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Isolation of caseins from whey proteins by microfiltration modifying the mineral balance in skim milk

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ABSTRACT

The objective of this work was to study the effect of different salts and salt concentration on the isolation of casein micelles from bovine raw skim milk by tangential flow microfiltration. Tangential flow microfiltration (0.22 μm) was conducted in a continuous process adding a modified buffer to maintain a constant initial sample volume. This buffer contained calcium chloride ($CaCl_2$), sodium phosphate (Na_2HPO_4), or potassium citrate ($K_3C_6H_5O_7$) in concentrations ranging from 0 to 100 m*M*. The concentrations of caseins and whey proteins retained were determined by sodium dodecyl sulfate-PAGE and analyzed using the Scion Image software (Scion Corporation, Frederick, MD). A complete isolation of caseins from whey proteins was achieved using sodium phosphate in the range of 10 to 50 m*M* and 20 times the initial volume of buffer added. No whey proteins were detected at 50 m*M* but this was at the expense of low caseins being retained. When lower sodium phosphate concentrations were used, the amount of caseins retained was higher but a small amount of whey proteins were still detected by sodium dodecyl sulfate-PAGE. Among the salts tested, calcium chloride at 50 m*M* and all volumes of buffer showed the higher retention of casein proteins. The highest casein:whey protein ratio was found at 30 m*M* $CaCl_2$, but no complete casein micelle isolation was achieved. Potassium citrate was the most ineffective salt because a rapid loss of caseins and whey proteins was observed at all concentrations and with low quantities of buffer added during the filtration process. Our results show the potential of altering the mineral balance in milk for isolation of casein micelles from whey proteins in a continuous tangential flow microfiltration system.

Key words: microfiltration, casein micelle, whey protein, mineral

INTRODUCTION

Milk proteins are well known for their nutritional value and for their potential application as functional ingredients to affect the physical and sensory attributes of a wide range of dairy food products. Caseins are one of the most important and complex proteins in bovine milk representing approximately 80% of the total protein fraction in milk. These proteins have excellent surfactant properties in emulsions and foams, gelling properties, and thermal resistance to denaturation (Fox and McSweeney, 1998) because of their lack of complex secondary and tertiary structure. However, they form colloidal particles (50–500 nm; the so-called casein micelle) composed of the proteins α_{S1}-, α_{S2}-, β-, and κ-caseins, and salts of Ca, P, Mg, and Zn (Fox, 2003). Casein isolates are mostly available as caseinates, derived from acid precipitation of milk and subsequent neutralization with NaOH, CaOH, or KOH, and commercialized as sodium, calcium, or potassium caseinates, respectively.

The wide particle size distribution of the various milk components (from nano- to micrometer scales) made separation based on size a convenient operation to obtain specific milk fractions (Korhonen and Pihlanto, 2007). Because of its relatively low operating cost, microfiltration using polymeric or ceramic membranes has been extensively used by the dairy industry to concentrate and isolate native caseins (Brans et al., 2004). However, electrophoresis (SDS-PAGE) of commercially available native casein isolates (e.g., micellar casein, American Casein Company, Burlington, NJ; micellnor, Kerry Dairy Ingredients, Kerry, Ireland) revealed that whey proteins remain in the final product and that complete isolation of the casein micelle remains a challenge (Figure 1). Methods were developed to obtain single fractions of casein (e.g., β-CN; Huppertz et al., 2006) but the effective isolation of the native casein micelle is still possible only at the laboratory scale (Rosenberg, 1995; Korhonen and Pihlanto, 2007). Membranes used in ultrafiltration usually have a pore size between 0.01 and 0.1 μm (or a molecular weight cutoff from 1 to 300 kDa; Ghosh, 2009) and those for microfiltration have a pore size from 0.1 to 1 μm. The ultrafiltration of skim

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milk using a molecular weight cutoff ≤300 kDa yields a permeate rich in water-soluble vitamins, minerals, lactose, and nonprotein nitrogen compounds and a retentate rich in colloidal minerals and proteins (caseins and whey; Rosenberg, 1995).

The mineral fraction of milk is relatively small (8–9 g/L; Gaucheron, 2005) and is mainly composed of the cations calcium, magnesium, sodium, and potassium, and the anions inorganic phosphate, citrate, and chloride. In milk, ions can be found free in the serum or in colloidal form associated with the caseins. Milk salts play an important role in the properties of dairy foods, because altering the balance of the mineral fraction in milk will affect the structure, stability, and functionality of casein micelles (Swaisgood, 1996; Gaucheron, 2005).

