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Air-water interfacial properties of enzymatically hydrolyzed wheat gluten in the presence of sucrose

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ABSTRACT

Enzymatically hydrolyzed wheat gluten proteins may be a valuable alternative to animal proteins as foaming agents in food. Studies of the air-water (A-W) interfacial properties of such hydrolysates in aqueous solutions contribute to the understanding of their functionality in food systems. We here studied the A-W interfacial characteristics of wheat gluten hydrolysates (GHs) in the absence and presence of sucrose. Sucrose increased ($P < 0.05$) the foaming capacity, which is the initial amount of foam formed, of GHs. This is probably related to an increased affinity of GHs for the A-W interface in the presence of sucrose, as could be observed by higher ($P < 0.05$) rates of diffusion to and adsorption at the A-W interface of the GH constituents in sucrose solution compared to those in water. Furthermore, the surface dilatational moduli of GH protein films at A-W interfaces were in most cases higher ($P < 0.05$) in a sucrose solution than in water. The latter could only partly be related to differences in foam stability. Surface hydrophobicity and intrinsic tryptophan fluorescence measurements revealed that protein conformational changes in the presence of sucrose might be at the basis of the observed differences. Another possibility is that the hydrophilic sucrose molecules in the bulk cause the more hydrophobic protein molecules to concentrate at the interface, more so than in water. In conclusion, it is crucial to investigate the foaming of plant protein hydrolysates in media more complex than water, as other non-surface-active food ingredients alter their interfacial behavior.

1. Introduction

Commercial wheat gluten is the co-product of the industrial wheat starch isolation and consists mainly of storage proteins (Van Der Borght, Goesaert, Veraverbeke, & Delcour, 2005). These proteins are often discharged in low-cost applications such as in animal feed systems, hence the clear interest from industry in alternative valorization routes (Day, Augustin, Batey, & Wrigley, 2006; Veraverbeke & Delcour, 2002). One of the main obstacles for a wide application of gluten proteins in food products is their low solubility in aqueous media (Delcour et al., 2012). Enzymatic hydrolysis not only strongly improves the solubility of said proteins but also induces emulsification and foaming properties (Adler-Nissen, 1976; Wouters, Rombouts, Fierens, Brijs, & Delcour, 2016).

Foams are structure and texture-defining in food products such as meringues, cakes, beer foam and coffee foam. They consist of a gaseous phase dispersed in a liquid, usually in the form of closely packed air bubbles in an aqueous phase. Foams have large air-water (A-W) interfaces which are thermodynamically unstable but can be stabilized by surface-active compounds such as proteins (Damodaran, 2005; Murray, 2007). Because of their amphiphilic nature, proteins have a certain affinity for an A-W interface. They adsorb at such interfaces, thereby lowering the surface tension and sterically preventing gas bubbles from approaching each other and possibly merging (Damodaran, 2005; Hunter, Pugh, Franks, & Jameson, 2008). After adsorption, proteins tend to mutually interact and form a visco-elastic film at the interface, thereby stabilizing the foam (Damodaran, 2005; Murray, 2007).

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The link between foaming and A-W interfacial properties has been studied for many food proteins, including β-caseins (Maldonado-Valderrama et al., 2008), and those of egg white (Davis & Foegeding, 2007; Pernell, Foegeding, Luck, & Davis, 2002; Yang, Berry, & Foegeding, 2009), whey (Davis & Foegeding, 2007; Pernell et al., 2002; Yang et al., 2009), and soy (Martínez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2009; Ruíz-Henestrosa et al., 2007). Such studies usually have included an evaluation of the adsorption kinetics to the A-W interface, the mechanical properties of an adsorbed protein film, and of course the foaming properties of protein solutions. In a recent paper, such study was performed on enzymatic wheat gluten hydrolysates (GHs). It was concluded that the foaming capacity (FC) was to some extent related to the rate of diffusion to and adsorption at the A-W interface, and that foam stability (FS) could be related to elasticity of the GH protein films (Wouters et al., 2016b, 2016a) The structural features responsible for their A-W interfacial behavior were studied in depth (Wouters, Rombouts et al., 2017). Furthermore, it was shown that the functionality of the GH constituents heavily depends on pH (Wouters, Fierens et al., 2017).

While many studies have focused on relatively simple experimental conditions (aqueous protein solutions), food systems are usually much more complex. For instance, sugars or alcohols, which are hydrophilic co-solvents in these aqueous solutions (McClements, 2002), can have a significant impact on protein functionality in food foams.

