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Influence of pH adjustment on physicochemical properties of microfiltration retentates of skim milk and rehydration properties of resulting powders

Ram R. Panthi [a], Francesca Bot [a], Sini N. Shibu [b, c], Dzianis Saladukha [b, c], Tomasz J. Ochalski [b, c], James A. O'Mahony [a, *]

[a] School of Food and Nutritional Sciences, University College Cork, Cork, Ireland
[b] Tyndall National Institute, University College Cork, Cork, Ireland
[c] Centre for Advanced Photonics & Process Analysis, Cork Institute of Technology, Cork, Ireland

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ABSTRACT

Effects of pH adjustment on physicochemical properties of microfiltration retentates of skim milk and rehydration of resulting micellar casein concentrate (MCC) powders were investigated. Aliquots of retentate (pH 6.9) were adjusted to pH 7.3, 7.6 or 7.6 followed by readjustment to pH 6.9 (6.9R) prior to powder preparation. The retentates with pH 6.9, 7.3, and 7.6 had casein micelle size of 179, 189 and 197 nm, respectively, while sample 6.9R had size of 183 nm, similar to retentate at pH 6.9. Higher retentate pH resulted in lower ionic calcium and higher conductivity, with sample 6.9R having higher values for both parameters than the pH 6.9 sample. The MCC powders displayed poorer wettability and enhanced dispersibility with increasing retentate pH. Interestingly, the 6.9R powder had the best wettability and dispersibility. This study demonstrated that pH-mediated modifications of the physicochemical properties of retentates improve the rehydration properties of resultant MCC powders.

1. Introduction

Microfiltratiton (MF) technology, as applied to skim milk, is used to selectively retain native casein micelles, while depleting whey proteins, lactose and minerals through the permeate stream. In the manufacture of micellar casein concentrates (MCC) and isolates, MF is supplemented with diafiltration (DF) to achieve greater permeation of those non-casein components, thereby increasing the proportion of casein in the retentate streams, which are generally dried to produce MCC powders (Crowley et al., 2018; Schokker et al., 2011). Global demand for casein-dominant powders (MCC, milk protein concentrate, MPC, and milk protein isolate, MPI) is increasing, due to their extensive use as functional (e.g., gelling, foaming and thickening) and nutritional (e.g., high protein and low lactose content) ingredients in formulation and development of new dairy-based products (Agarwal, Beausire, Patel, & Patel, 2015).

Casein-dominant powders often exhibit poor rehydration characteristics, which limit and challenge their applications as ingredients in food formulation (Crowley, Desautel, et al., 2015; Felix da Silva, Ahrné, Ipsen, & Hougaard, 2018). Complete rehydration of casein-dominant powders requires the powder particles to successfully progress through each of the wetting, sinking, swelling, dispersion and dissolution stages of rehydration, and it is well established that the principal rate-limiting step in rehydration of casein-dominant powders is dispersion (Crowley, Kelly, Schuck, Jeantet, & O'Mahony, 2016; Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010a). Poor dispersibility of spray-dried MPC powders has been attributed to the formation of hydrophobic hard 'skins' on the surface and physical entrapment of casein micelles within powder particles, the extent of which also increases with storage time and temperature (Fyfe et al., 2011; Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010b). Such compaction in the organisation of casein micelles results in retarded water diffusion into powder particles (Maidannyk, Lutjes, Montgomery, McCarthy, & Auty, 2019; Schuck et al., 2007).

To unravel the complex interlocking of casein micelles, as it affects rehydration of casein-dominant powders, a deep understanding of the physicochemical properties of the retentate and subsequent powder is fundamentally important (Felix da Silva

* Corresponding author. Tel.: +353 21 4903625.
E-mail address: sa.omahony@ucc.ie (J.A. O'Mahony).

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et al., 2018). Liu et al. (2019) reported that MPC powders manufactured from acidified skim milk (pH 5.4) subjected to combined UF and DF, followed by pH readjustment (pH-cycling) to 6.7, resulted in a large proportion of non-micellar casein entrapped between casein micelles, conferring improved solubility to the subsequent powder compared with powder prepared without pH alteration. Similarly, inclusion of up to 12% (% of total protein) sodium caseinate (Schokker et al., 2011) in MF retentate, nano-sized spacers containing lecithin in rehydrated MPC suspension (Bansal, Truong, & Bhandari, 2017) or addition of NaCl, whey proteins, citrate/phosphate to rehydrated micellar casein solution (Schuck et al., 2007) prior to drying, improved solubility of resultant powders. Disintegration of casein micelles from powders during rehydration can also be enhanced by chelating ionic calcium (McCarthy et al., 2017) or adding molecular spacers into the retentate prior to drying.

Modification of pH towards the alkaline range changes the properties of casein micelles and mineral equilibria in dairy-based systems (Duerasch, Wissel, & Henle, 2018; Lin, Kelly, O'Mahony, & Guinee, 2018). Major changes associated with pH adjustment of milk (up to ~ pH 8) result in swelling of micelles, as indicated by an increase in average casein micelle diameter, electrostatic repulsion and casein micelle hydration, with reduction in ionic calcium in the serum (Huppertz et al., 2017; Vaia, Smiddy, Kelly, & Huppertz, 2006) and dissociation of micellar casein components into the serum phase (Lam et al., 2018; Sinaga, Bansal, & Bhandari, 2016; Vaia et al., 2006). Alkaline pH-mediated modification of casein micelle size in milk is only partially reversed when pH is readjusted to the initial value (Sinaga et al., 2016). Wu, Fitzpatrick, Cronin, and Miao (2019) observed improved dispersibility of MPI powders in aqueous media upon adjustment of pH to the alkaline range up to pH 8.4. Although several studies have reported the effect of pH modification on the physicochemical properties of casein micelles (Vaia et al., 2006; Sinaga et al., 2016; Lam et al., 2018), to the best of the authors knowledge, no information is available on the influence of pH-mediated modification and/or pH restoration of MF retentate on rehydration properties of casein-dominant powders.

