Effect of change in pH, heat treatment and diafiltration on properties of medium protein buffalo milk protein concentrate

Ashwajit Tejram Patil¹ · Ganga Sahay Meena¹ · Neelam Upadhyay¹ ·
Yogesh Khetra¹ · Sanket Girdharbhai Borad¹ · Ashish Kumar Singh¹

Revised: 9 January 2019 / Accepted: 1 February 2019 / Published online: 18 February 2019
© Association of Food Scientists & Technologists (India) 2019

Abstract The demand of milk protein concentrate (MPC) powders is continuously increasing as high protein dairy ingredients. Presence of higher calcium and casein contents; heating, ultrafiltration (UF), diafiltration (DF) and spray drying of buffalo skim milk induces undesirable changes in milk proteins that causes problem of poor solubility in MPC powders. Therefore, this investigation was aimed to study the effect of change in pH (6.8-native, 7.0-neutral), heat treatments (74 ± 1 °C/15 s, 80 ± 1 °C/5 min, 85 ± 1 °C/5 min, 90 ± 1 °C/5 min) and DF on physicochemical, functional, reconstitution and rheological properties of medium protein buffalo milk protein concentrate (MP-BMPC) powder. Based on maximum ζ -potential and heat stability, UF retentate was selected, diafiltered and spray dried to obtain MP-BMPC powder. Despite having higher protein content, MP-BMPC powder exhibited markedly better functional (solubility, wettability, viscosity and emulsion stability) properties than buffalo milk protein concentrate 60. The interstitial air content, occluded air content, loose bulk density, packed bulk density, particle density and porosity values of MP-BMPC powder were 145.97 and 112.92 mL 100 g⁻¹ of powder, 0.21 g mL⁻¹, 0.30 g mL⁻¹, 0.55 g mL⁻¹ and 65.09%. Further, its specific surface area; particle size distribution (d_{10} , d_{50} , d_{90}); Sauter (D_{32}) and DeBroukere (D_{43}) mean values were 97.93 m² kg⁻¹; 34.32, 104.42, 218.58 μ m; 61.27 μ m and 117.99 μ m. The storage modulus (G') and loss modulus (G'') crossover temperature of UF and DF retentates were ~ 57.16 °C and 55.10 °C, respectively.

Rheological behaviour of UF, DF retentates and MP-BMPC solution were best explained by Herschel-Bulkley model. Fourier-transform infrared spectroscopy best described amide I, II and III regions in 1700–1400 cm⁻¹ and 1350–1200 cm⁻¹ wavenumber range.

Keywords Buffalo milk · Milk protein concentrate · Physicochemical · Functional · G' and G'' · FTIR

Introduction

Milk protein concentrate (MPC) are dairy based high protein powders which contain 40–89% protein content on dry matter basis. These MPC powders have unique caseins to whey proteins ratio which is identical to that of milk (Meena et al. 2017). MPC powders containing $\leq 40\%$, 60–70% and $\geq 80\%$ protein contents have been classified as low, medium and high protein powders, respectively (Sikand et al. 2011). Most common unit operations used during the production of MPC powders include centrifugal separation, pasteurization/heat treatments, ultrafiltration (UF) and diafiltration (DF) of skim milk and evaporation (optional) and spray drying of UF/DF retentates. UF retain and concentrate proteins present in skim milk by the selective permeation of water soluble milk constituents into permeate through UF membrane. Ultrafiltration leads to alteration in composition (mainly increases protein and total calcium contents but decreases lactose) of its retentate which induces variation in properties of MPC powders in comparison to skim milk powder (SMP) and whole milk powder (WMP). The upper limit of protein concentration in UF process is about 60% which is a function of total solid (TS) and viscosity of UF retentate and concentration polarization and fouling of UF membrane. Further

✉ Ganga Sahay Meena
gsmndri@gmail.com

¹ Dairy Technology Division, ICAR- National Dairy Research Institute, Karnal 132001, India

concentration of proteins are achieved by employing DF of UF retentate that facilitates a higher removal of soluble milk components into permeate.

Cow skim milk has been subjected to different temperatures (65–85 °C) and time durations (15 s–15 min) prior to its UF at either ≤ 10 °C or between 40 and 50 °C (Mao et al. 2012; Crowley et al. 2014; Patil et al. 2018; Meena et al. 2018; Udabage et al. 2012; Liu et al. 2014). Several changes have been reported to take place in casein micelles during UF process which include alteration in delicate salt equilibrium (Meena et al. 2017), dissolution of colloidal calcium phosphate (CCP) nanoclusters (Alexander et al. 2011), swelling of casein micelles due to movement of calcium from micelles and migration of caseins, particularly κ -casein from the micelle to the serum (McKenna 2000). Thereafter, aggregation of dissociated proteins during evaporation leads to migration of these protein to the air-droplet interface and their denaturation during spray drying (Bhaskar et al. 2003; Meena et al. 2018). Colloidal calcium also gets concentrated in retentate along with milk proteins during UF concentration of skim milk. Collectively, these changes may cause major problem of poor solubility in cow milk based MPC powders.

Market demand and uses of MPC powders are continuously rising in the form of protein rich dairy ingredients (Agarwal et al. 2015; Meena et al. 2017). According to Lagrange et al. (2015), the estimated production of MPC powders will be more than 40,000 MT by 2020. Most of the literature concerned with the production of MPC powders advocates the use of cow milk. However, information pertaining to the utilization of buffalo milk in MPC production is scarce. In countries like India where buffalo milk represents 53% of the annual milk production, manufacture of MPC powders using buffalo milk offers tremendous market opportunity (Patil et al. 2018). However, presence of higher calcium and casein contents in buffalo milk offers numerous challenges during its conversion into MPC powders. Schuck et al. (2013) reported that heating of skim milk can bind κ -casein with β -lactoglobulin (β -Lg) and results in improved heat stability of the casein micelle by increasing the electronegativity and decreasing the hydrophobicity, thus, leading to increased electrostatic repulsions.

