Heat stability of high-protein ultrafiltration retentate: Effect of concentration factor and stabilizing salts

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Abstract: Pasteurized cow skim milk (PCSM, 1×) was concentrated using ultrafiltration (UF) from $2 \times$ – $7 \times$ concentration factor (CF) to obtain retentates with different protein to total solids (TS) ratio. Alteration in chemical make-up led to poor thermal stability in UF retentates. Therefore, this study was aimed to investigate the changes in permeate flux, chemical composition, pH, viscosity and heat coagulation time (HCT) of UF/DF retentates as a function of CF. Impact of high-shear (20,000 rpm/ 5 min), diafiltration (DF) with 150mM NaCl solution and stabilizing salts on HCT of high-protein $7 \times$ UF retentates were also evaluated. With increase in CF; TS, protein, fat, ash, calcium contents and viscosity increased significantly ($p < 0.05$), while permeate flux, lactose content, pH and HCT of retentates significantly decreased ($p < 0.05$). Poor HCT (1 min) of $7 \times$ UF retentate was significantly increased ($p < 0.05$) up to 60 min by addition of stabilizing salts (NaH_2PO_4 , Na_2HPO_4 and $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$). Correlation was established between chemical constituents, pH, viscosity, HCT, permeate flux and concentration factor. Overall, this investigation established that identification of correct stabilizing salts via HCT-pH curve of PCSM and their appropriate addition converted thermally unstable (poor HCT) high-protein

($7 \times$ UF) retentates to thermally stable product. Hence, they could be now explored in number of food applications.

Keywords: Diafiltration, Heat stability, Protein concentration, Stabilizing salts, Ultrafiltration, Viscosity

Introduction

Membrane processing had enabled the dairy sector to separate, fractionate, concentrate and purify milk proteins without applying harsh conditions related to temperature and pH. Ultrafiltration (UF) is a well-established, pressure-driven membrane process that finds maximum use in dairy industry for concentration and purification milk proteins. At a particular temperature, a part of water soluble milk constituents (such as lactose, vitamins and minerals) are selectively allowed to pass through UF membrane into permeate under the influence of applied trans-membrane pressure (TMP) and membrane molecular weight cut off (MWCO). Contrary to this, water insoluble/ colloidal (fat, calcium and protein) milk compounds are rejected by UF membrane based on their size, shape, charge and MWCO and further concentrated in UF retentate with increase in concentration factor. Concentration factor is the ratio of initial weight or volume of feed to final weight or volume of retentate (Meena et al. 2016). At similar TS level, the chemical composition of concentrates produced by either reverse osmosis (RO) or by multiple effect evaporation was different than that of UF retentate (Meena et al. 2016). Diafiltration (DF) is accomplished in the same UF plant to increase protein purity which is otherwise not possible with UF process alone. Thus, both UF and DF process causes alteration in chemical composition of UF/DF retentates and the same is responsible for different physico-chemical, rheological and functional characteristics of those retentates compared to that of concentrated milks.

Heat coagulation time (HCT) also known as thermal stability is very important functional property of liquid and concentrated milks. During high-heat processing of milks, it plays a prominent role. Singh (2004) defined HCT as the resistance (as time in minutes) shown by liquid and concentrated milks prior to onset of their heat induced coagulation at 140°C and 120 or 130°C, respectively. Chemical composition, more precisely protein and

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calcium contents, pH and salt balance ratio have major influence on HCT of liquid and concentrated milks. HCT is mainly governed by stability of their milk proteins. As per Solanki and Gupta (2009), even slight change in delicate salt balance of milk, resulted from any process or additives, have major influence on their thermal stability.

HCT of liquid and concentrated milks (manufactured using multiple effect evaporators) has been mostly targeted in scientific investigations. Contrary to conventionally concentrated milks (maximum HCT between 6.4-6.6 pH), at similar protein or TS levels, UF and DF retentates exhibited entirely different HCT-pH profile. This was collectively governed by the extent of protein concentration, change in pH and alteration in delicate ionic equilibrium between serum and micellar proteins during skim milk concentration in UF/DF processes (Holt et al. 1981). Apart from intrinsic and extrinsic factor (Singh 1995), HCT of concentrated milks was also influenced by composition of salts (Sweetsur and Muir 1980) and beta lactoglobulin (β -Lg) as they showed inverse correlation with HCT (Muir and Sweetsur 1978). Singh and Creamer (1992) found liquid milks were more heat stable than concentrated milks on entire pH range.

Sweetsur and Muir (1980) evaluated the impact of pre-heating (90°C/10 min) treatment and observed that it caused slight increase in HCT of UF retentate, while doubled the HCT of ordinary produced concentrated milk. Partial removal of calcium from liquid or concentrated milks tend to increase their thermal stability. Ozimek et al (1988) observed non-significant differences between HCT of fresh and a week old samples of UF retentate. It was demonstrated in our earlier investigation that stabilizing salts significantly ($P < 0.01$) improved the HCT of homogenized 5 $\times$ UF retentate from 1.45 min (pH 6.41) to 120 min (at pH 6.5, 6.6, 7.0) and 80 min (pH 6.6), respectively (Meena et al. 2016). However, such studies have not been so far conducted on highly concentrated (such as 7 $\times$ UF/DF) retentates containing higher protein to TS ratio.

