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Probing the colloidal properties of skim milk using acoustic and electroacoustic spectroscopy. Effect of concentration, heating and acidification

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ABSTRACT

In colloidal systems physical–chemical changes are often a function of volume fraction and sample dilutions are critical. While most methods to characterize colloidal particles either require dilution or some disruption, acoustic spectroscopy can be performed *in situ*, without dilution. Objective of this work was to determine the effects of concentration, heating and acidification on the acoustic and electroacoustic properties of casein micelles in skim milk. The ultrasonic attenuation of skim milk increased with concentration of milk and frequency, and the average size of the colloidal particles calculated from the frequency dependence of attenuation was about 0.15 μm for both unheated and heated milk. When milk was concentrated by ultrafiltration, at 3× and 4× concentration (based on volume reduction), the calculated size deviated from that derived in undiluted or mildly concentrated milk, most likely because of increased particle–particle interactions. Electroacoustic measurements revealed a constant dynamic mobility of the particles in undiluted and concentrated milk, while lower mobilities were observed for milk diluted in permeate. The ζ-potential measured was significantly higher than the values measured using dynamic light scattering, with a value of −45.8 mV for casein micelles in unheated milk. With acidification, the ζ-potential decreased monotonically. Heating profoundly affected the change in charge with pH of the micelles, and it was concluded that the interaction of casein micelles with the whey proteins increased the surface charge of the casein micelles.

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1. Introduction

Casein micelles are a supramolecular aggregate of proteins interacting by hydrophobic and electrostatic interactions, linked together by calcium phosphate clusters [1,2]. At the natural pH and temperature of milk, casein micelles are stabilized primarily by κ-casein via steric interactions in addition to electrostatic and van der Waals forces [3]. This hairy κ-casein layer has been described as a salted polyelectrolyte brush [4] which ensures the stability of the micelles as long as it remains extended in solution. The means of controlling stability and destabilization of casein micelles are of great interest in dairy technology, as key to understand and manipulate processes and modify product structure.

Electrostatic forces play a significant role in the destabilization of casein micelle. By measuring the ζ-potential of the casein micelles, which is the electrokinetic potential of these soft and hairy colloidal particles, it is therefore possible to estimate the extent of their charge. When the absolute value of ζ-potential is sufficiently high, (with an absolute value approaching or above 25 mV), the system will be readily stable.

Milk processing strongly affects the surface properties of the casein micelles, causing changes in the reactivity of these protein particles. Concentration of milk by membrane filtration causes an increase in the volume fraction of the casein micelles, an increase in the colloidal minerals (those interacting with the casein micelles) while decreasing the water, soluble minerals, lactose and small molecules, which are removed in the permeate fraction. This process creates ionic unbalances which may cause changes in the polyelectrolyte brush and, as a consequence, to the processing functionality of the casein micelle. Thermal treatments also modify the functional properties of milk, by the changes occurring to the whey proteins present in solution. Whey proteins denature and interact amongst themselves and with casein micelles, forming aggregates via hydrophobic interactions and disulphide bridging. The location of these aggregates is either soluble in the serum phase or on the surface of the casein micelles, and this distribution depends on the value of the pH of the milk at the time of heating. At the natural pH of milk, a substantial amount of whey proteins is adsorbed on the surface of the casein micelles and the soluble aggregates are about 30 nm in diameter [5,6]. Acidification of milk causes the protonation of the carboxylic groups and a reduced electrostatic repulsion between the casein micelles. The hairy layer becomes decreasingly extended in the serum and eventually collapses on the surface of the micelle, whereas the micelles are

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gradually depleted of colloidal calcium phosphate leading to increased ionic strength in the serum phase [4,7,8].

The majority of analytical techniques used for the characterization of colloidal systems such as milk do not allow measuring at concentrated volume fractions, and this could alter the properties of the system under investigation. For example, the precision of conventional light scattering techniques, especially in the estimation of the ζ-potential, is largely confined to very dilute regimes. On the other hand, ultrasonic and electroacoustic methods are not affected by the turbidity of the medium, and changes occurring in milk can be observed *in situ*, without dilution.

The ultrasonic properties of food materials have been increasingly studied over the last few decades, for example, to determine food composition, follow phase transitions, crystal formation or interactions between different components [9,10]. In dairy foods, both transmission and resonance techniques have been used [11,12].

As the ultrasound propagates through the colloidal dispersion, ultrasonic energy is dissipated, and the extent of the loss is measured as ultrasonic attenuation. In addition to the intrinsic attenuation, which is a function of the material properties, colloidal particles drastically attenuate the ultrasonic wave because of viscoinertial losses, thermal absorption as well as scattering. Since these loss mechanisms are dependent on the thermophysical properties of the continuous phase and the various components present in the dispersion, it is possible to calculate the particle size distribution of a colloidal system from its attenuation spectrum [13].