Based on above, it was hypothesized that change in pH and applied heat treatments might reduce or prevent the undesired changes in casein micelles during UF and could improve the solubility and other functional properties of resultant MPC powder. Therefore, this study was undertaken to investigate the effect of change in pH, heat treatments and diafiltration on various properties of medium protein buffalo milk protein concentrate (MP-BMPC).

Materials and methods

Material

From the Cattle Yard of ICAR-National Dairy Research Institute, Karnal, India, fresh raw buffalo milk was collected, preheated (45 ± 1 °C) and subjected to cream separation in order to obtain buffalo skim milk (BSM). The total solids (TS), protein, fat, ash, calcium contents and pH value of this BSM were 109.7 g L^{-1} , 47.7 g L^{-1} , 1 g L^{-1} , 8.6 g L^{-1} , 1.6 g L^{-1} and 6.80, respectively. All chemicals used in the study were procured from Sigma-Aldrich (St. Louis, MO, US).

pH adjustment, heat treatments and ultrafiltration of BSM

The BSM samples of natural pH (6.8) and neutral pH (7.0, adjusted with 0.1 N NaOH) were heat treated at 74 ± 1 °C/15 s, 80 ± 1 °C, 85 ± 1 °C and 90 ± 1 °C for 5 min, respectively. Finally, these were concentrated up to $2.08 \times$ concentration factor to obtain UF retentates containing 0.60 ± 0.02 protein to TS ratio. Thus, total eight UF retentates were produced and referred as $2.08 \times$ UFR. Permeate flux means was calculated as: $FM = FF + 0.33 \times (IF - FF)$. The term IF, FF and FM denotes initial flux, final flux and flux means, respectively. Trials were conducted in the same UF plant adopting similar operational parameters as previously reported by Meena et al. (2018) and Patil et al. (2018).

Selection of UF retentate, its diafiltration and production of MP-BMPC powder

Changes in heat coagulation time (HCT) and ζ -potential of $2.08 \times$ UF retentates with change in pH and heat treatments were studied. Based on maximum ζ -potential and HCT, the most stable UF retentate was selected and diafiltered at 35 ± 1 °C to increase its protein content. In DF, reverse osmosis (RO) water was added in selected retentate and same quantity was removed as permeate. Retentate obtained after DF process was termed as DFR (protein to TS ratio = 0.70 ± 0.02) and spray dried adopting drying conditions reported by Patil et al. (2018) to obtain MP-BMPC powder that was packed and stored at 4 ± 1 °C until analysis. The flow chart of MP-BMPC powder production has been depicted in Fig. 1. It was manufactured ($n = 3$) and analyzed in triplicate ($n = 3 \times 3 = 9$).

Fig. 1 Production of MP-BMPC powder from buffalo milk

Methods

Compositional analysis and determination of physical, reconstitution and functional properties

TS, protein, fat, and ash contents, pH, HCT and ζ -potential values of BSM, $2.08 \times \text{UFR}$, DFR and reconstituted solution or MP-BMPC powder were measured/determined using official methods as previously reported by Meena et al. (2016, 2017, 2018) and Patil et al. (2018). Lactose contents were calculated by the subtraction of other constituents from TS. Calcium contents were analysed in atomic absorption spectrophotometer (AAS, Model No. AA-7000, Shimadzu, Kyoto, Japan) while pH and ζ -potential of BSM, $2.08 \times \text{UFR}$, DFR and reconstituted MP-BMPC powder solution were measured using a Eutech pH meter (Cyberscan 1100, Thermo Scientific) and Zetasizer Nano-ZS90 (Malvern Instrument Ltd., Malvern, Worcestershire, UK) at $20 \pm 1^\circ\text{C}$ and $25 \pm 1^\circ\text{C}$, respectively. For ζ -potential measurement, BSM and retentate samples were diluted with Mili-Q water in 1:100 ratio, while reconstituted MP-BMPC sample was diluted in 1:1000 ratio.

Physical properties such as interstitial air content (IAC), occluded air contents (OAC) particle density (PD) and porosity, loose bulk density (LBD); packed bulk density (PBD); flowability measured as angle of repose (θ°),

wettability (s); dispersibility; specific surface area (SSA); particle size distribution (d_{10} , d_{50} and d_{90}); volume mean diameter ($D_{3,2}$ and $D_{4,3}$); dispersion index; color values (L^* , a^* and b^*) and water activity (a_w) of MP-BMPC powder were determined using official methods as reported by Patil et al. (2018). Hydroxymethylfurfural of MP-BMPC was determined by the method of Keeney and Bassette (1959). The Carr index (CI) and Hausner ratio (HR) were calculated using following equations.

$$CI (\%) = \frac{PBD - LBD}{LBD} \times 100$$

$$HR = \frac{PBD}{LBD}$$

Functional properties (solubility, foaming capacity and foaming stability, emulsification activity index and emulsion stability; water and oil binding capacity (WBC, OBC) and buffer index (dB/dpH) of MP-BMPC powder were determined using official methods as previously reported by other researchers (Meena et al. 2017, 2018; Patil et al. 2018).

HCT (min) of BSM, $2.08 \times \text{UFR}$, DFR and reconstituted MP-BMPC solution was measured using an oil bath maintained at 140°C till 90 min or till the sample went coagulated (whichever was earlier). Method reported by Crowley et al. (2014) was used to reconstitute MP-BMPC powder.

Measurement of rheological properties

Apparent viscosity (50 s^{-1} , 20°C) and flow behaviour (1 to 1000 s^{-1}) of UF/DF retentates and 10% w/v solution of MP-BMPC were measured as per the method reported by Meena et al. (2017). The rheological data obtained were fitted to Bingham, Herschel-Bulkley and Power law rheological models. The equations for these models commonly reported in the literature are:

Bingham model : $\sigma = \sigma_B + \eta\dot{\gamma}$

Herschel – Bulkley model : $\sigma = \sigma_o + K(\dot{\gamma})^n$

Power Law model : $\sigma = K(\dot{\gamma})^n$

where, σ is the shear stress (Pa), σ_o is the yield stress, $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index ($\text{Pa} \cdot \text{s}^n$) and n is the flow behaviour index. The temperature sweep of DFR was performed in the range of 20 – 90°C at 1°C per min using a MCR 52 rheometer (Anton Paar, Germany) coupled to a cone and plate CP75-1° (SS) probe. The measurements were performed at a constant oscillation frequency of 1.592 Hz and 0.01% shear strain, respectively.

