The effect of commercial sterilization regimens on micellar casein concentrates

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ABSTRACT

This work focused on evaluating the effects of UHT sterilization and in-container retorting on the stability and physical properties of micellar casein concentrates (MCC). The study was performed on MCC obtained by membrane separation, with casein concentrations between 5 and 10%. The UHT and retorting regimens were designed to achieve the same microbial inactivation effect. Ultra-high temperature treatment was performed in a pilot-scale MicroThermics heating system (MicroThermics Inc., Raleigh, NC), and retorting in an FMC multipurpose laboratory retort (Steritort; FMC Corp., San Jose, CA). The heat-treated and the non-heat-treated MCC controls were evaluated for pH, mineral profile, ζ -potential, particle size, and rheological properties for up to 24 h after heat treatment. The treatments were performed in triplicate, and differences among samples were evaluated using statistical analyses. Retorting resulted in slight aggregation in the MCC, whereas UHT caused the formation of visible aggregates and coagulation. The UHT-treated MCC had higher viscosity than retorted MCC, and displayed predominantly solid-like rheological behavior, indicative of structure formation. These effects were, at least in part, attributed to a change in mineral equilibrium, which affected the stability of the casein micelles, but additional mechanisms such as κ -casein dissociation may also play a significant role in these heat-induced changes. Drying of MCC accentuated the observed instabilities, as dried and reconstituted micellar casein concentrates (R-MCC) were more unstable to UHT sterilization than the MCC that had not undergone drying. The results of this study provide valuable information about the sterilization behavior and physical properties of MCC, which can be useful to processors in the development and manufacture of shelf-stable casein-based products and beverages.

Key words: micellar casein, ultra-high temperature, retorting, mineral

INTRODUCTION

Casein ingredients are widely used in foods due to their high nutritive value, bland taste, as well as their physicochemical and functional properties (Southward, 1985; Chandan, 1997). These include exceptional water-binding capacity (Walstra, 1990), emulsifying and foaming properties (Phillips et al., 1994; Damodaran, 1997), and viscosity (Konstance and Strange, 1991). The number of new products containing casein or caseinates launched in the United States has grown, on average, by about 22% per year between 2000 and 2008 (Affertsholt, 2009).

Casein preparations traditionally used as food ingredients are obtained using methods that involve the destabilization or chemical modification of casein micelles (i.e., acidification, renneting, or coprecipitation). Developments in membrane filtration technology allowed the large-scale production of micellar casein concentrates (MCC; Le Berre and Daufin, 1996; Nelson and Barbano, 2005). In these preparations, micellar casein is closer to its native state than in the case of ingredients obtained by chemical methods. One of the potential applications of MCC obtained by membrane separation is the manufacture of shelf-stable, high-protein content beverages. Although casein micelles are remarkably stable to heat treatment, heating at high temperatures can lead to some changes at the micelle level. These changes depend on whether or not heating is done in the presence of all milk components, as well as on the heat treatment temperature.

Heating at temperatures above 90°C was observed to lead to an increase in casein micelle size, both in milk and milk protein concentrates obtained by UF (Mohammad and Fox, 1987; McMahon, 1996). High heat treatments can also lead to dephosphorylation of casein micelles and subsequently disrupt the native micellar structure, which is largely held together by calcium phosphate links (Pyne and McHenry, 1955; Belec and Jenness, 1962; Dalgleish et al., 1987; Singh, 2004).

It is known that calcium phosphate present in the diffusible mineral fraction of milk becomes less soluble during heat treatment (Holt, 1995), although the forms of calcium phosphate formed after heating have

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not been unequivocally identified (Visser et al., 1986; Nelson et al., 1989). Formation of insoluble calcium phosphate and its deposition onto casein micelles was identified as a major mechanism responsible for the instability of casein micelles in UHT-treated milks and UF-concentrated milks during storage (Wahlgren et al., 1990; Dalgleish, 1992). It has been reported that heat-induced precipitation of primary and secondary calcium phosphate as tertiary calcium phosphate is coupled with a concomitant release of protons, which partially contributes to the heat-induced acidification of milk; the subsequent decrease in pH was considered responsible for the dissolution of micellar calcium phosphate and potential destabilization of the casein micelles during heating (O'Connell and Fox, 2003).

Other observed effects of heat treatment include proteolysis, which was observed when sterilizing milk at 120°C for 30 min (Saidi and Warthesen, 1993) and protein dissociation, reported during heating of serum protein-free casein dispersions at temperatures above 110°C (Aoki et al., 1974). Heat-induced polymerization has also been reported in UHT-treated serum protein-free micellar casein dispersions (Zin El-Din and Aoki, 1993). As causes for heat-induced polymerization, cross-linking via Maillard reaction when lactose is present (Kato et al., 1988) or formation of thermally generated colloidal calcium phosphate linkages (Singh, 1994) were suggested.

Micellar casein concentrate is an emerging dairy ingredient, and very few reports exist regarding the effect of high heat treatment on the stability of serum protein-free, lactose-free micellar casein systems. To address this knowledge gap, the objective of this work was to evaluate the effect of commercial sterilization regimens (UHT and in-container sterilization) on the properties and stability of MCC obtained by membrane separation. In addition, the effect of spray drying on the heating behavior and stability of MCC was also investigated.

MATERIALS AND METHODS

Production of the MCC

The MCC used in this study were produced in the pilot plant at Cornell University (Ithaca, NY). As raw material, whole raw bovine milk from the Cornell University teaching and research dairy farm was used. This study was conducted during the months of June and July, when the average true protein content is the lowest in the year (Barbano, 1990). The production of MCC was replicated 3 times with different batches of raw milk.

The timeline for processing was as follows: a batch of raw whole milk (~965 kg) was received on d 1, and stored overnight in gravity separation tanks at 4°C. On d 2, the raw gravity-separated skim milk was collected, pasteurized, and centrifugally separated at 50°C, according to procedures described by Zulewska et al. (2009). The pasteurized skim milk was subjected to UF to reduce the total lactose content. Ultrafiltration was performed at 50°C using a polymeric spiral-wound membrane with a molecular weight cutoff of 10,000 Da. The UF retentate was rediluted with water purified by reverse osmosis (RO), cooled to 4°C, and stored overnight. On d 3, the diluted UF retentate was microfiltered (MF) to obtain a serum protein-reduced retentate and MF permeate, according to procedures described by Zulewska et al. (2009). Microfiltration was performed at 50°C using a graded permeability ceramic membrane with a nominal pore size of 0.1 µm. The retentate and permeate were collected separately, cooled to 4°C, and stored overnight. On d 4, the MF retentate was further diafiltered twice to produce a highly serum protein-reduced MCC. The third-stage MF retentate underwent a finishing MF stage designed to increase its protein content to 10.5%.

The resulting retentate was rapidly cooled to 4°C and stored overnight on ice. On d 5, the MF retentate was treated and analyzed according to the experimental design shown in Figure 1.

