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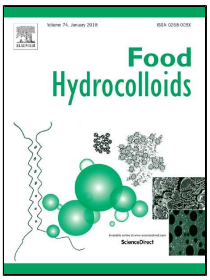

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HIGHLIGHTS

- Gluten hydrolysate (GH) solutions had high foaming capacity
- Egg white (EW) protein solutions had high foam stability
- Mixed solutions had both high foaming capacity and stability
- Nonetheless, the interface composition seemed to consist mainly of adsorbed GHs
- EW proteins may form a secondary interfacial layer by interacting with adsorbed GHs

Foaming and air-water interfacial characteristics of solutions containing both gluten hydrolysate and egg white protein

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ABSTRACT

Enzymatically hydrolyzed wheat gluten can be a viable alternative for traditional animal-based foam stabilizing proteins in food systems. Gluten hydrolysates (GHs) can be considered for (partially) replacing surface-active food proteins such as those of egg white (EW). We here studied the foaming and air-water (A-W) interfacial characteristics of mixed GH + EW protein solutions. GH solutions had much higher ($P < 0.05$) foaming capacities than EW solutions, while the latter had much higher ($P < 0.05$) foam stability than the former. When only one sixth of EW proteins was replaced by GHs, the foaming capacity of the mixtures was as high as or higher than that of the GH solutions. Furthermore, when half of the EW protein was replaced by GH, the mixtures still had high foam stability. It thus seems that both GH and EW proteins contribute positively to the foaming characteristics of the mixtures. However, measurements of the early stages of diffusion to and adsorption at the interface, plus measurements of surface dilatational moduli at the interface, both suggested that the adsorbed protein film consists primarily of GHs rather than of EW proteins. Nonetheless, FS was higher when EW proteins were present. Mixed GH + EW solutions have a higher resistance to coalescence than GH solutions. Therefore, it is hypothesized that EW proteins form a secondary protein layer below the A-W interface which is maintained by interactions with adsorbed GH constituents, thereby providing bubbles with an additional resistance to coalescence.

Keywords: Air-water interfacial properties; Gluten; Hydrolysates; Egg white proteins; Foam

1. INTRODUCTION

Food foams are dispersions of gas, usually air, in a continuous phase, usually water. They provide structure and texture to a wide variety of food products, such as meringues, cakes, and (chocolate) mousses (Foegeding & Davis, 2011; Foegeding, Luck, & Davis, 2006). Foams are thermodynamically unstable, but can be stabilized by proteins (Damodaran, 2005; Murray, 2007). Because of their amphiphilic nature, proteins can adsorb at air-water (A-W) interfaces. This results in a decrease of surface tension, in electrostatic and steric repulsion of adjoining gas bubbles at which they are adsorbed, and in the formation of a viscoelastic protein film around these gas bubbles (Damodaran, 2005; Hunter, Pugh, Franks, & Jameson, 2008; Murray, 2007). Mostly, animal proteins, such as those of hen egg white (EW), are used in food foam applications, because of their excellent functionality and desirable organoleptic properties. However, EW is rather expensive and its production has a significant environmental impact (Alexandratos & Bruinsma, 2006; Herrero et al., 2011; Lusk & Norwood, 2009). In contrast, the production of plant proteins is cheaper and more sustainable, but these proteins often lack functionality and/or solubility in aqueous media (Day, 2013). A notable example is the wheat gluten proteins, a co-product of the industrial starch isolation process (Van Der Borght, Goesaert, Veraverbeke, & Delcour, 2005). Their solubility can be substantially improved by controlled enzymatic hydrolysis, which also induces foaming properties (Adler-Nissen, 1977; Wouters, Rombouts, Fierens, Brijs, & Delcour, 2016a). In previous work by our group, the relationship between the foaming, structural and A-W interfacial characteristics (rate of diffusion to and adsorption at the interface, protein film properties) of such gluten hydrolysates (GHs) in water (Wouters et al., 2016b, 2016c; Wouters et

al., 2017d) was studied, including under conditions more relevant to food products (Wouters et al., 2017a; Wouters et al., 2017b; Wouters et al., 2017c). More specifically, the impact of pH (Wouters et al., 2017b) and the presence of common food constituents such as ethanol (Wouters et al., 2017c) or sucrose (Wouters et al., 2017a) on GH interfacial and foaming behavior were investigated. However, other surface-active constituents such as low molecular mass surfactants (LMMS) or other proteins may also be present in food systems. Numerous studies on mixed protein-LMMS interfaces have been published. Such interfaces are often not very stable because LMMS disrupt the way proteins stabilize interfaces and vice versa. Interested readers are referred to some excellent reviews on the matter (Maldonado-Valderrama & Patino, 2010; Miller et al., 2000; Rodríguez Patino, Rodríguez Niño, & Carrera Sánchez, 2007b; Wilde, Mackie, Husband, Gunning, & Morris, 2004; Wilde, 2000). Here, the focus is on mixed protein – protein interfaces.

