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Multiscale approach to characterize bulk, surface and foaming behavior of casein micelles as a function of alkalinisation

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ABSTRACT

Caseins display the major protein fraction in milk, and thus, significantly impact on milk's techno-functional properties such as foaming. As the micellar structure is mainly stabilized by hydrophobic as well as electrostatic forces, a gradual increase in pH value from 6.0 to 11.0 induced pronounced structural modifications. In consequence of alkalinisation, micelle size and composition changed. This had an impact on the solution properties such as turbidity. Furthermore, interfacial characteristics, e.g. surface pressure evolution and surface dilatational properties were affected. Different situations occurred regarding interfacial covering, which was also reflected in the resulting foam structures. The highest foam stability was obtained for pH 9.0. At this pH, highly voluminous casein micelles combined with a considerable amount of serum casein formed maximal viscoelastic surface layers. Micelle dissociation reduced viscoelasticity as well as foam stability, whilst drainage increased.

1. Introduction

Milk proteins, which are widely used for foam formation and stabilization purposes, display a group of various proteins with distinct structures, and therefore, different functionalities. For a better understanding of a complex product like milk, it is desirable to clarify the role of single ingredients, such as individual milk proteins or special protein structural units (e.g. casein micelles). Fractionation of milk proteins, for example, by means of membrane processes (Brans, Schroën, van der Sman, & Boom, 2004; Maubois, 1984; Tolkach & Kulozik, 2005) or ultracentrifugation (Heinrich, 2012; Marchin, Putaux, Pignon, & Leonil, 2007) is well established and facilitates detailed and sound investigation with regard to the distinct properties of the proteins. So far, focus of foam-related research was mainly on the small, globular whey proteins (e.g. α-Lactalbumin: 14.2 kDa, β-Lactoglobulin: 18.3 kDa), which are well known for their pronounced surface activity, the establishment of viscoelastic interfacial films and the formation of relatively stable foams (Damodaran, 2005; Engelhardt et al., 2012; Foegeding, Luck, & Davis, 2006; Lexis & Willenbacher, 2014; Richert, 1979). Besides whey proteins, larger structures such as casein micelles with an average diameter in milk of 150–200 nm (Dalgleish & Corredig, 2012; Kruif, 1998) are also able to diffuse to and to adsorb at air/water interfaces as shown by Kamath, Webb, and Deeth (2011) and Silva et al. (2013). In addition, with a share of approximately 80% of the total milk protein, caseins display the major protein fraction compared to whey proteins, and significantly influence the techno-functional properties of milk. However, detailed knowledge on interfacial behavior (e.g. interfacial dilatational rheology) of casein micelles in dependence of solution characteristics and combined with macroscopic foam properties is scarce (Baier, Schmitt, & Knorr, 2015; Kessler, Menéndez-Aguirre, Hinrichs, Stubenrauch, & Weiss, 2013; Silva et al., 2013; Zhang, Dalgleish, & Goff, 2004). Therefore, it is of high relevance to investigate the correlation between milieu-dependent solution properties and the manner in which casein micelles interact at the air/water interface.

As summarized in an ample number of publications, e.g. Silva et al. (2013), Dalgleish and Corredig (2012), Kruif, Huppertz, Urban, and Petukhov (2012), Fox and Brodkorb (2008), Marchin et al. (2007), Müller-Buschbaum et al. (2006) and McMahon and Brown (1984), casein micelles are dynamic structures, which are comprised of α_{S1}-, α_{S2}-, β- and κ-casein together with calcium phosphate, and include high amounts of water (3.5 g water/g protein) (Jeurnink & Kruif, 1993). For comparison purposes,