Composition Analyses of Milk and MCC

Lactose content was determined by infrared spectroscopy (LactoScope FTIR; Delta Instruments BV, Drachten, the Netherlands), the fat content by ether extraction (AOAC International, 2000; method 989.05), and the total nitrogen (TN) by the Kjeldahl method (AOAC International, 2000; method 991.20). The NPN was determined by Kjeldahl method (AOAC International, 2000; method 991.21), and the noncasein nitrogen (NCN) also by Kjeldahl (AOAC International, 2000; method 998.05). The true protein (TP) content was calculated as $TP = (TN - NPN) \times 6.38 (\%)$, and the casein content was calculated as $CN = (TN - NCN) \times 6.38 (\%)$. Casein as a fraction of true protein was calculated based on the TP and casein values.

Preparation of the MCC Dilutions

Microfiltered retentate was diluted with RO water to 4 fixed casein concentrations: 5, 7.5, 8, and 10%. The protein content of the retentate was estimated as 98% of the total protein measured by IR analysis (LactoScope FTIR; Delta Instruments BV) according

Figure 1. Experimental design. RO = reverse osmosis; MCC = micellar casein concentrates; R-MCC = reconstituted MCC.

to previous work by Hurt and Barbano (2010). The theoretical casein concentration in the MF retentate was calculated using the percent of true protein in the pasteurized skim milk (Table 1). The MF retentate was diluted with RO water to casein concentrations of 5, 7.5, 8, and 10%. The resulting MCC were then stirred for 15 min and kept under refrigeration at 4°C when not in use.

Preparation of the Reconstituted MCC

To evaluate the effect of spray drying on the sterilization behavior of MCC, a comparison between the

processing behavior of the MCC obtained by dilution of the MF retentate (referred to as MCC) and spray-dried and reconstituted MCC (referred to as **R-MCC**) at the same casein concentration (8%), was also performed. The procedure for obtaining the R-MCC is described below.

Spray Drying. A portion of the 8% MCC obtained as described above was kept refrigerated at 4°C overnight and then spray dried the following day (d 6) using a model 1 Niro Atomizer equipped with a FU11 atomizer rotating at 23,000 rpm (Niro Atomizer Inc., Columbia, MD). The feed rate into the spray dryer was 16 kg/h, the inlet air temperature was 200°C, and the outlet

Table 1. Mean (n = 3) chemical composition (% by weight) of skim milk and microfiltered (MF) retentate ± 1 SD

Item	Composition ¹							
	TP	CN	NCN	NPN	CN%TP	Lactose	Fat	pH
Skim milk	3.06 ± 0.05	2.48 ± 0.04	0.77 ± 0.02	0.19 ± 0.01	81.01 ± 0.16	4.78 ± 0.02	0.07 ± 0.01	6.49 ± 0.04
MF retentate	11.09 ± 0.06	10.31 ± 0.07	0.90 ± 0.04	0.12 ± 0.01	92.01 ± 0.33	0.18 ± 0.02	0.29 ± 0.02	6.78 ± 0.06

¹TP = true protein = total nitrogen (TN) – NPN; NCN = noncasein nitrogen × 6.38; CN = TN – NCN; NPN = NPN × 6.38; CN%TP = casein as a percentage of TP.

air temperature was 95°C. The powder collected during the first 10 min of the drying session was discarded. The dried MCC powder was collected and packaged in Lightblock Mylar bags (model PAKVF2.5MB; Impak Corp., Los Angeles, CA), which were subsequently stored at 21°C.

Reconstitution of the MCC Powders. The MCC powder was reconstituted to 8% casein concentration with RO water under high-shear mixing, at 40°C. A 10-min resuspension step was followed by a 20-min high-speed dispersion step to ensure homogeneity of the R-MCC. To achieve full hydration of the powder, the 8% R-MCC was cooled and then stored overnight at 4°C.

Preparation for Thermal Treatment

Each of the MCC dilutions was split into 3 portions: 1 was used as control, and the other 2 were heat treated by UHT and by retorting, respectively. The MCC for retorting was filled into 8-oz (237-mL) Mason glass jars (Ball Corp., Broomfield, CO). Special attention was paid to avoiding incorporation of air during the filling of the jars, to ensure a proper seal and avoid overflow of the jars during retorting. Each of the Mason jars was filled gravimetrically with 180 ± 1 g of product, using a peristaltic pump (Amicon LP-1 pump, Amicon Inc., Beverly, MA, with Cole-Palmer Masterflex 7015-81 pump head, Cole-Parmer Instrument Co., Vernon Hills, IL). All MCC samples were stored overnight at 4°C before the heat treatment.

Heat Treatments

On d 6, the MCC were subjected to continuous-flow UHT sterilization and batch retorting. The temperature-time combinations for the 2 treatments were selected to ensure an equivalent microbial inactivation effect by calculating the lethality factor F_0 according to Bylund (1995).

The calculated F_0 values for the hold times were 6.33 for the UHT treatment and 6.59 for retorting. Minimum F_0 values of 5 to 6 are typically used to achieve commercially sterile milk (Bylund, 1995). The F_0 value for the UHT treatment was calculated based on the average velocity of the product flowing through the UHT equipment.

UHT Treatment

The MCC (5, 7.5, 8, and 10% casein) and the R-MCC (8% casein) were heat treated under continuous-flow UHT conditions using a pilot-scale MicroThermics

heating system (model 25 HV; MicroThermics Inc., Raleigh, NC), equipped with an Ultra Clean Fill Hood/Sterile Product Outlet. The 4 MCC were treated sequentially, on 3 separate dates, and the three 8% R-MCC were all treated on a separate date.

The UHT unit was subjected to a cleaning-in-place cycle the day before the treatments, as well as between the treatments of individual MCC. At the beginning of each processing day, the clean fill hood underwent a 30-min steam sterilization cycle.

After the cleaning-in-place cycle, the UHT unit was heated up to the sterilization parameters using water in the product stream, until stability of the processing parameters (flow rate and temperatures in the product line, and steam pressure) was achieved. The MCC preparations were preheated from the refrigeration temperature to 40°C (104°F) in a steam kettle and immediately subjected to the sterilization treatment. The UHT treatment consisted of preheating the product to 90°C (194°F) in the first heater within 20 s, final heating of the product to 143.3°C (290°F) for another 20 s in the final heater, holding 141.7°C (287°F) for 3 s, and cooling to 38.3°C (101°F) for 20 s. The product flow rate through the UHT unit was 2 L/min.

Because a transition from water to product was used at the beginning of each processing run, the refractive index of the output stream was monitored by taking readings every 3 s using a portable refractometer (r²mini; Reichert Analytical Instruments Inc., Depew, NY), as an indication of sample concentration. Collection of UHT-treated samples was initiated when the refractive index of the output stream became stable (i.e., it remained unchanged after 3 consecutive measurements). The UHT-treated product was aseptically filled in presterilized polycarbonate bottles and quickly cooled in an ice bath before storing it under refrigeration at 4°C.