FTIR spectra acquisition

MP-BMPC powder was placed in direct contact with Diamond crystal cell Attenuated Total Reflectance crystal of Shimadzu IRAffinity-1. The absorption spectra of the MP-BMPC powder were acquired at resolution of 4 cm^{-1} and scan speed of 0.2 cm s^{-1} in the wavenumber range of 4000 – 500 cm^{-1} as per the method described by Patil et al. (2018).

Statistical analysis

Data ($n = 3$) were subjected to one-way analysis of variance (ANOVA) using SAS Enterprise guide (5.1, 2012), SAS Institute Inc., North Carolina, USA (SAS 2008). For the comparison of two samples mean values, unpaired two samples t test was used. Tukey's Studentized Range (HSD) test was used to compare mean values.

Results and discussion

Concentration of BSM, selection of UF retentate and its diafiltration

During $2.08 \times$ UF concentration of BSM, the calculated means of permeate flux was 20.99 LMH . TS, protein, fat, ash and calcium contents of $2.08 \times$ UFR were significantly increased ($P < 0.05$) than that of BSM (Table 1) due to

selective retention of these constituents by UF membrane. Such increase in TS, protein, fat, ash and calcium contents and change in flux during UF of BSM up to $2.08 \times$ were in agreement with the previous reports (Patil et al. 2018; Patel and Mistry 1997; Khatkar et al. 2014).

All eight $2.08 \times$ UFR samples produced from BSM of either 6.8 or 7 pH and heat treated at 74°C for 15 s , 80°C , 85°C and 90°C for 5 min were compared based on their ζ -potential and HCT values. The $2.08 \times$ UFR samples obtained from BSM (pH 6.8 or 7) and heat treated at 85°C for 5 min exhibited maximum ζ -potential and HCT values. Moreover, no-significant differences were observed in ζ -potential and HCT values of these two $2.08 \times$ UFR samples as $P > |t|$ (0.1644) value was higher than α (0.05) value (data not shown). Therefore, this $2.08 \times$ UFR sample was selected and subjected to diafiltration.

DF significantly increased ($P < 0.05$) the protein content and ζ -potential, but decreased lactose and calcium contents on dry matter basis in DFR than $2.08 \times$ UFR (Table 1). The increased protein content of DFR was attributed to dilution of $2.08 \times$ UFR during DF that reduced its viscosity and enabled higher permeation of lactose and salts into permeate. The DFR thus obtained had 0.68 protein to TS ratio (Table 1) and was spray dried to obtain MP-BMPC powder.

Chemical composition of MP-BMPC powder

The chemical composition of MP-BMPC powder had been shown in Table 1. The percent TS, protein, lactose, ash, fat and calcium contents of BM-MPC60 powder (Patil et al. 2018) were 98.12 , 58.47 , 27.49 , 8.24 , 3.93 and 2.63 while percent of these constituents in cow milk based CM-MPC70 powder were 95.58 , 70.05 , 15.59 , 2.04 , 7.90 and 2.66 , respectively. The presence of lower fat in BSM resulted in lower fat contents in MP-BMPC powder compared to the fat contents of BM-MPC60 and CM-MPC70 powders. However, its other constituents such as protein and ash contents in accordance with the values of CM-MPC70 powders as reported previously (Crowley et al. 2014; Huppertz and Gazi 2015; Meena et al. 2018).

Physical and reconstitution properties of MP-BMPC powder

Quality of MPC powders depends on their physicochemical, reconstitution, functional and rheological properties. These properties are function of feed composition and processing parameters. An increase in feed viscosity results in larger particle size, decreased porosity, higher densities and moisture content in final powder (Amaladhas and Emerald 2017). Different physical and reconstitution

Table 1 Chemical composition^d, pH, HCT, ζ -potential and G' & G'' cross over values of BSM, UF, DF retentates and MP-BMPC powder

Samples	TS (g 100 mL ⁻¹)	Protein	Lactose	Ash	Fat	Ca	pH –	HCT min	ζ -potential – mV	Cross over of G' & G'' °C
BSM	10.97 ^c ± 0.01	4.77 ^c ± 0.01 (43.48)	5.24 ^c ± 0.01 (47.77)	0.86 ^c ± 0.00 (7.83)	0.10 ^c ± 0.00 (0.91)	0.16 ^c ± 0.05 (1.46)	6.80 ^b ± 0.01	58.14 ^b ± 2.47	21.83 ^a ± 0.81	Absent
2.08 × UFR	15.92 ^b ± 0.01	9.95 ^b ± 0.03 (62.50)	4.50 ^b ± 0.04 (28.30)	1.27 ^b ± 0.01 (7.97)	0.20 ^b ± 0.0 (1.26)	0.44 ^b ± 0.07 (2.76)	6.78 ^c ± 0.03	24.68 ^c ± 1.16	17.13 ^c ± 0.55	53.40 ± 0.50
DFR	15.52 ^d ± 0.04	10.61 ^c ± 0.04 (68.36)	3.47 ^d ± 0.02 (22.35)	1.24 ^c ± 0.01 (7.99)	0.20 ^c ± 0.07 (1.29)	0.36 ^c ± 0.01 (2.32)	6.78 ^c ± 0.05	25.93 ^c ± 0.21	19.30 ^b ± 0.74	55.10 ± 0.45
MP-BMPC	96.98 ^b ± 0.05	66.30 ^a ± 0.60 (68.36)	21.68 ^b ± 0.03 (22.36)	7.75 ^b ± 0.01 (7.99)	1.25 ^a ± 0.02 (1.29)	2.25 ^b ± 0.03 (2.32)	7.15 ^a ± 0.05	90.00 ^a ± 0.00	ND	ND

^{abc}are significantly different at ($p < 0.05$) with each other row wise

^dMean ± S. E. (n = 3)

TS total solids, Ca calcium, HCT heat coagulation time, BSM buffalo skim milk, 2.08 × UFR 2.08 folds ultrafiltered retentate, DFR diafiltered retentate, MP-BMPC medium protein buffalo milk protein concentrate, ND not determined

Values given in parenthesis are mean values on dry matter basis

properties of MP-BMPC powder has been shown in Table 2.

