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Research Article

Effect of Calcium Chelators on Improving the Heat Stability of Liquid Micellar Casein Concentrate

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Abstract

Micellar casein concentrate (MCC) (18.5% w/w protein) were treated with varying concentration of sodium phosphate (SP) and trisodium citrate (TSC) in the range of 1 to 10 mM. The samples were analyzed for heat stability and particle size. The MCC samples treated with > 5 mM TSC showed significant improvement in the heat stability, and reduction in particle size as the TSC concentration increased from 7.5 to 10 mM. The MCC samples treated with TSC (7.5 and 10 mM) and SP (10 mM) remained in the liquid state after heat treatment at 140°C, whereas the control samples were coagulated. The improvement in the heat stability of MCC treated with TSC and SP solution was attributed to the ability of these calcium chelators to reduce the calcium ion activity by forming the complex with calcium and hence reducing the calcium induced aggregation of protein.

Keywords: *Micellar Casein Concentrate; Protein; Heat Stability; Calcium Chelator; Sodium Phosphate; Trisodium Citrate; Dynamic Light Scattering; Particle Size*

Introduction

Caseins are considered as one of the important functional and nutritional ingredient constituents in food applications. Casein based ingredients has a wide application for dairy, beverages, bakery, nutraceutical and meat industry to provide various functionalities such as emulsification, foaming, whipping and water-binding. Typical application of MCC is in high-protein formulation, low-lactose drinks and cheese making, including production of low-fat cheddar cheese. Nutritional value of casein and its textural properties further supports its application as a food ingredient [1-3].

Traditionally most of the commercial casein based ingredients are produced by rennet coagulation or acid precipitation, where casein micelles are destabilized and their native micellar structure is disrupted [1]. Through these treatments, casein micelles structure has been irreversibly changed from their native colloidal structure into spherical or linear aggregates [4,5]. Now with advanced development in microfiltration technology (MF) has made it possible to separate casein by microfiltration (MF) of skim milk where native micellar structure is maintained. Since the 1990s, MCC with casein levels ranging from 7 to 20% has been produced from skim milk using MF [6-10]. The retentate of skim milk in MF is called micellar casein concentrate (MCC). Using combination of MF, diafiltration with added water and ultrafiltration (UF) higher level of casein content in liquid MCC can be obtained. The liquid MCC are generally spray dried to produce powder.

MCC is an attractive ingredient to the food industry due to the partial removal of the serum phase (serum proteins, soluble minerals, lactose) components [11]. The powder form of MCC has a various advantages such as longer shelf-life without refrigeration, lower trans-

portation cost and ease of storage. However, Spray dried MCC powder are very difficult to re-solubilize [8,12]. The poor solubility can be a limiting factor for using these ingredients in few end product applications. Also it has been suggested in previous research that the spray drying may have some adverse effects on functional and flavor properties of dried ingredients [13]. Moreover, spray drying is the costly processing step in the manufacture of dried protein ingredients [14]. Also, The MCC powder needs to be reconstituted to use in food applications which is an extra step in the manufacturing process. Which required additional specialized high shear mixing system and also the additional hydration time in processing.

Having a liquid form of MCC would be advantageous to use for liquid food systems where high solubility and better sensory attributes are needed, beneficial to small scale manufacturer as they do not need additional specialized high shear mixer and hydration time in their processing and liquid MCC ingredients provides better sensory attributes over MCC powder [13]. Considering this, manufacture of liquid MCC could provide several benefits such as, save the cost of spray drying, provide improved flavor and functional properties, avoid the cost of installing additional mixing and hydration equipment, and time required for hydration.

These liquid MCC have to be heat treated to make them shelf stable, however poor heat stability can be a major constrain. There is general consensus that calcium chelators enhance heat stability of milk. There are various studies done to report the effect of calcium chelators on heat stability of milk, milk protein concentrate and MCC (5 - 10% protein) [11,15-18]. However to our knowledge no study has done so far to understand the effect of calcium chelator on heat stability of highly concentrated liquid MCC containing protein as high as 18.5%.