UF retentate acts as raw material or an ingredient during production of high-protein milks, *dahi*/curd (Meena et al. 2016), yoghurt (Yadav et al. 2018), dairy whiteners (Khatker et al. 2012) and milk protein concentrate (MPC) powders with different protein contents (Meena et al. 2017b). High-protein beverages which are preserved by conventional sterilization or ultra-high temperature treatments demands better thermal stability in high-protein liquid (retentates) and dried (MPC powders) ingredients (Meena et al. 2017a). Practically, proteins present in skim milk are concentrated using UF to achieve 0.60 protein to TS ratio in UF retentate. Its further purification with DF is necessary to obtain higher (>0.60) protein to TS ratio. Indeed, degree of protein concentration is directly proportional to increase in TS and calcium contents of UF/DF retentates which also exert detrimental effect on their HCT values.

Addition of stabilizing salts in concentrated milks to improve their heat stability is well- documented and commercially being explored in production of evaporated milks (Solanki and Gupta, 2009). These findings are not as such applicable on high-protein (6-7 $\times$) UF/DF retentates because of their higher protein and calcium contents at similar TS levels. Studies dedicated to improvement in HCT of such retentates are still scanty.

Therefore, present investigation has been aimed (i) to investigate the changes taking place in chemical composition, pH, viscosity and HCT of UF/DF retentates as well as in permeate flux as a function of concentration factor (1 $\times$ to 7 $\times$) and (ii) to evaluate the impact of high-shear treatment (20,000 rpm/ 5 min) and stabilizing salts such as monosodium phosphate (MSP- NaH_2PO_4), disodium phosphate (DSP- Na_2HPO_4) and trisodium citrate (TSC- $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) on HCT of high-protein 7 $\times$ UF/DF retentates.

Materials and Methods

Materials

Fresh cow milk of morning shift was obtained from cattle yard at Experimental Dairy of ICAR-national Dairy Research Institute (ICAR-NDRI), Karnal; it was heated to $40 \pm 1^\circ\text{C}$, centrifugally separated and, pasteurized at $73 \pm 1^\circ\text{C}/15\text{s}$ using a commercial scale milk pasteurizer (make-GEA Westfalia, capacity-10 KLPH). This pasteurized cow skim milk (PCSM) was collected in pre-sterilized stainless steel (SS) cans. All the chemicals used in present investigation were of analytical grade and procured from Sigma-Aldrich, (St. Louis, MO, US). Pre-serialized borosilicate glass bottles (make- BOROSIL®, capacity- 1000 mL) were used for the purpose of sample collection. For calcium estimation, only acid wash glassware were used.

Methods

Manufacturing of UF retentates (UFR) via concentration of PCSM

The SS cans containing PCSM were partially immersed in hot water, stored in a cheese vat at $80 \pm 1^\circ\text{C}$. For better heat transfer, PCSM was slowly stirred using a pre-sterilized SS plunger. Thereafter, this pre-heated PCSM was filtered through a clean muslin cloth and poured in the balance tank of a pilot scale UF plant (make- Tech Sep, France), equipped with a tubular heat exchanger, retentate and permeate flow meters, thermometer and different pressure gauges. This plant was also equipped with two tubular membrane modules containing total 1.68m² area of zirconium oxide (mineral) membrane. The MWCO of this UF membrane was 50 kilo Dalton. Before each run, the UF plant was properly sanitized and completely drained. Total 300 kg PCSM was used in each run.

Maintaining constant TMP of 1 bar, PCSM (1 $\times$) was concentrated to 2 $\times$, 3 $\times$, 4 $\times$, 5 $\times$, 6 $\times$ and 7 $\times$ concentration factors (ratio of initial

Fig. 1 Manufacturing of ultrafiltration (UF) - diafiltration (DF) retentates from pasteurized cow skim milk (PCSM).

weight of feed to final weight of retentate, usually denoted by $\times$) in UF plant at a constant temperature ($50 \pm 1^\circ\text{C}$). The weight of PCSM and permeate samples were determined using an electronic weighing balance. At any point of time, total weight of permeate subtracted from total weight of PCSM provided the actual retentate weight. At each concentration factor, the values of permeate flux in liter per hour per m^2 (LMH) were directly recorded from the permeate flow meter and used to calculate flux mean as per following equation (St-Gelais *et al.* 1991).

$$\text{Flux mean} = \text{Final flux} + 0.33 \times (\text{Initial flux} - \text{Final flux})$$

At each concentration factor, representative samples of $1 \times$ – $7 \times$ UF retentates were collected in pre-sterilized glass bottles. The $7 \times$ UF retentate ($7 \times \text{UFR}$) sample was divided into 3 parts. First part was treated as control, second part was used to evaluate the effect of different stabilizing salts while third was further subjected to diafiltration treatment as mentioned below.