The aim of this study was to investigate the influence of pH-mediated changes in the range 6.9–7.6, and pH readjustment from 7.6 to 6.9 of MF retentate of skim milk on the rehydration properties of resultant powders. Results from the present study will contribute to improving scientific understanding of the mechanisms by which such formulation strategies can be used to achieve improvements in rehydration properties.

2. Materials and methods

2.1. Preparation of microfiltration retentate

Low-heat skim milk powder with protein content of 36.1% (w/w) was provided by a local dairy company, which was reconstituted to 3.5% protein (w/w) in ultrapure water, to a batch size of 4 kg of skim milk with constant stirring for 3 h at 22 °C, followed by storage for 16 h at 4 °C. Following this, the skim milk was equilibrated at 50 °C for 2 h and diluted 1:2 with ultrapure water at 50 °C, as required for microfiltration (MF)/diafiltration (DF). The MF/DF process was performed at laboratory-scale, using a pressure-driven, tangential-flow device as described previously by Crowley et al. (2018). Briefly, a polymeric membrane (0.1 m^2 area) made from polyvinylidine fluoride (Durapore, Merck-Millipore, Carrigtwohill, Ireland) with 0.1 μm pore size was fitted in a Pellicon 2 mini holder (Merck-Millipore, Tullagreen, Carrigtwohill, Ireland) and the system was operated at a transmembrane pressure of 0.5 bar. Relevant hydrodynamic properties of the membrane were as reported by Crowley, Caldeo, et al. (2015). The temperature throughout processing was maintained at 50 °C by circulating water through an in-line plate heat exchanger.

Fractionation of skim milk was performed until the retentate was concentrated to a volume concentration factor (VCF) of ~3.3. The filtration process was stopped when total solids in the retentate reached 14%, as measured using a handheld refractometer (Atago™ R−5000 Refractometer, Atago Co., Ltd, Tokyo, Japan). An aliquot (~280 mL) of retentate was taken, without pH adjustment, and referred to as the control sample with unadjusted pH (sample R6.9). Other retentate aliquots were divided into three parts (~280 mL each) and pH was adjusted to pH 7.3 (sample R7.3), pH 7.6 (sample R7.6) and pH 7.6 (sample R7.6R) using 1 N NaOH. The pH was adjusted using a portable pH meter (SevenGo Duo pH/Cond meter SG23, Mettler Toledo, Mason Technology, Dublin, Ireland). The retentate samples were stored for 16 h at 4 °C without agitation, and on the following day samples were equilibrated at 25 °C for ~2 h, pH was measured and re-adjusted, if necessary, to the target of 7.3 or 7.6.

To investigate the reversibility of pH-induced changes in physicochemical properties, sample R7.6R was re-adjusted to pH 6.9, using 1 N HCl. On adjustment of pH of retentate samples, an upper limit of pH 7.6 was chosen to minimise changes in turbidity (Sinaga et al., 2016), which was also informed by preliminary viscosity measurements (data not shown) on retentate samples.

2.2. Production of micellar casein powder samples

Corresponding powder samples were produced from each of the retentates (i.e., R6.9, R7.3, R7.6 and R6.9R) using freeze-drying technology. Individual retentate samples were frozen at −20 °C, and subsequently dried in a vacuum chamber (Edwards, Davidson and Hardy Ltd., Dublin, Ireland). The freeze-dried powders obtained (i.e., P6.9, P7.3, P7.6 and P6.9R) were milled at 6000 rpm using a centrifugal mill (ultracentrifugal mill ZM 200, Retsch Centrifugal Mill, Carl Stuart Ltd., Dublin, Ireland) equipped with an 80 μm sieve. The sieve size was chosen to achieve a mean particle size similar to single stage spray dried powder. The milled powder samples were stored in air-tight tubes at 4 °C until analysed.

2.3. Composition of retentate and micellar casein powder samples

Total nitrogen content of all retentate and powder samples was determined using the Kjeldahl method and a nitrogen-to-protein conversion factor of 6.38 (IDF, 2001). Total solids content of retentates and powder samples was measured gravimetrically using oven drying at 103.5 °C for 12 h, following International Dairy Federation methodology (IDF, 1993).

2.4. Physicochemical properties of retentate samples

Concentration of ionic calcium [Ca^{++}] in retentate samples was determined using a Titrando 907 autotitrator, with Tiamo v2.2 software, equipped with a calcium ion-selective electrode (Metrohm Ireland Ltd, Co. Carlow, Ireland) according to the method of Crowley et al. (2018), with minor modifications. The probe was calibrated at 25 °C in imidazole and KCl buffer solutions of known calcium ion concentration (0.50, 1.00, 2.50, 5.00, 10.0 mM). Conductivity was measured using a Titrando autotitrator equipped with conductivity measuring probe, calibrated with standard KCl (12.88 mS cm^{-1}) solution at 25 °C with overhead stirrer and accompanying Tiamo v2.3 software (Metrohm Ireland Ltd) as described by Crowley, Desautel, et al. (2015). Size of casein micelles in retentate samples, after dilution in ultrapure water (0.25%, v/v), was measured using dynamic light-scattering with a Zetasizer Nano series HT instrument (Malvern Instruments Ltd.,

Worcestershire, UK) equipped with a He–Ne laser emitting at 633 nm and accompanying Malvern Zetasizer software v.7.02. Particle and dispersant (i.e., water) refractive indices were set at 1.46 and 1.33, respectively. Samples were measured at 25 °C after 120 s of temperature equilibration. A total of three measurements were taken for each individual replicate using a back-scattering configuration with a scattering angle of 173°.

Viscosity was measured using a controlled-stress rheometer (TA Discovery Hybrid 2 Rheometer, TA Instruments, Crawley, West Sussex, UK) equipped with a concentric cylinder geometry. Sample (25 mL) was loaded into the geometry and the gap between the cup and bub during measurement was 5920 μm. Viscosity was measured as a function of shear rate in the range 50–300 s^{-1} at 25 °C and results were presented in units of mPa s.