Objective of the Study

The objective of this study was to determine the optimum level of different calcium chelators to produce shelf stable concentered liquid MCC by improving the heat stability and preventing gel formation as a result of protein coagulation during high heat treatment such as UHT. We also aimed at measuring the particle size to understand the effect of different calcium chelators on heat induced protein aggregation.

Materials and Methods

Materials

Pasteurized skim milk was obtained from the SDSU dairy plant (SD, USA). Trisodium citrate and sodium phosphate were purchased from Fisher Scientific (NJ, USA).

Methods

Preparation of MCC sample and treatment of MCC sample with TSC and SP

Liquid MCC was manufactured at the Davis Dairy Plant (South Dakota State University, SD, USA). The MCC was produced from pasteurized skim milk (72°C for 20s) using microfiltration (polyvinylidene fluoride membranes, Parker FH 3838 OS01, 0.5 µm pore size, 11.4 m^2 surface area) in combination with diafiltration (100% diafiltration at 4 times volume reduction of feed skim milk). The final MCC sample obtained from this process had 20% protein w/w and 25% total solids.

The required amount of TSC and SP (calcium chelator) were mixed with the MCC (20% protein) to achieve treatment level of 1, 2.5, 5, 7.5 and 10 mM for both calcium chelator. No calcium chelators were added to control sample. The required amount of deionized water was added to all the samples to maintain same protein level. The final sample had 18.5% protein in all the treatment and control samples. These MCC samples adjusted to different level of TSC and SP were analyzed for heat stability and particle size measurement, and results were compared with control samples. Samples were allowed to equilibrate at room temperature for 1 hour. The pH of the solutions was

measured at 25°C using "All in One" pH/ATC electrode, pH 110 meter (EUTECH instruments, Singapore). The samples were adjusted to pH 6.7 by drop wise addition of either 1M NaOH or 1M HCL with continuous stirring. Sodium azide was added at a 0.02% rate to prevent bacterial growth. Samples were stored overnight at 4°C to ensure the equilibrium. These samples were brought to room temperature the next day using water bath set at 30°C and readjusted for minor pH change, if required.

Heat coagulation time (HCT) test

The heat stability refers to the ability of a product or sample to withstand very high temperature (such as UHT or retort sterilization) without visible flocculation, precipitation, coagulation and gelation. There is a direct relationship between HCT and heat stability, meaning that higher the HCT of the samples, higher the heat stability and vice-versa. Please note that the terms HCT and heat stability have been used interchangeably.

The HCT of MCC sample was measured using a method described by Sutariya and Patel [19]. For the purpose of this experiment, HCT test was performed at 140°C using oil bath. So, the HCT was defined as the time required for forming visible set precipitation or coagulation at 140°C. The maximum heating time allowed was 60 seconds. The 3 ml of control and test samples of MCC (18.5% protein) were filled in 8 mL wheaton glass tube (D- 17 mm × H- 61 mm). The tubes were sealed airtight and clamped on the rocker stand. These tubes were then submerged in an oil bath equipped with a rocker arrangement and variable speed was used to study HCT of MCC samples at 140°C. The rocker stand was submersed in an oil bath maintained at 143°C and put on rocker speed 3. Samples were monitored for the development of first visible precipitation, flocculation, coagulation or gelation. We choose temperate at 140°C because this the widely used in commercial continuous UHT application to manufacture liquid shelf stable dairy ingredients. Also we restricted the HCT time to maximum of 60 seconds which is many times more than the usual UHT heating time requirement at 140°C.

Particle size measurement

The particle size of the samples was measured using a method described by Meletharayil, Patel [20]. The samples were diluted to 120 times in a calcium imidazole buffer (5mM $CaCl_2$, 20 mM imidazole, 30 mM NaCl, pH 7.0) prior to measurement. The samples after HCT and control sample without heat treatment were analyzed for particle size measurement using photon correlation spectroscopy (PCS) at 25°C using a Malvern Zetasizer Nano-ZS (Malvern Instruments Ltd. Malvern, UK). Only the unheated control sample and test samples (heat treated with SP and TSC) in liquid state were analyzed for particle size to understand the level of heat induced protein aggregation. It was not possible to measure the particle size of the heat coagulated test samples as they were all gelled, hence we presented their visual image in figure 2 and figure 4. Also please note that the particle size determined with the Zeta sizer is only an apparent value as the measurements were not done on highly diluted solutions and at scattering wave vectors smaller than the inverse of the particle radii. Therefore the influence of interactions and polydispersity cannot be ignored.

