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The pressure-induced, lactose-dependent changes in the composition and size of casein micelles

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ABSTRACT

The effects of lactose on the changes in the composition and size of casein micelles induced by high-pressure treatment and the related mechanism of action were investigated. Dispersions of ultracentrifuged casein micelle pellets with 0–10% (w/v) lactose were subjected to high pressure (400 MPa) at 20 °C for 40 min. The results indicated that the level of non-sedimentable caseins was positively related to the amount of lactose added prior to pressure treatment, and negatively correlated to the size. A mechanism for the pressure-induced, lactose-dependent changes in the casein micelles is proposed. Lactose inhibits the hydrophobic interactions between the micellar fragments during or after pressure release, through the hydrophilic layer formed by their hydrogen bonds around the micellar fragments. In addition, lactose does not favour the association between calcium and the casein aggregates after pressure release. Due to these two functions, lactose inhibited the formation of larger micelles after pressure treatment.

1. Introduction

Casein is the major protein fraction in milk, comprising about 80% of the total milk proteins. Due to their relatively open conformation and abundant degree of conformational flexibility, caseins are categorised as rheomorphic proteins (Dalgleish & Corredig, 2012; Holt, Carver, Ecroyd, & Thorn, 2013). In milk, the caseins, together with calcium phosphate, form association colloids called casein micelles. Casein micelles, the primary building blocks of almost all milk-based products, are at the core of the dairy industry. The integrity of casein micelles is likely stabilised by hydrophobic interactions and electrostatic interactions, mainly through micellar clusters of calcium phosphate (Orlien, Boserup, & Olsen, 2010).

Different processing treatments are often adopted in the manufacture of milk-based products for various industrial purposes. The structure of the casein micelles changes depending on the processing operation, which gives milk a variety of technological properties (Fox & Brodkorb, 2008). Of those processing techniques, high-pressure treatment, an alternative to thermal treatment in inactivating microorganisms, has been shown to have the most potential, as it retains the sensory and nutritional quality of milk-based products (Goyal, Sharma, Upadhyay, Sihag, & Kaushik, 2013). However, when destroying spoilage microorganisms, high-pressure processing substantially alters the casein micelles, and therefore, modifies their technological properties, including optical or rheological properties and acid- or rennet-induced gel strength (Abbasi & Dickinson, 2001; Altuner, Alpas, Erdem, & Bozoglu, 2006).

After pressure treatment (above 200 MPa), the casein micelles are irreversibly disrupted into smaller particles (Anema, Lowe, & Stockmann, 2005). The mechanistic actions of high pressure-induced changes in casein micelles are not well established. The state of calcium and hydrophobic interactions has been suggested to play crucial roles in theses changes. Depending on the processing conditions, high-pressure treatment can influence the various types of forces to differing degrees, and consequently lead to the formation of different structures of casein micelles (Orlien et al., 2010; Regnault, Thiebaud, Dumay, & Cheftel, 2004).

The pressure-induced changes in casein micelles are also strongly dependent on the aqueous microenvironment (Huppertz & de Kruif, 2006; Merel-Rausch, Kulozik, & Hinrichs, 2007). The structure of casein micelles responds in different ways depending on the components present in the aqueous phase. When subjected to high pressure, the changes in the whey proteins, minerals and ethanol in the aqueous phase could easily alter the thermodynamic

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properties of casein micelles (Harte, Gurram, Luedecke, Swanson, & Barbosa-Canovas, 2007; Keenan, Young, Tier, Jones, & Underdown, 2001; Huppertz, Grosman, Fox, & Kelly, 2004). As a result, the pressure-modified casein micelles exhibit a different structure and different functional properties.

Lactose is an abundant disaccharide in the aqueous phase of milk, with some milks containing up to 7% lactose. The lactose content in milk significantly affects the stability of casein micelles. A study of the effects of lactose on the apparent micellar casein mass of casein micelles reconstituted from caseins demonstrated that 0.3 mol L^{-1} lactose reduced the mass by more than 45% of the value in lactose-free casein micelle dispersions (Mozersky, Farrell, & Barford, 1991). A recent study showed that the ion properties of milk varied with lactose concentration (in the range of 0–15% (w/w)). These variations of ion properties could lead to the conformational stability of casein micelles (Gao et al., 2010). It has been concluded that sugars can induce changes in the thermodynamic properties of proteins. Such changes could easily modify the biopolymer interactions, and consequently, the technological properties of milk (Dissanayake et al., 2013; Mancilla Canales, Hidalgo, Risso, & Alvarez, 2010). Our literature search led us to believe that lactose can influence the pressure-induced changes in casein micelles. However, little information is available on the effect of lactose in the aqueous phase on the high pressure-induced changes in the physicochemical characteristics of casein micelles.

Of all the physicochemical properties of casein micelles, the composition and size are the most important and most studied characteristics, since they affect key properties of a dispersed system (Anema et al., 2005; Glantz et al., 2010; Liu, Dunstan, & Martin, 2012). Therefore, the objective of this work was to evaluate the high pressure-induced changes in the composition and size of casein micelles, with special reference to the influence of lactose. Additionally, the main driving forces (hydrophobic interaction and calcium binding capacity of micellar fragments) for the formation of micelles after pressure treatment were evaluated to further explain the cause of changes in casein micelles.

