Alteration in physicochemical, functional, rheological and reconstitution properties of milk protein concentrate powder by pH, homogenization and diafiltration

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Abstract Concentration of milk proteins by ultrafiltration (UF) and diafiltration (DF) processes during manufacturing of milk protein concentrate (MPC) powders alter their natural milk protein stabilization system. Increasing calcium and protein contents often leads to poor functional properties in MPC powders. The pH adjustment using disodium phosphate (DSP, Na_2HPO_4) and DF with 150 mM NaCl solution of UF retentate were hypothesized to produce desirable changes in various properties of resulted MPC powders. Addition of Na_2HPO_4 followed by homogenization; DF of $5 \times$ UF retentate with 150 mM NaCl solution resulted in significant improvement in the dispersibility, wettability, flowability, solubility, heat stability, buffer index, emulsification and foaming and water and oil binding capacities of the MPC powders. The solubility of developed MPC powders was significantly higher than MPC-C powder in fresh as well as even after 90 days of storage at $25 \pm 1^\circ\text{C}$. Rheological behaviour of reconstituted MPC was best explained by Herschel Bulkley model. Scanning electron microscopy micrograph indicated that MPC powders were having smooth surfaced, intact and separate smaller particles compared to rough,

larger, infused aggregates with dents in MPC-C. Technological interventions applied are easier to adopt, cost-effective and efficient in producing excellent quality MPC powders that may find applications in wide range of novel food formulations.

Keywords Milk protein concentrate · Sodium chloride · Disodium phosphate · Diafiltration · Homogenization · Microstructure

Introduction

Milk protein concentrate (MPC) powders have 42–89% protein on dry matter basis and are produced employing a combination of several unit operations including ultrafiltration (UF), diafiltration (DF), evaporation (optional) and spray drying (Meena et al. 2018). Popularity and demand of MPC powders is growing worldwide as high protein ingredient in food and pharma sector. About 900 different products have been launched in market incorporating MPC and milk protein isolate (MPI) as key ingredient. MPC powders are being used in formulation of infant, medical, geriatric, sports nutrition and speciality foods. Global production of MPC powders is expected to cross 40,000 MT by 2020 (Meena et al. 2017b).

The composition of feed and thermal treatment, degree of protein concentration, treatments of UF/DF retentates and drying conditions are key determining factors influencing properties of MPC powders. Concentration of milk by UF and DF produces retentates with altered composition, disturbed ‘salt equilibrium’ and reduced protein stability, that may affects functionality of proteins adversely (Alexander et al. 2011). Further, swelling of casein micelles due to migration of calcium and κ -casein into

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serum phase (McKenna 2000), dissolution of colloidal calcium phosphate (CCP) nanoclusters (Alexander et al. 2011) and increase in total calcium also occur during UF and DF processes. Thereafter, aggregation of calcium-depleted proteins and their migration to air-droplet interfaces and denaturation during evaporation and spray drying occur. These changes have detrimental effect on most of the properties of MPC powders. Contrary to caseins, denaturation and interactions of whey proteins also occurs, but its role in lowering the solubility of MPC powders is still not clear.

Solubility of MPC powders is inversely proportional to their protein and calcium contents. Lower lactose content in UF and DF retentates fails to prevent extensive casein–casein interactions during spray drying and subsequent storage. Nitrogen solubility index and heat stability of commercial MPC powders (55–85% protein) have been reported in the poor range of 27–87% and 0–40 min, respectively (Huppertz and Gazi 2015). Application of these MPC powders caused problems of nugget formation in cheese; hardening of high protein bars and sedimentation in high protein beverages. Therefore, for the manufacturing of improved functionality MPC powders, enhancement in their solubility and thermal stability is pre-requisite and challenging as well.

