Effect of pH adjustment, homogenization and diafiltration on physicochemical, reconstitution, functional and rheological properties of medium protein milk protein concentrates (MPC70)

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Abstract Poor solubility is the major limiting factor in commercial applications of milk protein concentrates (MPC) powders. Retentate treatments such as pH adjustment using disodium phosphate (Na_2HPO_4), also responsible for calcium chelation with homogenization and; its diafiltration with 150 mM NaCl solution were hypothesized to improve the functional properties of treated MPC70 powders. These treatments significantly improved the solubility, heat stability, water binding, dispersibility, bulk density, flowability, buffer index, foaming and emulsifying capacity of treated powders over control. Rheological behaviour of reconstituted MPC solutions was best explained by Herschel Bulkley model. Compared to rough, large globular structures with dents in control; majorly intact, separate, smaller particles of smooth surface, without any aggregation were observed in SEM micrograph of treated powders. Applied treatments are easy, cost-effective and capable to improve functional properties of treated powders that could replace control MPC70 powder in various food applications where protein functionality is of prime importance.

Keywords Milk protein concentrate 70 · Sodium chloride · Disodium phosphate · Diafiltration · Homogenization · Powder properties

Introduction

Milk protein concentrates (MPC) powders are available with 42–90% protein content on dry basis and contains similar caseins to whey proteins ratio as present in milk. The blend of caseins and whey proteins offer multifunctional properties to MPC powders and make them a suitable ingredient for various food formulations. MPC powders are produced by fractionation and concentration of skim milk in ultrafiltration (UF); further purification of milk proteins in diafiltration (DF) followed by evaporation (optional) and spray drying. MPC powders have been classified as low, medium and high protein powders with < 40, 60–70 and > 80% protein content on dry matter basis. With increase in UF/DF folds, the concentration of proteins and colloidal calcium increases in retentate due to subsequent decrease in water soluble lactose and salts. Such changes in chemical composition leads to the altered HCT-pH profile of retentate and also causes variation in physical, reconstitution and functional properties of MPC powders compared to skim milk powder (SMP). Higher protein content and calcium ion activity are mainly responsible for the poor solubility and heat stability of MPC powders (Meena et al. 2017a). Poor solubility have detrimental effect on other functional properties and restricts the uses of MPC powders in different food formulation. Marella et al. (2015) reported that poorly soluble MPC80 led to different problems during product formulation such as nugget formation in cheese making, hardening of high-protein bars and settling of insoluble material as sediment during preparation of beverage mixes.

Improvement in solubility of MPC powders has been reported by several researchers employing various chemical, physical, and enzymatic methods (Meena et al. 2017b). However, improvement in functional properties along with

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treatment compatibility and adaptability with existing production lines, cost-effectiveness, desired product quality and commercial viability remain set of challenges. Meena et al. (2017a) produced four different types of MPC60 powders by individually employing (a) pH (6.6) adjustment with disodium phosphate (Na_2HPO_4 , DSP), (b) diafiltration with 75 mM concentration of NaCl and KCl each in 1:1 ratio (c) homogenization (17.24/3.45 MPa) and, (d) no treatment of 5 $\times$ UF retentate. The percent solubility values of these powders at 0 day were 92.92, 93.93, 95.43, 93.69 and that reduced to 77.37, 77.41, 78.66 and 67.39 after 2 months storage at 25 ± 1 °C. These approaches exhibited better solubility retention in MPC60 powders during storage when applied in isolation. However, the combined effect of pH adjustment and homogenization; diafiltration with only NaCl on various properties of MPC70 powders have not been studied so far. Moreover, improvement in functional properties of MPC70 is more challenging than MPC60 mainly due to decreased lactose and higher protein content. Therefore, it was hypothesized that stabilizing salt (disodium phosphate) addition that will leads to calcium chelation coupled with homogenization that may induce desirable structural changes in milk proteins present in UF retentate and, diafiltration of UF retentate with 150 mM NaCl solution which will facilitate the calcium decalcification from casein micelles or chelate the free calcium ions and will improve the solubility, heat stability and other properties of resultant MPC70 powders.

Hence, this study was undertaken to evaluate the combined effects of pH adjustment (6.6) using Na_2HPO_4 with homogenization and, DF of UF retentate with 150 mM NaCl solution prior to its spray drying on physicochemical, reconstitution, functional and rheological properties of MPC70 powders.

Materials and methods

Material

Fresh pasteurized (72 °C/15 s) cow skim milk (1 $\times$) containing 84.7 g L⁻¹ total solids (TS), 30.2 g L⁻¹ protein, 1 g L⁻¹ fat, 6.6 g L⁻¹ ash, 1.2 g L⁻¹ calcium and 6.67 ± 0.02 pH was procured from Experimental Dairy of National Dairy Research Institute, Karnal, India. All chemicals used in this investigation were procured from Sigma Aldrich, St. Louis, MO, US.