2. Material and methods

2.1. Sample preparation

Raw bovine milk was collected from a local farm (Sino-US Center for Dairy Research, China Agricultural University, Beijing, China). The milk was defatted twice by centrifugation at 3500×*g* for 20 min at 25 °C in a Feige centrifuge (TDL-5-A, Shanghai, China). Isolated casein micelles were obtained by ultracentrifugation at 70,000×*g* for 60 min at 25 °C in an Optima L-XP100 ultracentrifuge (Beckman, CA, US), using a type 45 Ti rotor. After ultracentrifugation, the supernatant was discarded and the pellet was collected and fully dispersed in lactose-free simulated milk ultrafiltrate (SMUF) with 0.02% (w/v) sodium azide for 10 h. The volume of SMUF was equal to that of the discarded supernatant. The composition of lactose-free-SMUF was 1.58 g L^{-1} KH_2PO_4, 1.20 g L^{-1} $K_3Citrate{\cdot}H_20$, 1.79 g L^{-1} $NaCitrate{\cdot}5H_20$, 0.18 g L^{-1} K_2SO_4, 1.32 g L^{-1} $CaCl_2{\cdot}2H_20$, 0.65 g L^{-1} $MgCl_2{\cdot}6H_20$, 0.30 g L^{-1} K_2CO_3, 0.60 g L^{-1} KCl, and adjusting the pH with NaOH to 6.6, based on the method of Beliciu and Moraru (2009). After the micelles were fully dispersed, solid α-lactose (Sigma-Aldrich, MO, USA) was added to the dispersion to reach 0%, 5% and 10% (w/v), respectively. Then all the samples (including the lactose-free samples) were stirred magnetically for 40 min to allow all the lactose to dissolve completely. Since a little dilution of the dispersion occurred due to the added lactose, the volume of the dispersions containing different concentrations of lactose were balanced by adding appropriate volumes of ultrapure water.

2.2. Pressure treatment

Samples (50 ml) were poured into polythene bags and vacuum sealed. High hydrostatic pressure was achieved at 400 MPa and 20 °C for 40 min in a HPP.L2-600 apparatus (Huatai Senmiao Corporation Biological Engineering Technology Ltd., Tianjin, China), using water as the pressure-transmitting medium. The pressure increase and release rate was 6.5 MPa/s and 20 MPa/s. After pressure release, the samples were stored at ambient temperature (~20 °C) for approximately 12 h for further analysis.

2.3. Size analysis

The size of the whole samples was determined at 20 °C using a Delsa-Nano C particle analyzer (Beckman, CA, US) according to the method of Beliciu and Moraru (2009). A total of 100 μl of samples was diluted in 10 ml of their corresponding ultrafiltrate. The ultrafiltrate was obtained using an ultrafiltration apparatus (type XX80EL005, Millipore Corporation, Billerica, US) using a 10 kDa cut-off membrane (type P2B010A01, Millipore Corporation, Billerica, US). The viscosity of the ultrafiltrate was determined using a thermostatised Ostwald's capillary viscosimeter at 20 °C. The refractive indexes of the ultrafiltrate were measured at 589.3 nm and 20 °C using a refractometer (Anton Paar Abbemat 500, Germany). The refractometer was calibrated with double distilled water. The viscosity and refractive index values of the ultrafiltrate introduced to the test conditions are shown in Table 1. After measurement, the size data were processed via the CONTIN algorithm, which permits the expression of diameter distribution in terms of intensity that could be read directly from the software package. In this case, the size of casein micelles corresponds to $d_{6,5}$ based on Eq. (1) (Porras, Martínez, Solans, González, & Gutiérrez, 2005).

$$\bar{d} = d_{6,5} = \frac{\sum_i n_i d_i^6}{\sum_i n_i d_i^5} \quad (1)$$

where $\bar{d}$ represents the average diameter of casein micelles. n_i represents the number of casein micelles with the diameter d_i.

The samples for the cryo-transmission electron microscopy (cryo-TEM) technique were created based on the method of Knudsen and Skibsted (2010). The samples (3.5 μl) were deposited on lacey carbon membranes (Lot BZ110223a, Zhongjing Keyi, Beijing, China) supported by a copper grid. After being mounted into a Gatan 626 cryo-holder pre-cooled by liquid nitrogen, the samples were transferred into a cryo-microscope (Philips Tecnai 20, Netherlands), and observed at 200 kV using a 2 K × 2 K Ultrascan 894 CCD camera (Gatan, US).

Table 1
Average viscosity and refractive index of the diluent.[a]

Solvents[b]	Lactose content (%)	Average viscosity (cP)	Refractive index
UF-0	0	1.015 ± 0.029a	1.335 ± 0.002a
	5	1.169 ± 0.030b	1.344 ± 0.000b
	10	1.364 ± 0.041c	1.348 ± 0.001c
UF-400	0	1.018 ± 0.010a	1.336 ± 0.003a
	5	1.191 ± 0.034b	1.343 ± 0.001b
	10	1.357 ± 0.027c	1.347 ± 0.002c

Data within a column followed by the same letters are not significantly different at $P < 0.05$.

[a] Mean ± standard deviation of 6 measurements from three independent experiments.

[b] UF-0 = casein-removed fresh permeate from the casein micelle dispersion; UF-400 = casein-removed fresh permeate from the casein micelle dispersion 12 h after pressurisation.