Retorting

An FMC multipurpose laboratory retort with Log-Tec process management system (Steritort; FMC Corp., San Jose, CA) was used to batch-sterilize the MCC. To verify the uniformity of temperature distribution inside the retort during processing, temperature data was collected in a preliminary trial. Thermocouples were attached to 4 jars in the center of the retort cage, and the heating profile was calculated from the recorded temperature data with a modified Ball's formula using CALSoft 32, version 1.0 software (TechniCAL Inc., Kenner, LA).

The filled jars were preheated in a water bath to 28°C (82.4°F) to ensure the same initial temperature for all

processing runs. The still cook mode with cascading hot water and a steam pressure of 30 psi (206.8 kPa) was used. The heating profile included a 17.5-min come-up step, a 9-min cooking step at an average product temperature of 120°C (248°F), and a 10-min cooling step to a final temperature of 41°C (106°F). At the end of the processing cycle, jars were taken out of the retort and placed on ice for 30 min.

Analytical Evaluation of the MCC Samples

Besides the composition analyses described above, all MCC samples were analyzed for pH, rheological properties, particle size, mineral profile, and ζ -potential. All analyses were performed at 0 (immediately after heat treatment), 12, and 24 h, with the exception of rheological characterization, which was only performed at 12 and 24 h after the heat treatment.

pH Measurements

The pH was measured in untreated samples immediately before the heat treatments, and in the sterilized samples at 0, 12, and 24 h after the heat treatments. All measurements were performed at 20°C using a freshly calibrated Fisher Scientific Accumet Excel XL20 pH meter (Fisher Scientific, Pittsburgh, PA).

Rheological Analyses

Large-Deformation, Steady Shear Rheological Analyses. An ARES strain-controlled rheometer with dual transducer in conjunction with the Orchestrator data collection and analysis software (TA Instruments, New Castle, DE) was used to evaluate the viscosity as a function of shear rate for the MCC. A parallel plate geometry with 50-mm-diameter Teflon plates and an interplate gap of 1 mm was used. A fixed 2-mL volume of the MCC sample was carefully loaded on the lower plate, avoiding the formation of air bubbles, and then the gap was set by lowering the upper plate. A relaxation time of 1 min was allowed before starting the measurements. Strain-controlled, steady-shear rate sweeps, with shear rates ranging from 1 to 10^3 s^{-1} were performed. The measured parameters were viscosity (η) and shear stress (τ) as a function of shear rate ($\dot{\gamma}$). All measurements were performed at 20°C; the temperature control was ensured using a Peltier system. To avoid dehydration during the measurements, the samples were thinly coated with mineral oil at the interplate gap. Additionally, an enclosure was placed around the tool to prevent variations in vapor pressure and humidity at the air-sample interface. All measurements were performed in duplicate.

Small-Deformation, Oscillatory Rheological Analyses. Oscillatory dynamic measurements were performed to obtain information about the structural characteristics and viscoelasticity of the MCC samples. The double-wall Couette geometry was used (interplate gap of 1 mm) and a constant temperature of 20°C during the measurement was maintained using a Julabo FS 18-MW heating and refrigerated circulator (Julabo USA Inc., Allentown, PA). For each analysis, 8 mL of MCC sample was loaded into the Couette, avoiding the formation of air bubbles. The gap was set and, to avoid dehydration during the measurement, the samples were thinly coated with mineral oil. An isothermal chamber was also secured in place above the geometry to prevent variations in vapor pressure and humidity at the air-sample interface. A relaxation step of 1 min was allowed after the sample reached the treatment temperature of 20°C. The rheological parameters measured were storage modulus (G'), loss modulus (G''), complex modulus (G^*), and the loss tangent ($\tan \delta = G''/G'$).

First, dynamic strain sweeps were conducted for each of the samples, to locate the linear viscoelastic region (**LVR**) of material response, which represents the range of strain where the rheological properties are independent of the applied strain. The strain sweeps were performed over the strain interval 0.05 to 10%, with a strain increment of 0.2%. Frequency sweeps were then conducted, to evaluate the viscoelastic properties of the MCC samples as a function of timescale. Frequency sweeps were conducted at 20°C, using a strain value in the LVR region, and a frequency range of 0.1 to 100 rad/s.

Particle Size Analysis

Dynamic light scattering (**DLS**) was used to evaluate the average particle size and particle size distributions in all MCC, following the procedure established by Beliciu and Moraru (2009). The analyses were performed using a 90Plus nanoparticle size analyzer equipped with a Peltier temperature control system (Brookhaven Instruments Corp., Holtsville, NY), at a fixed 90° angle and a wavelength of 658 nm.

Before analysis, the MCC samples were equilibrated in a water bath at 20°C. To prevent moisture loss from the MCC samples during the temperature equilibration step, the test tubes that contained the samples were covered with Parafilm.

To maximize the accuracy of the measurements, the MCC samples were diluted before the DLS analysis, using UF permeate from the same milk obtained during the UF step in d 2. According to Beliciu and Moraru (2009), milk UF permeate constitutes the ideal sol-

vent for measuring particle size distributions of casein micelles, as its chemical composition is very close to that of the milk serum. Before particle size analysis, the UF permeate was filtered using a 0.2- μm syringe filter (25mm SFCA syringe filter; Thermo Scientific, Waltham, MA) and equilibrated in a water bath at 20°C. The dilution ratio was adjusted for each sample so that the signal intensity was between 700 and 900 kilocounts per second, as recommended by the DLS equipment manufacturer.

The refractive index and viscosity of the UF permeate at 20°C were used to define the solvent. A viscosity of 1.154 cP at 20°C was measured for the UF permeate using an ARES strain-controlled rheometer (TA Instruments), equipped with a double-wall Couette geometry. The temperature during rheological analysis was maintained using a Julabo FS18-MW circulator (Julabo USA Inc.). The refractive index of the UF permeate was determined using a digital fiber-optic refractometer (Misco Products Division, Cleveland, OH); the measured refractive index of the UF permeate was 1.341.

The particle size measurements were performed at a constant temperature of 20°C. The disposable cuvettes were equilibrated in the temperature-controlled chamber of the particle size analyzer for 3 min and the diluted samples were pipetted in the cuvette and allowed a 2.5-min temperature equilibration step, after which the DLS measurement was promptly started.

Data collection and analysis was performed using the BIC software (Brookhaven Instruments Corp.), which converted the experimental data into size distributions. The software contains a dust filter algorithm that improves the quality of the measurements by rejecting data that are corrupted by scattering from random particles such as air bubbles or dust. The dust filter cutoff parameter was set at 30, which is the optimal value suggested by the manufacturer when the expected particle size is in the range of hundreds of nanometers. Each measurement consisted of 8 subsequent individual runs of 30-s duration. For each measurement, the relative particle size distribution, the intensity-weighted effective diameter ($\overline{d_{\text{eff}}}$), and the polydispersity index were determined. The measurements were performed in duplicate.