Different chemical, physical and enzymatic methods have been reported to overcome the problems of MPC powders (Meena et al. 2017b). However, most of these approaches desired further improvement to make them commercially viable. Role of stabilizing salts/calcium chelators is established in enhancing the thermal stability of liquid and concentrated milks. These salts have strong influence on calcium ion activity and colloidal calcium caseinate phosphate (CCP) system, but their type and levels required to achieve stability desired detailed investigation. Disodium phosphate (DSP, Na_2HPO_4) reacts with calcium in equimolar ratio and stabilizes casein micelles through its interaction with casein or CCP at ~ 6.7 pH. Meena et al. (2016) observed that heat coagulation time (HCT) of homogenized $5 \times \text{UFR}$ were above 60 min at 6.5, 6.6 and 6.7 pH values adjusted with 10% solutions of NaH_2PO_4 and Na_2HPO_4 . Sikand et al. (2012) observed that homogenization of reconstituted solution of MPC80 at 13800 kPa, improved its solubility because of reduction in mean particle size that facilitated the reconstitution. Reduction in calcium content of UF/DF retentates by ion exchange, CO_2 injection and DF with different salts have positive impact on solubility of MPC powders (Meena et al. 2017b). However, these interventions have been applied in isolation and their combined effect on quality characteristics of MPC powders have not been studied so far. Current investigation was conducted carried out to investigate the

effects of Na_2HPO_4 addition with homogenization and diafiltration in enhancing the quality of MPC powders.

Materials and methods

Material

Pasteurized ($72^\circ\text{C}/15$ s) cow skim milk (PCSM) was obtained from Experimental Dairy of ICAR-National Dairy Research Institute, Karnal, India. The mean values ($n = 9$) of total solids (TS), fat, protein, lactose, ash and calcium contents of PCSM were $7.85 \pm 0.02\%$, $0.05 \pm 0.01\%$, $2.83 \pm 0.09\%$, $4.29 \pm 0.10\%$, $0.68 \pm 0.01\%$ and $0.12 \pm 0.01\%$, respectively. Its natural pH was 6.60 ± 0.02 . Deionized water, acid washed glass wares and chemicals procured from Sigma-Aldrich, St. Louis, MO, US were used during the study.

Methods

Production of MPC powders

PCSM was ultrafiltered at $50 \pm 1^\circ\text{C}$ and diafiltered at $35 \pm 1^\circ\text{C}$ to $5 \times$ concentration factor (CF) as reported by Meena et al. (2017a). For each trial, 350 kg PCSM was used and retentate obtained was equally divided in three parts. The first part was treated as control. The pH of second part was adjusted to 6.60 by adding 10% solutions of Na_2HPO_4 prior to its homogenization at 2500 and 500 psi pressures in two stage, respectively. Whereas, third portion (20 kg) of retentate was diafiltered with the addition of 20 kg 150 mM NaCl solution. The DF process was completed with removal of 20 kg permeate. The TS, protein and pH values of control, pH adjusted and homogenized as well as diafiltered retentates were observed to be $21.97 \pm 0.09\%$, $21.19 \pm 0.06\%$, $22.92 \pm 0.08\%$; $14.13 \pm 0.10\%$, $13.09 \pm 0.12\%$, $14.00 \pm 0.09\%$; and 6.42 ± 0.02 , 6.60 ± 0.00 and 6.15 ± 0.02 , respectively.

Control, pH adjusted and homogenized, and diafiltered retentates were spray dried as suggested by Patil et al. (2018) to obtain MPC-C (control); MPC-T1 and MPC-T2 powders. The powders were packaged in airtight metalized polyester low-density polyethylene (LDPE) laminates and stored at $25 \pm 1^\circ\text{C}$ for their further evaluation. All experiments were carried out three times and samples were analysed for physico-chemical, rheological and functional properties in triplicate.

Chemical composition, physical, reconstitution, functional and rheological properties of MPC powders

PCSM and MPC powders were analyzed for TS, fat, ash, protein, calcium contents and pH values while only pH, protein and TS of retentates were determined using standard methods (AOAC 1998).

Loose bulk density (LBD, g/mL) and packed bulk density (PBD, g/mL); flowability (angle of repose (θ°), wettability (s); dispersibility (%); specific surface area (SSA, m^2/kg); particle size distribution (d_{10} , d_{50} and d_{90}); Sauter (volume) mean diameter ($D_{3,2}$ and $D_{4,3}$); dispersion index (Meena et al. 2017a); color values (L^* , a^* and b^*) and water activity (a_w) values of powders were determined following the method of Patil et al. (2018).

