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Interfacial properties, thin film stability and foam stability of casein micelle dispersions

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ABSTRACT

Foam stability of casein micelle dispersions (CMDs) strongly depends on aggregate size. To elucidate the underlying mechanism, the role of interfacial and thin film properties was investigated. CMDs were prepared at 4 °C and 20 °C, designated as $CMD_{4\,°C}$ and $CMD_{20\,°C}$. At equal protein concentrations, foam stability of $CMD_{4\,°C}$ (with casein micelle aggregates) was markedly higher than $CMD_{20\,°C}$ (without aggregates). Although the elastic modulus of $CMD_{4\,°C}$ was twice as that of $CMD_{20\,°C}$ at 0.005 Hz, the protein adsorbed amount was slightly higher for $CMD_{20\,°C}$ than for $CMD_{4\,°C}$, which indicated a slight difference in interfacial composition of the air/water interface. Non-linear surface dilatational rheology showed minor differences between mechanical properties of air/water interfaces stabilized by two CMDs. These differences in interfacial properties could not explain the large difference in foam stability between two CMDs. Thin film analysis showed that films made with $CMD_{20\,°C}$ drained to a more homogeneous film compared to films stabilized by $CMD_{4\,°C}$. Large casein micelle aggregates trapped in the thin film of $CMD_{4\,°C}$ made the film more heterogeneous. The rupture time of thin films was significantly longer for $CMD_{4\,°C}$ (>1 h) than for $CMD_{20\,°C}$ (<600 s) at equal protein concentration. After homogenization, which broke down the aggregates, the thin films of $CMD_{4\,°C}$ became much more homogeneous, and both the rupture time of thin films and foam stability decreased significantly. In conclusion, the increased stability of foam prepared with $CMD_{4\,°C}$ appears to be the result of entrapment of casein micelle aggregates in the liquid films of the foam.

1. Introduction

Milk proteins are widely used to produce aerated products in food industry, like e.g. aerated desserts, whipped cream, and cappuccino foam. Several factors can influence the stability of these products, including the interfacial properties of the adsorbed layer between the gas and liquid phase, and the bulk properties of the liquid films in between these interfacial layers, that separate the bubbles [1]. For relatively simple systems, like foams stabilized by low molecular weight surfactants, the interfacial properties of the air/water interface are often dominant for foam stabilization [2–4]. For more complex systems, which contain (mixtures of) proteins and/or colloidal particles, it depends on the detail of the system whether interfacial or bulk film properties are dominant in foam stability.

Caseins, which make up about 80% of cow milk protein and are predominantly present in the form of colloidal particles, termed casein micelles, exhibit good foaming properties [5,6]. In a recent study, we found that casein micelle dispersions prepared at 4 °C ($CMD_{4\,°C}$), with an average particle size of 500 nm and which contained aggregates of casein micelles, formed much more stable foams compared to foams made from CMD prepared at 20 °C ($CMD_{20\,°C}$) [7]. $CMD_{20\,°C}$ consisted predominantly of non-aggregated micelles. The aggregates present in $CMD_{4\,°C}$ appeared to play a crucial role in the increased foam stability. We hardly observed any differences between the dilatational properties of the air/water interfaces stabilized by the two CMDs. This was in line with another study that reported an improved foam stability in the presence of protein aggregates, without causing significant

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differences in the interfacial properties of the air/water interface [8]. In our previous study [7] we determined the dilatational properties at small deformations and constant frequency. However, insights in, large amplitude dilatations, which are more sensitive to subtle changes in the microstructure of the interface, is still lacking. Particularly when coarsening of the foam is predominantly driven by disproportionation, interfaces are subjected to large deformations, and dilatational moduli data determined at small deformations are therefore not a good indicator to establish whether surface properties play a role in foam stability.

The composition of air/water interfaces stabilized by CMDs is still largely unknown. CMD is a mixture of four kinds of caseins, peptides, small micelles, casein micelles and in some cases also casein micelle aggregates. Whether or not casein micelles adsorb at the air/water interface is still under debate. Borcherding et al. [9] indicated that casein micelles are not likely to be present at the air/water interface, while in another study, microscopic images of bubble ghosts of milk foam suggested that casein micelles were adsorbed at the air/water interface, possibly reversibly. In the present study we have investigated the adsorbed amounts at the air/water interface for both CMDs using ellipsometry.