Production of MPC70 powders

Pasteurized skim milk was concentrated to concentration factor 6 (6 $\times$) in UF process using a 50 kDa, pilot scale UF plant (Tetra Alcross M1, Tech-Sep, France) at 50 ± 1 °C

temperature and 1.0 kg/cm² transmembrane pressure as reported by Meena et al. (2016). The 6 $\times$ retentate was diafiltered with equal quantity RO water; after removal of exactly similar quantity water as permeate. One part of this retentate at its natural pH (no pH adjustment) was designated as 6 $\times$ DFR-C (control). Thereafter, pH of a part of 6 $\times$ DFR-C was adjusted to 6.6 by the addition of 10 g L⁻¹ DSP solution and homogenized (17.24/3.45 MPa) to obtain 6 $\times$ DFR-HDSP. Another part of 6 $\times$ DFR-C retentate was diafiltered with 150 mM NaCl solution to obtain 7 $\times$ DFR-NaCl. Control (6 $\times$ DFR-C), 6 $\times$ DFR-HDSP and 7 $\times$ DFR-NaCl retentates were dried in a pilot scale spray drier (SSP Faridabad, India; feed rate 110 kg/h; atomizer diameter-0.17 mm) at $185/85 \pm 5$ °C inlet–outlet air temperatures to obtain MPC70-C (control), MPC70-HDSP, and MPC70-NaCl powders. These powders were packed in metalized polyester low-density polyethylene (LDPE) bags and stored at 4 ± 1 °C until analysed. Powder samples were also stored at 25 ± 1 °C to evaluate their solubility after 60 days of storage. All powders were manufactured in triplicates.

Methods

Chemical analysis and determination of physical, reconstitution and functional properties of MPC70 powders

Total solids, fat, crude protein, total ash and calcium contents of skim milk, retentates and loose bulk density (LBD, g mL⁻¹), packed bulk density (PBD, g mL⁻¹), flowability measured in terms of angle of repose (θ°); wettability (min), dispersibility (%) and buffer index (dB/dpH) of MPC70 powders were determined adopting the standard methods as reported by Meena et al. (2017a). Calcium contents of the samples were analysed in an atomic absorption spectrophotometer (AAS, Model No. AA-7000, Shimadzu, Kyoto, Japan). Specific surface area (SSA, m²/kg), median particle size distribution (d_{10} , d_{50} and d_{90}), volume mean diameter (D_{32} and D_{43}) were determined using laser-light-scattering technique in a Malvern Mastersizer 3000 (Malvern Instruments Ltd., Malvern, Worcestershire, UK). Span was calculated from d_{10} , d_{50} and d_{90} values. The water activity (a_w) and color (L^* , a^* and b^*) values of MPC70 powders were estimated using Tristimulus spectrophotometer Hunter Lab model Colour Flex® (Hunter Associates Laboratory Inc., VA, U.S.A.), Aqua lab (U.S.A.). The pH of reconstituted (10 g powder, volume made-up to 100 mL) MPC70 samples were measured at 20 ± 1 °C using Eutech pH meter (make-Thermo Scientific, model-cyberscan 1100).

Solubility of MPC70 powders were determined using the method reported by Haque et al. (2012) with slight

modification as previously reported by Meena et al. (2017a). Foam capacity (FC) and foam stability (FS), emulsifying capacity (EC) and emulsifying stability (ES); water binding capacity (WBC) and oil binding capacity (OBC) of MPC70 powders were determined as per the methods reported by Shilpashree et al. (2015a, b). For emulsion capacity, high shearing action was performed instead of sonication.

All MPC70 powders were reconstituted adopting the method reported by Crowley et al. (2014) followed by measurement of their heat coagulation time (HCT) at 140 °C using an oil bath. Rennet coagulation time (RCT) of reconstituted (3.5% protein solution, stirring at 400 rpm, 25 ± 1 °C for 30 min) MPC70 samples was determined by the addition of rennet at the rate of 0.035 international milk clotting units (IMCU) per gram (Mucor Miehei, Sigma-Aldrich) as mentioned by Martin et al. (2010).

Measurement of viscosity and rheological properties

Apparent viscosity (50 s^{-1} , 20 °C) and flow behaviour ($1\text{--}1000 \text{ s}^{-1}$) of 100 g L^{-1} solution of MPC70 powders were measured as per the method mentioned by Meena et al. (2017a).

Scanning electron microscopy

The microstructure of MPC70 powders were studied by scanning electron microscopy (SEM) (EV018, 18th special edition, Zeiss, Tokyo, Japan) adopting the procedure reported by Shilpashree et al. (2015a, b).

Statistical analysis

The mean values of three replicates ($n = 9$) were subjected to one-way analysis of variance (ANOVA) using SAS Enterprise guide (5.1, 2012) developed by SAS Institute Inc., North Carolina, USA (SAS, 2008). The means were compared using Tukey's Studentized Range (HSD) test.

