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A non-invasive method for the characterisation of milk protein foams by image analysis

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ABSTRACT

An apparatus for the investigation of milk protein foams was introduced based on three jacket columns and exclusively image analysis. The method had a repetition coefficient <10%, and offered a high sample throughput and an expandable design. Sodium caseinate, micellar casein concentrate, whey protein isolate and whey protein concentrate foams were analysed as an application. Foaming properties depended on the protein, the composition of the preparations and the foaming conditions, e.g., stable foams at 20 °C were observed for micellar casein, while sodium caseinate showed a half-life of 22 min. At 50 °C, the stability of sodium caseinate decreased by about 70%. Additionally, a direct link between the foaming properties of sodium caseinate and its degree of enzymatic hydrolysis was found. No changes in foaming properties using Alcalase® 2.5L occurred up to a degree of hydrolysis of about 3%, while higher degrees of hydrolysis led to decreased foaming properties.

1. Introduction

Foam can be defined as a multiphase system consisting of air surrounded by a continuous liquid layer, called the lamella (Raikos, Campbell, & Euston, 2007). Food foams are complex systems including a mixture of gases, liquids, solids and surface active agents (surfactants) (Zayas, 1997). Different molecules, such as proteins, emulsifiers, solid crystals of fat or ice, present in a liquid phase or in a semisolid to solid matrix, can act as surfactants in foams (Campbell & Mougeot, 1999). Proteins are often used for the stabilisation of liquid food foams (Fains, Bertrand, Baniel, & Popineau, 1997), because of their strong adsorption to the gas/liquid interface as well as good steric and electrostatic stabilisation (Murray, 2007). Additionally, the lamellas of protein-stabilised foams tend to have some structural coherence due to interactions between the adsorbed molecules (Hall, 1996; Murray, 2007). The proteins have to diffuse from the continuous liquid phase to the gas/liquid interphase to stabilise the gas bubbles. The proteins undergo conformational changes during the adsorption at the interface and expose their hidden hydrophobic groups to the gas phase, while hydrophilic groups orient to the liquid phase. Finally, the proteins aggregate within the lamella, forming a network (Stressler, Ewert, Merz, Glück, & Fischer, 2015).

The gas bubble is stabilised, according to the steric hindrance or charge distribution of the lamella (Germain & Aguilera, 2014). The bubbles exist in a spherical or polyhedral shape depending on the gas/liquid-ratio of the foam (Damodaran, 2006). Regarding foam properties, the formation (foamability) and stability of the foam is of major concern (Raikos et al., 2007). Foam formation is influenced by the ability of the protein to diffuse quickly to and adsorb on the gas/liquid interface and the ability of the protein to reduce the surface tension. Foam instability is caused by drainage, Ostwald ripening (disproportionation) and coalescence (film rupture) (van der Ven, Gruppen, de Bont, & Voragen, 2002). The foaming properties of a protein preparation itself are determined by the compositional effects (content of protein, oil/fat, salt, sugar) as well as the structural and physical characteristics of the protein (electrostatic repulsion, molecular size, exposed hydrophobicity,

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amphiphilic nature, chemical reactivity, bulkiness) (Borcherding, Lorenzen, & Hoffmann, 2009).

Enzymatic hydrolysis is a possibility to modify the structural and physical properties of the proteins (Crowley et al., 2002). Proteolysis decreases the molecular weight and amount of secondary structure, increases the number of ionisable groups and the solubility near the isoelectric point, and can expose hydrophobic groups, which can improve the properties of the foam (Hall, 1996; Severin & Xia, 2005; Tavano, 2013). In contrast, peptides formed during the hydrolysis might destabilise protein foams by the displacement of proteins or by disturbing protein–protein interactions. Furthermore, hydrolysis leads to increased charge density, which might also negatively influence foam stability (van der Ven et al., 2002). In addition to the protein characteristics, the liquid phase (concentration of ions, carbohydrates, other surface active components), pH and temperature during foaming influences the foam properties (Waniska & Kinsella, 1979). Moreover, the properties of foams vary with the method and equipment used for their preparation (Halling, 1981).

While liquid foams are quite complicated systems, no standardised experimental procedure for foam formation and analysis exists so far. The methods of foam investigation differ mainly in the manner of dispersing the gas phase in the foaming solution (Lunkenheimer, Malysa, Winsel, Geggel, & Siegel, 2010) and the amount of protein needed to generate the foam (Waniska & Kinsella, 1979). Thus, foam may be produced mechanically by whipping, pouring or shaking the solution (Waniska & Kinsella, 1979). Because of such variability, comparisons of data on foaming properties from different laboratories are difficult to interpret and compare (Phillips, Haque, & Kinsella, 1987). While whipping is a common method for foam investigation (Phillips et al., 1990), the gas volume introduced in the liquid is not defined and a high amount of protein (3–40%) is needed (Waniska & Kinsella, 1979). Furthermore, the foam produced has to be removed from the foaming system for analysis (mostly gravimetric or density based measurement). While foams are dynamic systems of only transient stability, they do not respond well to invasive techniques, making whipping methods failure-prone for instable foams (Hall, 1996).