Protein profile of retentate samples and supernatant fractions derived from ultracentrifugation of retentate at 100,000×*g* for 1 h at 30 °C were analysed by reversed-phase high-performance liquid chromatography (RP-HPLC; Agilent 1220 Infinity II LC, Santa Clara, CA, USA) equipped with a C_{18} column (3.6 μm × 250 mm × 4.6 mm, Aeris Widepore, Phenomenex, Chesire, UK). Chromatograms were generated according to the method described by Bonfatti, Grigoletto, Cecchinato, Gallo, and Carnier (2008), with minor modifications as described by Bot, Crowley, and O'Mahony (2020). Briefly, aliquots (300 μL) of retentate were diluted with 10 mL of ultrapure water, whereas supernatant samples were diluted in a 1:1 ratio in ultrapure water prior to mixing with sample buffer containing 6 M guanidine hydrochloride, 0.1 M bis-Tris buffer, 5.37 mM sodium citrate and 19.5 mM dithiothreitol (pH 7) in a 1:1 ratio (v/v). After incubating for 1 h at 22 °C, samples were filtered using a nylon filter of pore size 0.20 μm and transferred to glass vials prior to injection into the HPLC system. Gradient elution was carried out with a mixture of solvents A (90% ultrapure water and 0.1% trifluoroacetic acid, TFA) and B (10% ultrapure water and 0.1% TFA in acetonitrile).

Moisture and protein contents of ultracentrifugal pellets (100,000×*g* for 1 h at 30 °C) were determined gravimetrically using freeze-drying and Kjeldahl methodology, respectively, using a nitrogen-to-protein conversion factor of 6.38 to calculate protein concentration (IDF, 2001). Protein hydration (g H_2O g $protein^{-1}$) was calculated using the measured data for moisture (%, w/w) and protein contents (%, w/w) of the pellet samples as described by Huppertz et al. (2017).

2.5. Morphology and rehydration properties of micellar casein powders

Scanning electron microscopy (SEM) images of MCC powders were obtained using a Jeol JSM-5510 (Jeol Ltd., Tokyo, Japan) scanning electron microscope. Powder samples were attached to sticky rubber on an aluminum stub, coated with gold and palladium (Au/Pd) up to 5 nm thickness. The voltage of 5 kV was accelerated and images were taken at magnifications of 200 × and 3000 ×.

Wettability of MCC powders was measured using the contact angle (θ) approach described by Crowley et al. (2018), using an optical tensiometer (Attension Theta, BiolinScientific Ltd., Espoo, Finland). Powder tablets (diameter 13 mm, height 5 mm) were formed by pressing powder samples with a load of 5000 kg using a manual hydraulic press (PerkinElmer, Buckinghamshire, UK). Change in contact angle was measured at 20 °C over 50 s after placing a drop of ultrapure water (10 μL) on the surface of the tablet.

Dispersion characteristics of the MCC powders were measured using a Malvern Mastersizer 3000 (Malvern Instruments Ltd.). Powder (2.5 g) was weighed and transferred to a glass beaker containing 200 mL of ultrapure water at 25 °C. To avoid floatation, powders were wetted by overlaying the dispersing water using a plastic Pasteur pipette. The suspensions were stirred at 500 rpm and samples were introduced into the dispersing unit of the instrument, with a stirrer operated at 1290 rpm, until a laser obscuration of 12 ± 1% was reached. Analysis of particle size distribution (PSD) of the samples was performed using the spherical model, as used for determination of casein micelle size (Crowley, Desautel, et al., 2015; Poste & Moss, 1972). Samples were measured at 60 and 90 min of rehydration. Data are presented on a volume based-PSD, with averaging of duplicate data from each measurement.

2.6. Raman spectral analysis of micellar casein powders

Molecular interactions between casein molecules (specific amide bonds I, II and III) within MCC powders were investigated using Raman spectroscopy in the spectral band between 1200 and 1800 cm^{-1} (Rodrigues Junior et al., 2016; Yazdanpanah & Langrish, 2013; Zhao et al., 2020). Powder samples (~10 mg) were placed on a glass slide and gently pressed with a spatula prior to acquiring spectra using a Horiba scientific XploRA^{TM}Plus Raman microspectrometer (Horiba UK Ltd. Northhampton, UK). The spectrometer was operated with an excitation wavelength of 785 nm through a 50 × confocal microscope objective, resulting in a laser spot size of ~3 μm in diameter at 4 cm^{-1} spectral resolution. For each sample, this procedure was repeated 5 times at different spots and the spectra were averaged. Spectra were recorded at 70 mW laser excitation power over a 5 s acquisition time, taking 10 scans from the 1200 to 1800 cm^{-1} spectral range. Spectra were baseline-corrected, normalised and analysed using OriginLab software (version 17, Silverdale Scientific Ltd, Stoke Mandeville, UK).

2.7. Statistical data analysis

Data were analysed using one-way ANOVA with a least-square difference (LSD) for multiple comparisons at 95% confidence interval using SPSS (IBM SPSS statistics for Windows, version 24, IBM Corp, Armonk, NY, USA). The results are the average of at least two measurements performed from triplicate trials (n = 3), unless otherwise stated.

3. Results and discussion

3.1. Influence of pH on composition of retentate

The influence of pH adjustment on composition and physicochemical properties, including protein concentration, total solids (TS) content, conductivity and ionic calcium concentration [Ca^{++}] of retentates is shown in Table 1. Retentate samples had 9.28% (w/w) protein and 11.8% (w/w) total solids, yielding concentrates with ~78% protein on a dry matter basis. The slightly, but not significantly ($P > 0.05$), lower levels of protein and TS for retentate adjusted to higher pH values may be attributed to the slight dilution effect with addition of NaOH and/or HCl for pH adjustment. Conductivity and [Ca^{++}] were greatly influenced by pH adjustment in retentate samples (Table 1). Conductivity values of samples R6.9, R7.3 and R7.6 were 1.90, 2.05 and 2.17 mS cm^{-1}, respectively, whereas [Ca^{++}] were 2.0, 1.32 and 1.06 mM, respectively. Higher and lower values of conductivity and [Ca^{++}], respectively, were recorded at higher pH conditions (i.e., 7.3 and 7.6) than at pH 6.9. For the retentate sample adjusted to pH 7.6 and restored to pH 6.9 (i.e., R6.9R) the conductivity and [Ca^{++}] values were significantly ($P < 0.05$) higher than for the R6.9 sample. Higher conductivity of retentate samples at alkaline pH, or after readjustment of pH to 6.9, can be attributed to addition of ions (Na^+ or H^+) during pH adjustment. The trend of lower [Ca^{++}] for retentate samples with