Diafiltration and high-shear treatment of $7 \times$ UF retentate

The 150mM NaCl solution was prepared in reverse osmosis (RO) water and used for the diafiltration of third part of $7 \times$ UF retentate in 1:1 ratio. Diafiltration was accomplished in same pilot scale UF plant via removal of exactly similar amount of permeate to the amount of added NaCl solution. The retentate thus produced

was referred as 7× DF retentate (7× DFR). A part of this was also subjected to high-shear treatment (20,000 rpm for 5 min) using a ULTRA-TURRAX® homogenizer (make: Ika, model: T18 digital). During high-shear treatment, sample temperature was maintained between 5-10°C by keeping it in an ice bank. In order to keep a check on microbial growth, NaN₃ (0.03% w/w) was added in all samples prior to their refrigerated storage (4±1°C), until their further analysis.

Chemical analysis and calcium determination

Standard gravimetric methods as reported in Indian standards (IS: 12333 1997 and IS: 1479 part III 1961) were used to determine TS and ash contents of PCSM and all retentate samples. Further, their crude protein contents were estimated adopting Macro Kjeldahl Method (IDF 1993) and multiplied with a constant factor (6.38) to get their actual protein contents. Gerber method (IS: 1224 part I 1977) was used to obtain fat contents of all these samples, while following equation was used to determine their lactose contents, respectively:

$$\text{Lactose content} = [\text{TS} - (\text{protein content} + \text{fat content} + \text{ash content})]$$

The standard AOAC (2005) method was used to determine calcium content of these samples using an atomic absorption spectrophotometer (make-Shimadzu; model- AA-7000).

pH measurement and its adjustment by stabilizing salts addition

To measure the pH of PCSM and different UF and DF retentates at 20±1°C, a Eutech pH meter (make-Thermo Scientific, model-cyberscan 1100) was used. First of all, pH of PCSM and 7× UF retentate were adjusted in the range of 6.0 to 7.2 (with a constant increment of 0.1 unit) using standard 0.1N HCl and 0.1N NaOH solutions. These pH adjusted PCSM samples were used to determine its HCT-pH curve (Figure 3.a) that was further explored to find suitable stabilizing salt (s) for improving the HCT of 7×UFR. Thereafter, 1.0% (w/v) solutions of NaH₂PO₄ (MSP), Na₂HPO₄ (DSP) and Na₃C₆H₅O₇ (TSC) salts were prepared in distilled water and used to adjust the desired pH of 7×UFR as shown in Figure 3.b. The 0.2-0.5 % mixture of MSP and DSP (2:1 w/w) salts with and without 0.7% TSC were directly added in 7×UFR in order to evaluate their effect on HCT via total 11 treatments (T1-T11) as shown in Table 2. In order to nullify the effect of buffering action of milk proteins, initially adjusted pH of each sample was re-checked and re-corrected after constant duration of 1 h at 20±1°C.

Determination of heat coagulation time (HCT)

Under standard test conditions, resistance shown by liquid and concentrated milk samples prior to onset their thermal coagulation is called HCT. In this investigation, HCT of PCSM was measured at 140°C while that of UF/DF retentates was measured at 120°C adopting the method reported by Khatkar et al. (2014).

Measurement of apparent viscosity

A Rheometer (MCR 52, Anton Paar, Germany) equipped with stainless steel cone plate CP75-1° probe was used to measure apparent viscosity (now onwards written as viscosity) of PCSM, UF and DF retentates at a fixed shear rate of 100 per second and temperature (20°C). Total twenty measurements were recorded for each sample. The analysis was conducted in triplicate for each sample.

Statistical analysis

One-way analysis of variance (ANOVA) and Pearson correlation were employed for statistical analysis of the obtained data using SAS Enterprise guide (SAS 2008). Using Duncan's multiple range test, the mean were compared (Duncan 1955).

Results and Discussion

PCSM concentration by UF and DF: effect on chemical composition, pH and HCT of UF/DF retentates and permeate flux

Table 1 shows the change in chemical composition, pH, and HCT of UF/DF retentates and permeate flux at each concentration factor (also denoted by '×') during PCSM concentration by UF process. Compared to TS, protein and ash contents of PCSM (1×), these components of all UF (2×-7×) retentates and DF (7×DFR) retentates were significant different (p<0.05) with each other and showed a significant increase (p<0.05) with increase in concentration factor. Similar increasing trend was also observed in fat contents of these UF retentates. The fat contents of 7×UFR and 7×DFR were statistically at par (p>0.05) with each other, but significantly higher (p<0.05) than the fat contents of PCSM and remaining UF retentates. Over PCSM (1×) sample on dry matter (DM or TS) basis (Table 1), the protein, fat, ash and calcium contents of 2×-7× UF retentates increased in the range of 52.34-74.72, 1.73-2.48, 6.40-7.96 and, 2.16-2.61%, respectively. Contrary to marked increase in above mentioned constituents, significant decrease (p<0.05) in lactose content, pH, HCT and permeate flux values were observed with increase in concentration factors in all retentates. The lactose content (on DM basis), pH and HCT of PCSM (1×) were 55.37%, 6.67 and 60 min (Table 1) which decreased to 14.25%, 6.32, 1 min and 13.30%, 6.24 and 1 min, respectively in 7×UF retentate and 7×DF retentate samples.

