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PII: S0260-8774(18)30479-5

DOI: https://doi.org/10.1016/j.jfoodeng.2018.11.003

Reference: JFOE 9454

To appear in: *Journal of Food Engineering*

Received Date: 12 April 2018

Revised Date: 17 July 2018

Accepted Date: 4 November 2018

Please cite this article as: Singh, J., Prakash, S., Bhandari, B., Bansal, N., Comparison of ultra high temperature (UHT) stability of high protein milk dispersions prepared from milk protein concentrate (MPC) and conventional low heat skimmed milk powder (SMP), *Journal of Food Engineering* (2018), doi: https://doi.org/10.1016/j.jfoodeng.2018.11.003.

Title: Comparison of ultra high temperature (UHT) stability of high protein milk dispersions prepared from milk protein concentrate (MPC) and conventional low heat skimmed milk powder (SMP)

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Declarations of interest: none

Abstract: This study compared the UHT (145 ^{o}C for 5 s) stability and fouling behavior of high protein milk dispersions prepared from reconstituted low heat skimmed milk powder (RSMP) and milk protein concentrate powder (RMPC). It was found that RMPC at 10 and 14% protein content was more UHT stable as compared to lower protein content RSMP (3.25, 6.5, 7, 7.5, 8 %). Matching the total solids and mineral composition of 7.5-RMPC with 7.5-RSMP by addition of minerals and lactose markedly reduced its UHT stability (UHT run-time reduced to 66 min from >120 min). The RP-HPLC analysis showed increased casein dissociation but similar whey protein aggregation in 7.5-RSMP as compared to 14-RMPC. UHT processing lead to formation of larger particles in case of 7.5-RSMP (1.84 μm D(0.9)) as compared to 14-RMPC (0.23 μm D(0.9)). It was observed that mineral environment affected protein interactions leading to the differences in UHT behavior of RSMP and RMPC.

Keywords: Milk powders; ultra high temperature processing; milk proteins; fouling; milk minerals

1 Introduction

Increased consumer awareness towards benefits of protein in diet and its positive effects on weight loss and muscle building has surged the demand for high protein weight loss diets (Friedman, 2004). There are various formats of high protein processed foods available in the market, but ready-to-drink (RTD) high protein beverages are notable departure from typical powder type sports supplements specifically targeted at body builders and sports people (Baxter et al., 2011). Due to their convenience, high protein beverages are also appealing to health conscious ordinary consumers. RTD high protein beverages based on milk proteins are processed liquid food products. Manufactures combine high protein dairy powders along with other ingredients to obtain a ready to drink product with desired protein, fat and carbohydrate content, amino acid profile and sensory attributes (Jelen, 2011, Baxter et al., 2011). The RTD high protein beverages are required to contain high protein levels without compromising product stability and quality. Food and drug administration (FDA) of the United States requires adding minimum 10 g protein per 240 ml of drink (~ 4.2%) to claim high protein beverage (Etzel, 2004).

There are several types of milk protein ingredients available in the market. Skim milk powder (SMP), whole milk powder, milk protein concentrate powders (MPC), casein and whey protein concentrate powders are widely produced and used as ingredients in a range of milk protein based beverages. MPCs are complete dairy proteins, containing casein and whey proteins in their original proportions found in milk and in their native state. Much of the caseins in concentrates are in micellar form and whey proteins are largely undenatured (Agarwal et al., 2015). The protein content of MPC varies from 42 to 85%, which is indicated by the number following MPC, e.g. MPC85. MPCs find application in the manufacture of food emulsions, cheese, yogurt, ice cream, health related products and various other dairy products (Kelly, 2011). There is growing popularity of MPC as a protein source in neutral pH RTD high protein beverages due to the fact that it is an excellent source of protein and can provide a milky flavor and opacity to the drink (Agarwal et al.,

2015). MPC are manufactured from skim milk by ultrafiltration and diafiltration process, followed by spray drying the retentate (Havea, 2006). The filtration process also partially removes lactose and mineral salts from the milk. Due to the significant differences in the composition of non-protein constituents of MPC and SMP, reconstituted MPC (RMPC) can provide more protein per total solids as compared to reconstituted SMP (RSMP) (Deeth and Hartanto, 2009). However, RMPC contains altered mineral environment as compared to RSMP due to ultrafilteration and diafiltration process used to concentrate milk proteins during MPC manufacturing process. MPC contains more calcium than SMP, however per unit of protein MPC contains less calcium (Kelly, 2011).

Generally, There are two types of high protein beverages based on milk protein ingredients: neutral pH (pH~6.8) and low pH acidic beverages (Beecher et al., 2008). MPC based beverages are mostly neutral pH beverages due to its high casein content (Agarwal et al., 2015). Neutral pH beverages are required to be commercially sterilized to make them shelf-stable for a longer storage period. UHT is a commonly used technology for thermally processing these products. There are less colour and flavour changes and minimal losses of nutrients during UHT due to very short holding time and faster heat transfer as compared to retort sterilisation (Burton, 1994). However, thermal processing of dairy products causes formation of deposit layers on heat transfer surfaces, which is known as fouling (Sadeghinezhad et al., 2013). Fouling is a result of heat-induced destabilisation of milk constituents during processing which can limit the processing time and incur costs of cleaning and processing down times. Fouling may also adversely affect product quality due to dislodgement of deposits and mixing with product. Fouling deposits has very low heat conductivity as compared to process surfaces and fouling layers can reduce the heat flow, which causes insufficient processing of product (Prakash et al., 2005). In addition, increased obstruction to fluid flow can cause pressure drops across the processing line. These issues may increase the energy requirements due to increased energy costs to maintain adequate processing condition. In a worst scenario UHT processing plant may also be required to be shut down for cleaning (Bansal and Chen, 2006). There

are several factors affecting fouling of heat transfer surfaces which can be broadly classified as product and processing factors (Deeth, 2010). Understanding the behaviour of a milk product during UHT processing can be of great importance in controlling fouling and increasing run-time of processing plant. Milk protein system consists of different types of proteins and a complex mixture of native whey proteins, whey protein aggregates and whey protein-casein complexes can be formed during heating of milk protein dispersions (Wijayanti et al., 2014).

The heat stability of milk proteins and their susceptibility to denaturation and aggregation and final composition of this mixture depends on the temperature and time of heating, pH, relative abundance of other proteins and salts in the food system (Singh, 2004). Difference in thermal stability of proteins coming from different milk protein ingredients can play an important role in determining UHT stability of the final product (Sikand et al., 2010). In case of high protein beverages, improved UHT stability can be achieved by choosing a milk protein powder based on knowledge about their heat stability. The differences in composition of SMP and MPC can cause these two protein dispersions to behave differently during UHT processing. A lot of research has been conducted on UHT stability of normal strength and concentrated RSMP, but not sufficient work has been previously reported on UHT processability of RMPC. More research is required on UHT stability of RMPC because of its increasing usage in formulation of UHT processed RTD high protein beverages (Agarwal et al., 2015).