Statistical analysis

All the samples were analyzed in triplicates. The results were analyzed by SAS (version 9.3). ANOVA was performed to examine the statistical difference between the samples, and the differences were considered significant when p values were less than 0.05.

Results and Discussion

For the samples with TSC treatments, the results of HCT are displayed in figure 1 and the HCT samples image displayed in figure 2. Control sample including test samples treated with 1, 2.5 and 5 mM TSC coagulated and formed gel during maximum HCT time of 60s (Figure 2). The sample treated with 1 mM TSC did not show significant improvement in heat stability compared to control sample (Figure 1). There was progressive improvement in heat stability of samples with 2.5 mM and 5 mM TSC (Figure 1). The samples treated with 7.5 mM and 10 mM TSC did not displayed any signs of coagulation for the maximum HCT of 60s and remained in liquid state (Figure 2). Only the samples treated with 7.5 mM and 10 mM TSC remained in liquid state and their mean average particle size were 630 nm and 272 nm respectively compared to 207 nm of non-heated control sample (Table 1).

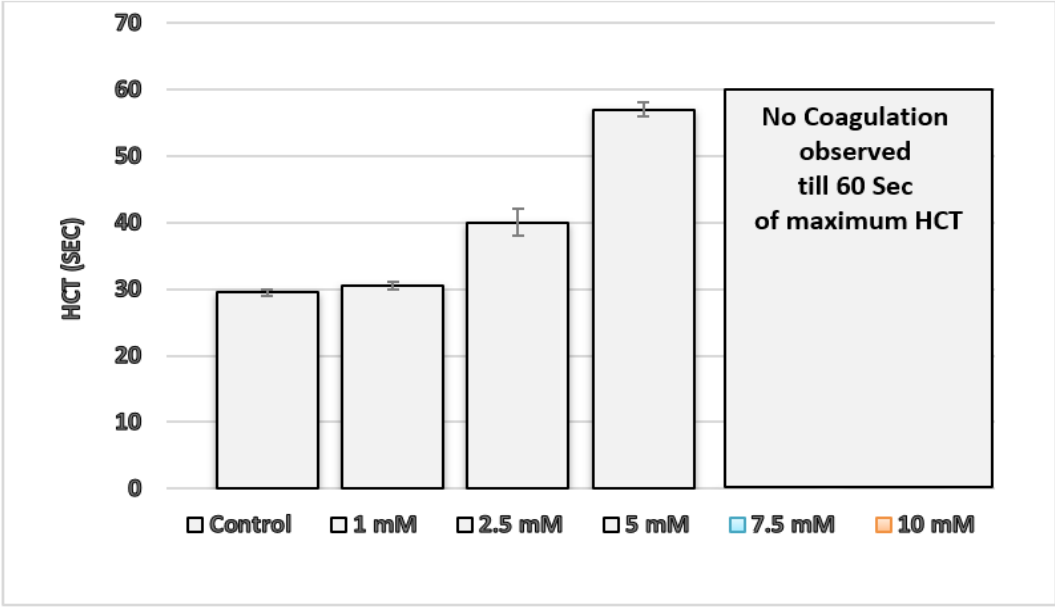

Figure 1: *Effect of TSC on HCT140°C of MCC (18.5% Protein). Heated control sample = 0 mM; MCC treated at different level of TSC; 1, 2.5, 5, 7.5 and 10 mM. (Error bars are the standard error of mean for mean values of triplicate analysis for each treatment)*