Results and discussion

Compositional analysis

Composition of retentates and MPC70 powders have been shown in Table 1. The pH (6.6) adjustment of 6×DFR-HDSP retentate with DSP solution significantly ($p < 0.05$) decreased its protein, fat and calcium contents, but increased its ash content on dry matter basis compared to 6×DFR-C retentate. Diafiltration with 150 mM NaCl significantly ($p < 0.05$) decreased the calcium content while, markedly increased protein, fat and ash contents of

7×DFR-NaCl retentate. Increase in TS and change in the cation profile of the milk during UF, collectively changed the pH of retentates. Calcium and pH play vital roles in expression of functional properties of milk proteins. Additional treatments, significantly ($p < 0.5$) decreased the calcium contents in treated retentates and powders; markedly increased their pH values compared to control retentate and powder (Table 1). This was attributed to the addition of DSP and NaCl salts in the retentates that released phosphate ions i.e. protonation of phosphate anions increased the pH whereas decalcification of casein micelles through the exchange of calcium ions with sodium ions during diafiltration and, calcium chelation as well as dilution effect induced by DSP salt collectively reduced the calcium contents of additionally treated powders.

Effect of additional treatments on functional properties

Solubility

Solubility is the property of prime importance in food formulations to impart desired functional attributes of milk proteins. It is mainly governed by the composition of powder. Poor solubility of control MPC70 was mainly attributed to its higher calcium content and lower pH, which favoured the protein aggregation during ultrafiltration and further denaturation and crosslinking of proteins during spray drying that resulted in the formation of poorly soluble aggregated powder particles as evident from SSA, particle size distribution, mean diameters (Table 2) and SEM micrograph (Fig. 3A). However, additional treatments significantly ($p < 0.05$) improved the solubility of treated MPC70 powders over control powder (Fig. 1A).

Increased solubility of MPC70-HDSP powder could be attributed to different reasons such as calcium chelation by DSP salt that caused calcium decalcification from casein micelles into soluble phase from casein micelles via dissociation as well as disruption of micellar structure of casein through solubilisation of calcium phosphate that might have released non-sedimentable, smaller casein aggregates (< 15 casein molecules) into aqueous phase. Such results have been also reported earlier by Pitkowski et al. (2008). Homogenization is supposed to disrupt the casein aggregates due to shearing and disperse them uniformly thereby might have enhanced the functioning of DSP and worked synergistically towards solubility improvement in MPC70-HDSP. DSP addition also increased the pH of MPC70-HDSP compared to MPC70-C that enhanced the net negative charge and electrostatic repulsive forces between the casein micelles and prevented their aggregation, which ultimately improved their stability. Thus, DSP added and homogenized retentate resulted

Table 1 Composition and pH of retentates and MPC70 powders

Samples	TS (g 100 mL ⁻¹)	Protein (g 100 mL ⁻¹)	Fat (g 100 mL ⁻¹)	Ash (g 100 mL ⁻¹)	Calcium (g 100 mL ⁻¹)	pH
Retentates						
6×DFR-C	26.78 ^b ± 0.10	19.64 ^b ± 0.14 (73.34)	0.57 ^b ± 0.02 (2.13)	2.22 ^c ± 0.10 (8.29)	0.75 ^a ± 0.01 (2.80)	6.36 ^c ± 0.07
6×DFR-HDSP	25.14 ^c ± 0.12	17.80 ^c ± 0.09 (70.80)	0.51 ^c ± 0.01 (2.03)	2.89 ^a ± 0.12 (11.50)	0.68 ^b ± 0.02 (2.70)	6.60 ^b ± 0.03
7×DFR-NaCl	28.44 ^a ± 0.16	21.25 ^a ± 0.16 (74.72)	0.75 ^a ± 0.09 (2.64)	2.50 ^b ± 0.16 (8.79)	0.35 ^c ± 0.01 (1.23)	6.69 ^a ± 0.02
Samples	TS (g 100 g ⁻¹)	Protein (g 100 g ⁻¹)	Fat (g 100 g ⁻¹)	Ash (g 100 g ⁻¹)	Calcium (g 100 g ⁻¹)	pH
Powders						
MPC70-C	95.58 ^a ± 0.08	70.05 ^b ± 0.02 (73.29)	2.04 ^b ± 0.01 (2.13)	7.90 ^c ± 0.00 (8.27)	2.66 ^a ± 0.01 (2.78)	6.49 ^b ± 0.04
MPC70-HDSP	92.30 ^c ± 0.05	65.33 ^c ± 0.01 (70.78)	1.87 ^c ± 0.01 (2.03)	10.61 ^a ± 0.00 (11.49)	2.50 ^b ± 0.01 (2.70)	6.95 ^a ± 0.06
MPC70-NaCl	93.90 ^b ± 0.07	70.16 ^a ± 0.01 (74.72)	2.50 ^a ± 0.01 (2.64)	8.20 ^b ± 0.00 (8.73)	1.15 ^c ± 0.00 (1.22)	6.92 ^a ± 0.08

Mean ± SE (n = 9), ^{abc}mean values with different superscripts in a column are significantly different with each other ($p < 0.05$). The values in parenthesis are on TS basis

in the formation of smaller powder particles without any aggregation as evident from SSA, particle size distribution (Table 2) and SEM micrograph (Fig. 3B) and exhibited significantly higher ($p < 0.05$) solubility than control powder. Conventional homogenization, addition of stabilizing and calcium chelating agents have been individually reported to enhance the solubility of casein rich powders, but this investigation had revealed that marked improvement in solubility of MPC70-HDSP powder was attributed to the collective effects induced in casein micelles by pH adjustment with DSP and homogenization.