When at least two different proteins coexist, they do not necessarily adsorb at an A-W interface in equal proportions. This phenomenon is referred to as competitive adsorption. It is controlled by several factors (Dickinson, 2011; Razumovsky & Damodaran, 1999). First, there is an energy barrier for adsorption at the A-W interface (Damodaran, 2004). Molecular properties of proteins such as their hydrophobicity determine how easily proteins can overcome such barrier . Wierenga, Meinders, Egmond, Voragen & de Jongh (2003) have shown that caprylated ovalbumin, which is more hydrophobic than its parent molecule, adsorbs more easily at an A-W interface than non-modified ovalbumin. In mixtures of proteins, their respective affinities for the interface therefore in part determine the ease with and the extent to which they adsorb at an interface. Second, a kinetic aspect should be considered. Small hydrophobic proteins diffuse more rapidly

towards the interface, which gives them an advantage in dominating the interface (Damodaran, 2004; Dickinson, 2011). Several studies have shown that the interface composition is mostly determined by this kinetic aspect. In mixed β-casein + lysozyme (Xu & Damodaran, 1994), lysozyme + bovine serum albumin (BSA) (Anand & Damodaran, 1995) and β-casein + BSA (Cao & Damodaran, 1995) systems, the extent of adsorption of the proteins at the A-W interface depends on their order of arrival at the interface. These studies also reported that, in sequential adsorption experiments, in which one protein was added only after the other one had already adsorbed at the A-W interface, no second protein displaced the first protein from the interface. This is in contrast with the so-called orogenic displacement mechanism in mixed LMMS-protein systems which was first introduced by Mackie, Gunning, Wilde & Morris (1999). It seems that most globular proteins adsorb strongly at interfaces, making their desorption in favor of other proteins unlikely (Dickinson, 2011). An exception to this is a mixed α_{s1}-casein + β-casein system in which both proteins displace each other from the interface under certain conditions (Anand & Damodaran, 1996). The latter was also found to be the case for the same protein mixture but at an oil-water interface (Dickinson, 1991). Furthermore, already adsorbed proteins affect the ability of other proteins to also adsorb at the A-W interface (Razumovsky & Damodaran, 1999). This was noted for a number of common food proteins (amongst which α-lactalbumin, β-lactoglobulin, α-caseins, β-caseins, BSA, lysozyme and ovalbumin). These proteins show Langmuir-like adsorption behavior at an A-W interface. However, binary mixtures of the same proteins no longer follow this model (Razumovsky & Damodaran, 1999, 2001). The effect was ascribed to thermodynamic incompatibility of proteins, which arises from unfavorable protein-protein interactions and differences in protein-solvent interactions, both of which depend on the intrinsic molecular

properties of the proteins (Damodaran, 2004; Polyakov, Grinberg, & Tolstoguzov, 1997; Polyakov, Popello, Grinberg, & Tolstoguzov, 1986; Razumovsky & Damodaran, 1999).

Thermodynamic incompatibility at A-W interfaces may also result in phase separation of adsorbed protein molecules in mixed protein films (Damodaran, 2004; Dickinson, 2011). For example, with mixtures of 11S soy globulin + β-casein, the acidic subunits of soy 11S globulins + β-casein, or mixtures of α-lactalbumin + β-casein, the interfaces do not show any signs of phase separation at the interface up to 24 h after adsorption. However, over a longer time frame (96 h), significant phase separation does occur, with β-casein always forming the more continuous phase of the film while the other protein occurs in dispersed patches (Sengupta & Damodaran, 2001). Similarly, in mixed β-casein + BSA films, separate regions of both proteins can be distinguished at the interface, pointing to interfacial phase separation (Sengupta & Damodaran, 2000). In contrast, Mackie, Gunning, Ridout, Wilde & Morris (2001) and Ridout, Mackie & Wilde (2004) reported that both proteins of a β-casein + β-lactoglobulin system formed homogeneous films at the interface with no signs of phase separation. Of importance regarding these varying observations is that the above mentioned studies by Damodaran and co-workers (Anand & Damodaran, 1995, 1996; Cao & Damodaran, 1995; Razumovsky & Damodaran, 1999, 2001; Sengupta & Damodaran, 2000, 2001; Xu & Damodaran, 1994) have used a radiolabeling method to study the adsorption and phase separation characteristics of mixed protein systems at the interface. It has been noted (Murray, 1997) that some radiolabeled proteins might display different surface activity than their native forms, which should of course be considered and may explain some of the contrasting findings in studies by different research groups above.

Mixed protein interfaces may also result in synergistic effects. For example, the cationic peptide protamine strongly improves the foaming characteristics of BSA, even though it does not display any surface activity itself. It has been suggested that electrostatic interactions between BSA and protamine lead to improved overall foaming properties (Glaser, Paulson, Speers, Yada, & Rousseau, 2007). In another study, mixed β-conglycinin + β-lactoglobulin films had higher interfacial elasticity values than did films of the separate proteins (Ruiz-Henestrosa, Martinez, Sanchez, Rodríguez Patino, & Pilosof, 2014). Furthermore, the addition of fish scale gelatin to EW protein improves the overall foaming properties, probably by strengthening the viscoelastic layer around the gas bubbles (Huang et al., 2017).

However, the most notable example of such synergistic effects is probably that encountered in hen EW, which contains a mixture of proteins, among which ovalbumin, ovotransferrin, ovomucoid, ovomucin, lysozyme and ovoglobulins. Many studies investigating the air-water interfacial or foaming characteristics of EW proteins have focused on its main protein, ovalbumin. However, the exceptional foaming properties of EW cannot merely be ascribed to the functionality of ovalbumin. They have been attributed to a cooperative effect exerted by its structurally different proteins (Dickinson, 1989; Dickinson, 2011; Lomakina & Mikova, 2006; Mine, 1995; Stevens, 1991). For example, recent studies have shown better foaming and A-W interfacial film properties in mixed lysozyme + ovalbumin systems than with the separate proteins (Le Floch-Fouéré et al., 2010; Le Floch-Fouéré et al., 2009). In this context, Damodaran, Anand & Razumovsky (1998) have described the formation of electrostatic complexes of lysozyme with other EW proteins at the A-W interface.