Functional properties such as solubility (%), foaming capacity and stability (%), emulsion capacity (%) and stability (min), water binding capacity (WBC in g/g protein) and oil binding capacity (OBC in g/g protein) were determined using the methods suggested by Shilpashree et al. (2015) while rennet coagulation time (RCT, measured till 210 min or till the sample went coagulated, whichever was earlier) and buffering capacity of prepared powders were estimated using earlier reported methods by Patil et al. (2018). MPC powders were reconstituted adopting the method of Crowley et al. (2014) to determine their HCT values at 140 °C.

Rheological properties

Apparent viscosity (50 s^{-1} , 20 °C) and flow behaviour ($1\text{--}1000 \text{ s}^{-1}$) of 10% w/v solution of MPC powders were measured using a Rheometer (MCR 52, Anton Paar, Germany) equipped with CP75-1° (SS) probe at 20 ± 1 °C. Data obtained were used to calculate the various rheological parameters.

Scanning electron microscopy

The microstructure of MPC powder were studied by scanning electron microscopy (SEM) (EV018, 18th special edition, Zeiss, Tokyo, Japan) by adopting the procedure reported by Shilpashree et al. (2015).

Statistical analysis

Data in triplicate ($n = 3 \times 3 = 9$) were subjected to one-way analysis of variance (ANOVA) using SAS Enterprise guide (5.1, 2012) developed by SAS Institute Inc. North Carolina, USA. Means were compared using Tukey's Studentized Range (HSD) test according to the method of Patil et al. (2018) and Meena et al. (2017a).

Result and discussion

Composition

The adjustment of pH (using Na_2HPO_4 solutions) and DF of $5 \times \text{UFR}$ with 150 mM NaCl caused significant difference ($P < 0.05$) in chemical composition (Table 1) of MPC-T1 and MPC-T2 powders. Applied approaches significantly increased ($P < 0.05$) ash contents, but decreased ($P < 0.05$) calcium contents of MPC-T1 and MPC-T2 compared to control powder (Table 1). Further, the pH values of MPC-T1 and MPC-T2 powders were significantly ($P < 0.05$) higher and lower than pH of MPC-C due to Na_2HPO_4 addition and DF with 150 mM NaCl. TS, protein, ash and lactose contents of MPC60 and MPC70 powders were reported to be varied in the range of 96–97.45, 60.13–70.05, 7.12–8.0 and 18–28.40%, respectively (Meena et al. 2016; Crowley et al. 2014).

The decrease in calcium content of MPC-T1 powder was attributed to the addition of Na_2HPO_4 that decreased its calcium content via inducing dilution effect compared to

Table 1 Composition of MPC powders

Parameters	MPC-C	MPC-T1	MPC-T2
Total solids (% w/w)	94.87 ^b	95.54 ^a	94.36 ^c
Ash (% w/w)	7.94 ^c (7.71)	10.00 ^a (10.47)	8.86 ^b (9.39)
Protein (% w/w)	60.90 ^b (64.19)	59.01 ^c (61.76)	62.54 ^a (66.27)
Fat (% w/w)	1.07 ^a (1.13)	1.04 ^b (1.09)	1.08 ^a (1.14)
Calcium (% w/w)	2.67 ^a (2.81)	2.62 ^b (2.74)	2.51 ^c (2.66)
pH	6.74 ^b	7.00 ^a	6.23 ^c

Means ($n = 9$) within the same row with different superscript differ (Tukey's HSD, $P \leq 0.05$). Values given in bold parenthesis are percent of total solids. Where-MPC-C is control powder produced without applying any treatment, MPC-T1 was produced from $5 \times \text{UFR}$ after adjusting its pH to 6.60 ± 0.00 using 10% solution of Na_2HPO_4 prior to its double stage homogenization (2500/500 psi), MPC-T2 was produced from diafiltered retentate obtained after DF of $5 \times \text{UFR}$ with 150 mM NaCl solution