Marked increase in the solubility of MPC70-NaCl over control was attributed to the DF treatment of retentate with 150 mM NaCl solution that exchanged calcium ions present in casein micelles with sodium ions and enhanced its ionic strength, pH and disturbed its micellar structure via decalcification. Thus, DF with NaCl enhanced the dissolution of colloidal calcium phosphate from the micelles, and enhance dissolution of caseins out of the micelles. The noticeable decrease in the calcium content and increase in pH value (Table 1) of MPC70-NaCl powder could easily explain its higher solubility over control. The increased solubility of MPC60 powder was also accorded to its lower calcium content (Meena et al. 2017a). Moreover, DF with NaCl has been reported to decrease calcium content and also reported to induce several changes in casein micelles such as it loosened casein structure, altered mineral composition and hydrophobicity of casein, modified/decreased protein–protein interactions, increased ionic strength and

favoured higher release of non-micellar casein and also lowered protein aggregation due to decreased disulphide bonds formation (Mao et al. 2012; Sikand et al. 2013). Although, MPC70-NaCl powder had bigger size particle than control (Table 2), but salt (NaCl) particles present within this powder might have provided pathways for water penetration and might have improved powder reconstitution behaviour. The efficacy of this treatment in solubility improvement is strongly supported by the SEM micrograph (Fig. 3C) of MPC70-NaCl powder that clearly showed absence of any aggregates in this powder compared to severe aggregation in control powder as shown in Fig. 3A. Such changes in casein micelles due to calcium depletion mediated by the addition of monovalent salts like NaCl or KCl as well as improvement in the solubility of casein powders and MPC80 were also reported by Schuck et al. (2002) and Sikand et al. (2013). After 60 days of storage, significantly lesser reduction ($p < 0.05$) was observed in the solubilities of MPC70-HDSP and MPC70-NaCl powders than control (Fig. 1A). Thus, effectiveness of the applied additional treatments was clearly advocated by the higher solubility retention in treated powders after 60 days at 25 ± 1 °C than control powder.

Heat stability

The heat stability of MPC powders containing 55–85% protein content were reported in the range of 0–40 min (Huppertz and Gazi 2015). Poor heat stability of MPC

Table 2 Physical and reconstitution properties of MPC70 powders

Samples	LBD (g mL ⁻¹)	PBD	a _w	Colour value		SSA (m ² kg ⁻¹)	Particle size distribution, (μm)			
				L*	a*		b*	d ₁₀	d ₅₀	d ₉₀
MPC70-C	0.30 ^c ± 0.02	0.41 ^c ± 0.01	0.25 ^b ± 0.02	88.92 ^a ± 0.02	- 1.21 ^b ± 0.02	11.99 ^a ± 0.01	66.77 ^b ± 0.24	52.42 ^b ± 0.28	114.95 ^b ± 0.07	225.51 ^b ± 0.23
MPC70-HDSP	0.38 ^a ± 0.02	0.51 ^a ± 0.01	0.36 ^a ± 0.03	89.02 ^a ± 0.01	- 1.31 ^c ± 0.02	11.97 ^a ± 0.01	87.75 ^a ± 0.30	41.33 ^c ± 0.10	86.95 ^c ± 0.10	167.88 ^c ± 0.13
MPC70-NaCl	0.33 ^b ± 0.01	0.46 ^b ± 0.02	0.37 ^a ± 0.00	87.25 ^b ±0.05	- 0.57 ^a ± 0.03	11.60 ^b ± 0.01	57.20 ^c ± 1.74	59.95 ^a ± 0.93	134.53 ^a ± 2.13	269.99 ^a ± 0.86
Samples	Mean diameter, (μm)			Flowability (θ°)		Dispersibility (%)	Wettability (min)			
	D ₃₂			D ₄₃						
MPC70-C	90.50 ^b ± 0.14			128.48 ^b ± 0.24		35.44 ^a ± 0.03	40.16 ^c ± 0.01	2.52 ^b ± 0.00		
MPC70-HDSP	68.79 ^c ± 0.17			96.88 ^c ± 0.14		32.36 ^b ± 0.10	60.43 ^b ± 0.05	38.90 ^a ± .00		
MPC70-NaCl	104.38 ^a ± 1.07			152.73 ^a ± 0.98		30.54 ^c ± 0.18	68.58a ± 0.24	2.10 ^c ± 0.00		
Mean ± SE (n = 9), ^{abc} mean values with different superscripts in a column are significantly different with each other (p < 0.05)										

powders could be attributed to its higher calcium content and lower pH. It is well known that reduction in pH of milk by any means results in its increased Ca²⁺ concentration that decreases its HCT. In this study also, maximum reduction in pH was observed in 6×DFR-C retentate that leads to minimum pH (Table 1) and HCT in reconstituted MPC70-C solution (Fig. 1B). However, additional treatments significantly improved ($p < 0.05$) the HCT values of MPC70-HDSP and MPC70-NaCl solutions to 13 and 35 times (Fig. 1B), respectively. Such marked increase in HCT of reconstituted MPC70-HDSP could be explained by its higher pH (Table 1) and decreased calcium content or Ca²⁺ concentration than reconstituted MPC70-C solution as a result of pH (6.6) adjustment of 6×DFR-HDSP retentate with DSP that have been reported to decrease Ca²⁺ concentration. The general destabilization effect of homogenization on HCT might be countered by DSP salt-led stabilization. Meena et al. (2017a) reported that reduced calcium ion activity, higher buffering capacity and increased pH values were attributed to the higher HCT values of the reconstituted MPC60-DSP powder (manufactured with only DSP addition) than control. Improvement in heat stability of micellar casein solutions were also observed upon the addition of calcium chelators by De Kort et al. (2012).