EW production is not very sustainable and EW is rather expensive. It is therefore important to consider plant-based alternatives such as the GHs which have already been discussed. As complete replacement of EW proteins in food products is difficult, we here studied the foaming, air-water interfacial (diffusion, adsorption and protein film) characteristics of mixed GH + EW protein solutions. This will render relevant mechanistic information on the interaction of these structurally different protein types at A-W interfaces and also be relevant for rational incorporation of GHs into food products as foaming agents.

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 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) from porcine pancreas and pepsin (EC 3.4.23.1) from porcine gastric mucosa were from Sigma-Aldrich (Bornem, Belgium), as were all other chemicals, solvents and reagents, unless otherwise specified. All filtrations were with Whatman (Maidstone, UK) paper filters (pore size 4-7 µm). Commercial dry EW powder with a protein content of 84.2% (on a dry matter basis) was obtained from Lodewijckx (Veerle-Laakdal, Belgium).

153 **2.2 Enzymatic hydrolysis**

154

155 Enzymatic hydrolysis of a 6.0% ($w_{protein}$/v) wheat gluten aqueous dispersion was carried out with
156 trypsin or pepsin at pH-stat conditions in a Titrino 718 device (Metrohm, Herisau, Switzerland) as
157 described earlier by Wouters et al. (2016b). For both enzymes, gluten was hydrolyzed to degrees
158 of hydrolysis (DH) 2 and 6. The DH reflects the percentage of initially present peptide bonds which
159 have been hydrolysed (see below). For tryptic hydrolysis, pH-stat conditions were 50 °C and pH
160 8.0. An enzyme to substrate ratio of 1:480 (DH 2) or 1:20 (DH 6) on protein mass basis was used.
161 For peptic hydrolysis, the reactions were carried out at 37 °C and pH 3.5. In this case, an enzyme
162 to substrate ratio of 1:1200 (DH 2) or 1:300 (DH 6) on protein mass basis was used. When the
163 desired DH was reached, the pH was adjusted to 6.0 and proteolysis was stopped by heating the
164 protein suspension for 15 min at 95 °C. The mixtures were then cooled to room temperature and
165 centrifuged (10 min, 12,000 *g*), and the supernatants were filtered over paper and then freeze-
166 dried. All further analyses, including those of protein contents (carried out as outlined in Section
167 2.1), were conducted on the dry supernatants of DH 2 or DH 6 tryptic (further referred to as T2
168 and T6, respectively) and peptic (further referred to as P2 and P6, respectively) hydrolysates.

169

170 **2.3 Determination of degree of hydrolysis**

171

172 DH is the percentage of peptide bonds hydrolyzed (h) relative to the total number of peptide
173 bonds (h_{tot}) per unit weight present in wheat gluten protein. It was calculated as reported earlier

(Wouters et al., 2016b) from the quantity of NaOH (trypsin) or HCl (pepsin) solution used to keep the pH constant during hydrolysis:

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

with X the consumption (mL) of NaOH or HCl solution needed to keep the pH during hydrolysis constant and M_x the molarity of the acid or base (respectively 0.50 and 0.20 M). The term 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 & Dambmann (2001) calculated the latter to be 8.3 meqv/g protein.

2.4 Foaming properties

Foaming properties were determined with a standardized stirring test identical to the one of Wouters et al. (2016b). An aliquot (50 mL) of T2, T6, P2 or P6 solutions (0.050%, and 0.150% $w_{protein}$/v), EW protein solutions (0.200%, 0.300%, 0.400%, 0.500% and 0.600% $w_{protein}$/v) and solutions containing GH + EW protein mixtures (0.050% $w_{protein}$/v hydrolysate with 0.250% $w_{protein}$/v EW protein or 0.150% $w_{protein}$/v hydrolysate with 0.150% $w_{protein}$/v EW protein) in deionized water (W) or in a 5.0% v/v ethanol solution (ES) was placed in a graduated glass cylinder (internal diameter 60.0 mm) in a water bath at 20 °C. After equilibration to this temperature for 15 min, it was stirred for 70 s with a propeller (outer diameter 45.0 mm, thickness 0.4 mm)

196 rotating at about 2,000 rpm. After stirring, the propeller was immediately removed and the glass
197 cylinder sealed with Parafilm M (Bemis, Neenah, WI, USA) to avoid foam disruption by air
198 circulation. The FC is the foam volume exactly 2 min after the start of stirring. FS is measured by
199 determining foam volume after 60 min and expressing it as percentage of the FC. Based on the
200 foam height and the cylinder internal diameter, foam volume was calculated and expressed in
201 mL. Mixtures of GHs and EW proteins are coded as in the following example: $T2_{0.050}EW_{0.250}$ is a
202 solution containing 0.050% $w_{protein}$/v T2 and 0.250% $w_{protein}$/v EW protein.
203
204 **2.5 Maximum bubble pressure method**
205
206 The rate and extent of diffusion to and adsorption at the A-W interface of T2, T6, P2 and P6
207 solutions (0.050% and 0.150% $w_{protein}$/v), EW protein solutions (0.150%, 0.300%, 0.500% and
208 0.700% $w_{protein}$/v) and solutions containing GH + EW protein mixtures (0.050% $w_{protein}$/v
209 hydrolysate with 0.250% $w_{protein}$/v EW protein or 0.150% $w_{protein}$/v hydrolysate with 0.150%
210 $w_{protein}$/v EW protein) were determined with the maximum bubble pressure method described
211 elsewhere (Wouters et al., 2016b) after filtration over paper filters as described in section 2.1. In
212 this method (Fainerman, Miller, & Joos, 1994), air bubbles are generated at a constant rate at 20
213 °C through a capillary (diameter 0.200 mm) in the liquid phase. When the bubble radius equals
214 the capillary radius (r_{cap}), the pressure in the bubble is maximal (P_{max}) and measured. This pressure
215 can be used in the following equation (with P_0 the initial hydrostatic pressure) to determine
216 surface tension (γ):