ζ -Potential Analysis

ζ -Potential analysis was performed using the Zeta-Plus option of the 90Plus nanoparticle size analyzer (Brookhaven Instruments Corp.). Before the measurement, samples were diluted using ultra-pure water filtered with a 0.2- μm syringe filter (25-mm SFCA syringe filter; Thermo Scientific). The diluted samples

were pipetted into a single-use cuvette and allowed a 2.5-min temperature equilibration step before initiating the test. Measurements were performed using a 35-mW solid state laser ($\lambda = 660 \text{ nm}$) in the high-precision mode, at 20°C, after setting water as solvent. Protein dilutions were adjusted to achieve an optimum ratio (0.1–0.5) between the instrument count rate and the reference count rate (1,454 kilocounts per second). The measurement consisted of 30 cycles per run, with an intercycle delay of 5 s. Data collection and analysis was performed using the BIC software (Brookhaven Instruments Corp.).

Mineral Profile Characterization

Micellar casein concentrate samples were evaluated for the total mineral content and for soluble minerals. To measure the concentration of soluble minerals, samples were ultracentrifuged and the mineral content in the clear supernatant was analyzed.

Ultracentrifugation Procedure. This step was performed using the procedure by Philippe et al. (2005). Equal volumes of 8.5 mL of sample were pipetted into four 10.4-mL polycarbonate centrifuge bottles with cap assemblies (model 355603; Beckman Instruments Inc., Palo Alto, CA). The bottles were ultracentrifuged at $100,000 \times g$ for 60 min at 20°C, using a Beckman L8M centrifuge (Beckman Coulter Inc., Brea, CA) with a standard 70.1 Ti rotor, at 38,200 rpm. At the end of the ultracentrifugation step, special care was taken to not resuspend the insoluble pellet while handling the rotor, removing the bottles and collecting the supernatant. Ten-milliliter syringes (Luer-Lok; Thermo Scientific) with 4-in stainless steel needles (Popper Blunt 304 Stainless Steel 20G; Popper & Sons Inc., New Hyde Park, NY) were used to collect 4 mL of clear supernatant from each bottle, without disturbing the pellet. The needle was only submerged once to avoid disturbing the thin fat layer formed at the top of the centrifugation bottle.

Mineral Composition Analysis. The mineral composition of the MCC and of the ultracentrifugation supernatants was tested at the Dairy One Forage Analysis Laboratory (Ithaca, NY). The following minerals were quantified: calcium, phosphorous, magnesium, potassium, and sodium. Four to five grams of each sample was weighed into a 50-mL calibrated CEM Xpress Teflon PFA vessel with fiberglass insulating sleeves (CEM Corp., Matthews, NC). The samples underwent a 30-min digestion step with 8 mL of nitric acid (HNO_3) and 2 mL of hydrochloric acid (HCl) at 1,600 W and 190°C in a CEM microwave accelerated reaction system (MARS; CEM Corp.) equipped with the MAR SXpress temperature control option. The ves-

Table 2. pH of micellar casein concentrates [MCC; mean ($n = 3$) $\pm$ 1 SD]

Type of MCC	pH		
	Control	UHT	Retorted
5.0% MCC	7.17 $\pm$ 0.06	7.17 $\pm$ 0.03	7.02 $\pm$ 0.08
7.5% MCC	7.10 $\pm$ 0.06	7.12 $\pm$ 0.03	6.96 $\pm$ 0.10
8.0% MCC	7.11 $\pm$ 0.08	7.12 $\pm$ 0.05	6.93 $\pm$ 0.13
8.0% R-MCC ¹	7.09 $\pm$ 0.02	7.09 $\pm$ 0.02	6.95 $\pm$ 0.03
10.0% MCC	7.08 $\pm$ 0.05	7.08 $\pm$ 0.05	6.91 $\pm$ 0.11

¹R-MCC = reconstituted MCC.

sels were then brought to 50 mL volume with 1.5 *N* HNO₃ + 0.5 *N* HCl solution to match standards and aspirated and analyzed with a Thermo Jarrell Ash IRIS Advantage HX Inductively Coupled Plasma (ICP) Radial Spectrophotometer (Thermo Scientific, Madison, WI). All mineral analyses were performed in duplicate.

Statistical Analysis

Data was analyzed with the JMP software (8.0; SAS Institute Inc., Cary, NC) using a mixed-model analysis, which included both fixed effects and random effects. Fixed effects included product source, casein content, treatment, and time after treatment; random effects included experimental replication, casein content and treatment, or combinations of these effects, depending on the parameters tested.

The effects of casein content and treatment on MCC mineral profile and functionality were assessed on the data obtained for the controls and the sterilized MCC immediately after processing (0 h); random effects included experimental replication and experimental replication $\times$ casein content. To assess the effect of experimental replication on MCC functionality, casein content was used as a random effect. The effect of time after processing was assessed for the sterilized MCC only; in this analysis, random effects included experimental replication, experimental replication $\times$ casein content, and experimental replication $\times$ casein content $\times$ treatment.

The effects of drying on MCC mineral profile and functionality were assessed using a restricted data set that included only the data for the 8% MCC and R-MCC (controls and sterilized samples at 0 h); random effects were experimental replication and experimental replication $\times$ product source. The effect of time after processing for these samples was assessed on the data set for the sterilized 8% MCC and R-MCC only (no controls); random effects included experimental replication, experimental replication $\times$ product source, and experimental replication $\times$ product source $\times$ treatment.

Statistical differences between values of different parameters were determined using the Tukey-Kramer honestly significant difference (HSD) test and the Student's *t*-test. Differences with $P < 0.05$ were considered statistically significant. Results were reported using the American Psychological Association (APA) style: F[df between groups, df within groups], at $P < 0.05$.

RESULTS

Chemical Composition of the Skim Milk and MCC

The mean chemical composition of skim milk and MF retentate is presented in Table 1. As a result of the combination of UF and 4 MF stages, more than 95% of the initial serum protein content was removed (Zulewska et al., 2009), and more than 96% of the initial lactose content was depleted.

The pH of the micellar casein concentrates varied significantly with casein content ($F[3, 22] = 8.98$, $P < 0.05$): the lower the casein concentration of the MCC, the higher the pH (Table 2). This was due to the dilution with RO water of the MCC samples, as the RO water had a pH_{20°C} of 7.65.

The pH values of untreated (controls) and UHT-treated samples were similar, whereas the retorted samples had significantly lower pH than the controls ($F[2, 22] = 65.71$, $P < 0.05$). The pH of the heat-treated samples did not change significantly within 24 h of processing. Experimental replication had a significant effect on the pH of all MCC: controls ($F[2, 6] = 20.72$, $P < 0.05$), UHT-treated ($F[2, 6] = 10.12$, $P < 0.05$), and retorted ($F[2, 6] = 27.16$, $P < 0.05$). This was due to the fact that the MF retentate obtained in the third replication was slightly more acidic (pH_{50°C} = 6.69) than the retentates obtained in the previous 2 processing runs (pH_{50°C} = 6.81 and 6.83 for replicates 1 and 2, respectively). This is important to note, as pH is an important factor for the heating stability of MCC.