The present work is focused on understanding the behavior of high protein milk dispersions prepared from MPC and compare with conventional low heat SMP during UHT processing. An understanding of UHT stability and fouling behavior of RMPC can be beneficial for controlling and minimizing heat induced fouling during use of MPC in commercially sterilized high milk protein beverages, and other UHT treated products.

2 Materials and Methods

2.1 Materials

Commercial MPC and low heat SMP (purchased from Real Dairy Australia Pty. Ltd, Australia) were used in the preparation of reconstituted milk protein dispersions for all but one experiment for which MPC-G from a different manufacturer was purchased from Maxum Foods, Queensland, Australia for comparison purposes. Lactose was procured from Bio-Strategy Laboratory Products Pty. Ltd., Queensland, Australia. Standards for pure proteins were bought from Bio-Rad, Australia. Other chemicals and reagents were purchased from Sigma-Aldrich Pty. Ltd., NSW, Australia unless otherwise stated. Simulated milk ultrafiltrate (SMUF) was prepared according to the recipe by Jenness and Koops (1962).

2.2 Compositional and quality analysis of milk powders

MPC and SMP were analyzed for total protein content and lactose content using Kjeldahl method (AOAC, 2005) and titrimetric method (AS, 1994), respectively. MPC and SMP were also analyzed for mineral composition using Inductively Coupled Plasma-Optical Emission Spectrometric (ICP-OES) analysis as described by Martinie and Schilt (1976).

MPC was also analyzed for its solubility according to Bansal et al. (2017). MPC solubility was analysed at rehydration temperature of 50°C, which was the temperature used for reconstitution of samples throughout this study. The solubility of each sample was calculated as follows:

Solubility (%) = (total solids per g of filtrate/ total solids per g of suspension) * 100

2.3 Undenatured whey proteins and electrophoresis of milk powders

Undenatured whey proteins were quantified by adjusting the pH of RMPC and RSMP to 4.6 using 2M HCl or 2M NaOH, followed by centrifugation at 4500 *g* at 20^0C for 15 min to precipitate serum caseins and denatured soluble whey proteins (García-Risco et al., 1999). Supernatants were analyzed for protein content using Kjeldahl method (AOAC, 2005). All measurements were performed in duplicates. Undenatured whey protein content was reported as percentage of total protein content of RMPC and RSMP.

Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis was performed under reducing (R SDS-PAGE) and non-reducing (NR SDS-PAGE) conditions following the method of Laemmli (1970). Precast polyacrylamide gels (4-20%), sample buffer and Precision Plus Protein™ Dual Xtra molecular weight standard were obtained from Bio-Rad Laboratories Pty. Ltd, NSW, Australia. All other preparations for SDS-PAGE analysis were based on the standard guidelines in the Bio-Rad manual (Catalog number 161-0993. Samples were mixed 1:1 with 2X sample buffer for NR SDS-PAGE analysis. For R SDS-PAGE analysis, samples were mixed with sample buffer containing 10% β-mercaptoethanol and heated at 95^0C for 5 min. 10 μg protein was loaded onto each well. Electrophoresis was carried out at 80 V for 30 min and then at 100 V. Bio-Rad Mini Protean Tetra Cell system (Bio-Rad Laboratories Pty. Ltd, NSW, Australia) was used to run the gels. The gels were stained overnight with a solution of 0.04% Coomassie Brilliant blue G250, 25% methanol and 10% acetic acid in water. The gels were scanned and analyzed using Bio-Rad GS-800 Calibrated Densitometer (Bio-Rad Laboratories Pty. Ltd, NSW, Australia).

2.4 Preparation of reconstituted milk protein dispersions

Calculated amounts of milk powders, lactose, SMUF and distilled water were mixed to achieve required (w/w) protein content (PC) and total solids (TS) of RMPC and RSMP. Table 1 shows different samples and sample codes used in this study. Suffix UN and UHT were used to denote unheated and UHT heated samples, respectively. Reconstituted protein dispersions were prepared by reconstituting milk powders in distilled water at 50±2 ^{0}C. The protein dispersions were kept under refrigeration overnight (~14 h) to ensure complete hydration of all powder particles. Protein dispersions were then allowed to reach room temperature. pH of protein dispersions were analyzed and adjusted to 6.8 using 2M NaOH or 2M HCl, if required. Milk protein dispersions were filtered to remove any undissolved particles.

2.5 Ethanol stability of reconstituted milk protein dispersions

Ethanol stability was determined by mixing equal volume (2 mL) of milk and a range of ethanol solutions (50 to 100% at 2% intervals) and carefully examining the sample for clotting when poured in a petri dish. The highest concentration of ethanol, which did not cause coagulation, was reported as ethanol stability for the sample.

2.6 Ionic Ca activity in reconstituted milk protein dispersions

Ca-ion activity in milk protein dispersions was measured using LAQUAtwin calcium ion meter (Horiba Instruments, Japan). The calcium ion meter was calibrated using 3.74 mM (150 ppm) and 49.90 mM (2000 ppm) Ca-ion activity standard solution before each experiment, according to manufacturer instructions. All measurements were performed at room temperature (23°C).

2.7 UHT processing of reconstituted milk protein dispersions

The samples were UHT processed using a bench top UHT plant as shown in Fig 1. The product temperature at inlet and outlet of sterilization section was measured by T-type thermocouples, which were connected to a data logger and the temperature data was recorded in a Microsoft Windows based data acquisition system, VISIDAQ (PCLD-8115, Advantech Co., Ltd., Taiwan). A complete description of bench top UHT plant can be found in Prakash (2007). The product was preheated to 95°C and held at this temperature for 8 s in the holding tube before heating to 145°C in sterilization section and held at this temperature for 5 s. The volumetric flow rate of the product in the beginning of the trial was 150 mL/min (2.5×10^{-6} m^3/s).

Indicators used to end the UHT run due to deposit formation were as described by Prakash (2007). The UHT run was stopped if the back pressure could not be maintained at 0.4 MPa and high back pressure triggered the over pressure valve. The experiment was also stopped in case the outlet temperature of sterilization section dropped below 120 ^{0}C. The other unlikely scenario to stop UHT run was blockage of product channel due to severe fouling. Unless otherwise stated, if none of the above factors stopped UHT processing, the experiment was terminated after 120 min has elapsed into the UHT run. All experiments were performed in duplicate and their average value is reported.

2.8 Fouling measurements

Changes in overall heat transfer coefficient (OHTC) were used to monitor fouling. The plot of OHTC versus run time of UHT plant from the start to the end run was used to monitor development of fouling during the UHT run. Equation 3.1 was used to calculate OHTC.

$$\mathrm{OHTC} = \frac{\mathrm{GC_p}\Delta\theta}{\mathrm{A}\Delta\mathrm{T_{lm}}} \qquad \text{eq (1)}$$

Where, G is the mass flow rate of the product in kg/s; C_p is the specific heat of product in J/kg^{0}C; $\Delta\theta$ is the temperature difference between the inlet and outlet of the UHT section, in ^{0}C; A is the

heat exchanging surface area of the tubing in m^2; ΔT_{lm} is the log mean temperature difference (LMTD) in ^0C calculated using the equation 3.2.

$$\Delta Tlm = \frac{(T_o - T_{mo}) - (T_o - T_{mi})}{\ln[(T_o - T_{mo})/(T_o - T_{mi})]} \quad \text{eq (2)}$$

Where T_o is the temperature of oil bath in ^0C; T_{mi} and T_{mo} are temperatures of milk at the inlet and outlet of the sterlisation section in ^0C.