The increased HCT of reconstituted MPC70-NaCl solution was mainly attributed to retentate decalcification induced by 150 mM NaCl solution during DF which significantly ($p < 0.05$) reduced the calcium content and increased pH of the MPC70-NaCl powder. Le Ray et al. (1998) reported that heat stability of casein micelles dispersions were improved upon addition of sodium chloride, sodium citrate and sodium phosphate salts due to increased steric repulsions and decreased micellar minerals. Similar explanations also hold true for the enhanced HCT of reconstituted MPC70-NaCl solution. Crowley et al. (2014) determined the heat stability of the reconstituted solution (35 g L⁻¹ protein) of MPC70 by adjusting its pH from 6.3 to 7.3 and observed very low HCT values in pH range without exhibiting any maximum or minimum, which gradually increased to maximum 18 min as the pH increased towards alkaline values (6.8–7.3). Thus, HCT values as observed for MPC70 powders manufactured applying additional treatments in this investigation were at par for reconstituted MPC70-HDSP solution and even better for MPC70-NaCl solution than the values reported by Crowley et al. (2014). Hence, it is evident (Fig. 1B) that decrease in calcium content and increase in pH collectively improved the heat stability of treated MPC70 powders over control.

Fig. 1 Functional properties of MPC70 powders **A** solubility at 0 and 60 days, **B** heat stability, **C** viscosity at 50 s^{-1} , **D** WBC and OBC, **E** FC and FS and **F** EA and ES. Mean $\pm$ SE ($n = 9$), ^{abcdef} mean values are significantly different with each other ($p < 0.05$)

Viscosity

The viscosity of treated and control powders exhibited significant difference ($p < 0.05$) with each other (Fig. 1C). Maximum viscosity was observed in control powder that could be attributed to the presence of larger casein aggregates because of its poor solubility. However, lower calcium content and presence of smoother (Fig. 3B) and bigger powder particles with increased particle size distribution and span (Table 2) could explain lower viscosity in MPC70-NaCl than control. Further, maximum solubility, higher pH, lesser calcium content and presence of smoother particles might be attributed for the minimum viscosity of MPC70-HDSP powder. Singh (2011) reported that viscosity of MPC, calcium caseinates, whey protein concentrate (WPC) and whey protein isolate (WPI) were similar to each other, but lower than that of sodium caseinate (NaCas).

Water binding capacity and oil binding capacity

Significant improvement ($p < 0.05$) in the WBC values of treated powders were observed over control, which could be attributed to their higher pH, lower calcium content and weak hydrophobic interactions. On the other hand, aggregation of powder particles at lower pH, higher calcium content and greater particle size reduced the number of

polar amino groups available for hydrogen bonding with water molecules resulting in lower WBC of control powder. Diafiltration with 150 mM NaCl resulted in altered ionic environment of milk proteins that enable better binding of salts and water molecules as well as induced competition between protein molecules and salt ions for water binding as also reported by Zayas (1997). This also resulted in reduced powder aggregation (Fig. 2C) due to enhanced steric repulsions and improved WBC of MPC70-NaCl.

The OBC values of MPC70 powders differed significantly ($p < 0.05$) with each other (Fig. 1D) although, OBC of control powder were noticeably higher and lower than MPC70-HDSP and MPC70-NaCl powders, respectively. In this investigation, applied treatments resulted in the altered composition of treated retentates (Table 1) that leads to lower LBD and higher d_{50} values (Table 2) as well as improved OBC values of control and MPC70-NaCl powders. It is well known fact that powders having lower density and smaller particle size absorb and entrap more oil and vice versa (Zayas 1997). Opposite to this, higher LBD and lower d_{50} value of MPC70-HDSP powder could easily explain its poor OBC compared to control and MPC70-NaCl powders.

Fig. 2 Titration curve (A) and buffer index (B) of alkalization-acidification with 0.1 N HCl and 0.1 N NaOH solution of MPC70 powders (0.5 g 100 mL⁻¹ protein solution)

Foam capacity and foam stability

The foam capacity and foam stability of MPC70 powders differed significantly ($p < 0.05$) with each other. Compared to control, significant ($p < 0.05$) improvement in foam capacities of additionally treated (MPC70-HDSP and MPC70-NaCl) powders were observed that might be collectively attributed to the open structure and less aggregation of casein micelles because of decalcification and formation of powder particles that could easily form thin interface between aqueous phase and air. Improvement in foaming capacity of MPC powders have been reported as a result of citrates and sodium chloride addition. The use of appropriate concentrations and salt combinations were emphasized for the betterment of foaming properties in MPC powders by Han and Vardhanabhuti (2011). However, powders prepared from additionally treated retentates had lower foam stability than control powder (Fig. 1E). Loss of hydrophobic interaction abilities in these treated powder resulted into poor foam stability. Greater hydrophobic linkages developed upon dissolving stabilized the air–water interface. Greater viscosity and yield stress value of control sample indicated the greater strength of protein in control and hence, could advocate the ability of control sample to stabilize the foam.