217 $$\gamma = \frac{(P_{max} - P_0).r_{cap}}{2}$$

Surface tension was determined as a function of surface age in a 5 ms to 10 s time frame. A typical profile was characterized by a constant surface tension (equal to that of water) up until a certain point, after which surface tension decreased linearly with the logarithm of surface age. The occurrence of such surface age region of constant surface tension depended on protein concentration. At sufficiently high protein concentrations, surface tension immediately decreased linearly with the logarithm of surface age. To characterize the moment at which surface tension started decreasing, a lag time was defined as the surface age when the surface tension had decreased to a value equal to or lower than 95% of the initial value, provided that there was an initial period in which surface tension was constant. This 95% value was chosen somewhat arbitrarily to allow systematic determination of lag times, rather than having to report visual estimates. The lag time is representative for the rate of diffusion (and possibly early stage of adsorption when surface tension had not yet decreased substantially) of proteins to the A-W interface. From the lag time onwards, surface tension thus decreased linearly as a function of the logarithm of surface age. A measure ($|S_{ST\text{-}t}|$) for the continuous adsorption and rearrangement of proteins at the interface was obtained by calculating the slope of the absolute value of this decrease of surface tension (starting from the lag time) as a function of logarithmic surface age.

2.7 (Oscillating) pendant drop measurements

Solutions of T2, T6, P2 and P6 (0.150% and 0.300% $w_{protein}/v$), EW protein solutions (0.150%, 0.300%, 0.500%, 0.700% $w_{protein}/v$) and solutions containing GH + EW protein mixtures (0.050% $w_{protein}/v$ hydrolysate with 0.250% $w_{protein}/v$ EW protein or 0.150% $w_{protein}/v$ hydrolysate with

0.150% $w_{protein}$/v EW protein) were filtered over paper filters as described in section 2.1 and introduced in a Theta optical tensiometer (Biolin Scientific Attension, Stockholm, Sweden) to create a pendant drop with a fixed volume of 8 µL. For every drop, the decrease in surface tension was measured over a 10 min time interval to assess protein adsorption and rearrangement at the A-W interface as described in Wouters et al. (2016b). During this period, images were taken at 1 frame every 7 seconds. Subsequently, a sinusoidal oscillation (50 cycles) was performed at a frequency of 1 Hz with an amplitude set at 1.00 in the OneAttension software (Biolin Scientific Attension), which corresponded to a volume change of ± 1 µL. During oscillation, images were recorded at 7 frames per second. From the drop shape analysis during oscillation, the surface dilatational modulus E could be determined. E is the variation in surface tension per unit relative change in surface area (A) (Lucassen-Reynders, Benjamins, & Fainerman, 2010; Lucassen-Reynders & Wasan, 1993).

$$E = \frac{d\gamma}{d\ln A}$$

where γ is surface tension. E is a viscoelastic complex quantity. It consists of a real surface dilatational elastic (E') and an imaginary dilatational viscous (E'') contribution, of which the latter is given by the product of a surface dilatational viscosity (η_d) and the frequency (ω) of the variation in A (Lucassen-Reynders et al., 2010; Lucassen-Reynders & Wasan, 1993).

$$E = E^{'} + iE^{''} = E^{'} + i\omega\eta_d$$

Surface dilatational elastic moduli E' are reported here. After each measurement, the device was thoroughly cleaned and the surface tension of pure water was checked to be 72.0 ± 0.5 mN/m, before initiating the next measurement.

2.6 Surface shear viscosity measurements

Surface shear viscosity measurements were performed with a two-dimensional Couette-type interfacial viscometer in a set-up similar to that described by Borbas, Murray & Kiss (2003), Murray and Dickinson (1996) and Burke, Cox, Petkov & Murray (2014). Solutions of T2, T6, P2 or P6 (0.300% $w_{protein}$/v), EW protein (0.300% $w_{protein}$/v) or GH + EW protein mixtures (0.050% $w_{protein}$/v hydrolysate with 0.250% $w_{protein}$/v EW protein or 0.150% $w_{protein}$/v hydrolysate with 0.150% $w_{protein}$/v EW protein) were filtered over paper as described in section 2.1 and placed in a circular dish. A circular biconical disc hanging from a wire (0.10 mm diameter) with a known torsion constant (3.4822 x 10^{-6} Nm/rad) was positioned with its bottom edge at the A-W interface of these protein solutions. A laser beam reflected off a mirror, mounted on the hanging disc, on a scale at a fixed distance from the disc. The dish containing the protein solution was able to rotate at a constant (shear) rate. When protein material adsorbed at the A-W interface and yielded a measurable surface shear viscosity, the rotation of the dish caused a deflection of the hanging disc at the interface, and thus of the laser beam. The motion of the laser beam on the scale, and thus the deflection of the disc over time was recorded digitally via a charge coupled device camera. The surface shear viscosity η as a function of time was then calculated as

$$\eta = \frac{\left(\frac{R_i^{-2} - R_0^{-2}}{4\pi}\right) \times K \times \theta_i}{\omega}$$

with R_i the radius of the disc (1.5 cm), R_0 the radius of the dish (7.5 cm), ω the angular velocity of the dish (1.270 x 10^{-3} rad/s) , K the torsion constant of the wire and θ_i the angle of rotation of the