For R-MCC, heat treatment had a similar effect on pH as noted for MCC, with retorting leading to signifi-

Figure 2. Rate sweeps [viscosity (η) vs. rate] for 10% micellar casein concentrates (MCC): control, retorted (after 12 h), and UHT treated (after 12 h). Data represent the average of all 3 experimental replicates and the error bars represent 1 SD.

cantly lower pH values than in the control and UHT-treated R-MCC ($F[2, 4] = 39.28$, $P < 0.05$; Table 2).

Rheological Properties of the MCC

Shear Viscosity. For all MCC, viscosity did change with shear rate, indicating non-Newtonian behavior. Figure 2 shows the viscosity versus shear rate graphs

for 10% MCC—both untreated and heat treated. Similar trends in apparent viscosity versus shear rate were observed for all other heat treatments (data not shown). Because most of the MCC behaved as non-Newtonian liquids, the term apparent viscosity will be used to describe their resistance to flow. To make direct comparisons among the different MCC, the apparent viscosity at a shear rate of 100 s^{-1} (η_{100}) was used. This value of shear rate was selected due to its association with stirring, pumping, pipe flow, spraying, and other processing operations, even mastication (Steffe, 1996; Carr et al., 2003).

The viscosity of MCC was affected by both casein concentration ($F[3, 22] = 42.37$, $P < 0.05$) and type of heat treatment ($F[2, 22] = 13.01$, $P < 0.05$). As seen in Figure 3, η_{100} increased with casein concentration for all treatments, as expected. An important observation is that, at equivalent casein concentrations, η_{100} of the UHT-treated MCC was much higher than that of the controls. The UHT-treated MCC appeared inhomogeneous and coagulated and this behavior was increasingly pronounced as casein concentration increased. This was also reflected in a large variability of η_{100} for the UHT-treated MCC. The η_{100} of the retorted MCC was lower than that of both UHT-treated samples and controls.

The viscosity of the untreated MCC did not vary significantly among the 3 replicates, but statistically significant differences among experimental replicates were observed after heat treatment. For retorted MCC,

Figure 3. Apparent viscosity at a shear rate of 100 s^{-1} for untreated and heat-treated micellar casein concentrates (MCC) and reconstituted MCC (R-MCC). Data represent the average of all 3 experimental replicates and the error bars represent 1 SD.

samples produced in the third experimental replication had significantly lower η_{100} than for the first 2 replications ($F[2, 6] = 6.36$, $P < 0.05$). In case of UHT treatment, MCC from the second and third experimental replications had significantly higher viscosity than those obtained for the first replication ($F[2, 6] = 17.92$, $P < 0.05$). For the UHT-treated MCC, viscosity did not change within 24 h after processing, whereas for the retorted MCC, viscosity decreased significantly after 24 h from the heat treatment ($F[1, 9] = 8.13$, $P < 0.05$).

These effects of heat treatments and experimental replication were also observed for the R-MCC (Figure 3). It must be noted also that R-MCC had a significantly higher η_{100} than the MCC with the same casein concentration (8%), both for the controls ($F[1, 8] = 119.88$, $P < 0.05$) and for the UHT-treated samples ($F[1, 8] = 10.53$, $P < 0.05$). No differences were observed between the viscosity of the retorted MCC and retorted R-MCC (at 8% casein).

Flow Behavior. The flow behavior of the micellar casein concentrates was characterized using the Herschel-Bulkley constitutive model:

$$\sigma = \kappa(\dot{\gamma})^n + \sigma_0, \quad [1]$$

where σ is the shear stress (Pa), κ is the consistency coefficient ($\text{Pa} \times \text{s}^n$), $\dot{\gamma}$ is the shear rate (s^{-1}), n is the flow behavior index, and σ_0 is the apparent yield stress (Pa).

The inhomogeneity of the UHT-treated MCC did not allow for an accurate application of the Herschel-Bulkley model. Therefore, this analysis was only performed for the controls and retorted samples.

All untreated and retorted MCC samples displayed shear thinning behavior ($n < 1$). The flow index of the control samples was significantly smaller than the flow index of retorted MCC ($F[1, 47] = 20.82$, $P < 0.0001$). The flow behavior of the retorted MCC did not change within 24 h of processing.

The same trends were observed for the R-MCC, for which n values were significantly smaller for the controls than the retorted samples ($F[1, 10.1] = 43.02$, $P < 0.0001$). Similar as for the MCC, the flow behavior of the retorted R-MCC did not change within 24 h of processing.

Most MCC manifested yield stress, but the calculated apparent yield stress values were very low. The effects of casein content, heat treatment, and time after treatment on yield stress were similar to the effects of these factors on apparent viscosity. Because the physical causes for the 2 physical properties are similar, yield stress data are not presented, to avoid redundancy.

Dynamic Rheological Properties. Small-amplitude oscillatory rheological testing was used to gain an insight into the structure of the MCC. First, strain sweeps were performed to identify the LVR for each type of sample. The viscoelastic behavior of all samples was relatively linear in the strain range studied (up to 5% strain). The 10% MCC (controls and sterilized, after 24 h from the treatment) had the highest values of G' and G'' of all samples, as they had the highest casein concentration (data not shown). Both the controls and the retorted samples showed very small values of G' and G'' , typically in the range of 10^{-1} to 10^{-2} Pa. The UHT-treated samples, on the other hand, had G' and G'' values higher by several orders of magnitude than the controls and the retorted MCC (10^2 to 10^3 Pa). It must be noted that the data for the UHT samples showed large variability, due to the differences among experimental replications and the samples' inhomogeneity. Despite the large variability of the data, the differences between the UHT samples on one hand and the controls and the retorted samples on the other hand were statistically significant.

Frequency sweeps were then carried out to evaluate the viscoelastic behavior of the MCC as a function of time. As shown in Table 3, controls and retorted MCC at all casein concentrations had a predominantly liquid-like behavior ($G' < G''$). For most of the UHT-treated samples, the solid-like behavior was predominant ($G' > G''$); this indicated structure formation in these samples, which is consistent with the visual observations. In the case of the first experimental replication, network formation ($G' > G''$) occurred at a casein concentration above 8%, for the second replication above 7.5%, whereas for the third replication, network formation was observed starting at 5% casein concentration. The untreated (control) and retorted R-MCC had liquid-like behavior ($G' < G''$), whereas the UHT-treated R-MCC displayed solid-like behavior ($G' > G''$; Table 3).

Particle Size and Aggregation Behavior of the MCC

The occurrence of particle aggregation in the concentrates was evaluated by determining the effective diameter. The formation of large, visible agglomerates and coagulation in all UHT-treated samples rendered the DLS method unusable for these samples and, therefore, particle size data are only available for the controls and the retorted samples.