Table 1
Composition and physicochemical properties of microfiltration retentates from skim milk at different pH values.[a]

Parameters	Unit	Retentate sample			
		R6.9	R7.3	R7.6	R6.9R
Protein	%, w/w	9.28 ± 0.35[a]	9.21 ± 0.29[a]	9.16 ± 0.21[a]	9.07 ± 0.29[a]
Total solid	%, w/w	11.82 ± 0.08[a]	11.78 ± 0.08[a]	11.70 ± 0.09[a]	11.66 ± 0.09[a]
Conductivity	mS cm^{-1}	1.90 ± 0.08[d]	2.05 ± 0.05[c]	2.17 ± 0.02[b]	2.83 ± 0.04[a]
Ionic calcium	mM	2.00 ± 0.07[b]	1.32 ± 0.06[c]	1.06 ± 0.01[d]	2.66 ± 0.05[a]
Casein micelle size	nm	179 ± 0.64[c]	190 ± 2.55[b]	197 ± 3.04[a]	183 ± 2.05[c]

[a] pH values were: 6.9 (R6.9), 7.3 (R7.3), 7.6 (R7.6) and pH readjusted from 7.6 to 6.9 (R6.9R). Ionic calcium and casein micelle size measurements were carried out from two independent trials (n = 2). Superscript letters, within a column, indicate statistically significant differences ($P < 0.05$).

higher pH observed in the present study was in agreement with previous studies on skim milk and milk protein concentrate suspensions (Ho et al., 2018; Vaia et al., 2006). Higher conductivity and lower [Ca^{++}] values for samples R7.3 and R7.6, compared to R6.9, are likely due to the fact that at alkaline pH, calcium ions are more extensively chelated by PO_4^{3-} from HPO_4^{2} (Ahmad, Piot, Rousseau, Grongnet, & Gaucheron, 2009), while sodium ions added during pH adjustment, through NaOH, contribute strongly to the high conductivity values.

Results from the current study showed that [Ca^{++}] in the R6.9R sample did not revert to the original concentration as measured in sample R6.9. A higher [Ca^{++}] in R6.9R, compared to that in the sample R6.9, indicates that during pH adjustment, the chelated Ca^{++} ions were not fully restored into the micellar casein form. Huppertz, Vaia, and Smiddy (2008) reported the reversibility of mineral equilibria in skim milk when reforming casein micelles following complete disruption at pH 10. This discrepancy observed in relation to the reversibility of Ca^{++} ions could be due to depletion of minerals in MF/DF retentate, developing different mineral equilibria between casein micelles and the serum phase than in skim milk.

3.2. Influence of pH on size of casein micelles in retentate

Size of casein micelles in the retentate samples were dependant on changes in pH (Sinaga et al., 2016). Retentate R6.9 had a casein micelle size of 179 nm and R7.6 had a size of 197 nm, while the sample after pH readjustment from 7.6 to 6.9 (R6.9R) had a casein micelle size of 183 nm, with no statistically significant ($P > 0.05$) differences between that measured for sample R6.9. The measured values of casein micelle size in the current study were similar to those reported for skim milk, with values between 178 and 194 nm and the smaller measured values for casein micelle size after pH restoration were consistent with previous findings (Sinaga et al., 2016). Adjustment of pH led to retention of the monomodal particle size distribution for casein micelles, in agreement with results reported by Lam et al. (2018). The monomodal particle size distributions observed in this study also suggests that casein micelles swelled, without disintegration, at alkaline pH (Duerasch et al., 2018; Kern, Fabre, Scher, & Petit, 2019; Lam et al., 2018; Sinaga et al., 2016; Vaia et al., 2006).

3.3. Influence of pH on protein profile and distribution in retentate

Concentration of κ-, α_S- and β-casein, and α-lactalbumin (α-lac) and β-lactoglobulin (β-lg) in the sera of ultracentrifuged retentate samples was 12.8, 24.7, 43.3 and 23.3 mg mL^{-1} in samples R6.9, R7.3, R7.6 and R6.R, respectively. These data demonstrate greater dissociation of casein proteins from casein micelles into the serum phase at higher pH, along with incomplete reversal on pH readjustment from 7.6 to 6.9. The corresponding individual proteins measured in ultracentrifugal serum fractions are shown in Fig. 1. Concentrations of κ-, α_S- and β-casein were higher in the serum fraction of R7.6 compared to other sera. Serum fractions of R6.9, R7.3 and R6.9R had similar concentrations of caseins, although the concentrations were slightly higher in R7.3 and R6.9R compared

