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Partial calcium depletion during membrane filtration affects gelation of reconstituted milk protein concentrates

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ABSTRACT

Milk protein concentrate powders (MPC) with improved rehydration properties are often manufactured using processing steps, such as acidification and high-pressure processing, and with addition of other ingredients, such as sodium chloride, during their production. These steps are known to increase the amount of serum caseins or modify the mineral equilibrium, hence improving solubility of the retentates. The processing functionality of the micelles may be affected. The aim of this study was to investigate the effects of partial acidification by adding glucono-δ-lactone (GDL) to skim milk during membrane filtration on the structural changes of the casein micelles by observing their chymosin-induced coagulation behavior, as such coagulation is affected by both the supramolecular structure of the caseins and calcium equilibrium. Milk protein concentrates were prepared by preacidification with GDL to pH 6 using ultrafiltration (UF) and diafiltration (DF) followed by spray-drying. Reconstituted UF and DF samples (3.2% protein) treated with GDL showed significantly increased amounts of soluble calcium and nonsedimentable caseins compared with their respective controls, as measured by ion chromatography and sodium dodecyl sulfate-PAGE electrophoresis, respectively. The primary phase of chymosin-induced gelation was not significantly different between treatments as measured by the amount of caseino-macropeptide released. The rheological properties of the reconstituted MPC powders were determined immediately after addition of chymosin, both before and after dialysis against skim milk, to ensure similar serum composition for all samples. Reconstituted samples before dialysis showed no gelation (defined as tan $\delta = 1$), and after re-equilibration only control UF and DF samples showed gelation. The gelation properties of reconstituted MPC powders were negatively affected by the presence of soluble casein, and positively affected by the amount of both soluble and insoluble calcium present after reconstitution. This work, testing the chymosin-induced gelation behavior of various reconstituted MPC samples, clearly demonstrated that a decrease in pH to 6.0 during membrane filtration affects the integrity of the casein micelles supramolecular structure with important consequences to their processing functionality.
Key words: milk protein concentrate, casein, calcium depletion, chymosin-induced gelation

INTRODUCTION

Milk protein concentrate (**MPC**) powders are manufactured from skim milk through membrane filtration and spray drying. The major components of these shelf-stable food ingredients are caseins, whey proteins, lactose, fat, and minerals, in varying proportions depending on the extent of concentration and the type of membrane separation employed.

For optimal utilization of MPC it is important to clearly understand their functionality after reconstitution. The details of the chymosin-induced gelation properties of reconstituted MPC powders have been previously reported (Ferrer et al., 2008; O'Mahony et al., 2009; Martin et al., 2010; Hunter et al., 2011), as MPC can be employed in the cheesemaking processes to increase cheese yield and productivity. The chymosin-induced gelation of casein micelles consists of 2 phases. In the initial phase, the enzyme chymosin specifically cleaves the Phe105-Met106 bond of κ-CN, which is present on the casein micelle surface. This reaction causes a reduction of the steric and electrostatic repulsion forces between micelles (Dalgleish, 1984; Sandra and Dalgleish, 2007). At a lower pH, a release of soluble calcium occurs, chymosin increases its activity (van Hooydonk et al., 1986), and a partial collapse of the κ-CN layer takes place due to a decrease in electrostatic repulsion (De Kruif and Zhulina, 1996). Once most of the κ-CN has been cleaved, the micelles are able to approach