The effective diameter measured in MCC was not affected by casein content, whereas retorting led to a statistically significant ($F[2, 22] = 8.44$, $P < 0.05$) increase in particle size (Figure 4). The particle size in the retorted samples remained constant within 24 h

Table 3. Viscoelastic behavior of the micellar casein concentrate (MCC) samples evaluated by frequency sweeps¹

MCC concentration	Control	Retorted	UHT
5% MCC	$G' < G''$	$G' < G''$	$G' < G''$ (rep. 3: $G' > G''$)
7.5% MCC	$G' < G''$	$G' < G''$	$G' > G''$ (rep. 1: $G' < G''$)
8% MCC	$G' < G''$	$G' < G''$	$G' > G''$
8% R-MCC ²	$G' < G''$	$G' < G''$	$G' > G''$
10% MCC	$G' < G''$	$G' < G''$	$G' > G''$

¹Storage modulus (G') > loss modulus (G'') indicates liquid-like behavior, whereas $G'' > G'$ indicates solid-like behavior; rep. = replicate.

²R-MCC = reconstituted MCC.

of the heat treatment (data not shown). Experimental replication significantly affected the measured in the untreated MCC ($F[2, 6] = 5.17$, $P < 0.05$) and in the retorted MCC ($F[2, 6] = 23.47$, $P < 0.05$), with for the third experimental replication being consistently higher than for the previous 2 replications.

The effective diameter values for the R-MCC are represented in Figure 4. For the 8% concentrates, no difference in particle size was observed between the retorted MCC and the retorted R-MCC, which indicates that spray drying did not affect aggregation trends in the retorted concentrates. However, it must be noted that for the untreated controls, the average diameter for R-MCC was significantly higher than for the MCC ($F[1, 4] = 72.99$, $P < 0.05$). The treatment and time after treatment had similar effects on particle size as for the MCC.

Colloidal Stability of the Concentrates

Casein micelle stability is governed by both electrostatic repulsion among the negatively charged micelles, and by steric hindrance caused by κ -CN. In this study, ζ -potential was used as measure of the electrical charge of casein micelles and as a relative indicator of the colloidal stability of the concentrates. If particles in a disperse system have large negative or positive ζ -potential values, they repel each other, which hinders aggregation and enhances the stability of the system; if particles have low absolute values of ζ -potential, they will agglomerate and the dispersion becomes unstable. Riddick (1968) defined the stability of dispersions with relation to ζ -potential as follows: (1) 0 to ± 3 mV: maximum agglomeration and precipitation; (2) $+5$ to -5 mV: strong agglomeration and precipitation; (3)

Figure 4. Average particle size (effective diameter) for untreated and heat-treated micellar casein concentrates (MCC) and reconstituted MCC (R-MCC). The values represent averages of all 3 experimental replicates, and the error bars represent 1 SD.

−10 to −15 mV: threshold of agglomeration; (4) −16 to −30 mV: threshold of delicate dispersion; (5) −31 to −40 mV: moderate stability; (6) −41 to −60 mV: fairly good stability; (7) −61 to −80 mV: very good stability; and (8) −81 to −100 mV: extremely good stability.

The ζ -potential values measured for the MCC were generally in the range of −20 to −30 mV (Figure 5). ζ -Potential was not affected by casein content, but was significantly affected by treatment ($F[2, 16] = 12.21$, $P < 0.005$). Both sterilization processes resulted in lower absolute values of ζ -potential, indicating a decrease in the electrostatic stability of the casein micelles after heat treatment. The ζ -potential of the heat-treated samples did not change within 24 h after processing (data not shown). Experimental replication only had a statistically significant effect on the ζ -potential of the retorted MCC ($F[2, 6] = 16.73$, $P < 0.05$). Retorted MCC from the third replication had significantly lower absolute ζ -potential values ($-24.5 < \zeta < -17.2$ mV) than for the previous 2 replications ($-29.9 < \zeta < -25.5$ mV). For the MCC obtained in the third replication, the absolute values of ζ -potential increased after 12 and 24 h after processing as compared with immediately after processing ($F[2, 18] = 6.02$, $P < 0.05$), whereas for the first 2 replications, the changes in ζ -potential after processing were not significant.

Drying seemed to affect the casein micelle surface charge, as untreated MCC had lower absolute values of ζ -potential as compared with untreated R-MCC ($F[1, 4] = 8.61$, $P < 0.05$). Yet, ζ -potential values measured in the sterilized 8% R-MCC were not significantly different than the values for sterilized 8% MCC (immediately after heat treatment). For the sterilized R-MCC, higher absolute values of ζ -potential were measured at 12 and 24 h after sterilization as compared with the values measured immediately after the treatment ($F[2, 22] = 6.31$, $P < 0.05$).

Mineral Profile of the MCC

Mineral Composition Before Heat Treatment.

The mean mineral composition of the MCC before the heat treatment (both total and soluble mineral content) is presented in Table 4. The decrease in total mineral concentration of the MCC with casein concentration was due to the dilution of the retentate with RO water. Due to repeated filtration and diafiltration, the total levels of K and Na (diffusible minerals) in the MCC were lower by 96 and 97%, respectively, than in skim milk. Experimental replication had a significant effect ($P < 0.05$) on the total mineral content of the MCC. The levels of total Ca, P, and Mg were significantly lower, whereas the levels of total K and Na were signifi-

Figure 5. ζ -Potential of untreated micellar casein concentrates (MCC), retorted MCC, UHT-treated MCC, untreated reconstituted MCC (R-MCC), retorted R-MCC, and UHT R-MCC. The values represent averages of all 3 experimental replicates, and the error bars represent 1 SD.

cantly higher for the third replication as compared with the first 2 replications. It is important to note that the Ca/P molar ratio in the MCC was significantly lower in the third replication as compared with the first 2 replications ($F[2, 18] = 6.01$, $P < 0.05$). The spray drying and reconstitution did not affect the total mineral profile of the MCC.

The soluble mineral content of the MCC decreased with decreasing casein concentration as a result of the dilution with RO water. For Ca, P, and Mg, which are all partly bound to the casein micelles, the soluble content as a fraction of total content was much lower in the MCC as compared with skim milk. In skim milk, the soluble fractions of Ca, P, and Mg represent, on average, 31, 54, and 65%, respectively, of the total content of these minerals (Holt, 1985). In the MCC, the soluble Ca, P, and Mg represented, on average, 8, 9, and 19%, respectively, of the total contents. For K and Na, which are essentially diffusible, more than 90% of their total content is present in soluble form in milk (Holt and Jenness, 1984), which was also observed for the MCC obtained in this study.

Experimental replication had a significant effect ($P < 0.05$) on the soluble mineral composition, with the soluble Ca, P, and Na being significantly lower for the third replication as compared with the first 2 replications. The Ca/P molar ratio in the supernatants of untreated MCC from the third replication was significantly higher than the Ca/P ratios for replications 1 and 2 ($F[2, 18] = 5.13$, $P < 0.05$).