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hydration of β-Lactoglobulin (dimeric structure) is only 0.02 g water/g protein (Pessen, Purcell, & Farrell, 1985). In the last few decades, various models have been proposed to describe the internal structure of casein micelles, e.g. coat-core (Waugh & Noble, 1965), submicellar (Walstra, 1999) and nanocluster models (Horne, 1998, 2006; McMahon & Oommen, 2008), but debate is still ongoing due to analytical limitations regarding non-invasive sample preparation and difficulties in exclusion of artefacts (Dalgleish & Corredig, 2012). In their review, Dalgleish and Corredig (2012) tried to combine several prominent models and assumed the native casein micelle to be of a sponge-like structure based on linked calcium phosphate/casein nanoclusters. General consensus prevails in terms of further intermolecular forces being responsible for the stabilization of the micellar structure. Thus, besides hydrogen bonding, attractive hydrophobic as well as repulsive electrostatic interactions play an important role. This is why the micellar structure can be altered significantly by a modification of pH value, for example. Thereby micelle size and molecular weight (Müller-Buschbaum et al., 2006) as well as the amount of single caseins (esp. α_S-casein and β-casein) in the serum phase (Ahmad, Piot, Rousseau, Grongnet, & Gaucheron, 2009; Post, Arnold, Weiss, & Hinrichs, 2012) are affected. Previous studies have demonstrated that alkaline pH induces significant structural modification up to the disruption of casein micelles (Ahmad et al., 2009; Vaia, Smiddy, Kelly, & Huppertz, 2006). The phenomenon of pH-related micelle destabilization is also known for the acidic region around pH 4.6, and widely used in dairy processing for various purposes (e.g. cheese manufacture or production of functional caseinate products) (Dalgleish & Corredig, 2012; Fox & Brodkorb, 2008). Acidification to the isoelectric region of micellar casein (isoelectric point (pI) ~ pH 4.6) leads to solubilization of inorganic phosphate and calcium ions (Le Graët & Gaucheron, 1999; Marchin et al., 2007). In addition, the κ-casein layer on the micelle surface collapses due to charge screening (pI = 4.47–5.81, depending on the peptide composition) (Holland, Deeth, & Alewood, 2004). This enables aggregation of the protein molecules (Dalgleish & Corredig, 2012), and thus, network formation in terms of gelation. Micelle disruption as consequence of alkalinisation was explained by the combination of modification of protein–protein as well as protein-minerals interactions. An alteration in ionization state of the protein molecules, i.e. increasing negative net charge from −20 mV (pH 6.7) to −24 mV (pH 10.8) (Ahmad et al., 2009), and changes in minerals equilibria, i.e. progressive demineralization of casein micelles (e.g. reduction in ionic calcium), lead to more favorable environmental conditions for the caseins in the milk serum. Thereby micelle disintegration is induced as described by Ahmad et al. (2009) and Vaia et al. (2006), particularly. The authors investigated skim milk or extracted casein micelles from bovine as well as buffalo milks (pH 7.0 to 11.0) by means of turbidity, particle size measurements and ultracentrifugation and assumed micelle disruption to occur around pH 9.0 and pH 9.7, respectively. Müller-Buschbaum et al. (2006) examined highly concentrated ($c_{protein}$ = 100 g/L) thin casein films in dependence of pH (5.15–9.35) by means of optical microscopy, atomic force microscopy and small-angle X-ray scattering (GISAXS). For comparison purposes, they also used dynamic light scattering to investigate casein behavior in solution and found casein micelle diameter to increase with increasing pH from 170 nm at pH 5.5 to 480 nm at pH 9.3. This observation was explained by a reduction of electrostatic repulsion between single caseins (esp. α_{S2}-casein: theoretical pI = 8.31, according to ProtScale®) with increasing pH leading to a higher aggregation number and thus an enlargement of casein micelle size. Further increase in pH was not investigated, and therefore, pH-induced micelle disruption was not mentioned by the authors. Differences in pH values causing micelle disruption could be due to variations in sample composition, sample preparation methods or environmental factors like protein concentration, ionic milieu and calcium content, for example. From literature, it can be recapitulated that variation of pH induces major changes in casein micelle size, and therefore, strongly impacts on their interaction behavior. However, implication of these structural modifications on techno-functionality of casein micelles and especially foaming has not been discussed, yet. Therefore, the objective of this research was to examine the effect of alkaline treatment on the structural state of casein micelles in relation to their surface properties as well as foam formation and stabilization behavior. Casein micelles were purified from bovine skim milk and exposed to alkaline treatment (pH 6.0–11.0) at a protein concentration of 1%, which is below the native concentration in milk (~2.6%) (Walstra, Geurts, Noomen, Jellema, & van Boekel, 1999) that was used in other publications (Ahmad et al., 2009; Vaia et al., 2006). As protein concentration plays a decisive role in surface covering, it was kept constant throughout the experiments to ensure transferability of the obtained results. Bulk, surface and foaming properties of this model system were studied with the attempt to generate a comprehensive knowledge of the underlying correlation between structural changes induced by alkalinisation and protein functionality. Nevertheless, the presented concept, experimental techniques and results are considered to be applicable and relevant for industrial issues.

2. Materials and methods

2.1. Casein fractionation and sample preparation

Micellar casein was produced from skim milk by an in-house diafiltration process and subsequent spray drying according to the methods described by Heinrich (2012) and Tolkach and Kulozik (2005). During diafiltration (7 diafiltration steps), MF-retentate was washed with UF-permeate, whereby the casein solution was slightly concentrated to a protein content of 45.1 g/L (Vario MAX cube, Elementar Analysesysteme GmbH, DE). Lactose (RP-HPLC) and salts (Na, K, Ca) (ELEX 6361, Eppendorf AG, DE) concentration were 41.8 g/L and 3.3 g/L respectively. The density of the casein solution was 1.035 g/cm^3 (DMA 4100M, Anton Paar GmbH, AT). The dry matter (CEM Smart Turbo, CEM Corporation, US) of the product prior and after spray drying was 9.9% and 95.0%, respectively. Relating to the total protein content, casein concentration was 93.9%, which was determined by RP-HPLC as described in Section 2.2.5. In addition to micellar casein, UF-permeate was obtained concurrently during diafiltration. It was subsequently frozen and kept at −18 °C until further use. Its lactose and salts concentration were 47.6 g/L and 3.6 g/L, respectively, Based on the casein concentration of the powder, solutions of 10 g/L micellar casein were achieved by dissolving the appropriate amount of powder in a mixture of UF-permeate and deionized water (Milli-Q Integral 3, Merck KGaA, DE) to maintain the milieu of skim milk. The mixing ratio was 3:1. The solutions were gently stirred at 20 °C for 12 h using a magnetic stirrer to ensure full hydration. The pH was then set to values of 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 with 1 M HCl (Bernd Kraft GmbH, DE) or 1 M NaOH (Gerbu Biotechnik GmbH, DE) and the samples were stirred for another 3 h at 20 °C prior to trials. Thereafter, all measurements were performed at 20 °C.

2.2. Solution properties

2.2.1. Turbidity

For turbidity measurements the Spectrophotometer 6305 (Jenway, Bibby Scientific Ltd., UK) was used. Transmission of the samples in dependence of pH (6.0–11.0) was determined at a