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Air–water interfacial properties of enzymatic wheat gluten hydrolyzates determine their foaming behavior

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ABSTRACT

Insight in the link between foaming and interfacial properties of proteins can increase their potential as functional agents in food systems. Here, foaming capacity and stability of structurally different peptic and tryptic wheat gluten hydrolyzates were related to the kinetics of their adsorption at an air–water interface as well as to the properties of a compressed protein film at this interface. Foams from degree of hydrolysis (DH, *i.e.* the percentage of cleaved peptide bonds) 2 hydrolyzates were more stable than those from their DH 6 counterparts, and this at all protein concentrations tested. However, at protein concentrations from 0.010% to 0.050% (w_{prot}/v), peptic DH 2 and 6 hydrolyzates had better foaming stability than their tryptic counterparts of the same DH. The opposite was observed when protein concentrations ranged from 0.050% to 0.150% (w_{prot}/v). These observations can in part be explained by the molecular mass composition of the samples and, more importantly, by high levels of hydrophobic peptides in the DH 2 samples. The calculation of an average elasticity (up to 20–25 mN/m) from the variation in surface pressure for a variation in surface area in Langmuir isotherms showed that DH 2 samples had higher elasticity than DH 6 samples, which was in agreement with their foaming stabilities at various protein concentrations. Additionally, although not usually considered in literature, it seemed there was a correspondence between surface pressure at different protein surface concentrations and foaming stability at different protein concentrations.

1. Introduction

Commercial wheat gluten mainly consists of the wheat storage protein. It is the co-product of the industrial isolation of starch from wheat flour (Van Der Borght, Goesaert, Veraverbeke, & Delcour, 2005). Despite the low cost and unique functional properties during bread making of these gluten proteins (Veraverbeke & Delcour, 2002), they are still often disposed of in low cost applications such as animal feed (Day, Augustin, Batey, & Wrigley, 2006). There is a clear interest from industry for alternative valorization routes. A hurdle in this context is the low solubility of gluten protein in aqueous media (Delcour et al., 2012). Partial hydrolysis increases its solubility and induces emulsifying and foaming properties (Adler-Nissen, 1976). Enzymatic hydrolysis is often preferred over acid hydrolysis due to preservation of nutritional quality (Adler-Nissen, 1985; Provansal, Cuq, & Cheftel, 1975) and a higher specificity during hydrolysis.

Foams consist of a gaseous phase dispersed in a liquid phase. They are important for food products such as meringue, angel food cakes and beer. Their most common form is a large amount of air bubbles separated by many aqueous layers. Air–water interfaces are thermodynamically instable but can be kinetically stabilized by surfactants (Damodaran, 2005; Murray, 2007). Proteins which diffuse, adsorb and arrange themselves at this interface can also act as surfactants, because they slow down the two major destabilization mechanisms of foam, i.e. disproportionation and coalescence (Damodaran, 2005). On the one hand, proteins can stabilize gas bubbles in dispersions by forming a steric barrier at the interface. Such barrier prevents bubbles from approaching each other and eventually merging (Hunter, Pugh, Franks, & Jameson, 2008; Murray, 2007). On the other hand, they lower the surface tension

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upon adsorption. Through mutual interactions, proteins form an elastic film around gas bubbles which stabilizes the foam (Damodaran, 2005). The adsorption kinetics of the proteins and the mechanical properties of protein films strongly affect foam formation and stability. The foaming capacity (FC) of proteins is influenced by the kinetics of their adsorption at the air–water interface (Rodríguez Patino, Carrera Sánchez, & Rodríguez Niño, 2008; Rodriguez Patino, Conde, et al., 2007). In contrast, foam stability (FS) is rather related to the strength and mechanical properties of the protein film around the air bubbles (Damodaran, 2005; Rodríguez Patino, Rodríguez Niño, & Carrera Sánchez, 2007). For some common globular food proteins, the relationship between structural, air–water interfacial and foaming properties has been documented. Maldonado-Valderrama et al. (2008) showed that β-casein foam formation is related to the rate of adsorption at the interface and the interface viscosity, whereas FS is rather linked to protein film elasticity. For egg white and whey protein, under some experimental conditions, interfacial characteristics are in line with their foaming properties (Davis & Foegeding, 2007; Karamoko, Danthine, Olive, & Blecker, 2013; Pernell, Foegeding, Luck, & Davis, 2002; Yang, Berry, & Foegeding, 2009). In contrast, Wierenga, van Norel, and Basheva (2009) showed that when lysozyme, β-lactoglobulin and ovalbumin were chemically glycated, this impacted their foaming but not their interfacial properties.