Spray drying and reconstitution had no statistically significant effect on the soluble minerals of the untreated MCC, except for Mg. Soluble Mg was present at a significantly higher level in the soluble fraction of R-MCC as compared with the MCC ($F[1, 8] = 37.73$, $P < 0.05$). The reason for this difference is not clear, but this is not expected to have a significant effect on the heating behavior of R-MCC.

Mineral Composition After Heat Treatment. Sterilization (both retorting and UHT) had no effect on the total mineral composition or on the overall Ca/P molar ratios of the MCC. Soluble Ca, P, and Mg levels were significantly lower ($P < 0.05$) in the sterilized MCC immediately after the treatment as compared with the untreated controls, whereas Na and K levels were not affected by sterilization. The concentration of soluble Mg was not affected by the type of heat treatment, but significantly lower levels of soluble Ca and P were recorded for the UHT-treated samples as compared with the retorted samples. The type of sterilization treatment also affected the Ca/P molar ratio in the soluble phase ($F[2, 22] = 42.88$, $P < 0.05$), both for MCC and R-MCC. The soluble Ca/P molar ratio was lower in the sterilized samples as compared with the

Table 4. Mean $\pm$ 1 SD for the total and soluble mineral composition ($n = 3$) of the untreated (control) micellar casein concentrates (MCC)

Type of MCC	Mineral composition (mM)							
	Ca		P		Mg		K	
	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble
5% MCC	42.2 $\pm$ 1.7	3.9 $\pm$ 0.6	31.1 $\pm$ 1.7	3.0 $\pm$ 0.4	2.5 $\pm$ 0.1	0.6 $\pm$ 0.2	2.1 $\pm$ 0.2	1.9 $\pm$ 0.5
7.5% MCC	65.4 $\pm$ 3.3	5.2 $\pm$ 0.5	48.5 $\pm$ 2.4	4.2 $\pm$ 0.5	3.9 $\pm$ 0.1	0.7 $\pm$ 0.0	2.8 $\pm$ 0.3	2.6 $\pm$ 0.1
8% MCC	72.2 $\pm$ 8.2	5.9 $\pm$ 0.6	53.6 $\pm$ 5.4	4.9 $\pm$ 0.6	4.2 $\pm$ 0.3	0.7 $\pm$ 0.1	3.1 $\pm$ 0.2	2.8 $\pm$ 0.1
8% R-MCC ¹	71.2 $\pm$ 2.4	5.7 $\pm$ 0.3	54.1 $\pm$ 1.8	4.8 $\pm$ 0.3	4.3 $\pm$ 0.2	0.8 $\pm$ 0.1	3.3 $\pm$ 0.2	2.6 $\pm$ 0.2
10% MCC	91.1 $\pm$ 5.5	7.5 $\pm$ 1.3	67.2 $\pm$ 2.7	6.0 $\pm$ 1.2	5.3 $\pm$ 0.2	1.0 $\pm$ 0.2	4.0 $\pm$ 0.2	3.1 $\pm$ 0.6
							1.0 $\pm$ 0.1	0.8 $\pm$ 0.2
							1.3 $\pm$ 0.2	1.1 $\pm$ 0.3
							1.4 $\pm$ 0.1	1.1 $\pm$ 0.2
							1.5 $\pm$ 0.1	1.2 $\pm$ 0.1
							1.8 $\pm$ 0.1	1.5 $\pm$ 0.3

¹R-MCC = reconstituted MCC.

untreated samples, with retorted samples having lower Ca/P ratios than the UHT-treated samples.

Time after heat treatment had a statistically significant effect on all soluble minerals ($P < 0.05$). The levels of soluble Ca and P in the heat-treated samples increased within 24 h after the heat treatment. In case of retorting, the level of soluble Ca reverted to pretreatment levels within 24 h, whereas for the UHT-treated samples, the soluble levels remained slightly lower than for the controls after 24 h ($F[2, 16] = 5.94$, $P < 0.05$). The soluble P level reverted to pretreatment levels after 24 h, both for the UHT-treated and the retorted samples. Although immediately after sterilization soluble Na and K levels were not different from the untreated MCC, 24 h after sterilization, the levels of both diffusible minerals became significantly higher in the heat-treated samples than in the controls.

Spray drying did not affect the soluble and total Ca, Na, and K, with similar levels being recorded for the sterilized R-MCC as for the sterilized MCC immediately after heat treatment. The Ca/P molar ratios in the heat-treated R-MCC were also similar to those calculated for the heat-treated MCC. The levels of soluble P and Mg in the sterilized R-MCC were significantly higher than in their MCC counterparts ($P < 0.05$). The type of treatment and time after treatment had similar effects on the soluble mineral fraction of R-MCC as for MCC. In case of the R-MCC, the soluble Ca, P, Mg, and the Ca/P ratio reverted to pretreatment levels within 24 h for both UHT and retorted samples ($P < 0.05$).

Figure 6 shows the levels of soluble minerals for all MCC and R-MCC, before and after the heat treatments, and Figure 7 illustrates the Ca/P ratios. Statistically significant differences cannot be identified from the standard deviations in these figures, as statistical analysis was performed on the entire data set, taking into account the different fixed and randomized effects.

DISCUSSION

Before discussing the changes observed in the MCC as a result of sterilization, it is important to note that the concentration of casein micelles by membrane filtration, although achieved using a physical process, will induce changes in the chemical composition of the medium surrounding the casein micelles. Specifically, as compared with the native environment of casein micelles, these changes are the removal of the majority of serum proteins by microfiltration and depletion of the lactose and soluble minerals from the serum phase by diafiltration. These changes will have effects on the buffering capacity of the casein micelles, and possibly on their hydration. It was reported before that concen-

tration of casein micelles by microfiltration increases the buffering capacity of retentates proportionally to their protein content (St Gelaïs et al., 1992; Le Graët and Gaucheron, 1999; Salaün et al., 2005). At the same time, dilution of the retentate will decrease the buffering capacity of the retentate (Abd El-Salam et al., 1982). In this study, dilution of the retentate took place both during the manufacture of the MCC (diafiltration with RO water), and as part of preparing the concentration series for the study (dilution with RO water). These dilution steps are very realistic for the commercial preparation and use of the MCC, but at the same time are likely to affect the stability of casein micelles to high heat treatment, and not necessarily in a predictable way.

The most significant observation of this study is that sterilization treatments induced structural changes in serum protein- and lactose-depleted MCC obtained by microfiltration. Retorting resulted in a visually homogeneous product, with a lower viscosity and significantly larger particle sizes than the untreated MCC, whereas UHT treatment resulted in a product with high viscosity, visible particle aggregation, and structure formation (coagulation). Spray drying of the MCC accentuated the particle aggregation in all sterilized samples, and the minor compositional differences in the MCC obtained in the 3 experimental replications had a significant effect on their heating behavior. Understanding the mechanisms behind these effects would be very beneficial in preventing these instabilities and in designing appropriate sterilization regimens for commercial applications of MCC. Based on the data presented in the previous section, some hypotheses regarding the heat-induced transformations in the MCC are formulated below.