Previous studies have reported that protein aggregates can have a large influence on foam stability. The presence of whey protein soluble aggregates [10–13], β-Lactoglobulin aggregates [14–16] and whey protein fibrils [17] was shown to improve foam stability, but other studies indicated that their presence can also decrease foam stability [18]. For whey protein soluble aggregates, foaming properties were found to be significantly influenced by the fraction, size, and surface hydrophobicity index of the aggregates [10]. More specifically, Dombrowski et al. [13] reported that these differences in particle characteristics had a significant impact on surface properties, i.e. surface tension, dynamics of protein adsorption and interfacial dilatational properties. According to Mahmoudi et al. [19], the adsorption behaviour of whey protein aggregates was very similar to that of non-aggregated protein, and essentially defined by the residual non-aggregated fraction of proteins. Aggregates were less likely to adsorb with increasing particle size. Whey protein hydrogel particles prepared by Lazidis et al. [20] also adsorbed at the air/water interface. The improved foam stability compared to non-aggregated protein dispersions was ascribed to a stiffer/stronger film which could slow down disproportionation [12], and drainage [20] and could reduce the coalescence rate [13]. In line with the above hypothesis, Gochev et al. (2014) [21] indicated that the viscoelastic characteristics of film surfaces stabilized by β-lactoglobulin solutions significantly affect film drainage and stability. Lexis and Willenbacher [22] found a unique correlation between foam rheological properties and surface viscoelasticity of corresponding β-lactoglobulin solutions. These studies indicate that surface properties will influence thin film stability and foam rheology.

Rullier et al. [14–16] conducted a thorough study on films and foams stabilized by mixtures of non-aggregated β-lactoglobulin and their aggregates, and found that film stability was dependent on the aggregate size and on the ratio between non-aggregated proteins and protein aggregates. For aggregate fractions ranging from 1 to 90% of the total protein concentration (1 g/L), gel formation in the thin film was observed. For higher fractions of aggregates, it was shown that the amounts of non-aggregated proteins were no longer sufficient to completely cover the interface, and as the larger aggregates did not reduce the surface tension at short times as much as the non-aggregated protein, the corresponding films and foams were less stable. For the fractions between 1 and 90%, the rheological properties of the interface were not determined. It is therefore hard to establish whether the immobilization of the films is the result of in-plane (surface) gel formation, cross-film (bulk) gel formation, or a combination of both. In conclusion, all these studies on whey protein aggregates attribute changes in foam stability, either to a difference in adsorption behaviour, surface dilatational properties, or thin film properties, but in none of these studies all of these properties were studied together. This makes it hard to establish conclusively what the dominant mechanism behind the increased foam stability is. This also counts for the CMD system. In the current study we more clearly establish the mechanism behind the improved stability of foams stabilized by $CMD_{4\,^\circ C}$. The foaming properties, interfacial properties and thin film stability of CMDs with different particle size distribution and of their supernatants were investigated. Samples were characterised for size distribution of the casein micelles (aggregates) and protein concentration. Sodium-caseinate was studied as a control. Frequency and strain amplitude dependence of the surface dilatational modulus of the CMDs were checked in large amplitude oscillatory dilatation. The absorbed amount of protein at the air/water interface for the different samples was determined by ellipsometry. Thin film properties including rupture time and morphology of the thin liquid film were studied by a microscope equipped with a Sheludko cell. Foam properties such as the foam half-life ($t_{1/2}$) and mean bubble diameter were obtained from bubble image analysis. By combining the above measurements, the relation between interfacial properties and foam stability as well as relationship between thin film stability and foam stability was examined to establish the mechanism behind the significantly improved foam stability of CMDs prepared at low temperature.

2. Materials and methods

2.1. Materials

Low-heated skim milk powder NILAC was obtained from NIZO food research (Ede, Netherland). Sodium caseinate (EM7-A9040445) was obtained from DMV International (Veghel, The Netherlands). Sodium azide was purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). Ultra-pure water (MilliQ Purelab Ultra, Darmstadt, Germany), free of surface active contaminants, was used in all experiments (>18.2 MΩ-cm, surface tension of 72.26 ± 0.4 mN/m at 20 °C).

2.2. Preparation of casein micelle dispersions (CMDs) and their supernatants

Skim milk was prepared (10%, w/w) by dissolving NILAC milk powder in MilliQ water and stirring overnight at room temperature. Sodium azide (0.02%, w/w) was added as a preservative. The reconstituted skim milk was ultracentrifuged (L-60 Beckman Ultracentrifuge, rotor type 70 Ti, Krefeld, Germany) at 100,000*g* for 90 min at 20 °C according to Huppertz and de Kruif [23]. The casein micelle pellets were separated from the serum phase and ground using a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany) at a frequency of 30 Hz for 10 min at room temperature. Subsequently, different amounts of the obtained casein micelle paste were redispersed in milk permeate either at 20 °C or at 4 °C for 60 h to obtain casein micelle dispersions (CMD) with a protein concentration of 2.5% (w/w). $CMD_{20\,^\circ C}$ denotes the CMD redispersed at 20 °C and $CMD_{4\,^\circ C}$ is the CMD made at 4 °C. The milk permeate was prepared according to Chen et al. [7]. For the thin film studies with homogenized $CMD_{4\,^\circ C}$, $CMD_{4\,^\circ C}$ samples with protein concentration of 2.5% (w/w) were homogenized at 20 MPa for 10 min with a homogenizer (Delta Instruments, Drachten, the Netherlands). For the preparation of the supernatants, CMDs and skim milk samples were ultracentrifuged for a second time according to the procedure described above. Their protein content was further analysed as described below.