282 disk. The fixed velocity (i.e., shear rate) was chosen so as to allow comparison with previous
283 measurements on other systems (Burke et al., 2014).
284
285 **2.8 Bubble disproportionation measurements**
286
287 Bubble disproportionation experiments were performed with a methodology thoroughly
288 described by Dickinson, Ettelaie, Murray & Du (2002) and outlined more briefly here. Solutions of
289 T2, T6, P2 or P6 (0.300% $w_{protein}$/v), EW protein (0.300% $w_{protein}$/v) or GH + EW protein mixtures
290 (0.050% $w_{protein}$/v hydrolysate with 0.250% $w_{protein}$/v EW protein or 0.150% $w_{protein}$/v hydrolysate
291 with 0.150% $w_{protein}$/v EW protein) were filtered over paper as described in section 2.1 and poured
292 into a stainless steel cell. Bubbles were introduced in the middle of the cell and allowed to rise to
293 the planar A-W interface at the top of the cell. They were then trapped in the circular opening of
294 a paraffin wax-coated mica sheet floating in the middle of this planar A-W interface. To avoid the
295 effects of the shrinkage of adjacent bubbles on the shrinkage of individual bubbles (Ettelaie,
296 Dickinson, Du, & Murray, 2003), only bubbles positioned at a distance of at least twice their own
297 radius from all other bubbles were considered. The bubbles were illuminated from below and
298 images captured using a microscope and a video camera. Bubble radii were determined with
299 ImageJ (NIH, Bethesda, MD, USA) image analysis software (Schneider, Rasband, & Eliceiri, 2012)
300 and plotted over time until the bubbles had shrunk to a size which could no longer be detected
301 with the microscope and camera set-up (< 10 μm) (see also Figure 1). To compare different
302 samples, the total shrinkage time of each air bubble was plotted as a function of its initial bubble
303 radius.

304

305 **2.9 Bubble coalescence measurements**

306

307 Bubble coalescence experiments were conducted in a set-up and methodology similar to the one
308 described in section 2.8 and described in detail previously (Murray et al., 2002; Murray, Dickinson,
309 Lau, Nelson, & Schmidt, 2005). As in section 2.8, solutions of T2, T6, P2 or P6 (0.300% $w_{protein}$/v),
310 EW protein (0.300% $w_{protein}$/v) and GH + EW protein mixtures (0.050% $w_{protein}$/v hydrolysate with
311 0.250% $w_{protein}$/v EW protein or 0.150% $w_{protein}$/v hydrolysate with 0.150% $w_{protein}$/v EW protein)
312 were filtered over paper as described in section 2.1 and poured into a stainless steel cell. Air
313 bubbles introduced in the solution rose to the planar A-W interface, where they were trapped in
314 the circular opening of a paraffin wax-coated mica sheet floating in the middle of this planar A-W
315 interface. The top of the steel cell was sealed using a rubber O-ring and a glass plate. In an
316 adjacent connected chamber of the steel cell, a steel piston was moved up or down in a controlled
317 way. Because the system was sealed off entirely, the pressure in the air phase above the planar
318 A-W interface could be decreased and increased again in a controlled manner by moving the
319 piston upwards and downwards, respectively. The pressure drop caused air bubbles to expand,
320 thereby stretching the adsorbed protein film at their surface. During this process, depending on
321 the strength of the protein film stabilizing them, some bubbles coalesced. A relatively short time
322 (1 to 2 min) after the pressure decrease had ceased, coalescence no longer occurred and the
323 remaining bubbles were stable (note, the bubbles were stable to coalescence if no pressure drop
324 was applied). At this point, the pressure was again increased to its initial value. The fraction of
325 coalesced bubbles for a given sample provides a very discriminating measure of the ability of

different systems to stabilize gas bubbles against coalescence (Murray et al., 2002). This procedure was performed at least 10 times for each sample. The total fraction of coalesced gas bubbles was then calculated for each sample. Figure 2 gives an example of a typical bubble coalescence measurement.

2.10 Statistical analysis

All determinations of foaming properties and oscillating pendant drop measurements were at least in triplicate. MBP measurements and surface shear viscosity measurements were carried out at least in duplicate. Error bars or values in all figures and tables represent the standard deviation from the means. All data was analyzed using statistical software JMP Pro 12 (SAS Institute, Cary, NC, USA). One way analysis of variance (ANOVA) was performed, followed by a Tukey multiple comparison test as post-hoc analysis to detect significant differences, both at a significance level P = 0.05.