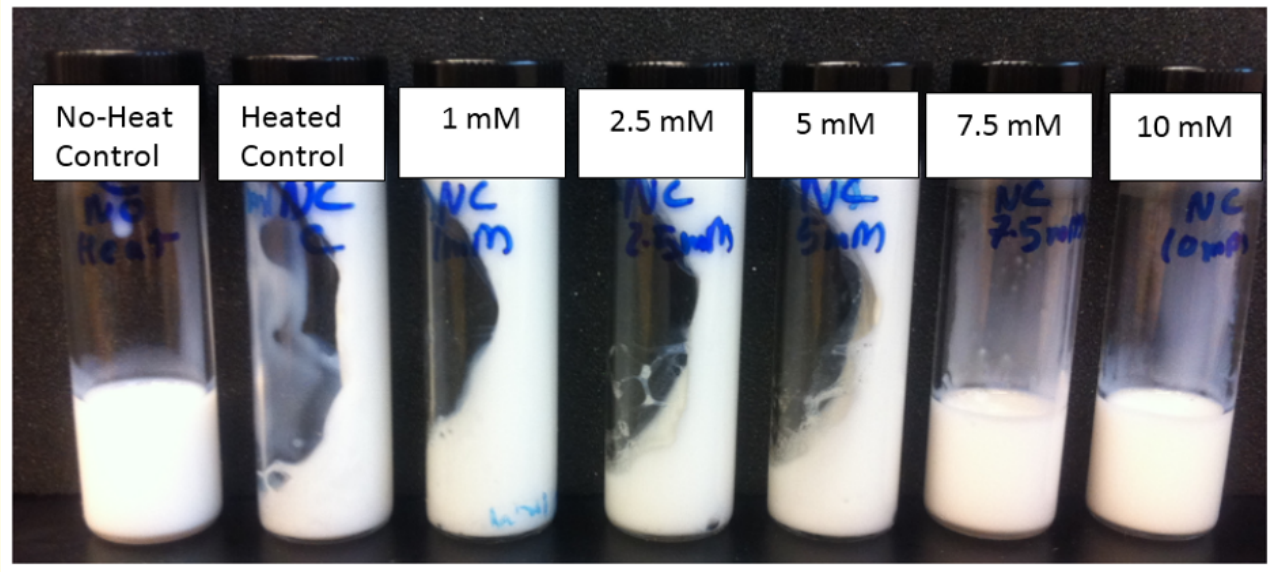

Figure 2: *Heat stability of MCC (18.5% Protein) samples treated with TSC. Non-heated control sample; Heated control sample; MCC treated at different level of TSC; 1, 2.5, 5, 7.5 and 10 mM.*

Similarly for the samples with SP treatments, the results of HCT are displayed in figure 3 and the HCT samples image displayed in figure 4. Control sample including test samples treated with 1, 2.5, 5 and 7.5 mM SP coagulated and formed gel during maximum HCT time of 60s (Figure 4). The sample treated with 1 and 2.5 mM SP did not show significant improvement in heat stability compared to control sample (Figure 3). There was progressive improvement in heat stability of samples with 5 mM and 7.5 mM SP (Figure 3). The samples treated with 10 mM SP did not display any signs of coagulation for the maximum HCT test time of 60s and remained in liquid state (Figure 4). Only the sample treated with 10 mM SP remained in liquid state and their mean average particle size was 414 nm compared to 206 nm of non-heated control sample (Table 1).