MPC-C (control) powder (Table 1). However, during diafiltration of $5 \times \text{UFR}$ with 150 mM NaCl solution, calcium ions were replaced with sodium ions that enabled higher permeation of soluble calcium ions into DF permeate, resulting lower calcium content in MPC-T2 powder (Table 1). Such reduction in calcium contents of MPC60, MPC70 and MPC80 powders, because of either Na_2HPO_4 addition or due to DF of UF retentates with 150 mM NaCl/KCl solution, is reported by several researchers (Bhaskar et al., 2003; Mao et al., 2012; Sikand et al. 2013; Meena et al. 2017a). The observed variation in pH of control, MPC-T1 and MPC-T2 powders (Table 1) could be attributed to the added Na_2HPO_4 and NaCl salts and differences in mineral permeation during their UF and DF. Similar decrease in pH of UF retentate was earlier also reported by Mao et al. (2012).

Physical properties

Physical properties of MPC-C, MPC-T1 and MPC-T2 powders are shown in Table 2. The LBD values of these powders were significantly different ($P < 0.05$) with each other being lowest for MPC-T2 and maximum for MPC-T1. PBD values of MPC-C and MPC-T1 powders showed no statistical difference ($P > 0.05$), but differed significantly ($P < 0.05$) with MPC-T2 powder. The variation in LBD and PBD values of these powders could be attributed to the difference in the TS values of the retentates from which they were produced and also induced variation in their particle sizes (Table 1). The a_w value of MPC-C was significantly higher ($P < 0.05$) compared to MPC-T1 and MPC-T2 powders that might be attributed to addition of sodium salts during their production. Lactose and mineral contents of these powders could also explain the varied a_w values as powder composition had been reported to have significant effect on its a_w value (Schuck 2011). Observed PBD values of these powders are in good agreement with the reported tapped density values of MPC60 and MPC70 powders (Crowley et al. 2014). Moreover, Schuck (2013) reported that bulk density of powders is a complex property that cannot be correlated to only a single factor as it is influenced by a number of other factors.

A significant difference ($P < 0.05$) was observed in SSA, D_{32} or D_{43} and d_{10} , d_{50} , d_{90} and span values of the three powders (Table 2). Applied treatments altered the TS and might have induced changes in casein micelles by lowering the calcium contents. Higher SSA values of MPC-T1 and MPC-T2 than MPC-C powder also indicated the significance of applied treatments. Sikand et al. (2012) and Mao et al. (2012) reported such decrease in particle size of reconstituted MPC80 powders as a function of applied homogenization and DF with 150 mM NaCl solution. The d_{10} , d_{50} , d_{90} ; SSA values of MPC-C, MPC-

Table 2 Physical properties of MPC powders

Sample attribute	MPC-C	MPC-T1	MPC-T2
Bulk density (g/mL)			
Loose bulk density (LBD)	0.38 ^b	0.40 ^a	0.29 ^c
Packed bulk density (PBD)	0.59 ^a	0.58 ^a	0.46 ^b
a_w	0.28 ^a	0.25 ^b	0.24 ^c
Specific surface area (m^2/kg)	109.80 ^c	110.80 ^b	112.70 ^a
Particle size distribution (μm)			
d_{10}	29.55 ^b	28.47 ^c	31.05 ^a
d_{50}	75.51 ^b	76.87 ^a	74.36 ^c
d_{90}	163.03 ^b	189.60 ^a	158.10 ^c
Mean (μm)			
D_{32}	54.65 ^a	54.14 ^b	53.23 ^c
D_{43}	87.87 ^b	97.92 ^a	85.95 ^c
Span	1.77 ^b	2.10 ^a	1.71 ^c
Flowability (θ°)	37.83 ^b	35.38 ^c	39.07 ^a
Wettability (min)	3.87 ^b	5.10 ^a	1.44 ^c
Dispersibility (%)	65.46 ^c	74.17 ^b	90.79 ^a
Color			
L^*	90.19 ^a	90.02 ^a	89.42 ^b
a^*	− 1.84 ^c	− 1.73 ^b	− 0.69 ^a
b^*	12.98 ^b	13.21 ^a	12.58 ^c