3. RESULTS AND DISCUSSION

3.1 Foaming properties

Figure 3 compares foaming characteristics of GH (0.050% or 0.150% $w_{protein}$/v), EW (0.300% $w_{protein}$/v) and mixed GH + EW ($GH_{0.050}EW_{0.250}$ or $GH_{0.150}EW_{0.150}$) protein solutions. A first striking observation is that even at a protein concentration six times as high as that of corresponding GH

348 solutions, an EW protein solution had much lower ($P < 0.05$) FC. In contrast, while FS of $EW_{0.300}$
349 was around 80%, that of the GH solutions at 0.150% $w_{protein}$/v ranged between 32% and 71%
350 depending, on the sample tested. A further increase in protein concentration of GH solutions from
351 0.150% to 0.300% $w_{protein}$/v (data not shown) did increase FS values. As also reported earlier, there
352 were no significant differences ($P > 0.05$) in FC between the different GHs, but DH 2 hydrolysates
353 had higher ($P < 0.05$) FS than DH 6 hydrolysates, explained by a higher average molecular mass
354 and the presence of some specific hydrophobic peptides in the former samples (Wouters et al.,
355 2016b; Wouters et al., 2017d).
356
357 Thus, overall, EW protein solutions had lower FC but higher FS than GH solutions. It is likely that
358 GH constituents because of their lower average molecular mass and higher molecular flexibility
359 diffuse to and adsorb at the A-W interface more rapidly than the large and bulky EW proteins and
360 thus have higher FC than EW proteins. In contrast, EW proteins can form stronger films at the A-
361 W interface than GH peptides, which explains the higher FS of the former. It remains to be
362 investigated how partial substitution of EW proteins by GHs would impact their foaming
363 characteristics.
364
365 Replacing 0.050% $w_{protein}$/v of EW protein by any of the GHs substantially increased the FC.
366 Indeed, values as high or higher than those of 0.050% $w_{protein}$/v solutions of the GHs were noted.
367 Similar results were obtained when 0.150% $w_{protein}$/v of EW protein was replaced by any of the
368 GHs. At a relatively low degree of EW protein substitution by any of the GHs ($GH_{0.050}EW_{0.250}$), FS
369 remained high as was the case for EW protein solutions. Even when half of the EW protein was

replaced by GHs, which intrinsically had lower FS than the former, this was still the case. It is of note that, while there were differences in FS between the different GH solutions, such differences were no longer noted in the mixed systems. Irrespective of which GH sample was used to replace EW proteins, the result was the same.

The presence of GHs, which had better FC than EW proteins, led to high FC values in the mixtures, while the presence of EW proteins, which had better FS than GHs, led to high FS values in the mixtures. These results suggest that both GHs and EW proteins are present at the A-W interface and play a role in stabilizing the interface. In the next sections the A-W interfacial properties of GH + EW protein mixtures are discussed in detail. Of note is that while such determination of A-W interfacial characteristics is surely to a large extent relevant for the foaming characteristics of protein solutions, it should still be kept in mind that there also differences between both types of analyses. In the foam tests, protein solutions are whipped while in surface tension and surface rheology measurements, protein molecules diffuse to and adsorb at the interface, without considerable energy input in the system. Thus, it cannot be guaranteed that the interfacial composition in these different tests is exactly the same. Nonetheless, investigating the A-W interfacial characteristics of mixed GH – EW protein solutions will yield important information to better understand their interplay at the interface.

3.2 Diffusion and adsorption characteristics at the A-W interface

As described in section 2.5, the early stages of protein diffusion to the A-W interface can be characterized by a lag time, i.e., the surface age at which surface tension had decreased to a value equal to 95% of its initial value. Figure 4 compares the lag times of 0.300% $w_{protein}$/v EW protein solution, 0.050% and 0.150% $w_{protein}$/v GH solutions and mixed GHs + EW protein ($GH_{0.050}EW_{0.250}$ or $GH_{0.150}EW_{0.150}$) solutions. The 0.300% $w_{protein}$/v EW protein solution had a significantly ($P < 0.05$) higher lag time than any of the GH solutions at 0.050% $w_{protein}$/v, or any of the mixed GH + EW protein ($GH_{0.050}EW_{0.250}$) solutions. Also, there were no statistically significant ($P > 0.05$) differences between the lag times of the different GHs (0.050% $w_{protein}$/v) and the different mixed GH + EW protein ($GH_{0.050}EW_{0.250}$) solutions. Furthermore, no lag times could be recorded for any of the mixed GH + EW protein ($GH_{0.150}EW_{0.150}$) solutions, indicating that even at very low surface ages, protein had adsorbed at the A-W interface. The same was true for all GHs at 0.150% $w_{protein}$/v.

After the lag phase, surface tension began to decrease, indicating protein adsorption and re-arrangement at the interface. The absolute value of the decrease of surface tension as a function of the logarithm of surface age was defined as the $|S_{ST\text{-}t}|$ value. It is a measure of the rate and extent of this continuous adsorption and rearrangement of proteins in a given sample (Figure 5). These rates of adsorption at the A-W interface showed trends which were very similar to those of the rates of diffusion in Figure 5. $|S_{ST\text{-}t}|$ values of EW protein solutions (0.300% $w_{protein}$/v) were significantly ($P < 0.05$) lower than those of any of the GH solutions (0.050% $w_{protein}$/v) and any of the mixed GH + EW protein ($GH_{0.050}EW_{0.250}$) solutions. The same was true when comparing $|S_{ST\text{-}t}|$ of EW protein solutions (0.300% $w_{protein}$/v) with the mixed GH + EW protein solutions