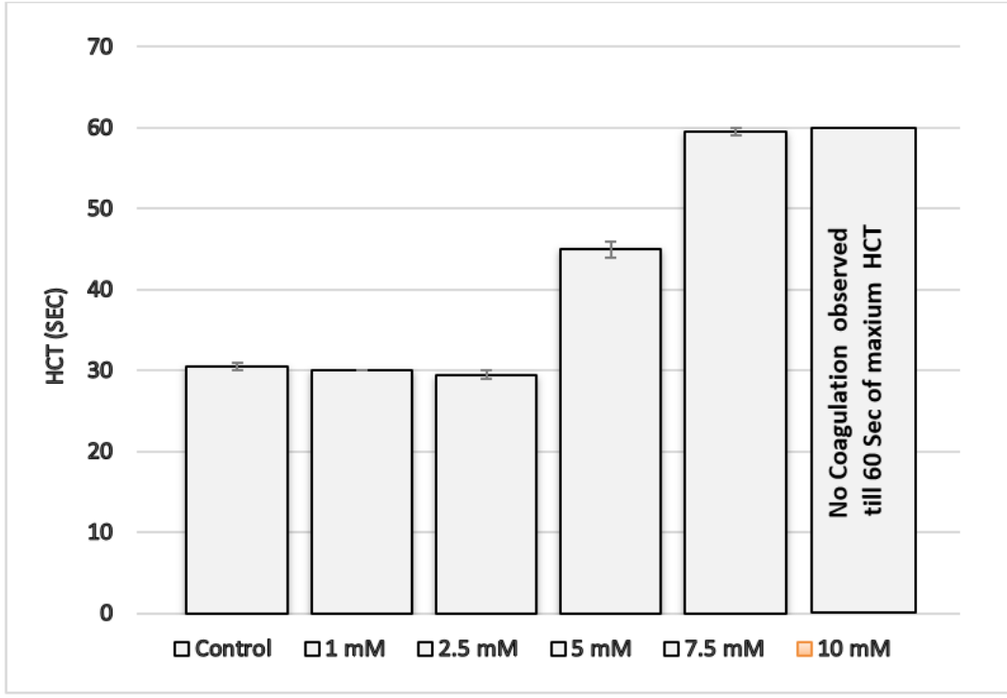

Figure 3: *Effect of SP on HCT140°C of MCC (18.5% Protein). Heated control sample = 0 mM; MCC treated at different level of SP; 1, 2.5, 5, 7.5 and 10 mM. (Error bars are the standard error of mean for mean values of triplicate analysis for each treatment)*

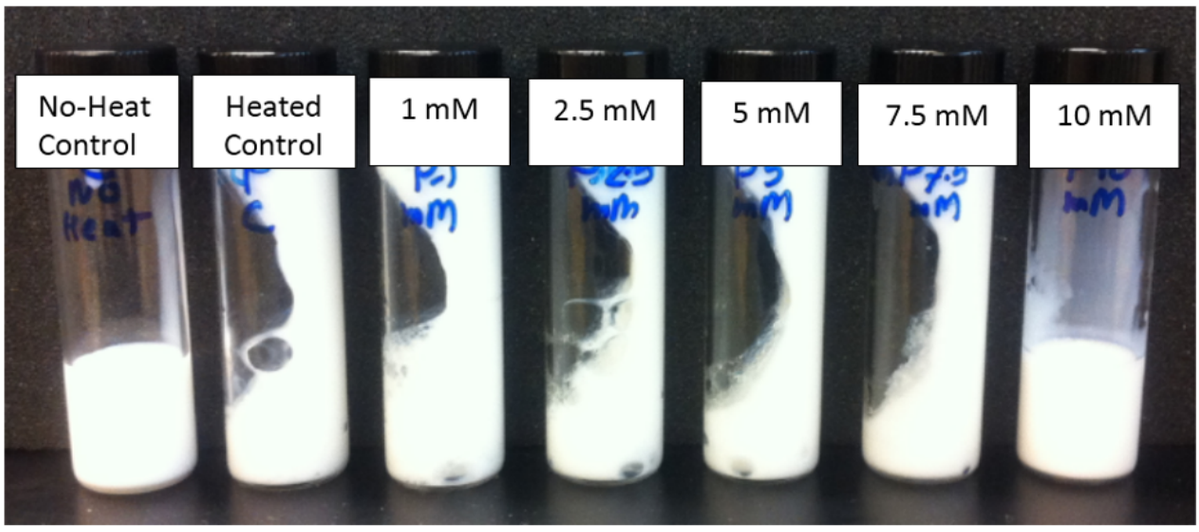

Figure 4: *Heat stability of MCC (18.5% Protein) samples treated with SP. Non-heated control sample; Heated control sample; MCC treated at different level of TSC; 1, 2.5, 5, 7.5 and 10 mM.*

Calcium Chelator	Unheated control	Heat Treated sample (140°C/60 Sec)					
		0 mM	1 mM	2.5 mM	5 mM	7.5 mM	10 mM
TCS	207[a]	--	--	--	--	630[b]	272[c]
SP	206[a]	--	--	--	--	--	414[d]

Table 1: *Particle size measurement of MCC measured post $HCT_{140°C}$ and treated with TSC and SP. Non-heated control sample; Heated control sample; MCC treated at different level of TSC & SP; 1, 2.5, 5, 7.5 and 10 mM.*

(--): Indicates samples were coagulated and hence not able to measures its practice size analysis.