Recently, aeration methods, in which gas is sparged through a tube or frit into the solution, have become more recognised for foam analysis due to their non-invasive nature and high reproducibility (Dombrowski, Dechau, & Kulozik, 2016; Fains et al., 1997; Lunkenheimer et al., 2010). Additionally, using aeration for foam analysis, an investigation of the foam forming and decay is possible, the gas volume introduced is well defined (Fains et al., 1997) and only low protein concentrations of 0.01–2% are needed (Waniska & Kinsella, 1979). Because of these advantages, new commercially introduced foaming instruments, such as the Dynamic Foam Analyzer (Krüss, Hamburg, Germany) or the FOAMSCAN® (Teclis, Lyon, France), are also based on aeration. A disadvantage of aeration methods so far is the need for complex measuring equipment. Especially the liquid height is often determined by conductivity measurement (Fains et al., 1997; Lunkenheimer et al., 2010) or diode array (Dynamic Foam Analyzer, Krüss). A conductivity measurement, as also used by the FOAMSCAN®, cannot distinguish between drainage and film rapture (Hall, 1996). Additionally, conductivity measurements, as well as a diode array, are not easy to apply and result in a high investment for the building of the foaming apparatus. Furthermore, these measurement devices limit the expandability of the foaming apparatus, making a high throughput measurement inapplicable. While the foam decay, especially, is a time-intensive analysis, an expandable design, where more than one sample can be measured at the same time, would be desirable for certain applications.

The aim of this study was to implement a novel, non-invasive, easy to build and cost-effective foaming method based on aeration with a defined gas (air). A high sample throughput and easy to use analytical system was introduced by using several columns for foaming simultaneously and image analysis exclusively for foam investigation. The method was applied to determine the foam characteristics of the four industrial relevant milk protein preparations: sodium caseinate, micellar casein concentrate (MCC80), whey protein isolate (WPI) and whey protein concentrate (WPC80). Furthermore, the influence of a hydrolysis on the foaming properties of sodium caseinate was demonstrated as an example.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade and obtained from Sigma–Aldrich GmbH (Schnelldorf, Germany), Carl Roth (Karlsruhe, Germany), AppliChem GmbH (Darmstadt, Germany) or Merck AG (Darmstadt, Germany). Sodium caseinate powder was purchased from FrieslandCampina (Amersfoort, Netherlands) and the protein content was 90.6% (w/w). The MCC80, WPI and WPC80 were purchased from Sachsenmilch (Leppersdorf, Germany). The protein content was 80% (w/w) for MCC80 and WPC80 and 90% (w/w) for WPI (for further specifications of the protein preparations see Supplementary material Table S1). Alcalase® 2.5L was a friendly gift from Novozymes (Bagsværd, Denmark).

2.2. Foam formation and analysis

2.2.1. Foaming apparatus and standard procedure of foam formation

The apparatus used for foam investigation (Fig. 1) consisted of three jacket columns (6.5 cm outer diameter, 4 cm inner diameter, 25 cm length) with a ceramic frit (P4; 10–16 μm), manufactured by Glasgerätebau OCHS Laborfachhandel e.K. (Bovenden, Germany), tempered with a controlled heating circulator (Julabo GmbH, Seelbach, Germany) during foaming. The whole apparatus was placed in front of a black background for improved contrast during the analysis of the images taken.

The columns were filled with distilled water (H_2O_d) and sparged at 1 L_{air} min^{-1} to moisturise the frits before the first run. The columns were emptied after 10 min and 100 mL of each test solution (sodium caseinate, MCC80, WPI or WPC80) was filled gently into each column with a volumetric pipette. While one of the main advantages of aeration for foam generation is the need of low protein concentrations, all test solutions had a concentration of 1% (w/v). All test solutions were diluted with tap water and stirred for 30 min at 20 °C before application. As soon as the solution in the columns reached the measurement temperature (20 °C or 50 °C), a Nikon D700 SLR camera with a AF Nikkor 24–85 mm f/2.8–4 d lens (Nikon, Tokyo, Japan) was started, taking an image of all three columns every 20 s automatically using the program Camera Control Pro 2 (Nikon). Foam formation was started by introducing 200 mL min^{-1} air for 1 min at 1 bar into the columns. The airflow was adjusted with a variable area flowmeter (Fischer & Porter GmbH, Ellershausen, Germany). Each column was sparged individually by adjusting the two cross valves (V1 and V2; Fig. 1) with an interval of 2 min. By sparging the columns individually, the same pressure and air in each column was ensured. A DigiMicro Profi reflected light microscope (dnt Drahtlose Nachrichtentechnik GmbH, Dietzenbach, Germany) was fixed on a stand and moved from column to column. A microscopic image of the foam directly above the liquid was taken 20 s after