412 ($GH_{0.150}EW_{0.150}$). In contrast, $|S_{ST\text{-}t}|$ values of the GH and the GH + EW protein solutions were
413 rather similar, although there were some minor but significant ($P < 0.05$) differences.
414
415 Thus, overall, the rates of diffusion and adsorption of EW proteins at the A-W interface were
416 lower than those of GHs. This is in agreement with the much lower FC values of EW protein
417 solutions than those of GH solutions (see section 3.1). Furthermore, both the rates of diffusion to
418 and adsorption at the A-W interface of mixed GH + EW protein solutions were dominated by the
419 presence of GHs. Partial substitution of EW proteins by GHs increased the rates of diffusion to
420 and adsorption at the A-W interface to values similar to those of the pure GH solutions. All this is
421 in agreement with the substantial higher FC of mixed GH + EW protein systems than of pure EW
422 protein solutions (see section 3.1). These results suggest that in these early stages of diffusion to
423 and adsorption of proteins to the interface, the interface composition is dominated by GH
424 peptides rather than by EW proteins.
425
426 **3.3 Surface dilatational elastic moduli**
427
428 Figure 6 compares E' values of a 0.300% $w_{protein}$/v EW protein solution, 0.300% $w_{protein}$/v GH
429 solutions and mixed GH + EW protein solutions ($GH_{0.050}EW_{0.250}$ or $GH_{0.150}EW_{0.150}$). E' values
430 reported here for pure GH protein solutions are lower than those reported earlier by our group
431 for similar samples (Wouters et al., 2016b; Wouters et al., 2017d) because of differences in
432 filtration procedures prior to analysis. However, for both filtration methods, similar trends were
433 observed. Most notably, Figure 6 shows that both DH 2 hydrolysates had significantly ($P < 0.05$)

higher E' values than both DH 6 hydrolysates , as was the case in earlier published work (Wouters et al., 2016b; Wouters et al., 2017d). E' of a 0.300% $w_{protein}$/v EW solution was significantly higher ($P < 0.05$) than E' of any of the GH solutions (0.300% $w_{protein}$/v) or any of the mixed GH + EW protein solutions. At the lowest degree of EW protein substitution, only $T6_{0.050}EW_{0.250}$ had significantly ($P < 0.05$) higher E' than $T6_{0.300}$. All other GH + EW protein mixtures had similar or even lower E' values than the GH solutions. This suggests that, already at this relatively low degree of substitution, the overall strength of the protein films for the mixtures was dominated by the presence of GHs. As already mentioned, the exception was $T6_{0.050}EW_{0.250}$, which had an E' value intermediate between those of $EW_{0.300}$ and $T6_{0.300}$. At a higher degree of EW protein substitution, E' of none of the GH + EW protein mixtures differed significantly from those of their respective GH solutions. Furthermore, $T2_{0.150}EW_{0.150}$ and $P2_{0.150}EW_{0.150}$ had significantly higher E' than $T6_{0.150}EW_{0.150}$ and $P6_{0.150}EW_{0.150}$, which is in line with the higher E' of pure DH 2 than of DH 6 hydrolysate films. This suggests that the interface at this point was again occupied by GH peptides rather than by EW proteins.

The above results are in line with observations made in section 3.2. There, it was concluded that GH components dominate the diffusion to and (early stages of) adsorption at the A-W interface in GH + EW protein mixtures. However, in section 3.1, high FS values were recorded for all mixtures, from which it would be expected that EW proteins, which intrinsically have better FS than any of the GHs, dominate the interface at later stages after adsorption. The trends in E' values did not support this hypothesis. Thus, the higher FS values of the mixtures could not be

attributed to an increase in surface dilatational elastic moduli due to the presence of EW proteins at the interface.

3.4 Surface shear viscosity

As dilatational experiments did not provide an explanation for the high FS values of GH + EW protein mixtures, measurements were performed to assess how the surface shear viscosity was affected when GH and EW proteins co-existed in solution. Figure 7 shows the surface shear viscosity over the course of one hour during constant shearing of the interface for 0.300% $w_{protein}$/v EW protein solution, 0.300% $w_{protein}$/v GH solutions and mixed GH + EW protein ($GH_{0.150}EW_{0.150}$) solutions, as described in section 2.7.

The surface shear viscosity of the 0.300% $w_{protein}$/v EW protein solution increased gradually over the course of one hour during constant shearing. However, for all GH solutions (0.300% $w_{protein}$/v) and all mixed GH + EW protein solutions ($GH_{0.150}EW_{0.150}$) surface shear viscosities were below the limit of detection, which was 0.70 mNs/m for the torsion wire used. Thus, EW proteins at the A-W interface formed strong protein films, illustrated by the relatively high surface dilatational moduli (see section 3.3) as well as high surface shear viscosity values. However, in mixed GH + EW protein systems, the hydrolysates, which provided no measurable surface shear viscosity on their own with the set-up used here, still seemed to dominate the interface.

3.5 Foam destabilization mechanisms

The foaming characteristics of EW protein + GH mixtures (section 3.1) suggested that proteins from both sources were present at the A-W interface, as both FC and FS were relatively high, which was ascribed to the occurrence of GHs and EW proteins at the interface, respectively. However, it was pointed out in sections 3.2 to 3.4 that GHs probably dominate the diffusion to and adsorption at the A-W interface in the early stages as well as the composition of the protein film in later stages after adsorption. Foams are mainly destabilized by disproportionation and coalescence (Damodaran, 2005; Pugh, 1996). Disproportionation is driven by the difference in pressure in gas bubbles of different sizes. The difference in pressure between the outside and inside of a smaller gas bubble is larger than that of a larger gas bubble (Damodaran, 2005). This means that the solubility of gas in the smaller bubble is higher than that in the larger one, which results in gas diffusion from the former to the latter bubbles. Eventually, this coarsens and destabilizes the foam (Damodaran, 2005; Gandolfo and Rosano, 1997). Coalescence refers to the merging of two adjoining gas bubbles (Damodaran, 2005). In what follows, the separate contributions of these two phenomena in the destabilization of air bubbles in mixed GH + EW protein solutions are discussed in an attempt to clarify the contradictory results in terms of FS and A-W interfacial properties of the mixtures.