Specific heat and density of reconstituted milk powders were calculated using the specific heat and density of protein, carbohydrate, fat, ash and water and mass fraction of these major components in the dispersion (Singh, 2006, Choi, 1986).

2.9 Heat coagulation time measurements

Heat coagulation time (HCT) of samples was measured at the temperature similar to UHT sterilization (145 ^0C) using the method described by Davies and White (1966). Glass vials (22.6 x 75.5 mm) containing 2 mL of sample were placed on a rocker and immersed in a temperature controlled oil bath for heating. The rocker speed was kept at ~8 revolutions per min. HCT was reported as time elapsed between putting the samples in the oil bath and appearance of first visible signs of coagulation.

2.10 Particle size distribution

Particle size distribution (PSD) of unheated and UHT processed protein dispersions were measured by dynamic light scatterring (DLS) using a Malvern Mastersizer 2000MU-A (Malvern Instruments Ltd, Malvern, United Kingdom) as described by Dumpler and Kulozik (2016). The refractive index of protein was set at 1.41 and for dispersant (distilled water) was 1.33. Particle absorption index

was kept as 0.001. Stirrer speed was set at 2000 rpm and laser obscuration was maintained between 10 and 11 during measurement. All measurements were performed at room temperature (23 °C).

2.11 Viscosity measurements

The apparent viscosity of unheated and UHT processed protein dispersions was measured using an AR-G2 Rheometer (TA Instruments Ltd., USA) equipped with 60 mm parallel plate geometry with interplate gap set at 300 μm during measurements. The temperature of Peltier plate was set at 20^0C and viscosity measurements were performed after samples were allowed temperature equilibration for 1 min. Apparent viscosity at a shear rate of 300 s^{-1} was analyzed because it normally falls under the typical range of shear rate encountered during pipe flow, mixing and stirring of liquid food products (Steffe, 1996).

2.12 Whey protein denaturation and heat induced dissociation of caseins

Unheated and UHT processed samples were ultra-centrifuged (Avanti JXN-30, Beckman Coulter, Australia Pty. Ltd., NWS, Australia) at 100,000g for 1 h at 20 ^{0}C. The supernatant was removed carefully and analyzed for non-sedimentable protein (NSP) content using Kjeldahl method (AOAC, 2005). The supernatants were further analyzed to quantify individual non-sedimentable proteins of interest using Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) (RP-HPLC-UV, Agilent 1100, Agilent Technologies Australia, Victoria, Australia) using method adopted from Wijayanti et al. (2013).

2.13 Statistical analysis

The data was analyzed using Microsoft Excel and Minitab 16 software package. Significant differences between average values of replicate measurements on each data point was analyzed by analysis of variance (ANOVA) using Tukey's HSD post hoc test at 95% confidence level.

3 Results and discussion

3.1 Compositional and quality analysis of milk powders

MPC contained on average 81.95 ± 1.77% (w/w) total protein and 6.26 ± 0.13% (w/w) lactose. The total protein and lactose contents of SMP on average were 32.64 ± 0.53% and 55.37 ± 0.53%, respectively. Amount of undenatured whey proteins in MPC (18.85 ± 0.55% w/w) and SMP (22.52 ± 0.21% w/w) were similar. This was also confirmed with SDS-PAGE analysis. Which showed that β-lactoglobulin (β-lg) and α-lactoalbumin (α-la) were present in their native form in the milk powders as shown in Fig 2. Total calcium content of MPC (2.19 ± 0.05% w/w) was significantly higher than that of SMP (1.46 ± 0.05% w/w), presumably due to the higher protein (casein) content of MPC binding higher amount of colloidal calcium. Solubility of MPC was found to be 85.19 ± 1.61%, which reached 100% at 50^0C. This ensured that the temperature used for reconstitution during this study was sufficient for complete rehydration of samples.

3.2 UHT processing of milk protein dispersions

Firstly, a UHT processing trial was conducted with 3.25-RSMP to establish baseline performance of the bench-top UHT plant. The run-time for this sample was very long and UHT run was terminated after 300 min elapsed and ~50 kg sample was exhausted. The samples did not show any excessive pressure development and UHT temperature was maintained at 145 ± 2^0C. After that UHT stability of concentrated milk protein dispersions (RMPC and RSMP at different protein concentrations) was analysed. 10-RMPC and 14-RMPC were also very stable during UHT processing and average run-times of bench-top UHT plant exceeded 300 min for 10-RMPC and averaged 280 min for 14-RMPC. These samples were processed without any major temperature drops or pressure fluctuations. The RMPC sample with 16% PC was not UHT processed due to high viscosity and gel like consistency before processing. For further experiments, to be able to process multiple samples in a day, the UHT run was terminated after 120 min has elapsed, if fouling did not interrupt

processing. Fig 3 and 4 shows average run times and changes in OHTC during UHT run, respectively, for all the milk protein dispersions used in this study.

For RSMP samples containing 6.5, 7 and 7.5% proteins, the UHT run lasted for 88, 72 and 23 min, respectively before fouling was observed. Excessive back pressure developed after this time, which triggered the over pressure valve and the milk was pumped back into the balance tank of the UHT plant. Sample containing 8% proteins could not be processed through the UHT plant, because excessive back pressure developed as soon as 8-RSMP passed through the UHT section of plant. This suggested that 8-RSMP was highly unstable under UHT conditions and fouled immediately. These results showed that total protein content in RSMP influenced its ability to be UHT processed. The UHT run decreased with increasing protein content. Although other studies do not report the effect of increased total protein directly, similar results were obtained by Kastanas (1996), Prakash (2007), when the total solids of milk were increased.

The RSMP containing 3.25% protein showed high OHTC values as compared to 6.5, 7 and 7.5-RSMP during UHT processing (Fig 4A). This can be attributed to low TS and high amount of water in the sample, which in turn leads to high values of specific heat (C_p) (Singh, 2006, Toledo, 2007, Choi, 1986). OHTC values are directly proportional to C_p as shown in eq. 3.1. On average, values of OHTC during processing of 6.5-RSMP, 7-RSMP and 7.5-RSMP were lower than 3.25-RSMP. This also suggests that increased total solids and increased viscosity play a role in decreasing turbulence and heat transfer during UHT processing.

