 2016).

WHC. The WHC of the yogurt decreased as centrifugation force was increased from 300 to 2,500 × *g* (Table 4). It was unaffected by heat treatment of the skim milk during MPC manufacture or by using milk permeate instead of water as a solvent ($P > 0.05$; Table 4). The results suggest that the effect of any differences in yogurt viscosity and microstructure due to skim milk heat treatment during MPC manufacture or solvent composition on WHC may have been overcome owing to structural collapse at the centrifugation forces applied (Harwalkar and Kaláb, 1986).

CONCLUSIONS

This study investigated the effects of milk heat treatment during the manufacture of MPC powder (~81% protein) and the solvent used for reconstitution on the composition, physicochemical properties, and functionality of the resultant MPC dispersions. The milk heat treatment during MPC manufacture affected rennet gelation, HCT as a function of pH at 6.2 to 7.2, and gel formation properties and consistency of model stirred skimmed yogurt to an extent dependent on the solvent composition. Ethanol stability was affected by solvent composition but not by heat treatment during MPC manufacture. These effects were associated with differences in whey protein denaturation between the MPC powders and in the composition, degree of κ-casein dis-

sociation, particle size and charge, and ionic calcium content of the resultant MPC dispersions. The results highlight the importance of the severity of milk heat treatment during the manufacture of MPC and the composition of the solvent used for reconstitution of the MPC powder when formulating beverages or semisolid food products. This study also highlights the need for model studies on the systematic effect of increasing the type, level, and combination of different components (e.g., salts, sugars) on the properties of aqueous-based MPC dispersions. The information gleaned should provide a more systematic insight into food formulation and the factors affecting protein aggregation in aqueous-based MPC dispersions.

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