Permeation of water soluble milk components into permeate through UF membrane along with subsequent concentration of colloidal milk constituents owing to their selective retention could easily explain such changes in concentration of milk components present in different UF/DF retentates. Similar changes in milk constituents were also reported by Meena et al (2016); Khatkar and Gupta (2014); Mistry (2002) during concentration of PCSM by ultrafiltration. Further, 2× to 7× concentration of PCSM (1×) markedly increased their calcium contents (on DM basis) in the

range of 2.16 to 2.61% compared to 1.42% calcium in PCSM. This is mainly attributed to concentration of milk proteins particularly casein. This is because only a part of soluble calcium present in PCSM passes into permeate through UF membrane, but at the same time its colloidal calcium, present in the form of colloidal calcium phosphate (CCP) got concentrated with concentration of caseins. Hence, the overall concentration of calcium enhances with increase in degree of protein concentration as shown in Table 1.

The increase in TS and ash contents seems to be responsible for significant ($p < 0.05$) decrease in pH values of $2\times$ to $7\times$ UF retentates as a function of concentration factors (Table 1). Ferrer et al. (2011) reported that during ultrafiltration of skim milk, concentration of different minerals (such as K^+ , Mg^{2+} and Ca^{2+}) also increases. Further, such rise in mineral contents induces changes in cationic profile of milk based on their association with milk proteins that ultimately reduces pH of UF retentates. Gradual decrease in pH of retentates during ultrafiltration was earlier also reported by Meena et al (2016); Ferrer et al. (2011). Apart from this, significant reduction ($p < 0.01$) was observed in pH of $7\times$ DFR than the pH of $7\times$ UFR. This was attributed to applied diafiltration of $7\times$ UFR with 150mM NaCl solution. Meena et al (2016) also observed decrease in pH of $5\times$ DF retentate.

Thermal stability of concentrated milk is majorly influenced by the concentration of its constituents and its delicate salt equilibrium. It is clearly evident from Table 1 that with increase in

concentration factor, chemical composition of different UF and DF retentates markedly changed. Furthermore, this also changed their pH and calcium contents which have prominent role in stabilization of milk proteins. Thus, alteration in chemical composition, decrease in pH and increase in calcium contents might have collectively disturbed the delicate salt equilibrium of UF and DF retentates and the same could be attributed to significant ($p < 0.05$) decrease in their HCT values as shown in Table 1. Indeed, higher calcium content and lower pH are detrimental for thermal stability of concentrated milk samples and the same can easily explain relatively lower HCT values of UF retentates subjected to higher concentration factors. Ozimek et al (1998) also reported that with increase in volume concentration ratio, a decreasing trend was observed in HCT values of UF retentates.

As expected a gradual and significant ($p < 0.05$) reduction was observed in permeate flux with the advancement of UF concentration (Table 1). Increase in TS and viscosity as well as decrease in pH of UF retentates acts as the prime factors promoting concentration polarization and fouling of UF membrane. Both concentration polarization and fouling are inversely proportional to permeate flux (Meena et al. 2016). In nutshell, reduction in stability of milk proteins have detrimental impact on permeate flux during ultrafiltration. The calculated mean flux values at each concentration factor are shown in Table 1 that clearly displayed a decreasing trend. Such reduction in permeate

Table 1 Chemical composition, pH, HCT of retentates and permeate flux at different concentration factors during ultrafiltration and diafiltration of PCSM