IAC and OAC values of MP-BMPC powder were shown in Table 2. These were markedly lower and higher than IAC (173.7 ± 2.98 mL 100 g⁻¹) and OAC (98.08 ± 2.23 mL 100 g⁻¹) values of BM-MPC60 (Patil et al. 2018) while noticeably higher than the values reported by Crowley et al. (2014) for CM-MPC70 powder. Different IAC and OAC values of MP-BMPC and BM-MPC60 powders might attributed to the heat treatment of BSM and composition of UF and DF retentates from which these powders were produced. The lower IAC value of MP-BMPC than BM-MPC60 was mainly attributed to its narrow particle size distribution (Table 2). Feed composition, aeration of feed, whipping before or during atomization, ability of feed to form stable emulsion and system opted for spray drying of retentate/concentrate (Meena et al. 2018) favours higher OAC contents in powders. The higher OAC value of MP-BMPC than BM-MPC60 was mainly attributed lower TS and fat contents of the feed/retentate as retentate with lower TS and fat generally foams more and favours higher OAC content in powders. OAC of MP-BMPC was supposed to decrease due to denaturation of milk proteins present in BSM as it was high heat treated (85 °C for 5 min), but opposite to this, other factors such as lowers TS, fat and viscosity of retentate might have increased its OAC value.

The LBD value (Table 2) of MP-BMPC powder was slightly higher than that of (0.18 mL 100 g⁻¹) BM-MPC60

(Patil et al. 2018) but, noticeably lower than 0.30 mL 100 g⁻¹ LBD value of CM-MPC70 (Meena et al. 2018). MP-BMPC and BM-MPC60 powders were manufactured from retentates containing lower TS contents (~ 15.52 and 18.63%) which have lower LBD values than SMP and CM-MPC60 powders, produced from 48–50% and 20–25% TS containing concentrated milk and UF retentates. Thus, lower TS containing feed resulted in lower LBD values in MP-BMPC and BM-MPC60 powders owing to their higher IAC and OAC and vice versa. High heat treatment of BSM and presence of lesser fat content in MP-BMPC (Table 1) could be attributed to its slightly higher LBD values than BM-MPC60 as severity of preheat treatment increases bulk density of milk powders. Other factors such as particle shape, size, surface characteristics, particle size distribution, SSA, IAC, OAC, fat and lactose contents, preheat treatment given to milk, feed viscosity and method of atomization (Schuck 2013) also influences LBD and collectively these could also be responsible for its lower LBD values.

Similarly, PBD value (as shown in Table 2) of MP-BMPC powder was slightly higher than (0.29 mL 100 g⁻¹) BM-MPC60 (Patil et al. 2018), but lower than reported PBD values in the range of 0.41–0.49 for CM-MPC60 and CM-MPC70 powders (Crowley et al. 2014; Meena et al. 2017, 2018). This is attributed to lower TS (lower viscosity and higher IAC and OAC values) of retentate from which MP-BMPC powder was manufactured. The mass of single particle per unit volume is known as PD that influenced by

Table 2 Physical, reconstitution and functional properties of MP-BMPC powder

Physical and reconstitution properties		MP-BMPC powder
Air contents (mL 100 g ⁻¹ powder)	Interstitial air	145.97 ± 1.24
	Occluded air	112.92 ± 0.51
Densities (g mL ⁻¹)	Loose bulk density (LBD)	0.21 ± 0.55
	Packed bulk density (PDB)	0.30 ± 0.84
	Particle density (PD)	0.55 ± 0.47
		65.09 ± 0.24
Porosity (%)		
Flowability	Angle of repose, θ°	43.19 ± 0.62
	Compressibility index (%)	37.00 ± 0.01
	Hausner ratio	1.58 ± 0.05
Wettability (s)		25.67 ± 1.20
Dispersability (%)		48.65 ± 0.47
Specific surface area (SSA, m ² kg ⁻¹)		97.93 ± 1.34
Particle size distribution (μm)	d ₁₀	34.32 ± 1.88
	d ₅₀	104.42 ± 2.75
	d ₉₀	218.58 ± 1.50
		61.27 ± 3.58
Average particle size/means	D _{3,2}	
	D _{4,3}	117.99 ± 1.45
Dispersion index (Span, %)		1.77 ± 0.02
Color values	L*	91.13 ± 0.07
	a*	− 3.21 ± 0.15
	b*	12.46 ± 0.04
Water activity (a _w)		0.20 ± 0.01
Hydroxymethylfurfural (HMF) μmol/L		583.94 ± 0.30
<i>Functional properties</i>		
Solubility (%)		82.50 ± 0.27
Heat coagulation time (min)		90.00 ± 0.00
Viscosity (mPa s)		21.30 ± 2.26
Water binding capacity (g per g protein)		4.82 ± 0.02
Oil binding capacity (g per g protein)		4.52 ± 0.08
Emulsification activity index (EAI, m ² g ⁻¹)		1.67 ± 0.01
Emulsion stability (ES, min)		840.00 ± 2.89
Foaming capacity (%)		35.88 ± 0.47
Foaming stability (%)		7.38 ± 0.84
Mean values ± S.E. (n = 9)		

feed viscosity, density of individual feed constituents and presence of OAC. The PD (0.55 g mL⁻¹) of MP-BMPC was lower compared to 1.09 g mL⁻¹ and 0.61 g mL⁻¹ PD values of CM-MPC70 (Crowley et al. 2014) and BM-MPC60 (Patil et al. 2018) because of its higher OAC value and lower TS of DF retentate as both air inclusion and lower TS favours decrease in PD. It is well established that increase in TS of feed results in denser powders as powder porosity decreases with increased TS and vice versa. Porosity indicates the void spaces present within powder. The porosity (Table 2) of MP-BMPC was lower (due to lower TS content of DF retentate) than the 70.62%, and 76.31% porosities of BM-MPC60 (Patil et al. 2018) and dairy whitener (Khatkar and Gupta 2012). Amaladhas and Emerald (2017) reported that increase in initial viscosity of

feed either by preheat treatment or by increasing the TS, will lead to larger particle size, increased densities and moisture content, and decreased porosity in the milk powder produced. This can easily explain the lower PD and porosity values of MP-BMPC than BM-MPC60, CM-MPC70 and dairy whiteners.

