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Changes in the physico-chemical properties of casein micelles during ultrafiltration combined with diafiltration

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ABSTRACT

The objective of this work was to determine if after membrane processing, the physical properties of casein micelles change. Milk was concentrated by ultrafiltration, and also subjected to various levels of diafiltration, by addition of water to the retentate. After the membrane concentration process, the retentates were diluted back to their original concentration, to study their physico-chemical properties. For better comparison, all the samples were dialyzed against the original milk, to obtain similar serum compositions. For the first time, the effect of different levels of diafiltration was studied. Diafiltration induced losses of colloidal calcium phosphate and caused changes in the turbidity parameter ($1/l^*$) measured by light scattering, as well as in the ultrasonic properties (velocity and attenuation) of the casein micelles. When tested in a similar serum environment, the reconstituted micelles after diafiltration showed a lower susceptibility to aggregation and the rennet induced gels had a lower storage modulus than those formed with the original milk, at the same protein concentration. This work brings for the first time evidence of the differences in the physical properties of the casein micelles as a function of membrane processing history.

1. Introduction

Milk protein concentrates prepared using membrane filtration have become an increasingly popular source of functional ingredients. The separation of solutes from milk occurs on the membrane surface, and as the composition of milk changes, the physical properties and functionality of milk protein concentrates also may change. During the process, concentration polarization at the membrane surface can rise to a point where the concentrated solutes form a gel layer on the membrane, and this irreversibly decreases the permeation flux and process performance, ultimately causing fouling of the membrane (Grandison, Youravong, & Lewis, 2000; Rinaldoni, Tarazaga, Campderros, & Perez Padilla, 2009). As the protein concentration increases, the permeate fluxes decline. To improve performance and achieve high concentration of proteins, water is then added to the concentrates to increase the flow and continue the separation (Brans, Schroen, van der Sman, & Boom, 2004). This common practice in dairy processing, known as diafiltration, results in a decrease of soluble ions and lactose from the concentrate, and a higher protein concentration in the final retentates (Mulhivill & Ennis, 2003).

Although diafiltration results in milk protein concentrates with high protein contents, this process alters the ionic environment surrounding the casein micelles, because of a dilution effect. The casein micelles are an assembly of four main proteins, α_{s1}-, α_{s2}-, κ- and β-casein, held together by equilibrium of hydrophobic and electrostatic interactions, as well as calcium phosphate bridges between the casein proteins. The integrity of the micelle is of great importance in dairy processing. It is widely accepted that the equilibrium between the colloidal calcium phosphate (CCP) associated with the micelles and the soluble calcium present in the serum phase is a key factor in determining the cheese making properties of the casein micelles (Choi, Horne, & Lucey, 2007). This equilibrium is modified during membrane filtration.

Previous studies have proposed that despite the losses of soluble calcium during diafiltration of milk, this process shows minimal effects on the levels of insoluble calcium (usually taken as micellar calcium) (Kulozik, 1998). The objective of this work was to determine if membrane filtration, and in particular, diafiltration, affects the physical properties of the casein micelles and their renneting functionality. The filtration parameters were kept constant and filtration was carried out at a low transmembrane pressure (TMP) and at 40 °C. Discontinuous diafiltration was used to optimize the

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efficiency of permeate removal and better control the volume of water added to the retentate. The milk was ultrafiltered until it reached a 2 times concentration (based on volume reduction) and then water was added to reach the original volume of milk, this process was carried out numerous times, to reach different levels of permeate reduction.

2. Materials and methods

2.1. Sample preparation. Membrane filtration

Fresh skim milk was obtained from Gay Lea Foods (Guelph, Ontario, Canada). Sodium azide (0.02 g/100 mL) (Fisher Scientific, Whitby, Ontario, Canada) was added and the milk was filtered four times through Whatman Fiberglass filters (Fisher Sci.) to eliminate residual fat globules. The milk was then kept at 4 °C until further use.

Membrane filtration was carried out using a tangential flow filtration system (PUROSEP LT-2, SmartFlow Technologies, Apex, NC, USA) with a polyethersulphone membrane (CONSEP 3000, 0.058 m^2 and 0.75 mm channel height, SmartFlow Technologies) with nominal molecular weight cut-off 10 KDa. The concentration step was carried out until half of the volume of permeate was recovered (measured using a graduated cylinder). The maximum level of concentration reached at all times was 2-times (i.e. half of the original volume, based on volume of permeate), as previous research demonstrated that this level of concentration does not show an effect on the physico-chemical properties and processing functionality of the casein micelles (Ferrer, Alexander, & Corredig, 2011). The system was operated with a transmembrane pressure (TMP) of 170 kPa and cross-flow velocity of 12 L min^{-1}. The retentate was kept at 40 °C using a water bath attached to a double-jacketed feed tank.