[a-d]: Mean (n = 3) values within same row not sharing common superscript are significantly different (P < 0.05).

No improvements in the HCT for the sample treated with low level of TSC (1 mM) and SP (1 mM and 2.5 mM) could be because, lower level of calcium chelators might not be able to chelate the minimum critical level of calcium required to show its effect on HCT. In comparison with non-heated control sample, the higher particle size of the heat treated samples with TSC (7.5 mM and 10 mM) and SP (10 mM) indicated that although this samples stayed in liquid state at the end of HCT there was some level of protein aggregation in these samples. The progressive improvement in the HCT and relative decrease in particle size with increase in concentration of calcium chelators could be the effect of decrease in calcium-ion activity with increasing calcium chelators concentration, and hence reduction in calcium induced aggregation [11].

It was clear from our results that both TSC and SP were effective in improving the heat stability of the MCC sample (18.5% protein) at level higher than 1 mM and 2.5 mM respectively. These results were supported by finding of de Kort Minor [11], Augustin and Clarke [15], Grossbier [16] and Ramchandran Luo [18], who reported improvement in heat stability of MCC (5 - 10% protein) and milk protein concentrate as a result of treatment with calcium chelating agents.

When compared the effectiveness of both SP and TSC in improving the HCT at same level (7.5 mM), the results displayed that TSC was more effective in reducing the HCT compared to SP at 7.5 mM treatment level. This differences can be explained by the fact that, different types of calcium chelator have a different affinity for calcium ions and hence each of them release a different amount of CCP from the micelle [21]. Moreover, release of CCP from the casein micelle depends on the degree of saturation of the calcium complexes formed in the solution [22]. Therefore, calcium chelators can affect the integrity of the micellar structure to different extents. Both SP and TSC chelate calcium ions from the casein micelle, but calcium phosphate precipitates in the casein micelle [23], whereas calcium citrate remains as stable, soluble complexes in the serum phase [24-26]. It has been reported by Fox and Hoynes [27] that precipitation of calcium phosphate complex on casein micelle reduced the protein change and, subsequently, the heat stability of the sample treated with SP. This observation explains relatively lower heat stability of sample treated with SP compared to TSC at same treatment level (7.5 mM).

During heat treatment, micellar casein stability decreases due to the dissociation of micellar κ-casein [28,29]. The dissociation of κ-casein exposes the calcium sensitive surface of the casein micelle, and calcium bridging plays an important role in heat induced aggregation [30]. To minimize these changes that can affect heat stability of milk, calcium chelators are of the effective tools used. These chelators affect the equilibrium of calcium in serum phase and associated with CCP and there by affecting heat stability of the casein micelle. Addition of TSC and SP decreases the calcium ion activity by chelating calcium from serum and CCP [11] and thereby protecting the caseins against calcium bridge induced aggregation.

Hence, calcium chelators could be an effective tool in the production of shelf stable liquid MCC (18.5% protein), by improving the heat stability of concentrated liquid MCC during heat treatment.

Conclusions

It was clear from our results that both TSC and SP were effective in improving the heat stability of the MCC sample (18.5% protein) at level higher than 1mM and 2.5 mM respectively. When compared the effectiveness of both TSC and SP in improving the HCT at same level (7.5 mM), the results displayed that TSC was more effective in reducing the HCT compared to SP at 7.5 mM treatment level. The chelators affect the equilibrium of calcium in serum phase and associated with CCP and thereby improving the heat stability of the casein micelle. Hence, calcium chelators could be an effective tool in the production of shelf stable liquid MCC (18.5% protein), by improving the heat stability of MCC during heat treatment.

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