Flowability is measured in terms of the angle of repose (θ°), CI and HR ratio. The θ°, CI and HR ratio of MP-BMPC powder have been shown in Table 2. This powder exhibited fair to passable flow (θ = 38–45°) as per Carr (1965) classification. Moreover, its flow characteristics were in agreement with that of BM-MPC60 (θ = 37.35 ± 1.10°) and CM-MPC70 (35.44 ± 0.03°) powders. However, MP-BMPC powder showed better flowability than CM-MPC70 as indicated by its CI =

50.5% values (Crowley et al. 2014). Flowability of powders have direct relation with their particle size and SSA (Schuck 2013) and presence of larger particle size improves flowability of powders while presence of smaller powder particles increases SSA that favours greater cohesive forces between particles and ultimately decreases powder flowability. As per Crowley et al. (2014), SSA values of CM-MPC70 powder were $380 \text{ m}^2 \text{ kg}^{-1}$, which was markedly higher than SSA value (Table 2) of MP-BMPC powder and this could easily explain its better flowability.

Wettability of MPC powders depends on density, particle size, surface area, surface charge, porosity, presence of moisture absorbing substances and hydrophilic (lactose), hydrophobic (fat) and amphiphilic (protein) character of the powder constituents. Wettability of MP-BMPC powder was markedly lower than 2.52 min and 70.2 s wettability values of CM-MPC70 (Meena et al. 2018) and BM-MPC60 powders (Patil et al. 2018), respectively. The wetting time of MP-BMPC powder was observed to be shorter than BM-MPC60 and CM-MPC70 powders which might be attributed its lower fat content (Table 1) content and lower IAC value (Table 2). The presence of higher protein load on particle surface was responsible for higher (more than 120 s) wetting index of MPC powders (Bouvier et al. 2013). As per Schuck (2013), the wettability of granulated and non-granulated high protein powders such as micellar casein and whey protein powders were 1, 3, 4 and 17 min, respectively and these values were markedly higher compared to wettability of MP-BMPC.

The percentage of the milk solids that get dissolved in water upon gentle mixing is known as dispersibility (Meena et al. 2018) and acts as a rate controlling step during reconstitution process of milk powders. The dispersibility (Table 2) of MP-BMPC powder was higher than $40.16 \pm 0.01\%$ dispersibility of CM-MPC70 powder (Meena et al. 2018) and might be attributed to its lower d_{90} values (Table 2). Dispersibility of powders have been reported to decrease with increase in the percentage of ($< 90 \mu\text{m}$) fine particles (Singh and Newstead 1992). Moreover, applied heat treatment leads to interaction of k -casein and whey proteins that prevented its interaction with other casein fractions responsible poor dispersibility and solubility of MPC powders. The dispersibility values of micellar casein isolates (MCI), milk protein isolate (MPI) and spray dried MPC powders have been reported to exhibit lower dispersibility index that ranges from 38 to 100% (Bouvier et al. 2013; Schuck 2013; Huppertz and Gazi 2015). Moreover, due to high protein content, whey and casein powders have inferior dispersibility than other milk powders (Ishwarya and Anandharamakrishnan 2017).

The SSA, d_{10} , d_{50} , d_{90} , D_{32} , D_{43} , span of MP-BMPC have been shown in Table 2 while that of CM-MPC70

powders were $66.77 \pm 0.24 \text{ m}^2 \text{ kg}^{-1}$; 52.42 ± 0.28 , 114.95 ± 0.07 , $225.51 \pm 0.23 \mu\text{m}$; 90.50 ± 0.14 , $128.48 \pm 0.24 \mu\text{m}$; and $1.51 \pm 0.01\%$ (Meena et al. 2018). The SSA and span values of MP-BMPC powder were higher while its d_{10} , d_{50} , d_{90} , D_{32} and D_{43} values were lower compared to CM-MPC70. According to Crowley et al. (2014), SSA and d_{10} , d_{50} , and d_{90} values of CM-MPC70 were $380 \text{ m}^2 \text{ kg}^{-1}$ and 14.9, 39.6, 72.1 μm , while the values of SSA; d_{10} , d_{50} , d_{90} ; D_{32} , D_{43} and span of BM-MPC60 (Patil et al. 2018) were $163.5 \pm 1.63 \text{ m}^2 \text{ kg}^{-1}$; $19.04 \pm 0.83 \mu\text{m}$, $74.34 \pm 0.71 \mu\text{m}$, $164.81 \pm 0.94 \mu\text{m}$; 36.69 ± 1.19 , $84.43 \pm 0.91 \mu\text{m}$ and $1.96 \pm 0.08\%$, respectively. This observed difference in SSA, particle size and mean values of MP-BMPC, BM-MPC60 and CM-MPC70 powders were attributed to the difference in TS contents of the UF/DF retentates.

Water activity plays vital role in decrease in solubility of MPC powders during their storage. The a_w of MP-BMPC powder (Table 2) was in agreement with that of BM-MPC60 (0.27) manufactured by Patil et al. (2018), but lower than a_w (0.25) of CM-MPC70 powder (Meena et al. 2018). The HMF content of BM-BMPC powder was $583.94 \pm 0.30 \mu\text{mol/L}$ (Table 2).

The lightness (L^*), redness (a^*) and yellowness (b^*) values of MP-BMPC powder are shown in Table 2 while these values of CM-MPC70 and BM-MPC60 powders were 88.92 ± 0.02 , -1.21 ± 0.02 , 11.99 ± 0.01 and 90.45 ± 0.01 , -3.28 ± 0.01 , 13.35 ± 0.01 , respectively (Meena et al. 2018; Patil et al. 2018). BM-MPC60 powder was manufactured from pasteurized milk while MP-BMPC powder was manufactured from skim milk hat treated at $85 \pm 1 \text{ }^\circ\text{C}/5 \text{ min}$ that can easily explain the existing difference in their L^* , a^* , b^* values. Different chemical composition of retentates/concentrates, severity of applied heat treatment, dryer type, stages and drying conditions employed during spray could easily explain the difference in color values of MP-BMPC and other powders. Generally, SMP have greyish-white color compared to yellowish-white color of high protein powders.

