Food Chemistry 141 (2013) 4081–4086

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Temperature-dependent dynamics of bovine casein micelles in the range 10–40 °C

Dylan Z. Liu [a], Michael G. Weeks [b], David E. Dunstan [a], Gregory J.O. Martin [a,*]

[a] Department of Chemical and Biomolecular Engineering, University of Melbourne, Parkville, Victoria 3010, Australia
[b] Dairy Innovation Australia Limited, Werribee, Victoria 3030, Australia

ARTICLE INFO

Article history:
Received 1 March 2013
Received in revised form 20 May 2013
Accepted 27 June 2013
Available online 4 July 2013

Keywords:
Casein micelles
Equilibrium
Dynamics
Temperature
Warming
Cooling
Skim milk

ABSTRACT

Milk is a complex colloidal system that responds to changes in temperature imposed during processing. Whilst much has been learned about the effects of temperature on milk, little is known about the dynamic response of casein micelles to changes in temperature. In this study, a comprehensive physico-chemical study of casein micelles in skim milk was performed between 10 and 40 °C. When fully equilibrated, the amount of soluble casein, soluble calcium and the pH of skim milk all decreased as a function of increasing temperature, whilst the hydration and volume fraction of the casein micelles decreased. The effect of temperature on casein micelle size, as determined by dynamic light scattering and differential centrifugation, was less straightforward. Real-time measurements of turbidity and pH were used to investigate the dynamics of the system during warming and cooling of milk in the range 10–40 °C. Changes in pH are indicative of changes to the mineral system and the turbidity is a measure of alterations to the casein micelles. The pH and turbidity showed that alterations to both the casein micelles and the mineral system occurred very rapidly on warming. However, whilst mineral re-equilibration occurred very rapidly on cooling, changes to the casein micelle structure continued after 40 min of measurement, returning to equilibrium after 16 h equilibration. Casein micelle structure and the mineral system of milk were both dependent on temperature in the range 10–40 °C. The dynamic response of the mineral system to changes in temperature appeared almost instantaneous whereas equilibration of casein was considerably slower, particularly upon cooling.

1. Introduction

During the processing of milk, temperature is an important variable that affects a wide range of physicochemical properties (Fox & McSweeney, 1998). Whilst the native temperature of bovine milk is 37 °C, it is usually stored cold (<10 °C) to control microbial growth. The temperature can then be varied over a wide range during processing. Whilst heat treatment of milk at temperatures above 70 °C can considerably alter the properties of milk (Livney, Corredig, & Dalgleish, 2003), changes in temperature below 50 °C also have a significant effect on the various components of milk, including the casein. Casein represents approximately 80% of the protein in milk and is predominantly present in the form of large hydrated assemblies called casein micelles (CM) (Dalgleish, 2011). CMs consist of four different species of casein proteins and calcium phosphate, and are in dynamic equilibrium with the milk serum (Walstra, 1990). In particular, calcium phosphate and casein are exchanged between the micelles and the serum, with the partitioning of these components being influenced by temperature (Davies & White, 1960; Rose, 1968).

The amount of calcium in the serum (soluble calcium) is known to decrease as a function of increasing temperature, with calcium shifting from the serum into the micelles as CCP (Davies & White, 1960; Rose & Tessier, 1959). In a comprehensive study of the effect of temperature on mineral balance in milk soluble calcium was shown to decrease very rapidly (<2 min) as a function of temperature on heating of milk from 4 to 20–90 °C (Pouliot, Boulet, & Paquin, 1989b). The reversibility on cooling of milk heated to 85 °C was considerably slower, taking up to 60 min to reach 90–95% reversal; however, the reversibility of milk warmed to 40 °C was not investigated (Pouliot, Boulet, & Paquin, 1989a). Whilst the rate of reversibility on cooling was attributed to the complex equilibrium between calcium phosphate and citrate salts, it has also been shown that the rate of exchange of calcium and phosphate between diffusible and colloidal phases is increased at higher temperature (Zhang, Fujii, & Aoki, 1996).

It is generally understood that casein dissociates from micelles at low temperature, and that β-casein is the predominant protein released (Creamer, Berry, & Mills, 1977; Downey & Murphy, 1970; Rose, 1968). Whilst the equilibrium between soluble and

* Corresponding author. Tel.: +61 3 8344 6613; fax: +61 3 8344 4153.
E-mail address: gjmartin@unimelb.edu.au (G.J.O. Martin).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.06.130

micellar casein is known to be affected by temperature, the rate at which equilibrium is reached after temperature change has not been properly investigated. In one study in which milk was cooled from 37 to 0 or 5 °C, β-casein release was shown to occur over within about 60 min (Creamer et al., 1977). In contrast, the concentration of soluble casein was found to continue to change at least 3 days after cooling milk from 20 °C to temperatures in the range 4–15 °C (Ali, Andrews, & Cheeseman, 1980). Casein released during storage at 4 °C has been shown to be completely reversible after 18 h of incubation at 20 °C (Davies & Law, 1983), however the kinetics were not investigated.