CF	TS	Protein	Fat	Lactose	Ash	Calcium	pH	HCT	Permeate
			Percent					(min)	flux LMH
1×	8.47 ^h ±0.01	3.02 ^h ±0.09 (35.66)	0.1 ^g ±0.00 (1.18)	4.69 ^a ±0.00 (55.37)	0.66 ^h ±0.01 (7.79)	0.12 ^g ±0.00 (1.42)	6.67 ^a ±0.02	60.00 ^a ±0.88	99 ^a ±0.58
2×	11.56 ^g ±0.06	6.05 ^g ±0.07 (52.34)	0.2 ^f ±0.00 (1.73)	4.58 ^b ±0.02 (39.61)	0.74 ^g ±0.01 (6.40)	0.25 ^f ±0.00 (2.16)	6.57 ^b ±0.01	60.00 ^a ±0.58	68.33 ^b ±0.88
3×	15.07 ^f ±0.02	9.07 ^f ±0.04 (60.19)	0.3 ^e ±0.00 (1.99)	4.52 ^c ±0.01 (29.99)	1.17 ^f ±0.01 (7.76)	0.35 ^e ±0.00 (2.32)	6.57 ^b ±0.00	60.00 ^a ±0.58	59.33 ^c ±0.88
4×	18.23 ^e ±0.04	12.1 ^e ±0.06 (66.37)	0.4 ^d ±0.00 (2.19)	4.38 ^d ±0.01 (24.03)	1.34 ^e ±0.00 (7.35)	0.43 ^d ±0.00 (2.35)	6.56 ^{bc} ±0.00	25.00 ^b ±0.88	43.67 ^d ±1.45
5×	21.63 ^d ±0.08	15.14 ^d ±0.08 (70.00)	0.51 ^c ±0.01 (2.36)	4.22 ^e ±0.00 (19.51)	1.76 ^d ±0.01 (8.13)	0.53 ^c ±0.00 (2.35)	6.51 ^c ±0.00	6.00 ^c ±1.2	23 ^c ±0.58
6×	25.01 ^c ±0.05	18.21 ^c ±0.06 (72.81)	0.6 ^b ±0.00 (2.40)	4.12 ^f ±0.00 (16.47)	2.08 ^c ±0.01 (8.32)	0.66 ^b ±0.01 (2.63)	6.44 ^d ±0.00	2.00 ^d ±0.00	16.67 ^e ±0.88
7×	28.28 ^b ±0.01	21.31 ^b ±0.07 (74.72)	0.7 ^a ±0.00 (2.48)	4.03 ^g ±0.01 (14.25)	2.25 ^a ±0.01 (7.96)	0.74 ^a ±0.00 (2.61)	6.32 ^e ±0.01	1.00 ^e ±0.00	12 ^e ±0.58
7×DF	29.23 ^a ±0.06	22.45 ^a ±0.03 (76.81)	0.7 ^a ±0.00 (2.39)	3.89 ^h ±0.03 (13.30)	2.19 ^b ±0.00 (7.49)	0.75 ^a ±0.00 (2.56)	6.24 ^f ±0.02	1.00 ^e ±0.00	8.67 ^f ±0.33

Mean ± S.E. (n=3), mean values with different superscripts ^{abcde} in a row are significantly different with each other ($p < 0.01$). Values shown in parentheses are on dry matter basis (% of TS). HCT of PCSM (1×) was determined at 140 °C while that of other retentates were determined at 120 °C.

Fig. 2 Change in viscosity (mPa.s) of UF-DF retentates as a function of concentration factor. Mean $\pm$ S.E. (n=3), mean values with different superscripts ^{abcdefgh} are significantly different with each other at $p < 0.05$.

flux with increase in UF concentration factors has been reported by Patil et al (2019); Uttamrao et al (2019); Patil et al (2018).

Effect on viscosity values of different samples

As a function of concentration factor, in comparison to PCSM, the changes in viscosity (at 100 per s shear rate and 20°C temperature) of 2×- 7× UF retentates and 7× DF retentate are shown in Figure 2. The viscosity values of all these samples were significantly different ($p < 0.05$) with each other. Further, a significant increase ($p < 0.05$) in viscosity values of these samples were observed with consecutive increase in concentration factors. Indeed, such increase in viscosity values of UF/DF retentates were expected owing to marked increase in their TS, protein and ash contents along with marked decrease in their pH values (Table 1). All these factors collectively enhanced the viscosity values of UF/DF retentates. Meena et al. (2016) also reported such increase in viscosity during skim milk concentration up to 5×. It was observed that during 1×- 5× concentration of PCSM, the increase in viscosity was in the range of 3.90-25.70 mPa.s (Figure

2), however, it drastically increased in the range of 567.80- 1391.8 mPa.s for 6× and 7× UF retentates and 7×DF retentate. This could be easily explained by their relatively higher TS, protein and mineral (particularly calcium) contents than that of PCSM and 2× to 5× UF retentates. Viscosity of UF/DF retentates plays a crucial role during their spray drying and directly influences physical, reconstitution and functional properties of [milk protein concentrate \(MPC\)](#) powders. Shinde et al (2020) reported that more than 150 mPa.s (20 °C and 50 per s) viscosity may create problems during atomization of UF/DF retentates. Hence, 6×, 7×UF retentates and 7×DF retentate might need additional treatment for their efficient atomization.