Emulsification capacity and stability

Additional treatments significantly improved ($p < 0.05$) the emulsification ability and stability of MPC70-HDSP and MPC70-NaCl powders over control (Fig. 1F), which might be attributed mainly to the dissociation of casein micelles that reduced casein aggregation via decreased calcium contents (Table 1) and higher stearic repulsions helped in the formation of fine and stable emulsion. As per Singh (2011), emulsification properties of aggregated powders such as MPC and calcium caseinate are poor while, Ye (2011) reported that NaCas, WPC and WPI form

stable emulsions at low protein-to-oil ratio (about 1:60) compared to much higher concentrations of MPC or calcium caseinate powders to form such stable emulsion. Further, MPC powders with reduced calcium contents exhibited better emulsification properties as the state of casein aggregation governs the emulsifying capability and adsorption behaviours of milk proteins in MPC powders as stated by Ye (2011). Thus, loss of aggregation and hydrophobic interactions among protein molecules resulted in enhanced surface activity at the oil–water interface and favoured better emulsification capacity and stability values in additionally treated MPC70 powders over control powder (Fig. 1E). Dispersion of aggregated proteins in the dissociating buffers have been also reported to improve the emulsifying ability of milk protein due to exposure of more groups (Euston et al. 2000).

Rennet coagulation time (RCT) and buffer index

In this investigation, reconstituted MPC70 samples did not coagulate upon rennet addition up to studied duration of 210 min, which may primarily be attributed to their low calcium content and weak hydrophobicity. Meena et al. (2017a) also observed similar rennet coagulation behaviour in different reconstituted MPC60 solutions. Martin et al. (2010) also reported that reconstituted MPC did not coagulate without external supplementation of approximately 2 mM calcium chloride. The buffer index of reconstituted MPC70 solutions changed with the relative change in pH (Fig. 2A, B). Compared to control, MPC70-HDSP and MPC70-NaCl solutions showed higher buffering index in majority of the pH range as the result of external addition of phosphate and sodium salts during retentate treatments. This enhanced the buffer index of treated powders as chelatants have both acid and basic groups that bonded with the protons and resisted the change in pH. The exposure of protein sites in the treated samples further increased the buffering capacity of proteins.

Effect on physical properties

Bulk density and water activity (a_w)

Because of additional treatments, the LBD and PBD values of control and treated powders exhibited significant difference ($p < 0.05$) between them. However, LBD and PBD were in broad agreement with the literature data (Table 2). The reported LBD value of MPC88 and PBD value of MPC70 were 0.30 and 0.49 g mL⁻¹. According to Crowley et al. (2014) the tapped density of MPC (MPC35–MPC90) powders were in the range of 0.29–0.59 g mL⁻¹. Additional treatments resulted in the altered composition of the treated retentates (Table 1) that caused variation in their physical properties. The drying of lower TS, protein and viscosity containing 6×DFR-HDSP retentates produced smaller particles in MPC70-HDSP powder as evident from SSA and d_{50} values (Table 2) and these smaller particles were mainly responsible for its higher LBD and PBD values than other powders because bulk density decreases with increase in particle size apart from different other factors. Higher viscosity of 6×DFR-C and 6×DFR-NaCl retentates due their higher protein contents also favoured feed foaming and resulted in bigger powders particles with higher interstitial air contents in control and MPC70-NaCl powders and ultimately decreased their LBD values. Trend similar to LBD was also observed in the PBD values of these powders. Moreover, bulk density of powders is a complex property that cannot be correlated to a single factor as it is affected by several other feed and processing factors (Schuck 2013).

The a_w values of MPC70-HDSP and MPC70-NaCl powders were identical and significantly higher ($p < 0.05$) than control. The observed deviation might be attributed to higher moisture contents of these powders due to added salts.

Particle size distribution and SSA

Additional treatments altered the chemical composition as well as viscosity of the retentates. Altered properties of the retentates might have led to different atomization behaviour of these retentates during spray drying and resulted in significant ($p < 0.05$) differences among the SSA, particle size distribution, D_{32} and D_{43} values of the powders (Table 2). Crowley et al. (2014) reported that SSA and particle size (d_{10} , d_{50} , d_{90}) values of MPC70 were 0.38 m² g⁻¹ and 14.9, 39.6, 72.7 (μm), respectively which are noticeably different than the values observed in this study and attributed to the composition and TS contents of the retentate from which all these MPC70 samples were produced. The pH adjustment and homogenization of 6×DFR-HDSP assisted calcium chelation and

disintegration of casein aggregates; decreased its TS, protein and calcium contents that collectively resulted in maximum SSA values in MPC70-HDSP powder via formation of high number of smaller particles compared to other powders. The span of control, MPC70-HDSP and MPC70-NaCl were 1.51, 1.46 and 1.56, respectively. The presence of higher TS in 6×DFR-C; TS and protein in 6×DFR-NaCl retentates were responsible for the lower SSA values in MPC70-C and MPC70-NaCl powders as retentates with higher TS (and also of higher viscosity) produced bigger size particles with lower SSA values. Similar trend was also observed for particle size and SSA values of MPC70–MPC90 powders by Crowley et al. (2014).