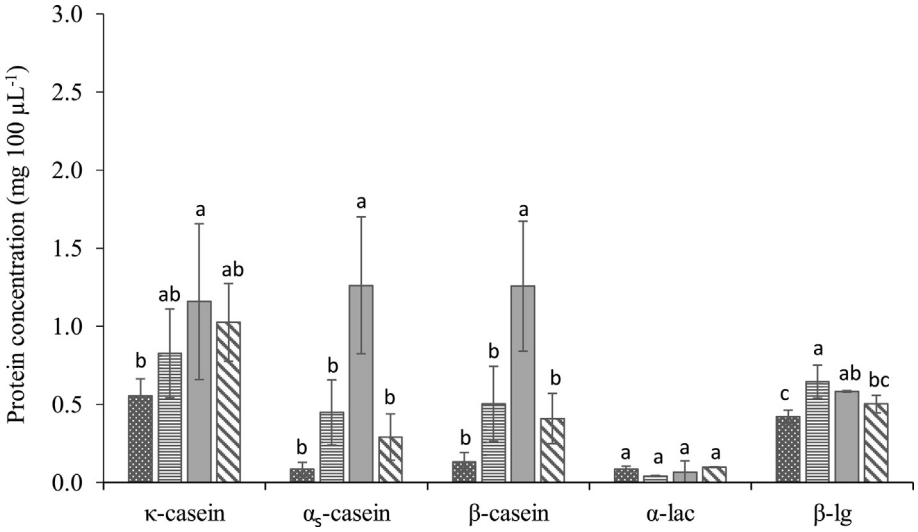

Fig. 1. Concentration of individual proteins in ultracentrifugal supernatants of microfiltration retentates R6.9 (▩), R7.3 (▤), R7.6 (▥), and R6.9R (▧) at pH 6.9, 7.3, 7.6 and pH readjusted from 7.6 to 6.9, respectively. The concentration of individual proteins in serum fractions was obtained after ultracentrifugation. Values are means ± standard deviation from three independent trials; different letters indicate significant differences ($P < 0.05$).

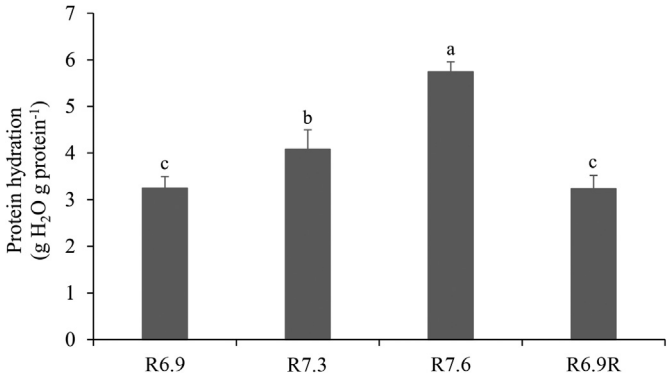

Fig. 2. Protein hydration of ultracentrifugal pellets obtained from microfiltration retentates R6.9, R7.3, R7.6, and R6.9R at pH 6.9, 7.3, 7.6 and pH readjusted from 7.6 to 6.9, respectively. Values are means ± standard deviation from three independent trials; different letters indicate significant differences ($P < 0.05$).

with R6.9 (Fig. 1). In relation to whey protein content in the serum fractions, α-lac was less influenced by pH adjustment than β-lg; indeed, the concentration of β-lg was significantly ($P < 0.05$) higher in R7.3 and R7.6 than in R6.9.

Concentration of caseins (κ-, α_S- and β-casein) in retentate R6.9 was 17.9, 42.4 and 36.7 mg mL^{-1}, of which 30.7, 1.71 and 3.54% of κ-, α_S- and β-casein, respectively, were measured in the corresponding serum fractions. The greater release of κ-casein compared with the other caseins is in agreement with previous studies on skim milk in the pH range 6.7–11.0 (Ahmad et al., 2009; Lam et al., 2018). Although actual concentrations of these individual proteins (except α-lac) were higher at alkaline pH conditions (Fig. 1), differences in proportions of proteins depended on the dissociation of individual caseins at higher pH. In particular, a greater release of α_S- and β-casein from native casein micelles occurs at alkaline pH, resulting in higher levels of non-micellar caseins in serum fractions (Duerasch et al., 2018).

These pH-mediated changes may be attributed to reduced hydrophobic interaction strength and increased electrostatic repulsions between amino acid residues (Huppertz et al., 2008). The larger casein micelle size measured at higher pH (Table 1) also suggests weak interaction within or between casein micelles. The lower [Ca^{++}], and larger casein micelle size, with higher pH is consistent with a mechanistic approach described previously for swelling of micelles (Vaia et al., 2006). However, changes in proportions of non-micellar casein in samples R6.9 to R7.6 were reversed after pH readjustment from 7.6 to 6.9 (i.e., R6.9R), as indicated by lower concentrations of non-micellar caseins in sera, with the concentrations being similar to those in sample R6.9. Because of the release of non-micellar caseins, and larger micelle size at higher pH (i.e., pH 7.3 and 7.6), it could be hypothesised that casein micelles in retentates at alkaline pH were more porous and surrounded by greater proportions of free, serum-phase α_S- and β-casein.

3.4. Influence of pH on protein hydration in retentate

Protein hydration, defined as the water attached to casein proteins by hydrogen bonding and that entrapped in spaces between casein proteins (Huppertz et al., 2017), is reported in Fig. 2. The pellets of samples R6.9, R7.3, R7.6 and R6.9R had hydration values of 3.25, 4.08, 5.74 and 3.23 g g^{-1} protein, respectively, demonstrating significantly ($P < 0.05$) higher casein hydration was obtained at alkaline pH. On readjustment of the pH 7.6 sample to pH 6.9 (R6.9R), similar hydration values to the original R6.9 sample were measured. Interestingly, protein hydration values measured in this study for retentate sample R6.9 were similar to those reported (~3.3 g of H_2O g^{-1} protein) for skim milk by Huppertz et al. (2017). The trend of high micellar protein hydration at higher pH observed in the present study was also in agreement with previous findings for milk (Ahmad et al., 2009; Lin et al., 2018). At alkaline pH, hydrogen from water bonds with negatively-charged amino acid residues of casein, making micelles more hydrated. Huppertz et al. (2017) reported that water associated with native casein micelles includes bound water, with ~15% in core with ~30% in the κ-casein outer layer, and ~55% entrapped water within the micelle structure, making casein micelles highly voluminous. Significantly ($P < 0.05$) higher micellar hydration in R7.3 and R7.6 indicates water associated with casein micelles and could be attributed to greater micelle voluminosity, release of non-micellar caseins and greater ionisation of amino acid residues.