Sucrose is present in food foams such as those of chocolate mousse and meringue. Foams stabilized by bovine serum albumin (BSA) (Guzey, McClements, & Weiss, 2003), whey (Phillips, Yang, Schulman, & Kinsella, 1989; Yang & Foegeding, 2010) and egg white (Raikos, Campbell, & Euston, 2007; Yang & Foegeding, 2010) proteins all have higher FS in the presence of sucrose. Its impact on the kinetics of adsorption at A-W interfaces has been described by several authors. Guzey et al. (2003) reported a decrease in adsorption rate of BSA (2.0% $w_{protein}$/v) in the presence of sucrose at concentrations ranging from 10 to 40% w/v. They ascribed the decreased adsorption rate in sucrose containing protein solutions to their high viscosity. A similar observation was made for concentrations of soy globulins ranging from 0.001 to 1% $w_{protein}$/v, which adsorbed more slowly at A-W interfaces when sucrose was present in concentrations ranging from 9 to 34% w/v (Ruíz-Henestrosa, Carrera Sánchez, & Rodríguez Patino, 2008). Antipova, Semenova, and Belyakova (1999) reported a decrease in surface activity of ovalbumin solutions (0.001% $w_{protein}$/v) in the presence of sucrose (in concentrations up to 25% w/v), which they ascribed to a higher hydrophilicity of the proteins in the bulk solutions. These observations are in contrast with the outcome of a study by Rodríguez Niño and Rodríguez Patino (2002). They noted an increase in the rate at which BSA (0.1% $w_{protein}$/v) adsorbs at the interface in the presence of 17% w/v sucrose. The latter observations were attributed to more compact folding of BSA in the presence of sucrose which promoted diffusion towards the interface. In another study, sucrose caused the surface activity of sodium caseinate to increase due to dissociation of sodium caseinate micelles. This rendered them more hydrophobic and prone to adsorb at the A-W interface (Antipova et al., 1999). Furthermore, the addition of sucrose resulted in an increase or decrease of the dilatational interfacial elastic modulus (E′) of an egg white or a whey (Yang & Foegeding, 2010) protein film, respectively. E' of BSA (Rodríguez Niño, Wilde, Clark, Husband, & Rodríguez Patino, 1997) and soy globulin (Ruíz-Henestrosa et al., 2008) protein films also decreased in the presence of sucrose (Rodríguez Niño et al., 1997). The above allows deducing that the impact of sucrose on the foaming and A-W interfacial behavior of protein solutions is complex to say the least.

Even though these studies were mostly conducted on model proteins under conditions (protein concentration, sucrose concentration) not necessarily directly relevant for food systems, they contribute to a more fundamental mechanistic understanding of food protein interfacial behavior in the presence of a common food constituent such as sucrose. Such studies are most relevant to better understand the foaming of food proteins in actual food systems. Up until this point, to the best of our knowledge, studies on the matter have focused mostly on animal derived proteins, but not so much on plant proteins, let alone on plant protein hydrolysates. Against this background, this paper deals with the A-W interfacial characteristics of enzymatically hydrolyzed wheat gluten proteins in the absence and presence of sucrose at varying protein concentrations. In terms of applicability, food products such as meringues generally consist solely of hen egg white proteins, water and sucrose, which means that this manuscript renders relevant information for the possible replacement of animal protein by a plant-based alternative such as gluten hydrolysates in meringue-like food products.

2. Materials and methods

2.1. Materials

Commercial wheat gluten was kindly provided by Tereos Syral (Aalst, Belgium). It contained 82.4% protein (N x 5.7) on dry matter (dm) basis when determined using an adaptation of AOAC Official Method 990.03 (AOAC, 1995) to an EA1108 Elemental Analyzer (Carlo Erba/Thermo Scientific, Waltham, MA, USA). Trypsin (EC 3.4.21.4; 13,000–20,000 units/mg protein with benzoyl L-arginine ethyl ester as a substrate under standardized conditions) from porcine pancreas and pepsin (EC 3.4.23.1; 3200–4500 units/mg protein with haemoglobin as a substrate under standardized conditions) from porcine gastric mucosa were from Sigma-Aldrich (Bornem, Belgium), as were all other chemicals (including analytical grade sucrose), solvents and reagents, unless otherwise specified.

2.2. Enzymatic hydrolysis

Enzymatic hydrolysis of a 6.0% ($w_{protein}$/v) wheat gluten aqueous dispersion was carried out as described in Wouters et al. (2016a) with trypsin or pepsin at pH-stat conditions in a Titrino 718 device (Metrohm, Herisau, Switzerland). For each enzyme, gluten was hydrolyzed to degrees of hydrolysis (DH) 2 and 6. The DH reflects the percentage of initially present peptide bonds which have been hydrolysed (see below). For tryptic hydrolysis, pH-stat conditions were 50 °C, pH 8.0 and an enzyme to substrate ratio of 1:480 (DH 2) or 1:20 (DH 6) on protein mass basis was used. For peptic hydrolysis, the reactions were carried out at 37 °C, pH 3.5 and an enzyme to substrate ratio of 1:1200 (DH 2) or 1:300 (DH 6) on protein mass basis was used. When the desired DH was reached, the pH was adjusted to 6.0 and proteolysis was stopped by heating the protein suspension for 15 min at 95 °C. The mixtures were then centrifuged (10 min, 12,000 *g*) at room temperature, and the supernatants filtered [Whatman (Maidstone, United Kingdom) paper filter, pore size 4–7 μm] and then freeze-dried. It should be noted that the heating procedure may have caused peptide conformational changes and possibly aggregation. The effect of limited aggregation of gluten hydrolysate constituents on their foaming properties has been addressed elsewhere (Wouters, Fierens et al., 2017). All further analyses, including those of protein contents (carried out as outlined in Section 2.1), were conducted on the dry supernatants of DH 2 or DH 6 tryptic (further referred to as T2 and T6, respectively) and peptic (further referred to as P2 and P6, respectively) hydrolysates.