



Short communication: Effect of pH on the heat stability of reconstituted reduced calcium milk protein concentrate dispersions

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ABSTRACT

This study aimed to investigate the heat stability of dispersions from reconstituted reduced-calcium milk protein concentrate (RCMPC) with 80% protein or more. The tested RCMPC powders were produced from skim milk subjected to CO₂ treatment before and during the process of ultrafiltration. The CO₂ injection was controlled to obtain 0 (control, no CO₂ injection), 20, 30, and 40% reduction in calcium levels in the RCMPC powders. The RCMPC powders were reconstituted to 10% (wt/wt) protein in deionized water. These dispersions were tested for heat stability in a rocking oil bath at 140°C at unadjusted, 6.5, 6.7, 6.9, and 7.1 pH. Calcium ion activity (CIA) and ionic strength measurements were carried out using a Ca ion-selective electrode and conductivity meter. Unadjusted pH of the dispersions varied from 6.8 in control to 5.96 in 40% RCMPC dispersions. The CIA of unadjusted dispersions ranged from 1.31 mM in control to 2.83 mM in 40% RCMPC. Heat stability, expressed as heat coagulation time (HCT) of unadjusted dispersions decreased as the level of Ca removal in powders increased (from 13.81 min in control to 0.46 min in 40% RCMPC) and was negatively correlated with the CIA of the dispersions. For control RCMPC dispersions, the minimum and maximum heat stability were observed at dispersion pH of 6.5 and 6.9, respectively, followed by a decrease at pH 7.1 (CIA was the lowest). Dispersions from 40% RCMPC and pH 7.1 had the maximum HCT of 30.94 min among all RCMPC dispersions at all pH values. From this study, it can be concluded that improved heat stability in high protein formulation beverages subjected to UHT processing could be achieved through calcium reduction in milk protein concentrates using CO₂ injection.

Key words: carbon dioxide injection, reduced-calcium milk protein concentrate, calcium ion activity, heat stability

Short Communication

Milk protein concentrates (MPC) are second-generation dairy ingredients that contain caseins and whey proteins in the same ratio as in starting milk. They are typically produced by the partial removal of water, lactose, and minerals from skim milk using membrane filtration, nanofiltration, and spray drying. Milk protein concentrates with varying protein concentrations from 40 to 90% are available for commercial sale. Milk protein concentrates with a protein content of ≥90% are referred to as milk protein isolates. A pleasant dairy flavor, relatively lower carbohydrate content, and functional properties such as low viscosity, high solubility, water binding, foaming, heat, and emulsion stability make MPC a preferred high-protein ingredient in several food applications (Augustin et al., 2011). Typically, MPC with protein content up to 50% are used in cheese, yogurt, dairy desserts, salad dressing, and sauces. In comparison, MPC80 and above (>80% protein on DM basis) are used in ready-to-drink nutritional beverages and bars (Patel et al., 2014). Higher-protein MPC powders such as MPC80 undergo a loss of solubility during shelf life. A strong negative correlation between calcium content and MPC solubility was established by Marella et al. (2015). Unit operations such as high-pressure homogenization, extrusion-porosification, microfluidization, and ultrasonication of liquid concentrate before spray drying, mineral modification by the addition of monovalent ions during diafiltration, and cation exchange are different options to improve the functionality of MPC powders. Injection of CO₂ before and during the UF process is an approach that has been studied to reduce the Ca content of the powders to improve MPC functionality (Marella et al., 2015).

Heat stability of milk is known to be influenced by ionic strength, milk salts, and milk proteins (Singh and Creamer, 1992). The reduction of Ca content to improve solubility during storage of MPC powders could also help with improving the heat stability of dispersions containing reduced-calcium MPC powders. High-protein beverage applications involve the reconstitution

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of MPC80 powders, followed by thermal treatments such as UHT and retort processing, making heat stability an important criterion for the selection of MPC powders. Several studies have established the role of calcium in the heat stability of milk and the relationship between heat coagulation time (HCT) and pH (Singh, 2004). High calcium ion activity (CIA) has a negative effect on the heat stability of unconcentrated and concentrated milk systems (Philippe et al., 2003; Sievanen et al., 2008). Alteration of ionic balance during the manufacture of MPC powders and changes to Ca equilibrium during reconstitution of MPC powders result in dispersions with a different ionic calcium content (Ca^{2+}) compared with skim milk (Crowley et al., 2014). The pH of the dispersion also influences the ionic Ca^{2+} in the reconstituted MPC dispersion. Therefore, reducing Ca content during MPC manufacture through CO_2 injection (to aid in superior reconstitution behavior during the powder shelf life) and adjusting the pH of the reconstituted dispersion may influence the heat stability of dispersions when reconstituted and subjected to high thermal treatments.