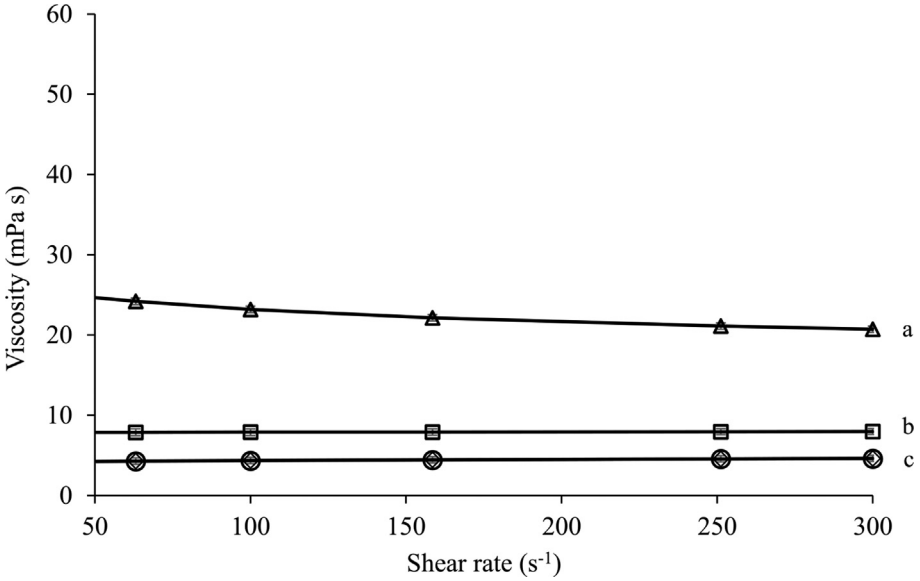

Fig. 3. Viscosity as a function of shear rate for microfiltration retentates R6.9 (○), R7.3 (□), R7.6 (△) and R6.9R (◇) at pH 6.9, 7.3, 7.6 and pH readjusted from 7.6 to 6.9, respectively. Values are means ± standard deviation from three independent trials; different letters indicate significant differences ($P < 0.05$).

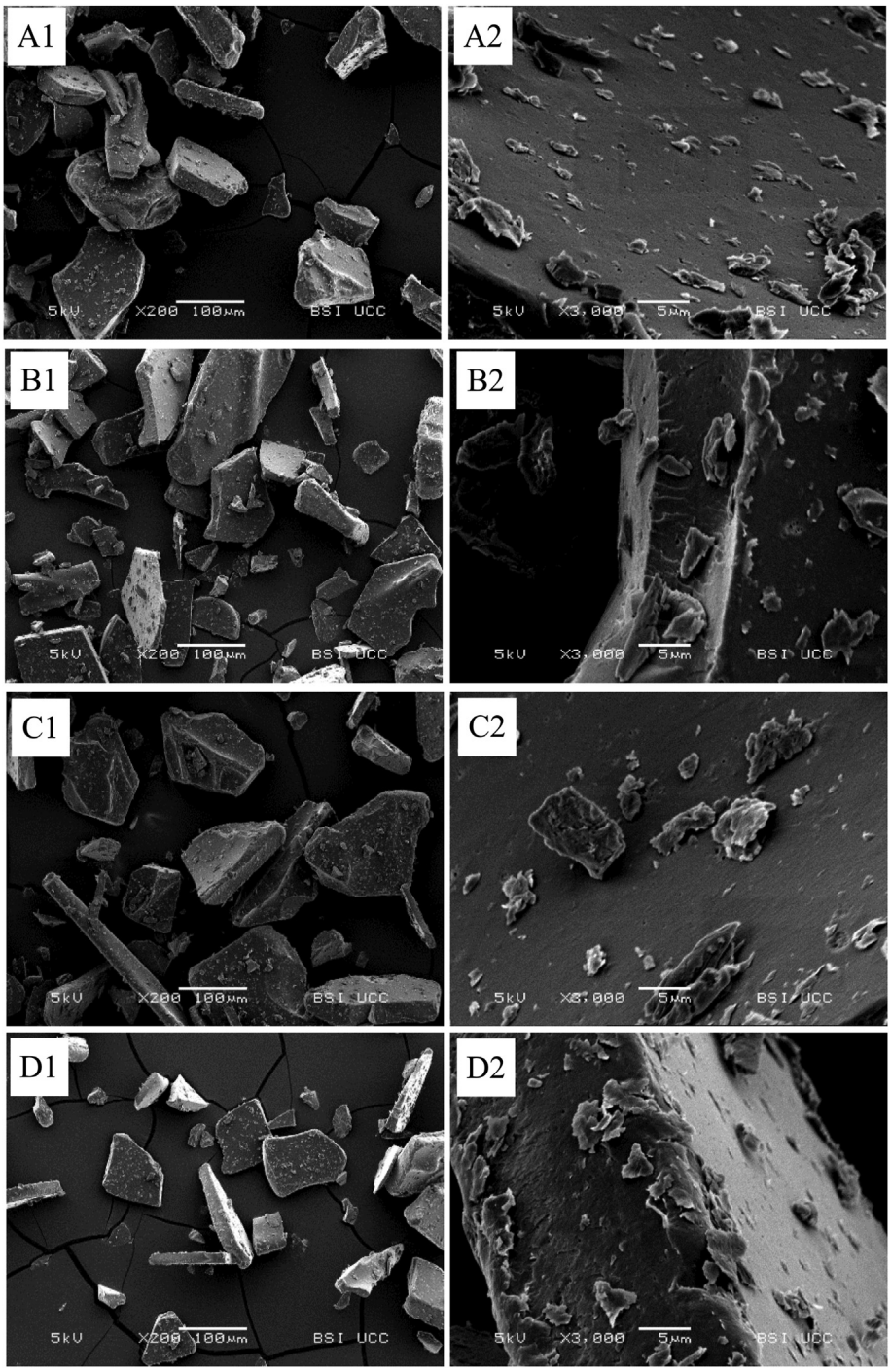

Fig. 4. Morphology of micellar casein concentrate powders P6.9 (A1), P7.3 (B1), P7.6 (C1), and P6.9R (D1), at 200 × resolution, obtained from microfiltration retentates at pH 6.9, 7.3, 7.6 and pH readjusted from 7.6 to 6.9, respectively. A2 to D2 shows the corresponding images at 3000 × resolution observed using scanning electron microscopy.