The OHTC vs run-time graph (Fig. 4A) shows that the OHTC remained almost constant for 3.25-RSMP throughout the run, whereas, for concentrated RSMP samples there was a gradual decrease in OHTC with increasing run-time after an induction period. The observations for 3.25-RSMP were consistent with results previously reported by Prakash (2007). This OHTC vs run-time behavior of

concentrated RSMPs was due to gradual fouling of heat transfer surfaces with milk solids, which offered more resistance to heat transfer as compared to clean surfaces, causing lower UHT temperatures. A fouling induction period of 25, 14 and 8 min was observed for 6.5-RSMP, 7-RSMP and 7.5-RSMP, respectively suggesting that fouling started faster in samples containing higher protein and higher total solids

In concentrated RSMPs milk proteins are relatively densely packed as compared to 3.25-RSMP. Increased protein content in concentrated RSMPs increases the chances of interactions between protein molecules leading to increase in amount of higher molecular mass β-lg aggregates (Bon et al., 1999). This can result in increased amount of voluminous Type A deposits (Tissier, 1984). These deposits will cause temperature drops and excessive fluctuations in back pressure to a point where back pressure could not be maintained at 0.4 kPa and UHT run had to be terminated. As 8-RSMP was extremely unstable to UHT processing, effect of protein content higher than 8% could not be studied using RSMP.

Further, RMPCs were processed to observe the effect of increased protein content on UHT behavior of high protein milk dispersions. RMPC samples showed high UHT stability as compared to concentrated RSMPs at much higher protein levels. RMPC with 8% protein was very stable during UHT processing (data not shown), therefore the amount of protein in samples was increased further. 10-RMPC and 14-RMPC samples showed no signs of fouling throughout the run-time of 120 min and there was an insignificant drop in OHTC (Fig 4B). The ethanol stability of 14-RMPC (86%) was significantly higher than that of 7.5-RSMP (54%) (Table 2). Heat stability behavior of milk protein dispersions when measured by HCT was also in agreement with their UHT stability. HCT for sample 7.5-RSMP (1.77 min) was low as compared to 14-RMPC (2.54 min) (Table 2). The UHT behavior of 14-RMPC-G prepared from MPC85 obtained from a different supplier showed similar UHT stability results. This was done to eliminate the possibilities of any differences

between UHT stability of reconstituted samples prepared from MPC85 manufactured by different manufacturers.

A distinguishing difference between RMPC and RSMP samples may be that RMPC samples had much lower TS (Table 1) than RSMP samples, which can lead to better heat stability under UHT conditions. High viscosities of concentrated samples can shift the fluid flow behavior from turbulent to laminar; which can cause low flow rates for layers of process fluid adjacent to the heat transfer surface, resulting in larger volume of material in contact with heating surface for longer period of time. This can lead to formation of larger volume of fouling deposits (Burton, 1994). However, 14-RMPC showed high UHT stability even though its viscosity was significantly ($P<0.05$) higher than 7.5-RSMP (Table 2). Therefore in order to look into the effect of total solids on UHT heat stability of milk protein dispersions, lactose was added to 7.5-RMPC (7.5-RMPC-LAC) to match TS of 7.5-RSMP.

7.5-RMPC-LAC showed a UHT run time of greater than 120 min (Fig 3). Also, the OHTC over the run time of 7.5-RMPC-LAC was similar to 14-RMPC and much higher than 7.5-SMP (Fig 4C). Ethanol stability and HCT of 7.5-RMPC-LAC were significantly ($P<0.05$) higher than that of 7.5-SMP (Table 2) and were similar to that of 14-RMPC. Hence, it could be concluded that 7.5-RMPC-LAC had much higher UHT stability than 7.5-SMP at same TS content and could be processed without fouling.

The results suggested that milk constituents other than proteins, such as milk minerals (in particular calcium), may be responsible for the differences in UHT stability and susceptibility to fouling of RMPCs and RSMPs. High calcium ion activity has been associated with decreased UHT stability of milk products (Singh, 2004). However, 14-RMPC had significantly ($P<0.05$) higher ionic calcium activity as compared to 7.5-RSMP (Table 2), which does not correlate with UHT stability of these

two high protein samples. Hence, calcium ion activity alone could not be the dominating factor explaining the differences between the UHT stability of these samples. Similar results on ionic calcium and heat stability behavior of milk protein concentrate suspensions (MPC80) was reported by Crowley et al. (2015) .

To investigate the synergetic effect of milk proteins, lactose and milk minerals on UHT stability of RMPC, an MPC dispersion (7.5-RMPC-LAC-SMUF) containing same amount of proteins, lactose and mineral salts as 7.5-RSMP was prepared. The amount of SMUF used in 7.5-RMPC-LAC-SMUF was calculated on the basis of matching total calcium of this sample to 7.5-RSMP, which also closely matched the amount of lactose and other milk minerals such as magnesium, phosphorous etc. in these two samples (Table 2). The calcium ion activity of 7.5-RMPC-LAC-SMUF was found to be 1.30 mM, which was very close to that of 7.5-RSMP (1.36 mM). During UHT processing, 7.5-RMPC-LAC-SMUF showed 66 min run-time on average (Fig 3). The sample showed an induction time of 21 min, after which frequent temperature and back pressure fluctuations were observed (Fig 4C) and the UHT run had to be terminated after 66 min due to fouling. The induction period of 21 min showed by 7.5-RMPC-LAC-SMUF was very close to total run-time of 23 min observed for 7.5-RSMP.

Ethanol stability and HCT of RMPC reduced markedly after addition of milk minerals; 7.5-RMPC-LAC-SMUF showed ethanol stability (59%) and HCT (1.51 min) similar to 7.5-SMP (Table 2). The study conducted by Crowley et al. (2015) on heat stability behavior of RMPCs containing 8.5% protein also showed that HCT of reconstituted MPC35 (composition closely matching to an SMP) at pH 6.8 was lower than that of reconstituted MPC80; however, UHT stability was not studied. Ethanol stability test is used to determine casein micelle stability. Ethanol collapses the κ-casein hairy layer on the casein micelle surface and its function of steric stabilization is lost, leading to

casein micelles coagulation (Horne, 1984). The ethanol stability results were similar to UHT stability of milk protein dispersions studied.

PSD data (performed on whole sample) of UHT processed samples demonstrated formation of larger size particles in 7.5-RSMP as compared to 14-RMPC (Fig 5). Almost all the particles in UHT processed 14-RMPC were of sub-micron size (0.23 μm D(0.9)) as compared to 7.5-RSMP (1.84 μm D(0.9)) as shown in Table 3. These differences in particle size were significant and were possibly due to the differences the mineral environment of the samples. The large particles could be formed from whey protein interactions amongst themselves and or with caseins to for whey protein-casein aggregates. The differences in the mineral environment can affect the size of whey protein aggregates formed during UHT treatment and can lead to formation of larger aggregates in case of RSMP and RMPC-LAC-SMUF (Havea et al., 2002, Crowley et al., 2015).

We further investigated the effect of mineral environment on protein dissociation in samples before and after UHT processing. RP-HPLC analysis was performed on supernatants of unheated and UHT processed 7.5-RSMP, 14-RMPC and 7.5-RMPC-LAC-SMUF samples (Fig 6). The RP-HPLC data showed that in all three samples, β-lg was completely aggregated after UHT processing (Fig 6A) and was absent from the non-sediemtable fraction. Additionally more than 75% of α-la was aggregated in all three samples (Fig 6B). Crowley et al. (2015) also showed that the difference in amount of non-sedimentable whey proteins in heated reconstituted MPC80 and MPC35 was not significant. But it is possible that the types of aggregates formed from these non-sedimentable whey proteins upon heating are responsible for differences in UHT stability of RMPC and RSMP samples as described above.