Means ($n = 9$) within the same row with different superscript differ (Tukey's HSD, $P \leq 0.05$). Where-MPC-C is control powder produced without applying any treatment, MPC-T1 was produced from $5 \times \text{UFR}$ after adjusting its pH to 6.60 ± 0.00 using 10% solution of Na_2HPO_4 prior to its double stage homogenization (2500/500 psi), MPC-T2 was produced from diafiltered retentate obtained after DF of $5 \times \text{UFR}$ with 150 mM NaCl solution

T1, MPC-T2 powders (Table 2) were different than that reported by Crowley et al. (2014) for MPC60 and MPC70 powders. It is because Crowley et al. (2014) produced MPC60 from UF/DF retentates containing noticeably higher ($\sim 35\%$) TS. Span of the distribution was more in MPC-T1, but less in MPC-T2 powders than in MPC-C (Table 2).

As per classification of Carr (1965), all these powders were fairly free-flowing ($\theta = 35^\circ\text{--}45^\circ$) and exhibited significant variation ($P < 0.05$) in their flowability with each other (Table 2). Flowability of MPC-C and MPC-T1 powders were better than MPC-T2 powder that could be attributed to their SSA values (Table 2) as powders having bigger particles (smaller SSA) exhibits better flowability.

The dispersability (65.46–90.79%) of different MPC produced were in agreement with earlier published values for MPC powders (Baldwin 2010; Huppertz and Gazi 2015). MPC-T1 and MPC-T2 powders had significantly improved ($P < 0.05$) dispersibility as compared to MPC-C (Table 2). Severe denaturation and interaction among milk proteins during spray drying of $5 \times \text{UFR}$ containing higher calcium and lower pH, could easily be the explanation for

poor dispersibility of MPC-C powder. Addition of Na_2HPO_4 resulted in higher pH (Table 1) and decreased calcium activity, while homogenization might have produced smaller particles in UF retentates. Collectively, these changes might have decreased interactions and aggregation of milk proteins during spray drying of the retentate and thereby improved the dispersibility of MPC-T1 powder.

Schuck (2013) reported that aggregated powders such as casein or MPC powders have poor wettability index (< 120 s) because of different surface composition than skim and whole milk powders and dairy whitener which normally have lower (15–60 s) wettability index. Wettability of MPC-C, MPC-T1 and MPC-T2 powders exhibited significant variation ($P < 0.05$) with each other (Table 2). Dissociation of casein micelles in the presence of NaCl during DF and presence of bigger powder particles could be reason for shorter wetting time of MPC-T2 and MPC-C powders. However, wettability of these powders was higher than SMP, WMP and dairy whiteners.

The a^* and b^* values of MPC-C, MPC-T1 and MPC-T2 powders differed significantly difference ($P < 0.05$) with each other (Table 2). L^* value of MPC-C and MPC-T1 powders was similar each other, but was higher than that of MPC-T2 powder. The variation in color values of MPC powders could be explained on the basis of their chemical composition and physico-chemical properties.

Functional properties

Treatments employed significantly decreased ($P < 0.05$) the calcium contents of MPC-T1 and MPC-T2 powders (Table 1) and subsequently improved ($P < 0.05$) their solubility if compared with control powder 25 ± 1 °C at zero and up to 90 days of storage (Table 3). MPC-T1 and MPC-T2 powders exhibited markedly higher solubility compared to earlier reported 27–81% and 68–88% solubility of 32 commercial MPC powders (Huppertz and Gazi 2015) and MPC55-MPC60 powders (Floris et al. 2007). This improvement in solubility of MPC-T1 powder clearly indicate positive effect of applied treatments in lowering the calcium contents and minimizing the protein–protein interactions. Increasing the pH towards neutrality usually improves solubility of milk proteins. Increase in pH and lowering of calcium content decreased the denaturation, aggregation and severe casein–casein interaction of milk proteins during spray drying. Moreover, homogenization might have broken the aggregates/clusters of milk proteins formed in retentate due to shearing action and might have facilitated reconstitution of MPC-T1 powder. Sikand et al. (2012) also observed improved solubility in reconstituted MPC80 solution after homogenization. Moreover, Broyard and Gaucheron (2015) have reported the formation of non-sedimentable small casein aggregates due to disruption of

casein micelle via solubilization and demineralization/dcalcification of calcium phosphate due to alteration in milk pH. Sun et al. (2016) reported that addition of emulsifying salts changed the ionic environment of milk that hindered the aggregation of casein micelles and exhibited positive effect on solubility of MPC80.