In addition to affecting the partitioning of calcium and casein, the size and hydration of casein micelles are also influenced by temperature. At high temperatures (i.e.>70 °C) attachment of denatured whey proteins and heat-induced aggregation are known to increase the hydrodynamic size of casein micelles (Anema & Li, 2003; Tran Le, Saveyn, Hoa, & Van der Meeren, 2008). In the range 0–50 °C, casein micelle size is also affected by temperature, but not due to interactions with whey protein. This temperature range is particularly important for processing such as the membrane filtration of milk. Dynamic light scattering measurements performed at 6, 20, and 50 °C showed the effect of temperature on micelle size to be different depending on the dilution solvent used (ultrafiltrate, SMUF, or water) (Beliciu & Moraru, 2009). In water or SMUF micelles were larger at increasing temperature. In UF permeate the micelles were approximately the same size at 6 or 20 °C but considerably smaller at 50 °C. Whilst UF permeate should be closest to the native environment of the casein micelles in undiluted milk, the permeates used in this study were not obtained at the same temperatures as the measurements and would therefore have an altered mineral content (Beliciu & Moraru, 2009). According to results obtained by differential centrifugation, cooling of milk to 4 °C decreases the population of larger micelles and increases the population of smaller micelles (Davies & Law, 1983; Ono, Murayama, Kaketa, & Odagiri, 1990). This effect was shown to be fully reversible upon re-warming to 20 °C after 18 h; however no indication of the kinetics of this reversal was provided (Davies & Law, 1983). It has also been found that the apparent voluminosity of casein micelles decreases with increasing temperature (Dewan, Bloomfield, Chudgar, & Morr, 1973; Snoeren, Klok, Van Hooydonk, & Damman, 1984; Walstra, 1979).

Detailed kinetic information on the effect of temperature on casein micelles has so far been limited by difficulties in measuring changes to casein micelles occurring over short time periods in native milk samples. In particular, direct real-time measurements of soluble casein, soluble calcium, micelle size, and micelle hydration are not possible using conventional methods. To obtain close to real-time information about changes to casein micelles, pH and turbidity measurements were employed in this study. These measurements have very short response times, are sensitive to small changes in casein micelle properties and able to be applied *in-situ* on milk samples at native concentrations. As these methods give indirect information about changes that are occurring within the skim milk system, the results need to be interpreted in the context of more comprehensive information on the equilibrium relationships underlying the various alterations as a function of temperature and an understanding of the physico-chemical properties of the skim milk system. The pH of skim milk results from the mineral system in the serum. Although complex, the predominant alteration effecting pH that will result from changes in the temperature is the exchange of calcium between the colloidal calcium phosphate (CCP) in the casein micelles and the soluble calcium in the serum (Fox & McSweeney, 1998):

$$Ca^{2+} + HPO_4^{2-} \underset{\text{cooling}}{\overset{\text{heating}}{\rightleftharpoons}} CCP + H^+$$

In this way, pH can be used as an indirect indicator of the equilibration of the mineral system, including the partitioning of calcium between the micelle and the serum. In skim milk the casein micelles are responsible for the majority of the measured turbidity and therefore any variation in turbidity can be attributed to changes in the casein micelles (Martin, Williams, & Dunstan, 2007). The turbidity can therefore be used as a real-time indicator of alterations to casein micelles. In this study a comprehensive study of temperature-dependent equilibrium changes to casein micelles in skim milk is combined with real-time measurement of dynamics between 10 and 40 °C.

Whilst much is already known about the general effect of temperature on casein micelles in bovine milk, most studies have examined only one selected aspect of the casein system (e.g. casein partitioning or micelle size). In addition, information on the dynamics of the casein micelle system in response to temperature changes is lacking. A detailed comparison of kinetic response to heating and cooling within the low temperature range has not been performed and highly time resolved information is yet to be obtained. In this paper a comprehensive investigation is performed on the effect of temperature on casein and calcium partitioning between the micelle and the serum as well as the size and hydration of casein micelles. This study examines both the equilibrium and kinetic aspects of calcium and casein alterations in response to temperature. By examining whether there is temporal correlation between calcium and casein alterations in response to temperature, a better understanding of the level of physical interconnection between these two phenomena can be gained.

2. Materials and methods

2.1. Skim milk samples

Pasteurised fresh skim milk containing 33 g/L protein, 52 g/L carbohydrate, and 1.5 g/L total fat was purchased from a local supermarket and stored at *ca* 10 °C and analysed within two days. For equilibrium studies, milk samples were held at the desired temperatures for at least one hour before commencing analysis.

2.2. Fractionation of casein by centrifugation

Centrifugation of skim milk samples was performed using an Ultra 90 ultracentrifuge fitted with a 90 Ti rotor (Beckman Instruments Inc., Palo Alto, CA). To account for differences in sedimentation velocity resulting from the dependence of viscosity on temperature, centrifugation times were adjusted for temperature (10 °C for 78 min; 20 °C for 60 min; 40 °C for 39 min) according to previously described calculations (Ono et al., 1990). Supernatants and pellets for determination of soluble calcium, soluble casein, and micelle hydration were obtained by centrifugation at 75,940*g*. Casein was fractionated into broadly different sizes based on differential centrifugation as follows: soluble casein = casein in supernatants obtained at 75,940*g*; 'small' CM = casein in supernatants obtained at 25,000*g* minus soluble CN; 'medium' CM = casein in supernatants obtained at 5700*g* minus small CN; and 'large' CM = casein in the pellets from centrifugation at 5700*g*. The casein content of the supernatants were determined by densitometric analysis of SDS–PAGE gels as previously described (Liu, Dunstan, & Martin, 2012).

2.3. Casein micelle hydration and soluble calcium measurement

Skim milk samples maintained at 10, 20, and 40 °C were centrifuged at 75,940*g* for 78, 60, or 39 min respectively. The hydration of casein micelles was determined by gravimetric analysis of