Significant differences were observed in the non-sedimentable caseins between 14-RMPC, 7.5-RSMP-UHT and 7.5-RMPC-LAC-SUMF before and after UHT treatment. This is interesting

because the stability of casein micelles during UHT processing could be another factor governing UHT stability of RMPC samples. It was observed from RP-HPLC data that unheated samples of 14-RMPC had significantly ($P<0.05$) higher amounts of dissociated caseins as compared to 7.5-RSMP and 7.5-RMPC-LAC-SMUF (Fig 6C-E).

Non-sedimentable protein content in all three samples was similar after UHT processing, however, when comparing unheated samples to UHT treated samples, it slightly increased in 7.5-RSMP and significantly ($P<0.05$) increased 7.5-RMPC-LAC-SMUF after UHT processing whereas it significantly ($P<0.05$) decreased in 14-RMPC (Fig 6F). This may suggest that casein micelles in 7.5-RSMP and 7.5-RMPC-LAC-SUMF were more unstable to UHT treatment than in 14-RMPC. It could be possible that in 14-RMPC the initially dissociated caseins might have deposited on heated surfaces initially (Santos et al., 2003), but there was not much further dissociation of caseins during UHT processing to participate in formation of large aggregates. In 7.5-RSMP higher dissociation of casein micelles during UHT treatment might have happened, bringing its post UHT non-sedimentable protein content almost similar to heated 14-RMPC. These UHT induced dissociated caseins might have led to formation of large aggregates due to whey-casein aggregation via κ-casein-β-lg interactions or aggregation of unstable casein micelles (Anema and Li, 2003, Ono et al., 1999). Smaller protein aggregates formation observed in UHT processing of MPC could be related to its altered mineral environment during manufacturing (Crowley et al., 2015).

The ethanol stability data (Table 2) also showed that 14-RMPC had higher ethanol stability than 7.5-RSMP, implying higher casein micelle stability in 14-RMPC. As RP-HPLC results (Fig 6E) showed that κ-casein content in all three UHT processed samples was similar it can be concluded that either electrostatic interactions between caseins or extent of collapse of κ-casein hairy layer and loss of steric stabilization during UHT processing of these protein dispersions were influenced by soluble salts in the serum phase (Horne, 2016). As stated above, mineral environment of RSMP appeared to be favorable to start rapid interactions of casein micelles as observed by Horne (1984),

Horne and Parker (1981) and indicated by low ethanol stability shown by RMPC with added SMUF (7.5-RMPC-LAC-SMUF).

It was observed that milk proteins behaved differently in different mineral environments. Milk protein dispersions prepared from RMPC formed submicron particles after UHT treatment as shown by PSD and dissociation of caseins was limited in RMPC mineral environment as compared to RSMP as shown by RP-HPLC and ethanol stability data. The similarity in UHT behavior of 7.5-RSMP and 7.5-RMPC-LAC-SMUF and drop in UHT stability of RMPC after addition of minerals was an indicator that total milk mineral environment plays a crucial role in UHT stability of high protein dispersions. Effect of UHT temperatures on milk protein stability, changes in protein state and their interactions with milk minerals during heating of milk protein dispersions prepared from these two different milk protein powders can be an important factor in determining their fouling behavior. This suggest that difference in mineral composition of MPC powder from SMP due to ultrafiltration process can be an important factor causing its high heat stability. During SMP manufacturing all the milk minerals are retained in the final product, however during membrane filtration process employed during manufacturing of MPC, free ions pass through the membrane and protein is retained, which increases the volume fraction of caseins and changes ratio between soluble and colloidal minerals (Dalgleish and Corredig, 2012). This also alters the casein inter-micelle interactions. Mineral composition of aqueous phase has also been found to have a significant role on physicochemical properties and heat stability of reconstituted casein micelles (Le Ray et al., 1998). The higher instability of 7.5-RMPC-LAC-SMUF compared to 7.5-SMP is being further investigated.

4 Conclusion

MPC is an important ingredient of milk protein based beverages, however, there is little known about their UHT stability. MPC reconstituted to 14% protein showed significantly higher UHT

stability as compared to SMP reconstituted at 7.5% protein, although the ionic calcium activity and viscosity of the former was higher than the later. The lower UHT stability of RSMP can be related to larger protein aggregate formation and destabilization of casein micelles in 7.5-RSMP at UHT temperatures. High UHT stability of milk protein dispersions made from high protein milk powder, such as MPC85, can be due to the ultrafiltration processing used during their manufacturing, which causes them to have a modified mineral composition as compared to SMP. The UHT instability of mineral readjusted MPC85 even at 7.5% protein concentration suggested that the total mineral composition is responsible for fouling of high protein SMP suspension. Further investigation is underway to explore the effect of changes in mineral composition on UHT behavior of MPC powders at different protein concentrations.

Acknowledgement

The authors acknowledge support provided by "Australian Government Research Training Program Scholarship" and "University of Queensland Centennial Scholarship".

Declarations of interest: none

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Figure Captions

Figure 1: Flow diagram of bench top UHT plant

Figure 2: SDS-PAGE analysis of milk powders, Lane: 1. Molecular weight standards, 2. Low Heat SMP (Non-Reduced SDS-PAGE), 3. MPC (Non-Reduced SDS-PAGE), 4. Low Heat SMP (Reduced SDS-PAGE) and 5. MPC (Reduced SDS-PAGE)

Figure 3: The average UHT run times on a bench top UHT tubular heat exchanger during processing of milk protein dispersions. Error bars represent standard deviation, n=2. Means with different letters are significantly different ($P<0.05$). Samples with ↑ did not foul in 120 min.

Figure 4: Variation in OHTC with run time for milk protein dispersions, (A) milk protein dispersions prepared using RSMP, (B) milk protein dispersions prepared using RMPC, (C) comparison of 7.5-RSMP with 7.5-RMPC with added lactose and SMUF. Representative data of duplicate runs is presented here.

Figure 5: Particle size distribution of UHT processed milk protein dispersions. Representative data of four measurements.

Figure 6: Effect of UHT processing of milk protein dispersions on non-sedimentable milk proteins (data shown as percentage of non-sedimentable protein of total individual milk protein present in the sample) . (A) β-lg , (B) α-la, (C) αs1-casein, (D) β-casein (E) κ-casein and (F) Total non-sedimentable protein. Error bars represent standard deviation, n=2. UN= unheated sample, UHT= UHT treated sample.