Functional properties of MP-BMPC powder

MPC powders delivers desired textural, functional and nutritional attributes to different food formulations owing to their unique whey protein to casein ratio. Functional properties of MP-BMPC powder are shown in Table 2. Solubility acts as prerequisite for the better expression of other functional properties of MPC powders. Solubility of MP-BMPC was $82.50 \pm 0.27\%$ (Table 2) and that was markedly higher compared to about 63.17% solubility of BM-MPC60 powder (Patil et al. 2018) and 71% solubility of CM-MPC70 (Meena et al. 2017). Different chemical composition of cow and buffalo skim milks is mainly

responsible for the difference in solubility values of MP-BMPC, BM-MPC60 and CM-MPC70 powders. Moreover, the calcium contents of these powders were 2.25, 2.63 and 2.66% respectively. Thus, presence of higher calcium in BM-MPC60 and CM-MPC70 powders was the major cause of their poor solubility as it favours severe casein–casein interactions which negatively affect their solubility. The dissolution of colloidal calcium phosphate (CCP) nanoclusters (Alexander et al. 2011), swelling of casein micelles and migration of κ -casein from the micelle into the serum (McKenna 2000) during UF/DF along with aggregation of dissociated proteins and their migration to the air-droplet interface followed by their denaturation in spray drying (Bhaskar et al. 2003; Meena et al. 2018) may be collectively responsible for the poor solubility and aggregation/clustering in (Shilpashree et al. 2015; Patil et al. 2018). High heat treatment of BSM ($85 \pm 1^\circ\text{C}/5$ min) enabled bonding of κ -casein with β -lactoglobulin (β -Lg) and might have improved stability of the casein micelle by increasing the electronegativity and decreasing the hydrophobicity. Thus, increased electrostatic repulsions might have reduced migration and aggregation of dissociated proteins at air-droplet interface and could have resulted in better solubility in SBM-MPC60 than BM-MPC60.

MP-BMPC powder solution was noticeably heat stable (Table 2) and its HCT was identical with that of BM-MPC60, but markedly higher than HCT (< 5 min) of CM-MPC70 (Meena et al. 2018) and other cow milk based MPC (55–85% protein) powders having their HCT values in the range of 0–40 min (Huppertz and Gazi 2015). This marked improvement in heat stability of MP-BMPC powder might attributed to heat treatment of skim milk at $85 \pm 1^\circ\text{C}/5$ min and its higher pH (7.15) as calcium ion activity decreases with an increase in pH and vice versa (Crowley et al. 2014) while HCT increases with decrease in calcium ion activity.

The viscosity of MP-BMPC powder (Table 2) was slightly higher than viscosity of BM-MPC60 (20.30 ± 0.05 mPa s) but lower than viscosities of CM-MPC60 (~ 33.50 mPa s) and CM-MPC70 (~ 27 mPa s) powders, respectively (Meena et al. 2017, 2018) in identical test conditions. The BM-MPC60 and MP-BMPC powders solutions exhibited lower viscosities compared to CM-MPC60 and CM-MPC70 powder solutions. The d_{10} values of these powders were 34.32, 19.04, 38.82 and 52.42 μm , respectively. This can easily explain the existing variation in their viscosity values as higher number of smaller powder particles have stronger particle–particle interactions and enhance the viscosity (Meena et al. 2017). Moreover, treatment of skim milk at $85 \pm 1^\circ\text{C}/5$ min from which MP-BMPC was produced also advocates its

higher viscosity than BM-MPC60 that was produced from only pasteurized skim milk.

Water binding of proteins is influenced by different factors such as protein concentration, charge, pH, temperature, ionic strength, physicochemical and surface properties of casein, presence of other hydrophilic and hydrophobic constituents in food such as polysaccharides, lipids and salts, rate and length of heat treatment (Zayas 1997). WBC and OBC values of BM-MPC powder are shown in Table 2. The WBC and OBC values of BM-MPC60 and CM-MPC60 powders were 5.49 ± 0.05 , 5.18 ± 0.05 and 5.22 , 3.38 g g^{-1} protein, respectively while these were lesser than 3.5 g g^{-1} protein for CM-MPC70 powder (Patil et al. 2018; Meena et al. 2017, 2018). The lower WBC values of MP-BMPC than BM-MPC60 might be attributed to its lower porosity and higher bulk density as well as in presence of more undenatured whey proteins in BM-MPC60 that possess a higher water binding power. As per Zayas (1997), powders having smaller particle size absorb and entrap more oil and this could easily explain the poor OBC values of MP-BMPC than BM-MPC60 because of its higher SSA values (more small particles) as shown in Table 2.

Different factors such as ionic strength, particle size, protein, calcium concentration and pH of powders affects their emulsifying capacity (Mao et al. 2012). The EAI (Table 2) of MP-BMPC powder was higher than $1.65 \pm 0.01 \text{ m}^2 \text{ g}^{-1}$ EAI of BM-MPC60 powder. This can be easily explained by higher SSA values (Table 2) of SBM-MPC60, also advocated by $D_{3,2}$ value (Table 2) as smaller the size, the better is the protein as an emulsifying agent (Ye 2011). Moreover, limited denaturation of whey proteins also favours their emulsifying properties in food systems. MPC and calcium caseinate are aggregated powders which possesses much lower emulsifying ability than whey protein and sodium caseinate, needs higher concentration to form stable emulsion (Singh 2011; Ye 2011). The ability to resist any alteration in emulsion properties and structure over the studied time period is known as emulsion stability. ES of proteins depends on the type and concentrations, viscosity, pH and temperatures of continuous and dispersed phases, applied heat treatment, high pressure and conditions of homogenization. The ES (Table 2) of MP-BMPC was slightly higher than ES (775.32 ± 1.92 min) of BM-MPC60 (Patil et al. 2018).