To avoid any initial sample dilution with water, the retentate tank was filled with skim milk, the pump was started and at least 1 L of milk was discarded immediately after exiting the membrane outlet. The milk was then recirculated at low TMP to fill the membrane and permeate compartment. After permeation of at least 200 mL of permeate, the whole system was drained. Then the tank was filled with a new batch of milk, and the experiment started. The membranes were then cleaned according to manufacturer's instructions (Ferrer et al., 2011).

Three levels of diafiltration were achieved by replacing approximately 10%, 50% and 100% of the volume of permeate with MilliQ water. These samples are referred to as 10DF, 50DF and 100DF, respectively. The milk was concentrated by removing 10% of its original volume as permeate, and the volume of permeate removed was replaced with MilliQ water. After recirculating the retentate for 2 min a small sample was collected (10DF). The addition of water resulted in a volume fraction for the retentates comparable to those of the original skim milk. The residual retentate was then concentrated removing half of the original volume as permeate, and the volume of permeate removed replaced with water (50DF). The 2 times volumetric concentration and redilution was then repeated 4 consecutive times, and a sample was collected (100DF). The volume reduction never reached more than a 2× concentration. A corresponding permeate sample was collected for each retentate. The first 200 mL of permeate were discarded before collection. The entire membrane filtration process was repeated three times, with three different skim milk batches.

A portion of the samples (10DF, 50DF and 100DF) was dialyzed (nominal cut-off of 6–8 kDa, Millipore Corp., Bedford, MA, USA) against 33 volumes of raw skim milk for 24 h at 4 °C, to reach comparable compositions of the serum phases, for renneting experiments. After dialysis the protein content of the dialyzed milk was measured (Bio-rad DC protein assay, Bio-Rad Laboratories, Mississauga, Ontario, Canada), and if necessary, the protein concentration was adjusted by adding permeate. This ensured a comparable volume fraction of caseins in all the samples.

2.2. Light scattering

The apparent diameter and ζ-potential of the casein micelles were measured immediately after diafiltration and after 24 h of storage at 4 °C using a dynamic light scattering (DLS) instrument (Zetasizer, Nano-ZS, Malvern Instruments, Malvern, UK) by diluting each sample in its own permeate about 1000 times, to avoid multiple scattering. The measurements were performed at 23 °C.

The light scattering properties of the various samples were also measured undiluted using diffusing wave spectroscopy (DWS). This technique, optimal for turbid samples, can be employed not only to measure the size of the casein micelles *in situ*, but also to investigate the turbidity (positional correlation) of the colloidal particles via the value of the photon transport mean free path, l^*. A detailed description of the theory, the differences from traditional light scattering and the experimental set up can be found elsewhere (Ferrer et al., 2011; Weitz, Zhu, Durian, Gang, & Pine, 1993).

As viscosity and refractive index of the milk serum are necessary parameters for DLS and DWS experiments, they were measured experimentally. Viscosity of the different permeates was determined using a calibration curve of aqueous solutions of lactose. Viscosities were obtained using a Cannon Fenske viscometer for transparent liquids using about 7 mL of sample at 23 °C. For each level of lactose concentration, 10 replicates were measured and the kinematic viscosity (η_k) was calculated (Swindells & Ullmann, 1959)

$$\eta_k = C^*t \quad (1)$$

where C is a constant (cSt/s) related to the Cannon viscometer and t is the time taken by the sample to flow a set distance. The dynamic viscosities (η) in centipoises were then calculated using the following equation:

$$\eta = \eta_k{}^*\rho \quad (2)$$

where ρ were the densities (kg m^{-3}) of the different serums. Refractive indices of the various permeates were measured using an Abbe refractometer (Carl Zeiss Canada Ltd, Toronto, Ontario, Canada) at 20 °C. A value of 1.39 was used for the casein micelles.

2.3. Determination of soluble, total calcium and phosphate

The concentration of calcium in skim milk, DF retentates, as well as the corresponding centrifugal supernatants and permeates were determined by non-suppressed ion chromatography (Rahimi-Yazdi, Ferrer, & Corredig, 2010). Skim milk and retentate samples were centrifuged at 25,000 × g using a Beckman Coulter Optima™ LE-80K Ultracentrifuge with rotor type 70.1 Ti (Beckman Coulter Canada Inc., Mississauga, Ontario, Canada) for 30 min at 20 °C. Samples, serum phases from the centrifugation and permeates from UF-DF cycles were frozen at −18 °C, until further analysis.

For the determination of soluble calcium (defined as the total calcium in serum after centrifugation) and calcium in permeates, different amounts of centrifugal supernatants or permeates were diluted with HPLC water with the addition of HCl 1 mol L^{-1} (final concentration 2 g L^{-1}). For the determination of total (soluble plus insoluble) cations 666 μL of sample, 200 μL of HCl (1 mol L^{-1}) and 466 μL of HPLC water were mixed in 1.5 mL eppendorf microcentrifuge tubes. The samples were centrifuged at room temperature for 15 min at 4500 × g (Eppendorf centrifuge 5415D,

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