Table 1: Description of reconstituted milk protein dispersions used

Sample	Ingredients	Protein content (%w/w)	Total solids (% w/w)
3.25-RSMP	SMP	3.25	10.00
6.5-RSMP	SMP	6.50	20.00
7-RSMP	SMP	7.00	21.53
7.5-RSMP	SMP	7.50	23.07
8-RSMP	SMP	8.00	24.61
10-RMPC	MPC	10.00	12.27
14-RMPC	MPC	14.00	17.17
16-RMPC	MPC	16.00	19.63
14-RMPC-G	MPC-G	14.00	17.17
7.5-RMPC-LAC	MPC and Lactose	7.5	23.07
7.5-RMPC-LAC-SMUF	MPC, lactose and mineral salts	7.5	23.07

Table 2: Calcium ion activity, ethanol stability, viscosity, HCT, lactose and major milk minerals of selected samples

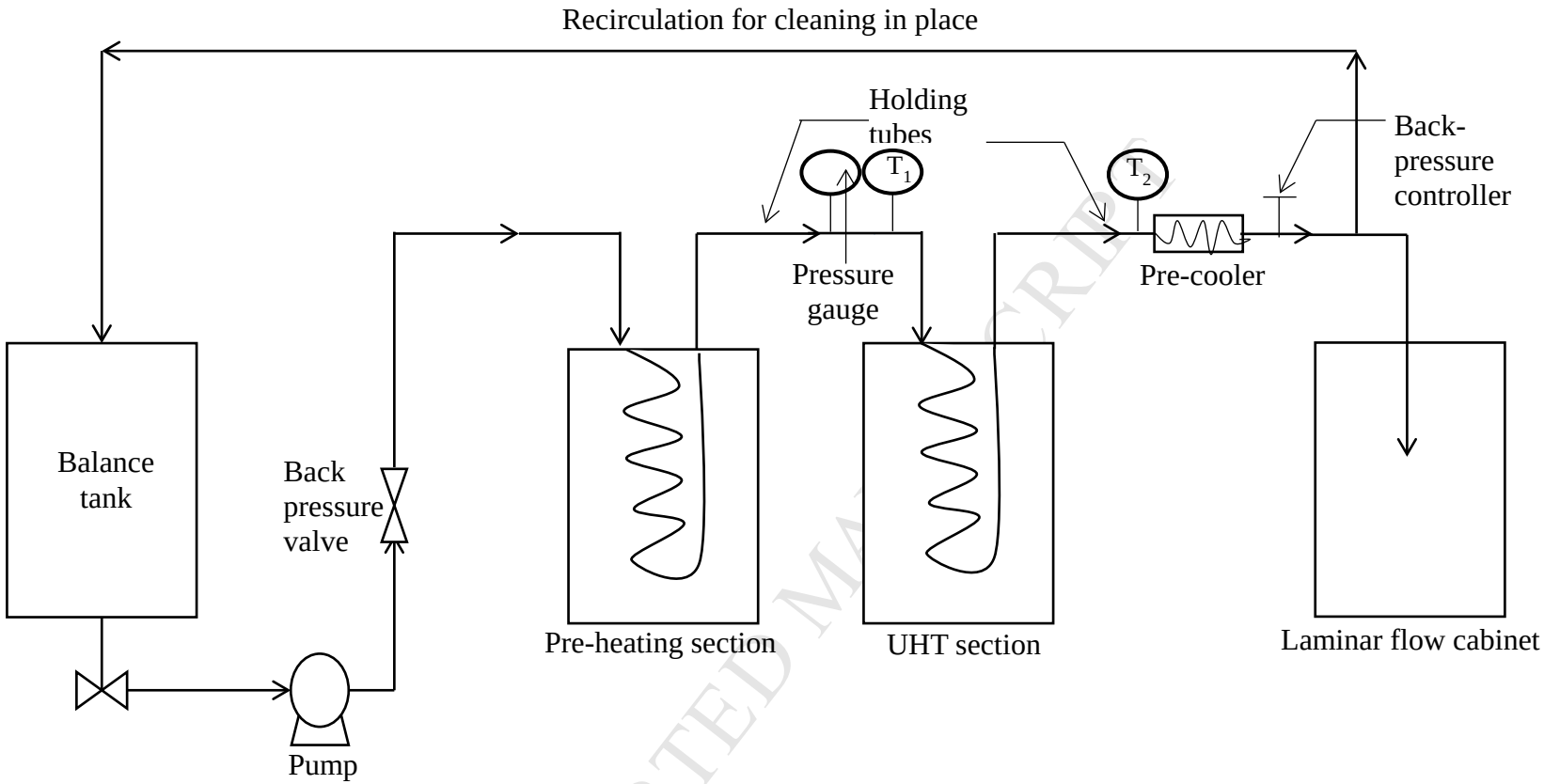
Sample	Calcium ion activity* (mM)	Ethanol Stability* (%)	Viscosity* (mPa.s)		HCT* (min)	Lactose (% w/w)	Ca# (% w/w)	P# (% w/w)	Mg# (% w/w)	Cl# (% w/w)	K# (% w/w)
	UN	UN	UN	UHT							
7.5-RSMP	1.36±0.01^{b}	54.00±0.00^{d}	6.79±0.64^{c}	12.97±1.66^{b}	1.77±0.16^{c}	12.69	0.34	0.24	0.03	0.14	0.30
14-RMPC	1.98±0.02^{a}	86.00±0.00^{b}	32.43±0.89^{a}	34.74±4.36^{a}	2.54±0.11^{b}	0.86	0.38	0.25	0.02	0.03	0.06
7.5-RMPC-LAC	2.00±0.13^{a}	88.00±0.00^{a}	8.85±0.49^{c}	5.50±0.03^{c}	2.82±0.16^{a}	14.73	0.20	0.14	0.01	0.01	0.03
7.5-RMPC-LAC-SMUF	1.30±0.02^{b}	59.00±0.89^{c}	19.20±2.82^{b}	7.53±0.46^{bc}	1.51±0.10^{d}	12.56	0.36	0.28	0.04	0.35	0.58

*All results are expressed as the mean ± standard deviation (n = 4). Means in a single column with different superscripts are significantly different (P<0.05)
#Values derived from mineral composition of milk powders used to prepare the samples. (except for sample 7.5-RMPC-LAC-SMUF values measured using ICP-OES)
UN= unheated sample, UHT= UHT treated sample

Table 3: Comparison of volume weighted mean diameter, Surface weighted mean diameter and particle size distribution for 7.5RSMP and 14-RSMPC