Selection of stabilizing salts via HCT-pH curve of PCSM

The UF and DF retentates obtained through the concentration of PCSM [act](#) as intermediate product. In liquid form these are used in different applications such as standardization of cheese milk, manufacturing of plain *dahi* (Meena et al. 2015), yoghurt (Yadav et al. 2018), high-protein milks, high-protein beverages,

Fig. 3. The HCT-pH curve of PCSM at 140°C (a); effect of NaH₂PO₄ & Na₂HPO₄; NaH₂PO₄ & Na₃C₆H₅O₇ on HCT of 7×UF retentate at 120°C (b). Mean±S.E. (n=3) with similar superscripts ^{A&c} are at par ($p>0.05$) while those with different superscripts ^{abc} are significantly different with each other at $p<0.05$.

liquid dairy whiteners (Khatkar et al. 2014) and high-protein yoghurts. Further, through spray drying, they are converted to high-protein powders such as dairy whiteners (Khatkar et al. 2014), low-lactose powders (Solanki and Gupta 2014) and MPC powders containing protein in the range of 42-89% (Meena et al. 2017b) and milk protein isolates with >90% protein content. However, the major problem with high-protein UF/DF (6×-7×) retentates is their poor HCT (Table 1). Hence, tailoring and maintaining good heat stability in such retentates still remains as a key challenge and acts as a major obstacle in production of high-quality, high-protein products.

Thermal stability of UF retentates could be improved by the addition of stabilizing salts (Meena et al. 2016), however, the type of salts and their levels depends on the typical characteristics of a particular retentate. Selection of the type of stabilizing salts to be added is not a straight forward choice always and varies between addition of acidic (MSP-NaH₂PO₄) or basic phosphates (DSP-Na₂HPO₄) and citrates (such as TSC-Na₃C₆H₅O₇). Deeth and Hartanto (2009) recommended the use of MSP, if the natural pH of the milk is higher than the pH of maximum stability. Furthermore, addition of Na₂HPO₄ or Na₃C₆H₅O₇ was suggested if the natural pH of the milk is lower than the pH of maximal stability (Singh et al. 1995).

In current investigation, this was verified by adjusting the pH of PCSM (natural pH-6.6) from 6.0 to 7.2 and recording their HCT

values at each pH level as shown in Figure 3.a. It is clear from HCT-pH curve (Figure 3.a) that PCSM showed maximum HCT at pH 6.7 and the same was markedly higher than its HCT at natural pH (6.6). These results are in good agreement with the earlier findings reported by Singh (2004). The HCT-pH curve (Figure 3.a) clearly demonstrated that the natural pH of the PCSM was lower than the pH of maximal stability, hence, Na₂HPO₄ and Na₃C₆H₅O₇ were selected to improve the heat stabilities of high-protein 7× UF/DF retentates.

Improvement in HCT of 7×UF retentate: Effect of stabilizing salts addition

As per Table 1, the 7×UF retentate had poor (1 min) HCT at its native pH (6.23). The HCT of 7×UF retentates whose pH was adjusted with 0.1N HCl and 0.1N NaOH solutions ranged from 2-5 min (for pH values 6.0-6.6) and 60 min for higher pH values (data not shown). This demonstrated a lead that the samples adjusted to pH values higher than natural pH of 7×UF retentate, markedly increased its HCT values. The pH of 7×UF retentate was also adjusted from 6.60 to 7.0 as shown in Figure 3.b using MSP (only to decrease pH, if required) and DSP as well as MSP and TSC solutions. As evident in Figure 3.b, the adjustment of pH with MSP and DSP solutions, significantly increased ($p<0.05$) the HCT of 7×UF retentate in the range of 42 - 60 min in the studied pH range. Thereafter, the test was discontinued. Further, the adjustment of pH with MSP and TSC solutions, significantly

increased ($p < 0.05$) the HCT of 7×UF retentate to 60 min in similar pH range (Figure 3.b). Hence, Figure 3.b clearly indicate that in similar pH (6.60-7.0) range, the combination of MSP and TSC was more efficient in HCT improvement of 7×UF retentate compared to MSP and DSP salts. Observed marked improvement in HCT of 7×UF retentate could be attributed to stabilizing salts induced protection of milk proteins via increasing pH leading to decrease in calcium ion activity as both favor enhancement in HCT. In 6.1-7.0 pH range, changes in HCT values of homogenized 5× UFR samples were investigated by Meena et al. (2016) and it was concluded that its HCT (1 min at pH 6.41) was markedly enhanced in the range of 80-120 min in 6.5-7.0 pH range adjusted with DSP and TSC salts. Le Ray et al (1998) described that addition of sodium phosphate, sodium citrate and sodium chloride protected reconstituted casein micelles dispersions against heat coagulation by decreasing the amount of miceller minerals and enhancing stabilizing forces such as steric repulsions. Hence, similar effects could also explain increase in HCT values of 7×UF

retentate at different pH values in present investigation. Indeed, observed variation in HCT values of 7×UF retentate emphasizes the importance of pH and salt balance and because of this reason, addition of the suitable stabilizing salt (s) in optimum levels is crucial as well as helpful in achieving most desired pH and salt balance ratio in studied 7×UF retentate. Khatkar et al. (2014) also reported similar findings during conversion of UF retentate into dairy whiteners.