Electroacoustic analysis can also be performed by coupling acoustic and electric fields, and can be employed for the characterization of the charge properties of the colloidal particles. In electroacoustics, an electrical input and an acoustic response (electrokinetic sonic amplitude) [14], or conversely an ultrasonic input and an electrical response (colloid vibration current) [15] can be implemented to derive the particle's ζ-potential. With the colloid vibration current (CVI) method, which is employed in the present research, an ultrasound wave causes slight vibrations in the particles by means of planar wave compressions, the motion of particles relative to the liquid disturbs their double layers, and as a result an electroacoustic signal is measured as colloid vibration current (CVI) [16]. From the CVI, and knowing the density contrast between the particles and the continuous phase, electrophoretic mobility and ζ-potential can be derived.

In most of the earlier investigations on casein micelles, ζ-potential was measured using light scattering-based techniques [7,17], and the analysis can be carried out only under diluted conditions. Typically, a ζ-potential of about −20 mV has been reported at the natural pH of milk (∼pH 6.7). The ζ-potential of casein micelles in undiluted milk using electroacoustics has also been reported [18]. These investigators used electroacoustic spectroscopy on commercial and reconstituted skim milk, to study the pH dependence of the dynamic mobility of the casein micelles. The ζ-potential was calculated, under low surface conductivity and thin double layer assumptions, using the electrokinetic sonic amplitude (ESA) theory [14]. The values obtained by these authors agreed with those reported in the literature measured under diluted conditions using DLS. The authors clearly pointed out the critical importance of sample preparation and the use of the appropriate continuous phase background for the analysis of the ζ-potential of casein micelles in skim milk. In native milk, the casein micelles are in fact in ionic equilibrium with the surrounding serum, and the serum composition will affect the colloidal calcium phosphate present as well as the κ-casein polyelectrolyte layer surrounding the casein micelles.

The objective of this work was to determine the colloidal properties (ζ-potential and size) of casein micelles in skim milk using acoustic and electroacoustic spectroscopy, to measure changes *in situ*, without dilution, as a function of concentration, as well as after heating and during acidification.

2. Materials and methods

Fresh pooled bovine milk was obtained from the Research Station (Elora, Ontario, Canada) of University of Guelph. Sodium azide (0.02% by wt) (Fisher Scientific, Fair Lawn, NJ, USA) was immediately added to minimize bacterial growth. Skimming of milk was carried out by centrifugation (20 min at 18,000*g*, 4 °C) (supplied with a J2-21 rotor, Beckman-Coulter, Mississauga, Ontario, Canada), followed by vacuum filtration using 110 mm glass microfibre Whatman filters (934-AH™) (Florham Park, NJ, USA). Skim milk was kept refrigerated (4 °C) at all times.

Skim milk was concentrated up to 4× (based on volume reduction, by measuring the volume of permeate) using a lab-scale ultrafiltration unit with a molecular mass cut-off of 10 kDa (Millipore Corporation, Billerica, MA, USA). Both permeate (the continuous phase transmitted through the filter) and retentate (the concentrated portion) fractions were collected. Different dilutions were prepared by addition of permeate (from the ultrafiltration) to skim milk. This allowed the preparation of milk with different volume fractions of casein micelles but identical ionic composition of the continuous phase.

2.1. Heat treatment and acidification

Skim milk was heated to 85 °C using a water-bath and kept at this temperature for 20 min. Aliquots of both heated and unheated skim milk samples were acidified adding varying amounts of glucono-δ-lactone (GDL) (0–1% w/w) (Sigma–Aldrich Inc., St. Louis, MO, USA). The samples were kept gently stirred using a magnetic stirrer and incubated at room temperature (22 °C). The pH of the system was recorded at 30 min intervals, for a total duration of 5 h. When the change in pH was less than 0.02 pH units in 30 min, the system was assumed to be in equilibrium and casein micelles were separated from the serum within an hour. The pH of milk was adjusted to 7.2 using a concentrated NaOH solution (2 M). All experiments were carried out in triplicate.

2.2. Separation of caseins micelles from their serum fraction

Two different methods were used to determine ζ-potential: dynamic light scattering (DLS) (Zetasizer Nano, Malvern Instruments, Worcestershire, UK), and electroacoustic spectroscopy (DT-1200 Acoustic and Electroacoustic Spectrometer, Dispersion Technology, Bedford Hills, NY, USA). The electroacoustic device can analyze ζ-potential of casein micelles in skim milk with no need for dilution; however the background signal corresponding to the sample needs to be subtracted. On the other hand, dilutions of approximately 1:500–1000 are needed for DLS analysis, and for this reason, an appropriate continuous phase (with the appropriate the ionic equilibrium after the GDL reaction) needed to be separated for these experiments.

To obtain the corresponding serum, each sample was centrifuged at 95,000*g* for 30 min (OptimaTM LE-80 K supplied with a Ti-45 rotor, Beckman-Coulter, Mississauga, Ontario, Canada). The centrifugal supernatant was filtered using nylon syringe filters (0.2 μm, Fisherbrand, Ireland). The protein concentration in the ultracentrifuged serum fractions was determined indirectly using the Dumas method (Leco FP-528 Nitrogen Analyzer, St. Joseph, MI, USA). The nitrogen content was converted to protein concentration by a conversion factor of 6.38 [19]. All the nitrogen content analyses were carried out in triplicate.

For the electroacoustic analysis, the milk sera were used in the determination of the background electroacoustic signal and then ζ-potential of the undiluted skim milk was determined, as a

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