3.5. *Influence of pH on viscosity of retentate*

The viscosity profiles of retentate samples at 25 °C as a function of shear rate are shown in Fig. 3. Samples R6.9, R7.3, R7.6 and R6.9R had apparent viscosity values of 4.60, 7.97, 20.7 and 4.63 mPa s, respectively. The viscosity value (20.7 mPa s) measured for the sample adjusted to pH 7.6 corresponds to the apparent viscosity of skim milk evaporated to ~35% TS in conventional systems (Bista, Hogan, O'Donnell, Tobin, & O'Shea, 2019). Higher viscosity values were measured in R7.3 and R7.6 than in R6.9, and viscosity of R6.9R was similar to that observed in R6.9. High viscosity in retentate with higher pH was attributed to micelle swelling (Table 1) and release of non-micellar caseins into the serum phase (Fig. 1). Lower viscosity values in R6.9R coincided with smaller casein micelle size and loss of non-micellar caseins from the serum fractions (Fig. 1). Results from the current study

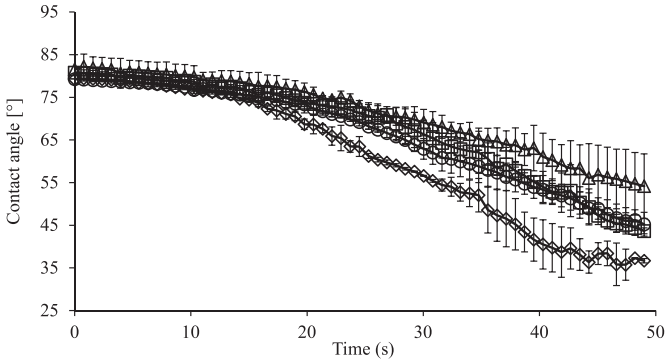

Fig. 5. Contact angle developed between a deposited drop of deionised water on the surface of a compressed disc of micellar casein powders P6.9 (○), P7.3 (□), P7.6 (△) and P6.9R (◇) obtained from microfiltration retentates at pH 6.9, 7.3, 7.6 and pH readjustment from 7.6 to 6.9, respectively. Values are means ± standard deviation from two independent trials.

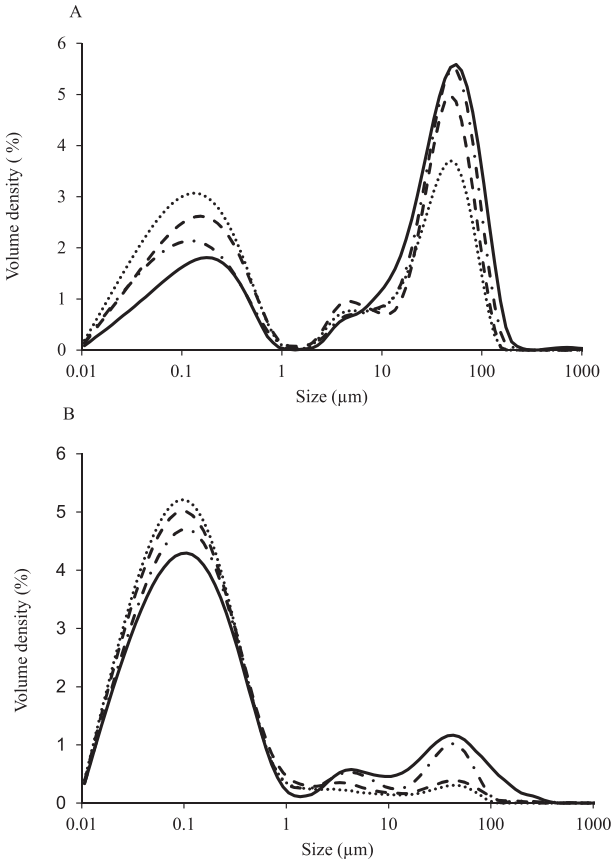

Fig. 6. Particle size distribution data of micellar casein powders P6.9 (▬), P7.3 (▬ ▪), P7.6 (▬ ▬) and P6.9R (▪▪▪▪) obtained from microfiltration retentates at pH 6.9, 7.3, 7.6 and pH readjustment from 7.6 to 6.9, respectively, after (A) 60 min and (B) 90 min of rehydration in water at 25 °C.

are in agreement with McCarthy et al. (2017), who observed an increase in apparent viscosity (3–40 mPa s) in MPC retentate (protein content of 8.14%, w/w) at alkaline pH (6–8). Previous studies also observed increased viscosity in skim milk (~3.3% protein) from 1.4 to 3.5 mPa s on increasing pH from 6.7 to 9.5 (Ahmad et al., 2009; Lam et al., 2018) or in MPC retentate (16.5% protein, w/w) from 8 to 38 mPa s on increasing pH from 6.7 to 7.0 at 45 °C (Ho et al., 2018).

3.6. Influence of retentate pH on physicochemical properties of powders

3.6.1. Morphology of micellar casein concentrate powders

On average, the protein and moisture content of powders was 73.5% (w/w) and 3.93% (w/w), respectively and, as expected, no significant ($P > 0.05$) differences were observed between samples prepared after pH adjustment in retentate. Morphological properties of the powders at magnifications of 200 × and 3000 × are reported in Fig. 4. All powders displayed an appearance of broken particles, having sharp edges, with a range of sizes. As expected, all the powders had no air vacuoles or void spaces with typical 'skin-layer' as normally observed in spray-dried MPC powder (Mimouni et al., 2010b). Since freeze-dried powders are prepared by sublimating ice into vapour in a vacuum, this manufacturing procedure yielded a more compact and homogeneous powder structure than spray drying (Kavli, 2018).