Flowability

Based on angle of repose (θ) values, the flowability of control powder was fair ($\theta > 35^\circ$) compared to good flowability ($\theta = 31^\circ$ – 35°) of MPC70-HDSP and MPC70-NaCl powders without any visible lumping. Moreover, θ values of these powders exhibited significant difference ($p < 0.05$) among them because of applied additional treatments. The presence of bigger powder particles as indicated by either lower SSA or higher d_{50} values (Table 2) could easily explain better flow properties of MPC70-NaCl powder over control powder. The calculated values of Carr's compressibility index given by $[(PBD - LBD)/PBD] \times 100$ and Hausner ratio (PBD/LBD) of control, MPC70-HDSP and MPC70-NaCl powders were 26.82, 25.49, 28.26 and 1.36, 1.34, 1.39, respectively. Thus, according to compressibility index and Hausner ratio, only MPC70-HDSP powder had passable flow that might be attributed to its lowest fat content (Table 1) compared to poor flow properties of remaining powders. In both treatments, salt component is believed to interfere with the hydrophobic interactions in casein-rich powders and might have contributed in the flowability improvement of the treated powders compared to control MPC70 powder.

Dispersibility

Dispersability of the powders can be defined as the percentage of the milk solids that get dissolved in water upon gentle mixing. MPC powders with dispersibility index $\geq 95\%$ were only considered as dispersible in the literature. The dispersibility of the studied MPC70 powders differed significantly ($p < 0.05$) with each other (Table 2) and in broad agreement with the values (38–100%) reported by Huppertz and Gazi (2015) for MPC55–MPC85 commercial powders, however, powders studied in this investigation were not dispersible as their dispersibility

ranged from 40.16 to 68.58% only. Further, casein-based powders such as micellar casein and MPC powders have been reported as poorly dispersible powders as only 38, 5.1, 25.6, 10 and 66% dispersability were observed in MPC85 (Bouvier et al. 2013), MCI, MPI, NaCas and WPI powders, respectively by Schuck (2013).

In this investigation, additional treatments significantly improved ($p < 0.05$) the dispersibility of MPC70-HDSP and MPC70-NaCl powders over control powder as shown in Table 2. The poor dispersibility of the control powder was attributed to the lower pH and higher calcium content in the 6×DFR-C retentate that favoured protein aggregation in it during UF process and leads to severe calcium-casein, casein-casein interactions as well as aggregation, denaturation and hydrophobic crosslinking between caseins during spray drying. The diafiltration with 150 mM NaCl markedly increased the pH and decreased the calcium content and casein aggregation in 7×DFR-NaCl retentate that led to lesser casein-calcium-casein interactions and aggregation during spray drying and improved the solubility and dispersibility of MPC70-NaCl powder over control. Moreover, higher viscosity of 6×DFR-NaCl retentate owing to its more protein content leads to formation of bigger particles (d_{50}) without aggregation as evident from SEM image (Fig. 2C) due to reduced calcium content also accorded to its higher dispersability as increase in the particle size of dairy powders promotes powder dispersibility. Moreover, increased pH and ionic strength, decreased calcium content and presence of smaller casein micelles due to pH adjustment with DSP and homogenization of 6×DFR-HDSP retentate also decreased the strength of covalent linkage that lead to decrease in casein-casein (hydrophobic) interactions and aggregations during spray drying and collectively improved the dispersibility of MPC70-HDSP powder over control.

Wettability

It is the ability to absorb water by powder particles through the penetration of liquid. Shorter wetting index is preferred

for powders. Because of the additional treatments used in this investigation, wettability of MPC70 powders differed significantly ($p < 0.05$) with each other and was in the range of 2.10–38.90 min. These powders were also observed to be non-wettable as casein or MPC powders with wettability index < 120 s were only considered wettable in the literature. Although, observed wettability of produced powders were in broad agreement with other high protein powders (casein and MPC powders) exhibiting relatively poor wettability due to their surface composition (Bouvier et al. 2013). However, wettability index of MPC85, MCI, MPI, NaCas and WPI was also > 120 s (Bouvier et al. 2013). Further, wetting time of granulated and non-granulated micellar casein and whey protein powders were 1, 3, 4 and 17 min, respectively (Schuck 2013). Compared to control powder, MPC70-NaCl powder exhibited maximum wettability (minimum wetting time) that could be accorded to its large particle size (D_{32} or D_{43}) and addition of NaCl, which weakened the hydrophobic interactions between caseins and thus, provided pathways for water migration and transportation within the powder particles. Further, DF with NaCl increased the hygroscopic nature of MPC70-NaCl that also favoured its improved wettability. Smaller D_{32} or D_{43} values and higher SSA could be responsible for poor wettability of MPC70-HDSP powder as smaller particles fills the voids present between bigger particles and restricts the migration of water and enhanced its wetting time. The presence of larger powder particles in control powder could be accorded to its shorter wetting time.