The diafiltration of UF retentate with 150 mM NaCl significantly ($P < 0.05$) improved the solubility of MPC-T2 powder, because of the marked reduction in its calcium content. Such reduction in calcium have been also associated with increased ionic strength, weakened covalent bonds and loosened micelles structure due to the exchange of calcium with sodium ions with a higher release of non-micellar casein and reduced micellar interactions (Sikand et al. 2013). Changes in casein micelles such as altered protein–protein interactions, modified casein hydrophobicity, reduced disulphide bonds formation and protein aggregation were also related to a reduction in calcium content (Mao et al. 2012).

After 90 days storage at 25 ± 1 °C, MPC-C, MPC-T1 and MPC-T2 powders showed significant ($P < 0.05$) lowering in their solubility (Table 3). Maximum (32.40%) reduction was observed in MPC-C powder compared to only 20.31% and 13.87% reduction in MPC-T1 and MPC-T2 powders, respectively. It reflecting that the applied treatments were capable of improving and retaining higher solubility in treated MPC powders.

Proper heat stability in MPC powders is desired during the production of high protein beverages, recombined milk, soups and sauces, and products for enteral and clinical nutrition. The heat stability of commercial MPC powders containing 55–85% protein has been reported to be in the range of 0–40 min (Huppertz and Gazi 2015). Heat stability of MPC-T1 powder was significantly higher ($P < 0.05$) than MPC-C and MPC-T2 powders (Table 3). DSP addition increased the pH leading to decrease in calcium ion activity and micellar salts and enhanced steric repulsive forces in MPC-T1 as compared to MPC-C. The HCT values of reconstituted MPC60 solutions at 6.3, 6.7, 7 and 7.3 pH have been reported to be about 1, 3, 13 and 18 min, respectively (Crowley et al. 2014). The MPC-T2 had minimum HCT values compared to MPC-C and MPC-T1 powders that could be attributed to higher calcium ion activity at its lower pH. DF with 150 mM NaCl solution resulted in increased TS and protein contents, but decreased the pH value of MPC-T2 powder. Mao et al. (2012) also noticed such reduction in pH of retentates obtained after DF of UF retentates with 50, 100 and 150 mM NaCl containing DF water during the manufacture of MPC80 powders.

A significant difference ($P < 0.05$) was observed in the viscosity of the reconstituted MPC samples (Table 3) obviously due to different chemical composition, mainly in

Table 3 Functional properties of MPC powders

Sample attribute	MPC-C	MPC-T1	MPC-T2
Solubility (%)			
Fresh (0 day)	91.95 ^b	94.38 ^a	97.45 ^a
After 90 days	59.55 ^c	74.07 ^b	83.58 ^a
Heat coagulation time (min)	20.00 ^b	36.00 ^a	6.00 ^c
Foaming properties (%)			
Foam capacity	48.28 ^b	95.88 ^a	48.25 ^b
Foam stability	46.37 ^b	70.18 ^a	36.62 ^c
Emulsification properties (%)			
Emulsifying capacity	40.00 ^a	35.65 ^b	41.17 ^a
Emulsifying stability	36.00 ^b	33.42 ^c	36.12 ^a
Viscosity (mPa s) at 50 s ⁻¹	24.20 ^c	31.37 ^b	65.60 ^a
Water binding capacity (g/g of protein)	3.92 ^c	5.61 ^a	4.48 ^b
Oil binding capacity (g/g of protein)	3.35 ^c	3.59 ^b	4.73 ^a
Rennet coagulation time (min)	210.00 ^a	210.00 ^a	210.00 ^a