Studies on the effect of pH on the heat stability, often measured as heat coagulation time, of concentrated milks have shown that heat stability is reduced with decreasing pH (Singh and Creamer, 1991). Although this work did not investigate the effect of pH on the heat stability of MCC, some changes in pH were observed across experimental replications. These differences could not be avoided, and they may have contributed to the differences in the behavior of the different MCC replicates during heat treatments. The different stability of MCC and R-MCC to retorting and UHT treatment suggests that the heat-induced changes in the MCC are a function of temperature.

The decrease in pH of 0.15 to 0.2 units after retorting is consistent with previous reports. For instance, Berg and van Boekel (1994) reported a decrease in pH of 0.2 to 0.3 units after retorting of milk. According to Pyne and McHenry (1955), 3 different causes could be responsible for the decrease in pH during extended heat-

Figure 6. Time dependence of the soluble mineral content for untreated (controls), UHT-treated, and retorted micellar casein concentrate (MCC) samples. The values represent averages of all 3 experimental replicates, and the error bars represent 1 SD.

ing of milk at high temperatures: (1) thermal oxidation of lactose to organic acids, (2) dephosphorylation of casein, and (3) precipitation of the tertiary calcium phosphate, with a concomitant release of protons. As the MCC used in this study contained very low levels of lactose, it is safe to assume that the contribution of the last 2 mechanisms to the decrease in pH was more substantial than that of lactose.

The decrease in the absolute value of ζ -potential in the sterilized MCC and R-MCC as compared with the untreated controls indicates a reduced negative charge at the surface of the casein micelles, which could have been caused by a combination of κ -CN dissociation from the casein micelle and calcium phosphate precipitation

on the surface of the κ -CN-depleted micelles. It has been reported before that when heating serum protein-depleted milk at temperatures above 110°C the soluble casein content increased with both increasing heating time and casein concentration (Aoki et al., 1974). A subsequent study will be carried out to evaluate the fate of casein following sterilization of MCC. The lower absolute value of ζ -potential for the retorted concentrates for the third experimental replicate as compared with the other replicates can be explained by the lower pH of the untreated MCC from this processing replication.

Precipitation of calcium phosphate on the surface of the micelles is also supported by the reduction in

Figure 7. Calcium-phosphorus molar ratios in the soluble phase for untreated micellar casein concentrates (MCC) and reconstituted MCC (R-MCC), UHT-treated MCC and R-MCC, and retorted MCC and R-MCC immediately after the treatment (time = 0 h). The values represent averages of all 3 experimental replicates, and the error bars represent 1 SD.

soluble calcium and phosphorus in the MCC as a result of sterilization. The different Ca/P molar ratios in the soluble phase of MCC treated by retorting and UHT suggests the formation of different forms of insoluble calcium phosphate ($\text{Ca}_x[\text{PO}_4]_y$) in the sterilized MCC subjected to the 2 types of heat treatments (O'Connell and Fox, 2003). The higher rate of calcium resolubilization in the retorted MCC as compared with the UHT-treated MCC may be caused by the slightly lower pH in the retorted samples, as low pH values are known to increase the solubility of calcium phosphate.

Another observation is that the 2 sterilization processes affected differently Ca and Mg, 2 minerals that are partially associated with the casein micelles. Whereas soluble Ca levels were significantly lower in the UHT-treated than in the retorted MCC, no difference was observed between the soluble Mg levels between the 2 treatments. It has been shown before that calcium phosphate can form cross-links in artificial casein micelle systems, whereas magnesium phosphate does not have this cross-linking ability (Kaminogawa et al., 1977; Aoki et al., 1987). Thus, it is reasonable to hypothesize that some of the calcium phosphate may have participated in the cross-linking and subsequent aggregation of casein micelles during the UHT treatment, which prevented it from returning into the soluble phase.

The fact that the viscosity of the retorted samples was lower than of the controls could be due to the lower negative charges of the casein micelles in the retorted

MCC, which decreased the repulsion among casein micelles, thus causing a lower resistance to flow of these samples. These findings are in agreement with reports by Anema and Klostermeyer (1997) and Horne (1998). In case of the UHT-treated MCC, the apparent viscosity of the UHT-treated samples was more than 10-fold higher than of the untreated controls, due to the coagulation that took place during this treatment. This was even more significant in case of the UHT-treated R-MCC, which had apparent viscosity 2-fold higher than their UHT-treated MCC counterparts. Similar observations were made from the dynamic rheology data, which showed the transition from a liquid-like behavior before UHT to a solid-like behavior after UHT, particularly in the higher concentration MCC.

Overall, the results of this study indicate that sterilization treatments led to a partial insolubilization of minerals responsible for micellar stability (Ca, P, and Mg) and a reduction in surface charge of the casein micelles, which resulted in significant instability and aggregation phenomena, particularly in the UHT-treated samples. The observed increase in particle size could occur as a result of either aggregation of the native micelles or the destructuring of the micelles, followed by the formation of casein aggregates that are different than the native micelles.

It has been reported before that when milk is heated above 120°C, dissociation of κ -CN from the micelle takes place (Singh and Creamer, 1991; Singh and Latham, 1993). The κ -CN-depleted micelles become more sensitive to calcium and calcium phosphate, and heat-induced aggregation is essentially induced by calcium bridging (Singh, 2004). It is possible that more significant κ -CN dissociation from the micelles occurred in case of the UHT treatments as compared with retorting of the MCC, which led to less aggregation in the retorted MCC as compared with the UHT-treated MCC. The dissociation of casein micelles as a result of sterilization treatments is the subject of an ongoing investigation. Whether or not the removal of lactose may affect the stability of the micelles is not clear and may also require further analysis.

CONCLUSIONS

Commercial sterilization affected the stability, viscosity, and flow behavior of MCC obtained by membrane separation. The observed heat-induced changes were largely attributed to the redistribution of minerals, mainly calcium and phosphorus, at the micelle level as a result of heat treatments, and the loss in solubility of calcium phosphate. Additionally, dissociation of caseins may have occurred, but this has yet to be proven. The results of this study provide useful infor-

mation about the sterilization behavior and physical properties of MCC obtained by membrane separation. Retorting of MCC resulted in a more stable product than UHT treatment. The drying and reconstitution of MCC resulted in products that were significantly more unstable to UHT treatment as compared with the concentrates that had not been subjected to drying before the heat treatment. The differences in behavior observed among experimental replicates suggest that even small compositional and processing differences in the manufacture of the MCC can lead to significant changes in their sterilization stability, which is very important to consider in practical applications.

A full elucidation of the mechanisms responsible for the destabilization of MCC as a result of UHT treatment may provide solutions for avoiding this undesirable effect, without the addition of nondairy stabilizers.

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