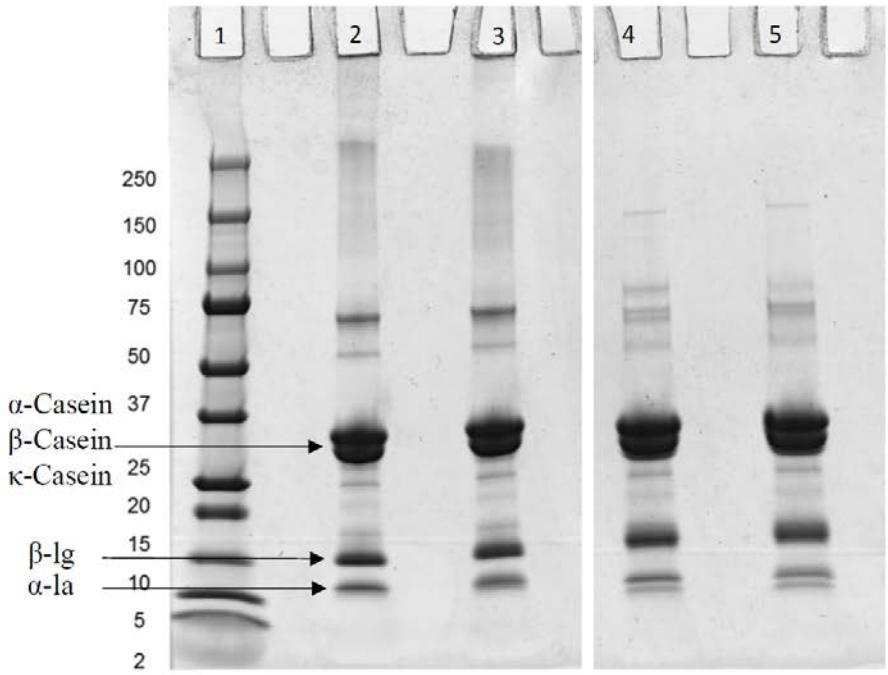
Sample	D[4,3] (μm)	D[3,2] (μm)	D(0.1) (μm)	D(0.5) (μm)	D(0.9) (μm)
7.5-RSMP	1.12±0.50	0.16±0.01	0.08±0.00	0.19±0.01	1.84±1.38
14-RMPC	0.50±0.17	0.12±0.00	0.08±0.00	0.13±0.00	0.23±0.00
7.5-RMPC-LAC-SMUF	22.43±2.99	1.40±0.30	0.62±0.10	1.39±0.29	74.71±4.49

All results are expressed as the mean ± standard deviation (n = 4). Means in a single column with different superscripts are significantly different ($P<0.05$).



T_1 - Thermocouple at the inlet of UHT section
T_2 - Thermocouple at the outlet of UHT section

Figure 1: Flow diagram of bench top UHT plant



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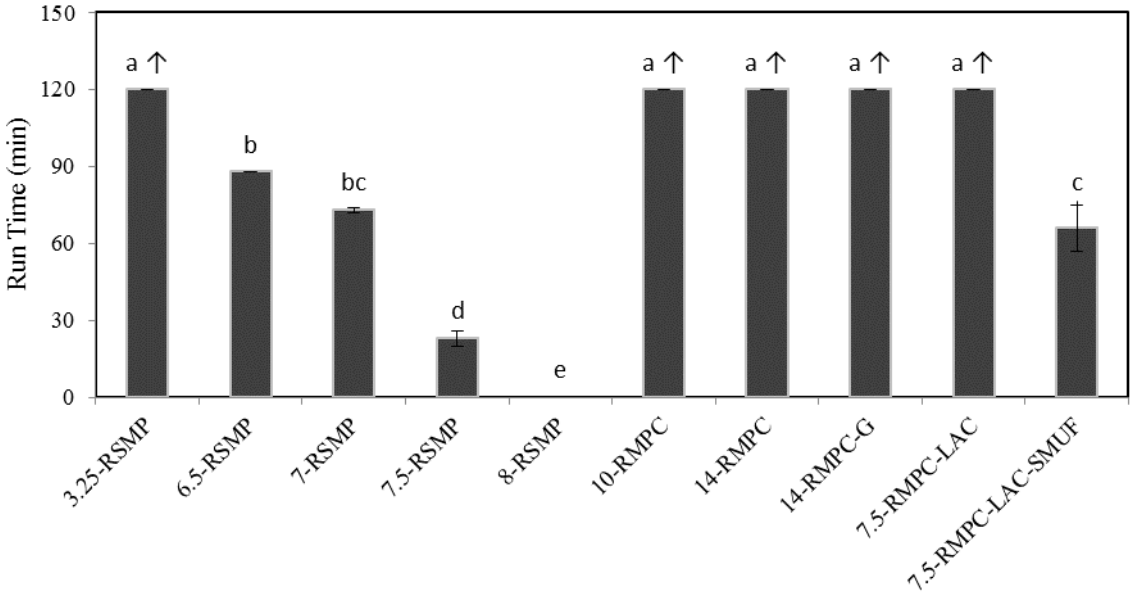

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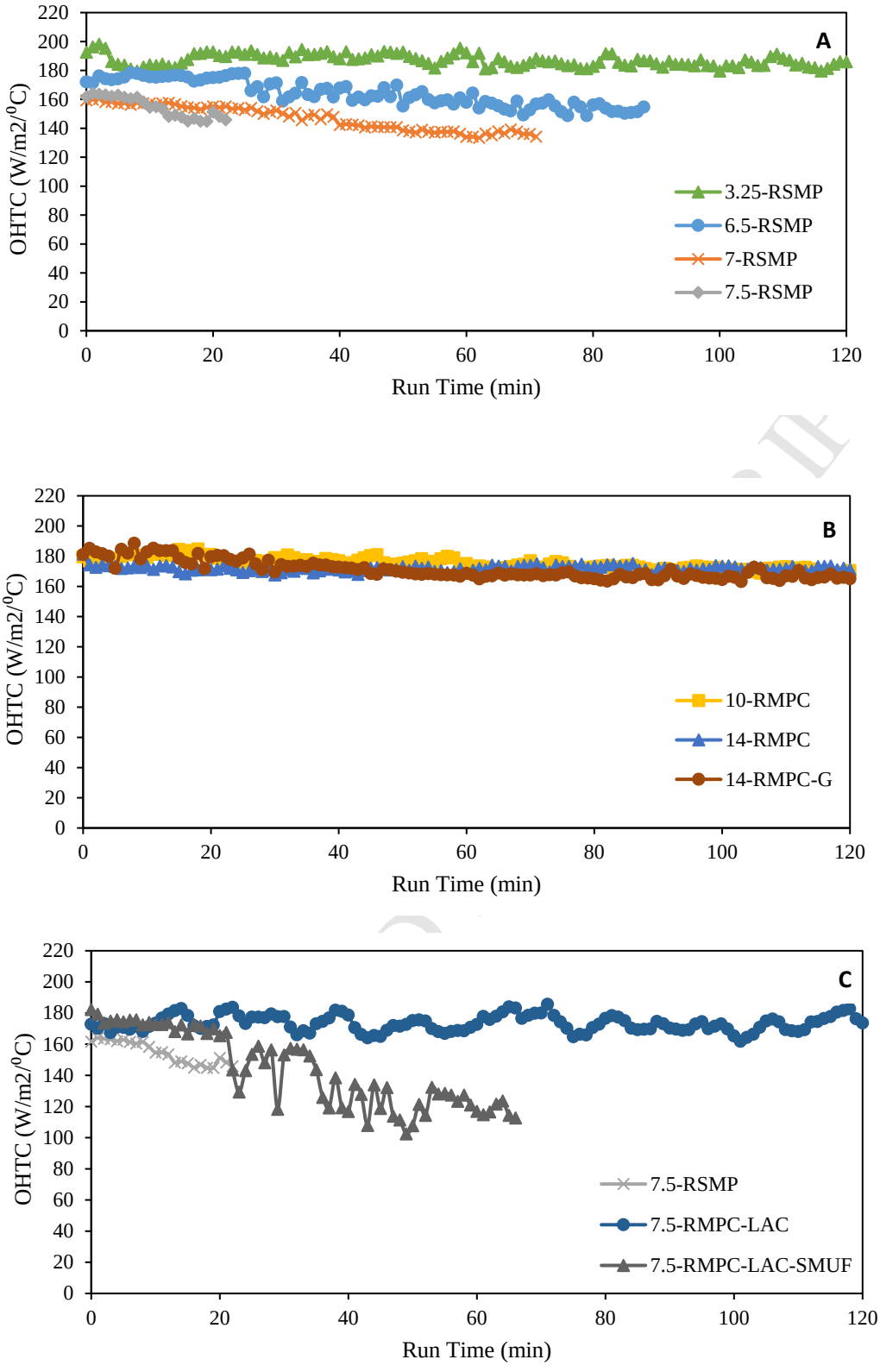