Means (n = 9) within the same row with different superscript differ (Tukey's HSD, $P \leq 0.05$). Where-MPC-C is control powder produced without applying any treatment, MPC-T1 was produced from $5 \times$ UFR after adjusting its pH to 6.60 ± 0.00 using 10% solution of Na_2HPO_4 prior to its double stage homogenization (2500/500 psi), MPC-T2 was produced from diafiltered retentate obtained after DF of $5 \times$ UFR with 150 mM NaCl solution

protein and calcium contents (Table 1) and physical and reconstitution properties of powder particles (Table 2). Significantly higher ($P < 0.05$) SSA of MPC-T1 and MPC-T2 than MPC-C powder (Table 2) could be responsible for their higher viscosity, as large number of smaller particles with improved solubility might have facilitated the hydration of proteins to disperse them in solution. Addition of phosphate was also associated with the enhanced viscosity of caseins (Matheis et al. 1983) because of increase in water absorption capacity of proteins.

The WBC of treated samples were significantly higher ($P < 0.05$) than that of MPC-C probably owing to phosphate addition led phosphorylation of caseins that decreased its surface hydrophobicity and thereby enhanced the WBC. Similar observations were also reported by Matheis et al. (1983). Mao et al. (2012) reported improved WBC in caseins upon addition of phosphate groups and NaCl, respectively. A significant increase ($P < 0.05$) in OBC values of treated samples were observed over MPC-C (Table 3) that could be attributed to their density and smaller particle size as evident from higher SSA values (Table 2). It is well-known fact that powders having lower density and smaller particle size absorb and bind more oil and vice versa.

A significant variation ($P < 0.05$) was observed in foam stability of all powders. The marked increase in the foaming capacity of MPC-T1 powder may be attributed to DSP addition as calcium replacing additive from casein. Moreover, higher pH could be responsible for its increased electronegativity and foaming properties (Table 3). Such

improvement in foaming has been earlier reported by Matheis et al. (1983). Foam capacity of MPC-C and MPC-T2 powders were at par with each other. However, foam stability of MPC-T2 powder was the least owing to lower pH value. Emulsifying activity and stability of MPC-C and MPC-T2 powders were significantly higher ($P < 0.05$) than MPC-T1. Addition of Na_2HPO_4 and NaCl were responsible for the poor emulsification properties of MPC-T1 and MPC-T2 powders than MPC-C powder. Earlier, Matheis et al. (1983) have observed decrease in emulsification capacity upon addition of 7.4 phosphate groups per mole of caseins.

No-significant ($P > 0.05$) difference was observed in the RCT values of reconstituted solutions of MPC powders and noticed no visible coagulation on rennet addition was observed till 210 min. The higher stability of reconstituted MPC solutions towards rennet coagulation could mainly be attributed to their lower calcium content as it is necessary for the completion of rennet action. Hence, external addition of about 2 mM calcium chloride was reported in the literature to induce rennet coagulation in MPC powders.

The buffer index values of MPC-T1 and MPC-T2 powders were higher than MPC-C powder in the investigated pH range as shown in Fig. 1. Higher buffer index values of MPC-T1 and MPC-T2 powders were mainly contributed by acid or basic groups of phosphate and sodium salts as they bind the protons to resist the change in pH (Broyard and Gaucheron 2015). Buffer index of casein micelles (5% wt/wt, pH-5.8) solution was also reported to

Fig. 1 Buffer index during alkalization-acidification with 0.1 N HCl and 0.1 N NaOH solution of MPC-C (control), MPC-T1 and MPC-T2 powders ($0.5 \text{ g } 100 \text{ mL}^{-1}$ protein solution). Where-MPC-C is control powder produced without applying any treatment, MPC-T1 was produced from $5 \times \text{UFR}$ after adjusting its pH to 6.60 ± 0.00 using 10% solution of Na_2HPO_4 prior to its double stage homogenization (2500/500 psi), MPC-T2 was produced from diafiltered retentate obtained after DF of $5 \times \text{UFR}$ with 150 mM NaCl solution

increase with the addition of Na_2HPO_4 (Mizuno and Lucey 2005).