In lack of scientific literature, HCT of 7×UF retentate containing stabilizing salts (as mentioned above) was not possible. Therefore, observed results have been compared with earlier reported studies on UF retentates concentrated to lower concentration factors. Meena et al (2016) evaluated the changes in heat stability of homogenized 5×UF retentate as a function of pH adjustment from 6.1 to 7.0 pH range using DSP and TSC salts. Both salts significantly ($P < 0.01$) improved the HCT of this retentate from 1.45 min (native pH-6.41) to 80-120 min in studied range (6.5-

Table 2 Effect of high shearing and stabilizing salts addition on HCT of 7×DF retentates

Treatments	Code	HCT (min, at 120 °C)
7×UFR (produced by Ultrafiltration of PCSM)	T1	1.00 ^d ±0.00
7×DFR (produced by diafiltration of 7×UFR with 150mM NaCl solution)	T2	1.00 ^d ±0.00
T2+High-shear (20,000 rpm/ 5 min) treated 7×DFR	T3	1.00 ^d ±0.00
T2+ Addition of 0.2 % mixture of MSP and DSP (2:1 w/w)	T4	1.00 ^d ±0.00
T2+ Addition of 0.3 % mixture of MSP and DSP (2:1 w/w)	T5	1.00 ^d ±0.00
T2+ Addition of 0.4 % mixture of MSP and DSP (2:1 w/w)	T6	1.00 ^d ±0.00
T2+ Addition of 0.5 % mixture of MSP and DSP (2:1 w/w)	T7	1.00 ^d ±0.00
T4+ 0.7% TSC	T8	28.00 ^a ±0.01
T5+ 0.7% TSC	T9	25.00 ^b ±0.06
T6+ 0.7% TSC	T10	20.00 ^c ±0.04
T7+ 0.7% TSC	T11	20.00 ^c ±0.04

Mean ± S.E. (n=3), mean values with different superscripts ^{abcd} in a column are significantly different with each other ($p < 0.05$).

Table 3 Correlation between concentration factor (CF) chemical constituents and physical properties (pH, HCT, viscosity) and permeate flux during ultrafiltration of pasteurized cow skim milk

	CF	TS	Protein	Fat	Lactose	Ash	Calcium	pH	HCT	Flux	Viscosity
CF	1.000										
TS	0.996**	1.000									
Protein	0.997**	1.000**	1.000								
Fat	0.991**	0.999**	0.998**	1.000							
Lactose	-0.997**	-0.991**	-0.993**	-0.987**	1.000						
Ash	0.977**	0.990**	0.988**	0.992**	-0.974**	1.000					
Calcium	0.991**	0.998**	0.997**	0.998**	-0.985**	0.989**	1.000				
pH	-0.955**	-0.936**	-0.940**	-0.923**	0.952**	-0.892**	-0.930**	1.000			
HCT	-0.899**	-0.919**	-0.915**	-0.928**	0.891**	-0.930**	-0.916**	0.734*	1.000		
Flux	-0.962**	-0.973**	-0.973**	-0.979**	0.959**	-0.965**	-0.979**	0.871**	0.942**	1.000	
Viscosity	0.843**	0.802**	0.808**	0.775*	-0.849**	0.755*	0.787*	-0.925**	-0.560	-0.688*	1.000

Pearson correlation denoted by ** and * are significant at the $p < 0.01$ and $p < 0.05$ levels, respectively

7.0) of pH. HCT of 10% and 20 % fat (% of TS) containing cow skim milk based UF retentates were only 14 min that enhanced to 69 and 68 min upon addition of 0.5% mixture of MSP and DSP (2:1 w/w), respectively. Apart from this, addition of 0.4 % mixture of MSP and DSP (2:1 w/w) salts caused marked improvement in thermal stability of medium and full fat homogenized liquid dairy whiteners (Khatkar and Gupta 2012; Khatkar et al. 2014). Addition of 0.5% mixture of MSP and DSP (2:1 w/w) caused notable improvement in HCT of 5.48×UF retentate containing 21.28% TS. The maximum HCT (61 min) was observed at pH 6.72, however, addition of this salts mixture to higher levels gradually decrease its HCT.

Improvement in HCT of 7×DF retentate: Effect of stabilizing salts addition

The pH and HCT of 7×DF retentate were significantly lower ($p < 0.05$) than that of 7×UF retentate as shown in Table 1. In order to bring the desired changes in techno-functional properties of milk proteins particularly in casein, UF retentates were intentionally subjected to either diafiltration with NaCl/KCl solutions or high-shear treatment. Such diafiltration decreases calcium content of DF retentate via exchanging sodium and calcium ions during this process, while high-shear treatment induces physical modifications in casein structure and markedly improve functional properties (particularly solubility) of resultant MPC powders. However, these approaches have detrimental effect on heat stability of retentates as shown in Table 2. Homogenization have been reported to decrease the heat stability values of liquid, evaporated and concentrated milks (Sweetsur and Muir 1980) and UF retentate (Meena et al. 2016).