The FC and FS properties majorly depend on level of protein denaturation, pre heat treatment, pH, surface activity of proteins and influence of ionic environment and have importance during selection of high protein ingredients for their end use in mousse, ice-cream, whipped topping, frozen desserts, meringues, bakery and certain confectionary products and even in espresso coffee (Meena et al. 2017). Percent FC and FS of MP-BMPC as shown in

Table 2 were lower than that of BM-MPC60 (39.81 ± 0.22 , 19.51 ± 1.40), CM-MPC60 (82.74 ± 0.03 , 55.86 ± 0.01) and CM-MPC70 (~ 31.70 and ~ 30.50) powders (Patil et al. 2018; Meena et al. 2017, 2018). Protein solubility had significant contribution in foaming behavior (Zayas 1997). Foaming of milk proteins has been known to decrease with increased denaturation of whey proteins. MP-BMPC and SBM-MPC60 powders were manufactured from the skim milks treated at $72^\circ\text{C}/15\text{ s}$ and $85 \pm 1^\circ\text{C}/5\text{ min}$, respectively. Thus, denaturation of whey proteins was higher in MP-BMPC powder, so its FC and FS were lower than that of BM-MPC60.

Buffering capacity of MP-BMPC powder

The pH of reconstituted MP-BMPC solution varied in 2–10 pH range with 0.5 unit increments. The pH values were changed with the addition of acid and base and relative changes in its dB/dpH values are shown in Fig. 2. The dB/dpH values of reconstituted MP-BMPC solution were ranged between 0.0018 and 0.0396 which increased with increase in solution pH from acidic side to alkaline side. These values are in good agreement with previously reported values of buffer index values of BM-MPC60 (Patil et al. 2018).

Rheological properties of SBM-MPC60 powder solution and data modeling

The crossover temperature of G' and G'' was about 57.16°C and 55.10°C for selected $2.08 \times \text{UFR}$ and DFR samples (Table 1). For both retentates, a gradual decrease was observed in G' and G'' values between 20 and 56°C , indicating a solid-like behaviour. Till 55°C , the elastic component G' values were lower than the values of G'' , but

thereafter values G' as well as viscous component G'' were increased. However, values of elastic component G' were higher compared to G'' values in 57 – 90°C temperature range. Modelling of rheology data revealed that Herschel-Bulkley model best explained the rheological behaviour of DFR and reconstituted solutions of MP-BMPC. The coefficient of determination (R^2) of Herschel-Bulkley model for DFR and MP-BMPC solution were 0.967 and 0.987, respectively (Table 3). The production of MP-BMPC from high heat treated skim milk ($85 \pm 1^\circ\text{C}/5\text{ min}$) led to higher protein denaturation in $2.08 \times \text{UFR}$ and DFR. This higher protein denaturation can easily explain higher viscosity of MP-BMPC than BM-MPC60 and CM-MPC70 powders. Further, smaller particle size (Table 2), higher SSA and pH (in alkaline side) of MP-BMPC that usually decreases the calcium ion activity could easily explain the reduction in its viscosity and yield stress compared to CM-MPC70 (Fig. 2).

FTIR spectral acquisition of MP-BMPC powder

The manufactured MP-BMPC powder had 66.30% protein content. The FTIR spectra of this powder (Fig. 3) showed a similar trend as has been previously reported by other researchers (Kher et al. 2007; Patil et al. 2018). The amide I and amide II regions were best described in 1700 – 1400 cm^{-1} wavenumber range, while amide III regions were described in 1350 – 1200 cm^{-1} range. The protein content of the prepared powder was higher than that reported by Patil et al. (2018) and the same was confirmed on the basis of higher absorbance obtained by FTIR spectra in different regions responsible for absorption by amides.

Conclusion

This study envisaged the effect of pH, heat treatments and diafiltration of buffalo skim milk on properties of MP-BMPC powder. UF retentate with maximum ζ -potential and heat stability was selected, diafiltered and spray dried to obtain MP-BMPC60 powder. Heat treatment ($85 \pm 1^\circ\text{C}/5\text{ min}$) of BSM induced interactions between κ -casein and β -Lg which increased electrostatic repulsions within casein micelles and improved their heat stability. This might also have decreased their aggregation and denaturation during spray drying. Applied DF decreased total calcium contents in MP-BMPC powder. All these changes collectively improved the solubility, wettability, viscosity and emulsion stability of MP-BMPC0 compared to BM-MPC60 powder. Herschel Bulkley model explained the rheological behaviour of ultrafiltered, diafiltered retentates and reconstituted MP-BMPC solutions. This investigation has established that applied interventions

Fig. 2 Buffer index of acidification-alkalization with 0.1 N (i.e. $1.0 \times 10^{-4}\text{ g equivalent per m}^3$) HCl and 0.1 N (i.e. $1.0 \times 10^{-4}\text{ g equivalent per m}^3$) NaOH solution of MP-BMPC powder (0.5% protein solution)

Table 3 Rheological properties of diafiltered rete MP-BMPC powder

Samples	Bingham			Herschel Bulkley				Power law		
	σ_0 (Pa)	η (mPa.s)	R^2	σ_0 (Pa)	K (Pa.s)	η (mPa.s)	R^2	k	n	R^2
SDFR	0.710	72.66	0.955	0.521	0.05	0.593	0.967	0.372	0.281	0.957
MP-BMPC	1.004	10.76	0.974	0.830	0.049	0.687	0.987	0.544	0.274	0.977

Where, σ is the shear stress, σ_0 -yield stress, η -viscosity, k -consistency index, n flow behaviour index and R^2 is coefficient of determination

Fig. 3 Spectra of MP-BMPC powder in the wavenumber range 500–4000 cm^{-1}

markedly improved functional properties of MP-BMPC powder. Hence, MP-BMPC powder can be used as a high protein ingredient in several food formulations to improve their textural and nutritional properties.

Acknowledgements The first author is thankful to the Director of National Dairy Research Institute, Karnal, India, for providing financial assistance in the form of Institutional Fellowship for carrying out this research work.