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one another and aggregate in the presence of calcium (Fox et al., 2000; Sandra et al., 2011). The equilibrium between soluble and colloidal calcium in the micelles is critical to the formation of chymosin-induced gels (Choi et al., 2007). Ferrer et al. (2008) reported that mineral equilibrium in MPC solutions plays a crucial role in the aggregation of chymosin-induced MPC gels. Furthermore, Kuo and Harper (2003) reported that chymosin-induced gels made with MPC56 were stiffer than those prepared with MPC85 tested at equivalent protein levels. It was recently demonstrated (Sandra and Corredig, 2013) that the presence of nonmicellar casein in the soluble phase may have a negative effect on the chymosin-induced gelation properties of MPC powders. Partial calcium depletion by the addition of sodium chloride (Sikand et al., 2013) chelating agents, cation exchange chromatography, or acidification can affect functional properties of MPC (Bhaskar et al., 2001). However, the effect of mineral distribution and the state of casein micelles on the technological properties of MPC is still not fully understood and the molecular mechanisms behind such effects have not been fully elucidated. Solubilization of colloidal calcium phosphate from casein micelles during membrane filtration (e.g., through acidification) may affect the structural organization of these protein particles. The aim of our study was to investigate the effects of addition of glucono-δ-lactone (**GDL**) to skim milk during membrane filtration on the structural changes of the casein micelles by studying their chymosin-induced gelation behavior after reconstitution, as chymosin-induced gelation is affected by both the calcium equilibrium and their supramolecular structure.

MATERIALS AND METHODS

Materials

Pasteurized skim milk was obtained from Producer's Dairy Foods Inc. (Fresno, CA). Analytical grade reagents were from Sigma-Aldrich Chemical Ltd. (St. Louis, MO). Glucono-δ-lactone was purchased from Roquette America, Inc. (Geneva, IL). Ultrapure water (Milli-Q Ultrapure Water Purification Systems, Billerica, MA) was used to prepare all the solutions.

Preparation of MPC65 and MPC80

The MPC powders were manufactured in duplicate, either by UF (65% protein; **MPC65**) or by UF followed by diafiltration (80% protein; **MPC80**) using pasteurized (72°C for 16 s) skim milk. The GDL (3.25 g/L) was added to cold milk (4°C) under continuous stirring to reach pH 6.0 in a time period of ~5 h before membrane filtration. Ultrafiltration began at 5.8 ± 1.5°C (mean ± SD). During UF, the temperature was allowed to increase in such a way that, by the end of the UF process, the temperature was 20 ± 1.6°C. Controls were prepared at the native milk pH (~pH 6.6). It is important to note that this research studied the effect of pH adjustment (to 6.0) only before starting the UF, and no pH control occurred throughout the UF or diafiltration (**DF**) steps. The MPC65 and MPC80 powders were manufactured in the pilot plant of Dairy Products Technology Center at California Polytechnic State University (San Luis Obispo) with a cross-flow membrane pilot-plant unit (R12 model, Niro Inc., Hudson, WI) equipped with dual 10-kDa cut-off, spiral-wound, polyethersulfone membranes (Snyder Filtration, Vacaville, CA). The liquid MPC was spray dried with a pilot Niro Filtermat Spray Dryer (Niro Inc.) to approximately 3.5% moisture, and the obtained MPC powders were immediately collected and upon cooling to ambient temperature (23 ± 2°C) sealed in airtight bags for further analysis. Total protein present in the powders was determined by Kjeldahl.

Powder Rehydration

The MPC powders were reconstituted in Ultrapure water (Milli-Q Ultrapure Water Purification Systems) to a final protein concentration of 3.2% (wt/wt) using a household kitchen blender for 6 min at high speed. Water was heated to 66°C using a heat-stir plate before reconstitution. Each reconstituted sample was then divided into 2 equal portions; one portion was stored in a tightly sealed container at 4°C overnight. The other portion of the rehydrated samples (prepared as described above) was dialyzed against skim milk at 4°C, also overnight, to allow enough time for full rehydration and obtain samples with similar rehydration history before and after dialysis. Dialysis was carried out using an 8,000-Da cut-off membrane (Spectra/Por, Spectrum Laboratories Inc., Rancho Dominguez, CA) to restore, as much as possible, the original milk serum composition and be able to compare the gelation behavior of the casein micelles between treatments. The control samples were named **UFC** and **DFC** before and **UFC-D** and **DFC-D** after dialysis and the GDL-treated samples were named **UFG** and **DFG** before and **UFG-D** and **DFG-D** after dialysis for UF and DF samples, respectively.

Characterization of Rehydrated Samples

The protein was measured by the Dumas method (Leco FP-528; Leco Corp., St. Joseph, MI) and the pH was measured using an Accumet pH meter model 925