3.6.2. Raman spectral analysis of micellar casein concentrate powders

Normalised intensity of the Raman spectra for P6.9, P7.3, P7.6 and P6.9R are shown in Supplementary Fig. 1. Assigned peaks in the Raman spectra provide information for vibrations of amide bonds in caseins, and thereby provide insights into molecular interactions (Rodrigues Junior et al., 2016; Yazdanpanah & Langrish, 2013). Raman peaks in the amide I region (1656 cm^{-1}) are mainly due to the C=O bond, while in the amide II region (1551 cm^{-1}) these are due to C–N and N–H bonds and in the amide III region (1242 cm^{-1} and 1278 cm^{-1}) the peaks are due to the combination of C–C and C–N bonds between caseins (Rodrigues Junior et al., 2016; Yazdanpanah & Langrish, 2013; Zhao et al., 2020). Powders had similar molecular vibrations at given amide regions as indicated by the similar appearance of peak intensities, despite being prepared from pH-adjusted retentate. This demonstrates that there were no major changes in the secondary structure of the caseins (e.g., α-helix, β-sheet, β-turn) (Yazdanpanah & Langrish, 2013) between powders prepared from pH-adjusted retentates.

3.7. Wettability of micellar casein concentrate powders

Changes in contact angle for water droplets on the surface of powder tablets prepared for samples P6.9, P7.3, P7.6 and P6.9R are shown in Fig. 5. Initially (at t = 0 s), samples P6.9, P7.3, P7.6 and P6.9R had contact angles of 79.3, 80.8, 81.5, and 79.2°, respectively, with no major differences ($P > 0.05$) among samples. A marked difference in contact angle, as influenced by pH adjustment of retentate, was observed after 20 s. The calculated negative slope of contact angle as a function of time over the first 20 s of wetting for powders P6.9, P7.3, P7.6, and P6.9R was 0.97, 1.11, 0.77 and 1.23, respectively, suggesting water droplets spread more quickly on the powder surface prepared from retentate with pH readjusted from 7.6 to 6.9 (R6.9R), compared with other samples. Moreover, at 50 s, samples P6.9, P7.3, P7.6, and P6.9R had contact angles of 45.2, 43.7, 54.2, and 36.7°, respectively. The results from the current study show that sample P6.9R wetted more rapidly and extensively than other samples, while sample P7.6 had the poorest wettability. The observed differences in wettability among powder samples can be related to the composition of serum phase of the retentate from which powder was prepared. Powders with faster wettability had high levels of [Ca^{++}] and lower levels of α_S-and β-caseins in the serum fractions (Table 1; Fig. 1).

3.8. Dispersion properties of micellar casein concentrate powders

In the early stages of powder dispersion, a peak in the micron-sized range (1–1000 μm) corresponds to primary powder particles and the appearance of a peak in the nanometer-sized (0.01–1 μm) region indicates dispersion of casein micelles from powder particles (Crowley, Desautel, et al., 2015). After rehydrating powder for 60 min, the percentage of total volume in the nanometer-sized region was higher in sample P6.9R, followed by P7.6, P7.3 and P6.9, thereby indicating greater release of casein micelles from the powder particles in the respective order (Fig. 6A). The volume percentage of the nano-sized peak was higher after 90 min of rehydration compared with that measured at 60 min, and the percentage of total volume of the peak between samples at 90 min was similar to that measured at 60 min (Fig. 6B). The percentage of total volume of primary powder particles in the micron-sized region decreased with the release of casein micelles. The result showed that pH adjustment improved dispersibility compared with the sample without pH adjustment (P6.9), with the greatest dispersibility shown by P6.9R.

The better rehydration characteristics of P6.9R compared with the other powder samples suggests that the ionic environment in the serum phase, (i.e., higher [Ca^{++}] and conductivity; Table 1) plays an important role in powder rehydration. Higher [Ca^{++}] in P6.9R indicates partial solubilisation of colloidal calcium phosphate from casein micelles, which may have reduced the number of casein–casein interactions within micelles in P6.9R. Data from the present study show that wettability and dispersibility of MCC powders are strongly influenced by physicochemical properties of the corresponding retentates, and can be modulated readily by changes in pH. Powders prepared from retentate R6.9R had improved wettability and dispersibility compared with all other samples. Improvements in powder dispersibility are consequences of changes in non-micellar casein content, [Ca^{++}] and protein hydration as influenced by pH adjustment.

4. Conclusion

Adjustment of skim milk microfiltration retentate pH from 6.9 to 7.6 resulted in larger casein micelle size, higher conductivity, protein hydration and viscosity, with lower ionic calcium concentrations and higher levels of non-micellar caseins in ultracentrifugal serum fractions. Readjustment of retentate pH from 7.6 to 6.9 (R6.9R) restored casein micelle size, protein hydration and viscosity to values similar to those measured in the retentate without adjustment (R6.9), whereas conductivity and ionic calcium concentrations were signficantly higher in R6.9R than in R6.9. Raman spectroscopy and scanning electron microscopy revealed similar molecular interactions between caseins and surface structures, respectively, in the resultant micellar casein concentrate powders. Adjustment of retentate to higher pH resulted in powders having slow wetting but higher dispersibility, while pH readjustment from higher pH to initial level improved both properties. These results demonstrate that it is possible to significantly alter rehydration properties of casein-dominant powders by pH-mediated modulation of physicochemical properties of membrane filtration retentates.

Credit author statement

Ram R. Panthi: Planned and designed experiment, conducted lab work, analysed and interpreted results along with the preparation of manuscript draft.

Francesca Bot: Conducted HPLC analysis of the samples, analysed and interpreted results and provided feedback on manuscript.

Sini N. Shibu: Conducted Raman spectroscopic analysis of the samples, reviewed texts related to Raman spectroscopy.

Dzianis Saladukha: Analysed Raman spectra of the MCC powders and reviewed texts related to Raman spectroscopy.

Tomasz J. Ochalski: Provided feedback on Raman spectroscopy and assisted in interpretation of the spectra.

James A. O'Mahony: Led the research project as principal investigator and looked after every aspect of research from planning, managing and organizing, and reviewing manuscript.

Declaration of competing interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idairyj.2020.104953.

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