The aim of our study was to evaluate the effects of different levels of Ca reduction in MPC powders (RCMPC), by controlling CO_2 injection during their manufacture, on the heat stability of the resultant reconstituted MPC dispersions. In the reported study, heat stability and CIA of RCMPC80 dispersions were studied at 4 levels of calcium reduction (0, 20, 30, or 40%) of RCMPC80 powders and 5 levels of dispersion pH (unadjusted or pH 6.5, 6.7, 6.9, or 7.1). The RCMPC powders were produced 3 times from a fresh lot of skim milk. The RCMPC80 powders were prepared from skimmed pasteurized milk (72°C for 15 s) at $\sim 10^\circ\text{C}$ by injecting CO_2 prior to and during UF using 10-kDa spiral-wound polyethersulfone membranes (Marella et al., 2015). Skim milk was injected with 2,200 ppm (mg/L) of dissolved CO_2 before UF, and the level was maintained during the UF process with additional injection. The retentate was spray dried and stored until further use. The composition of the RCMPC80 powders is given in Table 1. The RCMPC80 powders were reconstituted to 10% protein in demineralized water,

stirred for 20 min, and left overnight in a refrigerator. Sodium azide was used as a preservative (0.02%, wt/wt). Samples were equilibrated to 30°C in a water bath following overnight storage. Apart from a sample set that was unadjusted for pH, the pH of the dispersions was adjusted to either 6.5, 6.7, 6.9, or 7.1 using 0.1 M NaOH or 0.1 M HCl solution. Samples were allowed to equilibrate for 2 h, followed by minor, if any, readjustments of pH. The CIA and ionic strength of the all the RCMPC80 dispersions were measured using an Orion Versa Star calcium ion-selective electrode and an Orion Versa Star conductivity module attached to an Orion Versa Star meter (Thermo Fisher Scientific, Waltham, MA). The instrument was calibrated, and standard curves for ionic strength and CIA were developed using KCl at 10, 20, 40, 60, and 80 mM concentrations and CaCl_2 at 0, 0.25, 0.5, 1.0, 2.0, and 3.0 mM levels, respectively, and the same were used for calculations. Heat stability of RCMPC80 dispersions was determined by HCT, the time in minutes required for the dispersion to visually coagulate at 140°C (Singh, 2004). To measure HCT, 3 mL of dispersion in a securely capped glass tube was placed on a clamp frame connected with the rocking arm in an oil bath maintained at 140°C , and the time taken to develop visual coagulation was recorded (Sutariya and Patel, 2017; Pandalaneni et al., 2018).

Reducing the Ca content of MPC powders by 40% markedly improved the heat stability of the dispersions at pH values of 6.9 and 7.1. The mean HCT and CIA values of RCMPC80 dispersions with different Ca reduction and at different pH levels are summarized in Table 2. The unadjusted pH of the RCMPC80 dispersions was observed to decrease with greater reduction of Ca (6.72 at 0% Ca reduction to 5.96 at 40% Ca RCMPC80) and resulted in an increase in CIA of dispersions that ranged from 1.17 to 2.83 mM. Injection of CO_2 to milk decreases the pH (Ma et al., 2003), increasing soluble calcium that, during the process of ultrafiltration and diafiltration, is partitioned into the permeate.

Control MPC80 dispersions had maximum and minimum HCT at pH 6.9 (20.29 min) and 6.7 (13.02 min),

Table 1. Composition of reduced-calcium milk protein concentrate (RCMPC) powders; values are means of 3 replicate experiments

RCMPC	Protein (%, wt/wt)	Lactose (%, wt/wt)	Ash (%, wt/wt)	Calcium (%, wt/wt)
Control	77.02	6.41	6.88	2.16
20% Ca reduction	77.57	6.46	5.81	1.84
30% Ca reduction	77.82	6.88	4.95	1.48
40% Ca reduction	78.08	7.93	4.67	1.34

Table 2. Mean ($n = 3$) heat coagulation time (HCT) and calcium ion activity (CIA) values of reconstituted reduced-calcium milk protein concentrate (>80% protein on DM basis) powder dispersions (10% protein) at different pH levels¹