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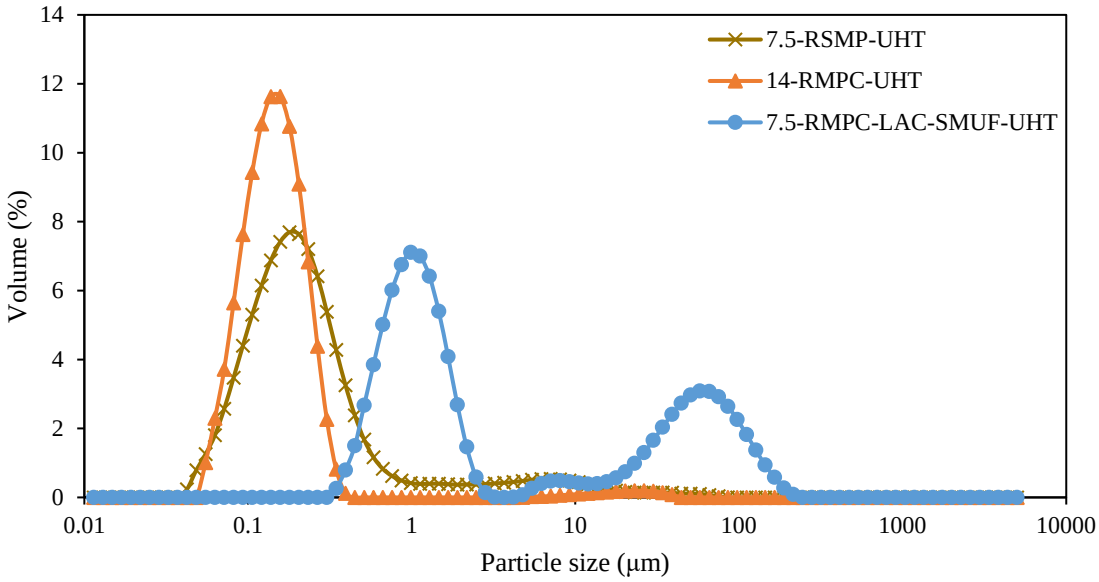

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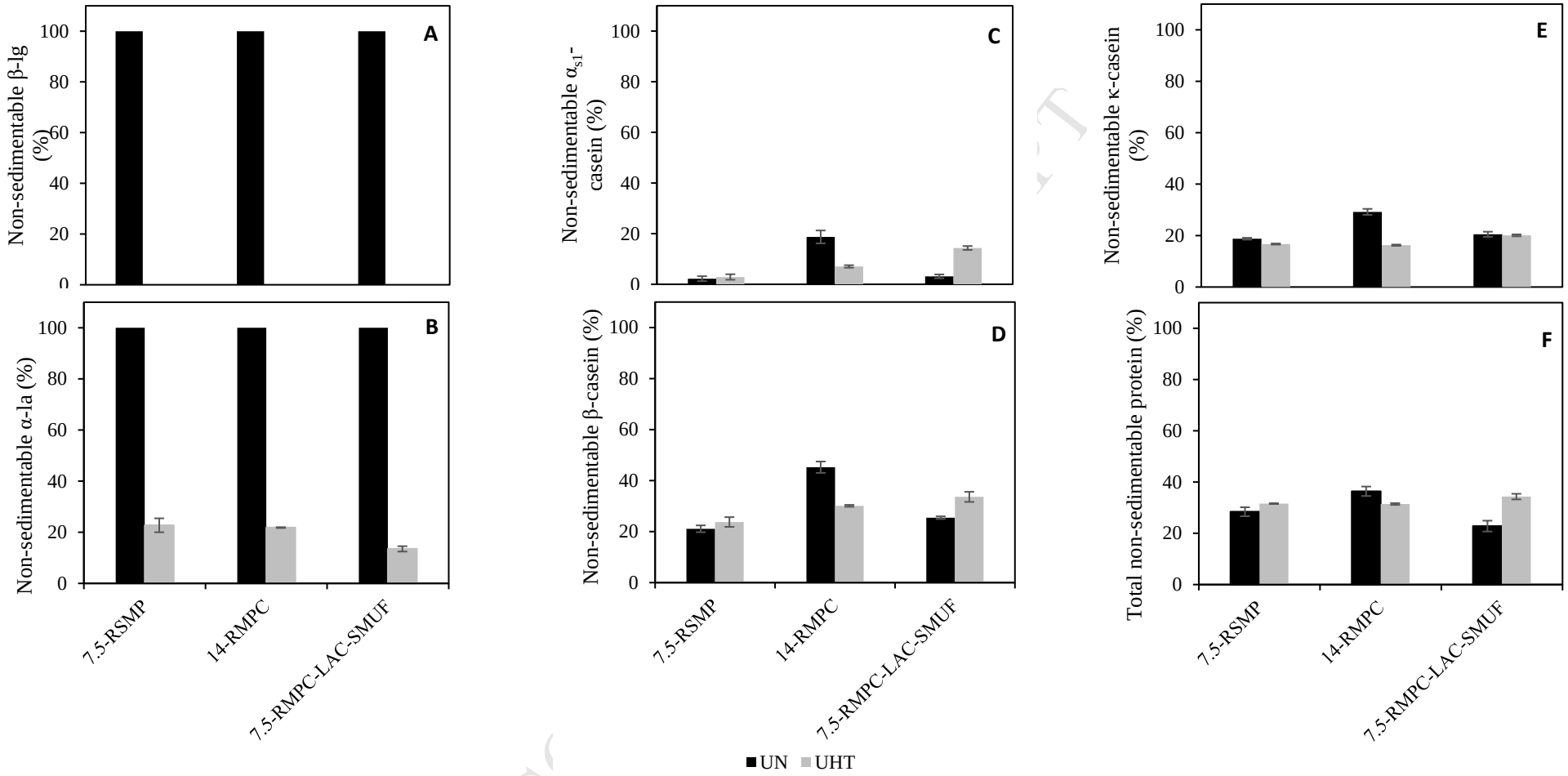

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Highlights:

- MPC has higher UHT stability than SMP even at higher protein content.
- Higher viscosity and higher ionic Ca did not cause fouling in MPC.
- Total mineral balance affected the UHT behaviour of high protein milk dispersions.
- Larger protein aggregates caused lower UHT stability of SMP.