Therefore, the effect of MSP and DSP (2:1 w/w) salts mixture on HCT of 7×DFR with and without TSC have been studied by different treatments as shown in Table 2. It was observed that HCT of T1, T2 and T3 samples were at par ($p > 0.05$) with each other that further did not improve upon even 0.2-0.5% (T4-T7) addition of MSP and DSP (2:1 w/w) salts mixture. Contrary to this, further addition of 0.7% TSC in T4, T5, T6 and T7 led to significant improvement ($p < 0.05$) in HCT values in treated samples (T8-T11). The HCT values of these samples were in the range of 20-28 min, respectively. Such variation in HCT values underlines the vital importance of salt balance and pH over the control on heat stability of 7× DF retentates as well. This is clear from Figure 3.b and Table 2 that the addition of MSP, DSP and TSC were capable in enhancing the heat stability of 7× UF retentate and 7× DF retentate samples. Although, the extent of rise in HCT values was higher in 7× UF retentates than 7× DF retentate.

Hence, findings of this investigation will be of prime importance to dairy sector in improving the thermal stability of high-protein containing UF/DF retentates. This will also widen the applications of liquid retentates in different food applications apart from helping in improving the physical, reconstitution and functional

properties of dairy based high-protein powders manufactured from them.

Correlation between concentration factor, chemical composition, pH, HCT, viscosity of retentates and permeate flux during PCSM concentration by ultrafiltration

During concentration of PCSM by ultrafiltration, TS ($r = +0.996, p < 0.01$), protein ($r = +0.997, p < 0.01$), fat ($r = +0.991, p < 0.01$), ash ($r = +0.977, p < 0.01$), calcium ($r = +0.991, p < 0.01$) and viscosity ($r = +0.843, p < 0.01$) showed positive correlation, while lactose ($r = -0.997, p < 0.01$), pH ($r = -0.955, p < 0.01$), HCT ($r = -0.899, p < 0.01$) and permeate flux ($r = -0.962, p < 0.01$) showed negative correlation with concentration factor as shown in Table 3. Almost similar correlation was observed between TS and remaining parameters, while protein content showed positive correlation with fat ($r = +0.998, p < 0.01$), ash ($r = +0.988, p < 0.01$), calcium ($r = +0.997, p < 0.01$) and viscosity ($r = +0.808, p < 0.01$), however, it was negatively correlated with lactose ($r = -0.993, p < 0.01$), pH ($r = -0.940, p < 0.01$), HCT ($r = -0.915, p < 0.01$) and permeate flux ($r = -0.973, p < 0.01$), respectively. The ash content revealed positive correlation with calcium ($r = +0.989, p < 0.01$) and viscosity ($r = +0.755, p < 0.05$), but showed negative correlation with pH ($r = -0.892, p < 0.01$), HCT ($r = -0.930, p < 0.01$) and permeate flux ($r = -0.965, p < 0.01$). Apart from this, calcium content was negatively correlated with pH ($r = -0.930, p < 0.01$), HCT ($r = -0.916, p < 0.01$) and permeate flux ($r = -0.979, p < 0.01$) and positively correlated with viscosity ($r = +0.787, p < 0.05$). The pH was positively correlated with HCT ($r = +0.734, p < 0.05$) and permeate flux ($r = +0.871, p < 0.01$), however it was negatively correlated with viscosity ($r = -0.925, p < 0.01$), while HCT was positively correlated with permeate flux ($r = +0.942, p < 0.01$). Furthermore, correlation of fat and lactose with other parameters has been shown in Table 3, respectively.

Conclusion

Concentration of PCSM by ultrafiltration caused noticeable increase in colloidal milk components and viscosity, however this also decreased the pH and permeate flux as a function of concentration factor. Such alteration in chemical composition disturbed the delicate salt equilibrium and led to gradual decrease in HCT up to 3× concentration, however, marked increase in TS, protein and calcium contents along with decrease in pH, drastically reduced the HCT of UF (5× - 7×) and 7× DF retentates. Even, applied diafiltration with 150mM NaCl solution and high-shear (20,000 rpm/ 5 min) treatment were found detrimental for HCT. Tailoring and maintaining good heat stability in high-protein retentates is acts a key challenge and major hurdle in production of high-quality products from such retentates. Combined use of stabilizing salts, identified from classical HCT-pH curve of PCSM at appropriate levels restored desired equilibrium between milk constituents and markedly improved thermal stability of high-protein UF/DF retentates. Establishment of correlation between

concentration factor, milk constituents, pH, viscosity, HCT and permeate flux provided better understanding about thermal stability of retentates. In nutshell, this investigation had established that poor heat stability of high-protein containing UF/DF retentates can be restored through the addition of suitable stabilizing salts in appropriate amounts. Further, such retentates with improved thermal stability could be explored in number of liquid products and they can also result in high-protein powders with improved physical, reconstitution and functional properties.

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