In the context of protein foaming, literature for wheat gluten hydrolyzates almost exclusively reports on the relationship between their molecular mass (MM) distribution and hydrophobicity on the one hand and foaming properties on the other. Publications on wheat gluten evidently agree that hydrolysis strongly increases its solubility over a wide range of pH values. Furthermore, the degree of hydrolysis (DH, *i.e.* the percentage of cleaved peptide bonds) of gluten hydrolyzates critically impacts the foaming properties. Hydrolysis up to a DH 5 improves foaming, while excessive hydrolysis worsens it (Drago & González, 2000; Kong, Zhou, & Qian, 2007; Linares, Larre, Lemeste, & Popineau, 2000). Enzymatic hydrolysis followed by ultrafiltration over membranes with different MM cut-offs demonstrated that the bigger and more hydrophobic fractions generally foam better than the more hydrophilic ones (Berot, Popineau, Compoint, Blassel, & Chaufer, 2001; Popineau, Huchet, Larre, & Berot, 2002; Wang, Zhao, Bao, Hong, & Rosella, 2008). Agyare, Addo, and Xiong (2009) and Babiker, Fujisawa, Matsudomi, and Kato (1996) reported that transglutaminase treatment of hydrolyzed gluten improves its foaming properties by cross-linking smaller peptides into larger chains. Hardt, van der Goot, and Boom (2013) found that increasing the gluten concentration up to 60% during hydrolysis does not impact foaming properties of the resulting hydrolyzates.

Equilibrium surface tensions of gliadin hydrolyzates have been determined, but the link with foaming properties was not clear (Thewissen, Celus, Brijs, & Delcour, 2011). Very recently, the colloidal stability and adsorption behavior at the air–water interface of different wheat gluten hydrolyzates obtained by chemical hydrolysis were investigated (Fuentes-Prado & Martínez-Padilla, 2014). However, no links to functional properties were made.

It remains to be investigated whether and, if so, which air–water interfacial properties of enzymatic wheat gluten hydrolyzates can be related to their functional properties. For other proteins, the link between adsorption kinetics and interfacial film mechanical properties on the one hand, and foam formation and stability on the other hand, significantly contributes to better understanding the foaming potential. Against this background, we here studied adsorption kinetics and protein film properties of different enzymatic wheat gluten hydrolyzates and related them to structural and foaming properties.

2. Materials and methods

2.1. Materials

Commercial wheat gluten was from Tereos Syral (Aalst, Belgium). It contained 82.4% protein (N x 5.7) on dry matter basis when determined using an adaptation of the AOAC Official Method (AOAC, 1995) to an EA1108 Elemental Analyzer (Carlo Erba/Thermo Scientific, Waltham, MA, USA). Trypsin (EC 3.4.21.4) from porcine pancreas and pepsin (EC 3.4.23.1) from porcine gastric mucosa were from Sigma–Aldrich (Bornem, Belgium) as were all chemicals, solvents and reagents (unless indicated otherwise).

2.2. Enzymatic hydrolysis

A 6.0% ($w_{protein}$/v) wheat gluten aqueous dispersion was incubated with trypsin or pepsin at pH-stat conditions in a Titrino 718 device (Metrohm, Herisau, Switzerland). For each enzyme, hydrolyses were performed until DH 2 and DH 6. 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. For peptic hydrolysis, these conditions were 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. When the desired DH was reached, pH was adjusted to 6.0 and proteolysis was stopped by heating the protein suspension for 15 min at 95 °C. The hydrolyzates were then centrifuged (10 min, 12,096 g) at room temperature and supernatants were filtered and freeze-dried. All further analyses, including those of protein contents (carried out as outlined in Section 2.1), were conducted on the freeze-dried supernatants of DH 2 or DH 6 tryptic hydrolyzates (further referred to as T2 and T6, respectively) and of peptic DH 2 or DH 6 hydrolyzates (further referred to as P2 and P6, respectively).

2.3. Determination of DH

DH was defined as the percentage of the number of peptide bonds hydrolyzed (h) to the total number of peptide bonds per unit weight present in wheat gluten protein (h_{tot}). DH was then calculated from the amount of base (trypsin) or acid (pepsin) used to keep pH constant during hydrolysis, using the formula:

$$DH\ (\%) = \frac{h}{h_{tot}} = \frac{X \cdot M_x \cdot 100}{\alpha \cdot M_p \cdot h_{tot}} \quad (1)$$

With X the consumption (mL) of acid or base needed to keep the pH during hydrolysis constant and M_x the molarity of the acid or base. α is a measure for the degree of dissociation of the α-NH_3^+ (neutral or alkaline conditions) or α-COOH group (acidic conditions). Under the given conditions, for tryptic hydrolysis α is 0.89 (Adler-Nissen, 1985), whereas for peptic hydrolysis it is 0.29 (Diermayr & Dehne, 1990). M_p is the mass of protein used, h are hydrolysis equivalents [milli-equivalents (meqv)/g protein] and h_{tot} is the theoretical number of peptide bonds per unit weight present in gluten protein. Nielsen, Petersen, and Dambmann (2001) calculated the latter to be 8.3 meqv/g protein.

2.4. Foaming properties

Foaming properties were determined with a standardized whipping test based on Caessens, Gruppen, Vissers, van Aken and Voragen (1997). An aliquot (50 mL) of T2, T6, P2 or P6 solution [0.010%, 0.025%, 0.050%, 0.100% and 0.150% (w_{prot}/v)] was placed in a graduated glass cylinder (internal diameter 60.0 mm) in a water bath at 20 °C. After temperature equilibration, it was whipped for