Rheological behaviour

Flow behaviour of all reconstituted MPC ($10 \text{ g } 100 \text{ mL}^{-1}$) solutions were best described as Herschel–Bulkley model that suggest requirement of minimum shearing force to initiate the flow (Table 4). MPC-T2 sample exhibited the

higher yield stress and viscosity as compared to the control sample which might be attributed to lower pH of MPC-T2. However, MPC-T1 sample showed lower yield stress and viscosity as compared to the control sample that may be due to better calcium chelation by Na_2HPO_4 and application of 2-stage homogenization in achieving better reconstitution and solubility properties.

Morphological characteristics

The SEM micrographs of the three powders are shown in Fig. 2a–c, respectively. In MPC-C powder (Fig. 2a), most of the particles contained wrinkled and dents of variable depth and sizes, in which smaller particles were entrapped. This powder also contained clusters of collapsed and infused particles advocating higher interactions between calcium and proteins. The MPC-T1 powder contained much of smooth surfaced, intact particles of variable size and without intensive aggregation (Fig. 2, b). Na_2HPO_4 addition lowered calcium and increased the pH in powder and thereby reduced the intensity of calcium and casein interactions. Moreover, applied homogenization enabled the formation of smaller particles in it supported by its higher SSA values. DF with 150 mM NaCl solution also decreased calcium content and dissociated the casein micelles via exchange of calcium ions with sodium ions and resulted in smaller powders particles with the smooth surface, without much aggregation in MPC-T2 powder as visible in Fig. 2c. However, all these micrographs, being of fresh powders, exhibited similarity of many accounts.

Table 4 Rheological properties of reconstituted MPC samples

Rheological models	MPC-C	MPC-T1	MPC-T2
Bingham			
σ_0 (Pa)	1.01	1.26	2.74
η (mPa s)	5.38	8.83	7.33
R^2	0.995	0.988	0.994
Herschel–Bulkley			
σ_0 (Pa)	0.866	0.836	2.77
K (Pa s)	13.35	50.49	6.49
η (mPa s)	0.865	0.739	1.02
R^2	0.999	0.999	0.995
Power law			
k	0.277	0.323	1.098
n	0.422	0.466	0.290
R^2	0.906	0.948	0.822

Where σ is the shear stress, σ_0 -yield stress, η -viscosity, k is consistency index, n is flow behaviour index and R^2 is the coefficient of determination. Where-MPC-C is control powder produced without applying any treatment, MPC-T1 was produced from $5 \times \text{UFR}$ after adjusting its pH to 6.60 ± 0.00 using 10% solution of Na_2HPO_4 prior to its double stage homogenization (2500/500 psi), MPC-T2 was produced from diafiltered retentate obtained after DF of $5 \times \text{UFR}$ with 150 mM NaCl solution

Fig. 2 Scanning electron micrograph of **a** MPC-C (control), **b** MPC-T1, and **c** MPC70-T2. Where-MPC-C is control powder produced without applying any treatment, MPC-T1 was produced from $5 \times$ UFR after adjusting its pH to 6.60 ± 0.00 using 10% solution

of Na_2HPO_4 prior to its double stage homogenization (2500/500 psi), MPC-T2 was produced from diafiltered retentate obtained after DF of $5 \times$ UFR with 150 mM NaCl solution

Conclusion

Adjustment of $5 \times$ UF retentate pH to 6.6 using Na_2HPO_4 , prior to its homogenization, significantly ($P < 0.05$) decreased the calcium content of MPC-T1 powder and might also have prevented the severe calcium-casein and casein-casein interactions. The homogenization facilitated the formation of smaller particles and thus improved reconstitution of MPC. Moreover, combined use of Na_2HPO_4 and homogenization can substantially improve physical (SSA, LBD and PBD), reconstitution (dispersibility and flowability) and functional (solubility, heat stability, foam capacity and foam stability and water binding) properties of MPC powder significantly. Diafiltration with 150 mM NaCl solution also delivered promising results in lowering calcium content that markedly enhance the dispersibility, wettability, PBD, SSA, solubility, emulsification and foaming properties, viscosity, water and oil binding capacities of MPC powder.

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