Ca reduction (%)	HCT (min)					CIA (mM)				
	UA	pH 6.5	pH 6.7	pH 6.9	pH 7.1	UA	pH 6.5	pH 6.7	pH 6.9	pH 7.1
0	13.8 ^{b,A}	14.13 ^{b,A}	13.02 ^{bc,B}	20.29 ^{a,A}	8.37 ^{c,C}	1.17 ^{b,B}	2.09 ^{a,A}	1.24 ^{b,A}	1.09 ^{b,A}	0.8 ^{c,A}
20	6.53 ^{b,B}	11.7 ^{ab,A}	20.73 ^{a,A}	20.35 ^{a,A}	8.54 ^{b,BC}	1.84 ^{a,B}	1.47 ^{ab,B}	1.15 ^{bc,A}	1.08 ^{bc,A}	0.74 ^{c,A}
30	4.83 ^{c,B}	13.54 ^{b^c,A}	25.27 ^{a,A}	27.76 ^{a,A}	24.25 ^{ab,AB}	1.92 ^{a,B}	1.42 ^{b,B}	1.07 ^{bc,B}	1.13 ^{bc,A}	0.78 ^{c,A}
40	0.46 ^{d,C}	10.41 ^{c,A}	21.18 ^{b,B}	17.86 ^{bc,A}	30.94 ^{a,A}	2.83 ^{a,A}	1.38 ^{b,B}	1.02 ^{bc,B}	0.95 ^{bc,A}	0.68 ^{c,A}

^{a-d}Mean values within the same row corresponding to HCT and CIA not sharing a common superscript are significantly different ($P < 0.05$).

^{A-C}Mean values within the same column corresponding to HCT and CIA not sharing a common superscript are significantly different ($P < 0.05$).

¹UA = unadjusted dispersion pH from 0, 20, 30, and 40% reduced-calcium milk protein concentrate (>80% protein on DM basis) powders were 6.72, 6.28, 6.17, and 5.96, respectively.

respectively. A local minimum and a maximum in HCT are typical for Type A milk. The HCT decreased as the pH increased to 7.1. A decrease in CIA was observed in dispersions as the pH of the dispersions increased from 6.5 to 7.1 (from 2.09 mM to 0.8 mM). For dispersions from 20% RCMPC80, a similar trend to that of the control MPC dispersion was observed. Heat stability was least at pH 7.1, and maximum HCT was seen in the pH range of 6.7 to 6.9. The CIA also decreased as the pH of dispersions increased (from 1.84 mM to 0.74 mM). A minimum observed in HCT of control and 20% reduced RCMPC dispersions at pH 7.1 could be due to extensive dissociation of κ -caseins from the micelles combined with sufficiently high Ca ion activities (Crowley et al., 2014). Dispersions from 30% RCMPC80 powders showed no significant difference in HCT from pH 6.7 onward. The CIA of the dispersions decreased with increasing pH. Dispersions from 40% RCMPC80 powders showed an HCT profile with a maximum at pH 6.7 and a minimum at pH 6.9, with a further increase in HCT with an increase in pH. The CIA decreased with an increase in pH, a trend observed across all RCMPC dispersions. In milk, it is hypothesized that HCT is influenced by 2 different reactions: colloidal reaction and chemical cross-linking (Walstra et al., 2005). This hypothesis can be extended to MPC dispersions from 40% RCMPC80 powder, where it can be assumed that colloidal reaction drives the rate-determining step for heat coagulation due to dissociation of casein micelles and depletion of κ -caseins up to a pH of 6.9. At pH values >6.9, increased micellar charge and low Ca^{2+} activity could lead to an increase in HCT.

To summarize, in RCMPC powder dispersions, an interplay of colloid reactions (CIA, ionic composition, and dissociation of caseins) induced by a combination of CO_2 treatment and UF processes and chemical cross-linking can lead to differences in casein aggregation

behavior on heating. With an increase in Ca removal during the manufacture of MPC, casein micelle dissociation and changes to the serum component (decrease in soluble calcium, phosphates, and citrates) can influence the heat stability of RCMPC dispersions. The increase in the CIA of the unadjusted pH RCMPC80 dispersions with increasing Ca reduction (and corresponding pH shift toward the acidic side) can be explained by a lower pH. The CIA of control MCP80 (0% Ca reduction) was highest at pH 6.5 (2.09 mM) and continued to decrease with an increase in dispersion pH, (0.80 mM at pH 7.1). A similar trend of decreasing CIA values with increasing pH was observed in the case of RCMPC80 dispersions across levels of calcium reduction (20, 30, and 40% reduction), with CIA being the lowest at pH 7.1 (0.74, 0.78, and 0.68 mM for 20, 30, and 40% Ca reduction, respectively).

Thermal stability during processing (UHT and retort) as well as storage stability of beverages containing MPC is an important functional attribute for consumers and processors. As the application of MPC powders in high-protein dairy beverages is on the rise, this study provides valuable insights into the thermal behavior of Ca-reduced MPC powders in high-protein dispersions. An increase in heat stability with the use of 30% Ca-reduced MPC powder and pH adjustment to ≥ 7.1 in formulations can help with an increased run time of thermal processing equipment (an important economic consideration) apart from potentially improved storage stability in high-protein beverages.

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