References

- Agarwal S, Beausire RL, Patel S, Patel H (2015) Innovative uses of milk protein concentrates in product development. *J Food Sci* 80(S1):A23–A29
- Alexander M, Nieh MP, Ferrer MA, Corredig M (2011) Changes in the calcium cluster distribution of ultrafiltered and diafiltered fresh skim milk as observed by small angle neutron scattering. *J Dairy Res* 78:349–356
- Amaladhas PH, Emerald FME (2017) Physicochemical and Sensory properties of dried dairy products. In: *Handbook of drying for dairy products*, pp 203–228
- Bhaskar GV, Singh H, Blazey ND (2003) Milk protein products and processes. US Patent US 2003/0096036
- Bouvier JM, Collado M, Gardiner D, Scott M, Schuck P (2013) Physical and rehydration properties of milk protein concentrates: comparison of spray-dried and extrusion-porosified powders. *Dairy Sci Technol* 93(4–5):387–399
- Carr RL (1965) Evaluating flow properties of solids. *Chem Eng* 72(2):163–169
- Crowley SV, Gazi I, Kelly AL, Huppertz T, O'Mahony JA (2014) Influence of protein concentration on the physical characteristics and flow properties of milk protein concentrate powders. *J Food Eng* 135:31–38
- Huppertz T, Gazi I (2015) Milk protein concentrate functionality through optimized product-process interactions. *New Food* 18(1):12–17
- Ishwarya SP, Anandharamakrishnan C (2017) Spray drying. In: *Handbook of drying for dairy products*, p. 56
- Keeney M, Bassette R (1959) Detection of intermediate compounds in the early stages of browning reaction in milk products I. *J Dairy Sci* 42(6):945–960
- Khatkar SK, Gupta VK (2012) Physicochemical and functional quality attributes of dairy whitener prepared from ultrafiltration process. *J Food Process Pres* 38:1145–1154
- Khatkar SK, Gupta VK, Khatkar AB (2014) Studies on preparation of medium fat liquid dairy whitener from buffalo milk employing ultrafiltration process. *J Food Sci Technol* 51(9):1956–1964
- Kher A, Udabage P, McKinnon I, McNaughton D, Augustin MA (2007) FTIR investigation of spray-dried milk protein concentrate powders. *Vib Spectrosc* 44(2):375–381
- Lagrange V, Whitsett D, Burris C (2015) Global market for dairy proteins. *J Food Sci* 8(S1):A16–A22
- Liu DZ, Weeks MG, Dunstan DE, Martin GJ (2014) Alterations to the composition of casein micelles and retentate serum during ultrafiltration of skim milk at 10 and 40 °C. *Int Dairy J* 35(1):63–69
- Mao XY, Tong PS, Gualco S, Vink S (2012) Effect of NaCl addition during diafiltration on the solubility, hydrophobicity, and disulfide bonds of 80% milk protein concentrate powder. *J Dairy Sci* 95:3481–3488
- McKenna AB (2000) Effects of processing and storage on the reconstitution properties of whole milk and ultrafiltered skim

- milk powders. Ph.D. thesis, Massey University, Palmerston North, New Zealand
- Meena GS, Singh AK, Borad S, Raju PN (2016) Effect of concentration, homogenization and stabilizing salts on heat stability and rheological properties of cow skim milk ultrafiltered retentate. *J Food Sci Technol* 53(11):3960–3968
- Meena GS, Singh AK, Arora S, Borad S, Sharma R, Gupta VK (2017) Physico-chemical, functional and rheological properties of milk protein concentrate 60 as affected by disodium phosphate addition, diafiltration and homogenization. *J Food Sci Technol* 54(6):1678–1688
- Meena GS, Singh AK, Gupta VK, Borad S, Arora S, Tomar SK (2018) Effect of pH adjustment, homogenization and diafiltration on physicochemical, reconstitution, functional and rheological properties of medium protein milk protein concentrates (MPC70). *J Food Sci Technol* 55(4):1376–1386
- Patel RS, Mistry VV (1997) Physicochemical and structural properties of ultrafiltered buffalo milk and milk powder. *J Dairy Sci* 80:812–817
- Patil AT, Meena GS, Upadhyay N, Kheta Y, Borad S, Singh AK (2018) Production and characterization of milk protein concentrates 60 (MPC60) from buffalo milk. *LWT-Food Sci Technol* 91:368–374
- Schuck P (2013) Dairy protein powders. In: Smithers GW, Augustin MA (eds) *Advances in dairy ingredients*. Wiley and Institute of Food Technologists, Hoboken, pp 1–29
- Schuck P, Le Floch-Fouere C, Jeantet R (2013) Changes in functional properties of milk protein powders: effects of vacuum concentration and drying. *Dry Technol* 31(13–14):1578–1591
- Shilpashree BG, Arora S, Chawla P, Tomar SK (2015) Effect of succinylation on physicochemical and functional properties of milk protein concentrate. *Food Res Int* 72:223–230
- Sikand V, Tong PS, Roy S, Saona LER, Murray BA (2011) Solubility of commercial milk protein concentrates and milk protein isolates. *J Dairy Sci* 94:6194–6202
- Singh H (2011) Functional properties of milk proteins. In: Fuquay JW, Fuquay JW, Fox PF, McSweeney PLH (eds) *Encyclopaedia of dairy science*, vol 3, 2nd edn. Academic Press, San Diego, pp 887–893
- Singh H, Newstead DF (1992) Aspects of proteins in milk powder manufacture. In: Fox PF (ed) *Advanced dairy chemistry*, vol 1, proteins, 2nd edn. Elsevier Applied Science, New York, pp 735–765
- Udabage P, Puvanthiran A, Yoo JA, Versteeg C, Augustin MA (2012) Modified water solubility of milk protein concentrate powders through the application of static high pressure treatment. *J Dairy Res* 79:76–83
- Ye A (2011) Functional properties of milk protein concentrates: emulsifying properties, adsorption and stability of emulsions. *Int Dairy J* 21(1):14–20
- Zayas JF (1997) *Functionality of proteins in food*. Springer, Berlin, pp 76–133

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.