Rheological properties

Flow behaviour of all reconstituted MPC70 (10 g 100 mL⁻¹) solutions was best described as Herschel Bulkley flow behaviours (Table 3). Control sample exhibited the maximum yield stress that is in accordance to its poor solubility. The control sample could not bind water owing to its poor solubility and formed aggregates and hence low HCT, very high yield stress and viscosity were

Table 3 Rheological parameters modelling of reconstituted MPC70

Samples	Bingham			Herschel Bulkley				Power law		
	σ_0 (Pa)	η (mPa s)	R^2	σ_0 (Pa)	k	η (mPa s)	R^2	k	n	R^2
MPC70-C	5.696	14.1	0.945	3.478	344.20	0.540	0.994	2.340	0.281	0.933
MPC70-HDSP	1.101	14.6	0.963	0.658	70.0	0.760	0.999	0.268	0.557	0.971
MPC70-NaCl	3.711	15.3	0.946	0.258	163.5	0.653	0.998	1.174	0.372	0.954

σ is the shear stress (Pa), σ_0 yield stress, η viscosity (mPa s), and k consistency index (Pa s), n flow behaviour index and R^2 is coefficient of determination

Fig. 3 Scanning electron micrograph of **A** MPC70-C, **B** MPC70-HDSP, and **C** MPC70-NaCl

exhibited. The MPC70-HDSP sample had the least viscosity which supported the hypothesis of better calcium chelation phenomenon by DSP during homogenization. Among the studied powders, minimum yield stress was observed in reconstituted MPC70-NaCl solution.

Scanning electron microscopy

The SEM micrograph (1000 $\times$) of control, MPC70-HDSP and MPC70-NaCl powders have been presented in Fig. 3A–C, respectively. Microstructure of control powder depicted mostly collapsed and infused, rough globular structures with dents of variable depth and sizes and particles with large clusters or aggregates (Fig. 3A). Shilpashree et al. (2015a, b) also observed predominance of collapsed globular structures having coarse appearance with a distinct tendency to aggregate in SEM micrographs of native MPC80 powder. Such aggregation of powder particles in MPC powders is mainly attributed to their high calcium content and processing induced changes in casein micelles such as alteration of delicate salt equilibrium between colloidal and soluble phases of milk (Alexander et al. 2011), swelling of casein micelles as calcium moves from micelles into serum phase (McKenna 2000); dissolution of colloidal calcium phosphate (CCP) nanoclusters (Alexander et al. 2011) and migration of caseins, particularly κ -casein, from the micelle into the serum (McKenna 2000) with increase in calcium content. All these changes collectively disturbs the natural protein stabilization mechanism of milk and promote aggregation. Micrograph of MPC70-HDSP indicated mostly intact, smoothed surface, individual particles of variable size without any aggregation. Changes induced in casein micelles due to addition of DSP because of its calcium chelation action and impact of high shearing during homogenization could be responsible for smaller size particles as supported by higher SSA value (Table 2) than control and these particles were also trapped in dents of large particles. Similarly, smooth surfaced, majorly individual particles of different

sizes without any aggregation were seen in the micrographs of MPC70-NaCl powder, which could be explained by calcium reduction due to exchange of calcium ions with sodium ions and casein solubilization during DF with 150 mM NaCl solution.

Conclusion

This investigation was aimed to study the effect of pH adjustment, homogenization and diafiltration on various properties of MPC70 powders. Additional treatments such as addition of DSP to adjust pH followed by homogenization as well as DF using 150 mM NaCl significantly ($p < 0.05$) decreased the calcium content, thereby might have prevented severe casein–casein aggregations. Different processing induced changes in casein micelles such as disturbed salt equilibrium, swelling and decalcification of casein and migration of κ -casein micelle into serum phase with concentration of calcium in UF/DF processes and aggregation of milk proteins during evaporation and drying have adverse effect on the functional properties of MPC powders. Applied interventions were capable to reduce the calcium content of the treated UF/DF retentates and decreased this undesirable aggregation of milk proteins particularly caseins and resulted in improved physical (bulk density, flowability, dispersibility) and functional (solubility, HCT, water binding, foam and emulsifying capacity) properties in MPC70-HDSP and MPC70-NaCl powders over control. The efficacy of applied interventions was also advocated by microstructure of the treated powders. Hence, this investigation established that addition of disodium phosphate combined with homogenization; diafiltration of retentates with 150 mM NaCl solution can produce MPC70 powder with improved functional properties that can replace native MPC70 powder in various commercial food formulations, where functionality of milk protein is of prime importance.

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Compliance with ethical standards

Conflict of interest The authors declare that they do not have any conflict of interest.

Informed consent Written informed consent was obtained from all study participants.

Human and animal rights This study does not involve any human or animal testing.

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