An effective protocol for the isolation of the native casein micelles would be beneficial in the study of casein micelle structure–function properties and in the development of novel ingredients based on casein isolates (Brans et al., 2004; Mier et al., 2008). Because it is known that altering the mineral fraction of skim milk modifies the interactions between caseins and whey proteins, the purpose of this study was to evaluate the effect of ionic strength of milk relative to 3 salts and to establish the potential use of tangential flow microfiltration for the complete isolation of native casein micelles from bovine milk. Ionic strength was modified by changing the concentrations of calcium chloride ($CaCl_2$), sodium phosphate (Na_2HPO_4), and potassium citrate ($K_3C_6H_5O_7$), commonly used in the dairy industry or naturally present in milk.

MATERIALS AND METHODS

Raw milk was obtained from the dairy farm of The University of Tennessee (Knoxville) and skimmed by centrifugation (1,500 × *g*, 15 min) using a Sorvall RC-5B Plus centrifuge (Thermo Scientific, Waltham, MA) equipped with a SLA-1500 rotor (Kendro, Newtown, CT). Sodium azide (0.02% wt/vol; Fisher Scientific, Fair Lawn, NJ) was added to the skim milk to prevent bacterial growth.

A 20 m*M* imidazole buffer solution was prepared and modified by adding $CaCl_2$, Na_2HPO_4, or $K_3C_6H_5O_7$ at concentrations of 10, 30, 50, or 100 m*M*. The ionic strength for $CaCl_2$ and Na_2HPO_4 solutions ranged from 0.03 to 0.30 (10 to 100 m*M*), whereas that for $K_3C_6H_5O_7$ solutions ranged from 0.06 to 0.60 (10 to 100 m*M*). The pH was adjusted to 6.8 with 1 *N* HCl and the solutions were stored at 4°C overnight to reach equilibrium. All chemicals were of analytical grade and purchased from Fisher Scientific.

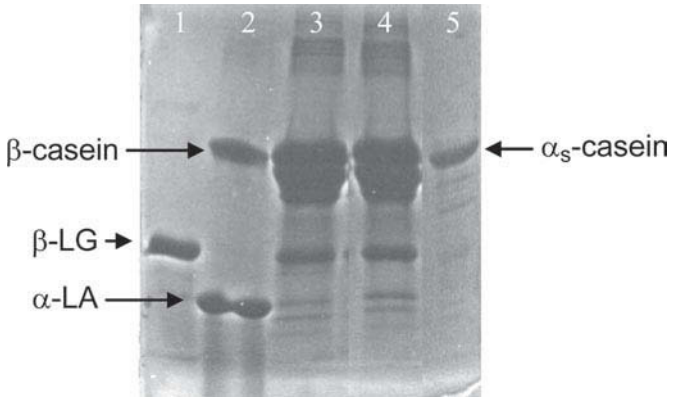

Figure 1. Sodium dodecyl sulfate-PAGE of 2 commercial native casein isolates. Lane 1 = β-LG standard; lane 2 = β-CN and α-LA standards; lane 3 = commercial casein isolate (Micellnor, Kerry Dairy Ingredients, Kerry, Ireland); lane 4 = commercial casein isolate (Micellar Casein, American Casein Company, Burlington, NJ); and lane 5 = α_S-CN standard.

Filtration was done using a 0.22-μm tangential flow microfiltration cartridge (Pellicon XL50, Millipore, Billerica, MA) with a filtration area of 50 cm^2 and a hydrophilic polyvinylidene fluoride membrane connected to a variable flow peristaltic pump (Vera, Barnat, Barrington, IL). Preliminary exploratory tests showed strong whey protein retention using filtration membranes with a small pore size (30, 100, and 300 kDa; 0.1 μm) and loss of caseins with larger pore size membranes (0.45 μm). Each modified buffer solution was initially added to skim milk (1:1, vol/vol), and then buffer solution was added during the filtration process to keep a constant volume of retentate. The retentate was recirculated during filtration and permeates were disposed. Experiments were conducted at room temperature and the microfiltration process was ended when 20 volumes of the modified buffer solution were circulated through the original skim milk sample (1:20, vol/vol) and the final and initial volumes of retentate were the same. Samples of retentates (0.5 mL) were collected at regular intervals during the microfiltration process and stored at −40°C until analyzed by SDS-PAGE.

For SDS-PAGE, the retentate samples were thawed at room temperature and diluted (1:4, vol/vol) in an SDS reducing buffer for SDS-PAGE according to Laemmli (1970). A vertical electrophoresis unit (Mini-Protean 3 Cell, Bio-Rad Laboratories, Hercules, CA), in conjunction with a power supply (PowerPack 300, Bio-Rad Laboratories), was used. The samples were loaded (20 μL) in precast gels (Ready gels, 15% Tris-HCl, Bio-Rad Laboratories) and run for 30 min at 200 V. The gels were then stained with a 0.25% (wt/vol) solution of Coomassie Brilliant Blue R-250 (Bio-Rad