Figure 8 shows the time needed for air bubbles of different initial sizes formed in solutions of 0.300% $w_{protein}$/v of EW protein, 0.300% $w_{protein}$/v of T2, T6, P2 and P6, or in mixed GH + EW protein solutions ($GH_{0.150}EW_{0.150}$) to shrink to a size (< 10 µm) no longer detectable in the set-up used. Of course, the shrinkage time increased with initial bubble radius for all samples analyzed. Moreover, the evolution of bubble radius over time does not follow a linear course. Indeed, as also reported

in the paper first describing this method (Dickinson et al., 2002), larger bubbles shrink disproportionately more slowly than smaller bubbles. As a result, normalizing shrinkage times of air bubbles for differences in initial bubble radius is not possible and the plots shown in Figure 8 are best suited to assess differences in the disproportionation of different samples. The left part of Figure 8 shows that bubbles formed in 0.300% $w_{protein}$/v EW protein solution generally needed more time to shrink than similarly sized bubbles in any of the GHs solutions at 0.300% $w_{protein}$/v. This observation is in line with the higher FS (see section 3.1) and surface dilatational moduli (see section 3.3) of EW protein than of GH solutions. Interestingly, the right hand side of Figure 8, which compares a 0.300% $w_{protein}$/v EW protein solution with mixed GH + EW protein ($GH_{0.150}EW_{0.150}$) solutions, shows a pattern which is very similar to the one on the left hand side. This suggests that the disproportionation of gas bubbles introduced in a mixed GH + EW protein solution is dominated by the GH constituents, rather than by the EW proteins. That FS readings of mixed GH + EW protein solutions were higher than those of GH solutions (see section 3.1) could thus not be attributed to an increased resistance to disproportionation.

These observations can be understood better by considering that proteins slow down disproportionation in two ways. First, by lowering surface tension, the difference in pressure between the inside and outside of the gas bubbles is reduced, thereby delaying the diffusion of gas through the liquid films. Second, the formation of a viscoelastic film around gas bubbles may prevent gas from easily diffusing into the liquid films between gas bubbles (Damodaran, 2005; Dickinson et al., 2002). Thus, both mechanisms depend directly on the protein layer adsorbed at the interface. As shown in sections 3.2 to 3.4, the interface in GH + EW protein mixtures is

dominated by GH constituents rather than by EW proteins. Therefore, it makes sense that the disproportionation of gas bubbles stabilized by GHs alone or by GH + EW protein mixtures is very similar. In contrast, coalescence, while also depending on the adsorbed protein layer and the viscoelastic film around gas bubbles, also depends on steric and electrostatic effects caused by proteins at the surface of gas bubbles (Damodaran, 2005).

Figure 9 compares the fraction of bubbles, formed in solutions of 0.300% $w_{protein}$/v of EW protein, 0.300% $w_{protein}$/v of T2, T6, P2 and P6, or mixed GH + EW protein ($GH_{0.150}EW_{0.150}$) solutions, which coalesced after they had been subjected to the controlled pressure drop. First, it is to be noted that there were no notable differences in the overall initial bubble size distributions of the different samples (data not shown). Thus, none of the differences in bubble coalescence between different samples could be attributed to differences in their initial bubble size distributions. None of the bubbles formed in a 0.300% $w_{protein}$/v EW protein solution coalesced. In contrast, 11%, 22%, 14% and 50% of the bubbles produced in 0.300% $w_{protein}$/v T2, T6, P2 and P6 solutions, respectively, coalesced upon pressure drop. The higher resistance to coalescence of air bubbles stabilized by EW proteins than of those stabilized by GHs is in line with the higher FS of the former. Furthermore, DH 2 hydrolysates had higher stability against coalescence than DH 6 hydrolysates, which is in agreement with the higher FS of the former. The fractions of coalesced bubbles in the mixed GH + EW protein solutions were 6%, 11%, 4% and 8% for $T2_{0.150}EW_{0.150}$, $T6_{0.150}EW_{0.150}$, $P2_{0.150}EW_{0.150}$ and $P6_{0.150}EW_{0.150}$, respectively. These values are intermediate between those of the pure EW protein solutions and the GH solutions at 0.300% $w_{protein}$/v. Thus, the resistance to

coalescence of air bubbles in the mixed GH + EW protein solutions was considerably higher than that of the pure GH solutions (0.300% $w_{protein}$/v).

All this suggests that the higher FS of mixed GH + EW protein solutions compared to that of GH solutions alone can be attributed to an increased resistance to coalescence of air bubbles due to the EW proteins. However, results from sections 3.2 to 3.4 indicated that the A-W interface is dominated by adsorbed GH constituents, rather than by EW proteins, both in the earlier and later stages after creating the interface. An explanation of these observations may be that, even though EW proteins apparently do not easily displace adsorbed GHs from the A-W interface, they can interact with the adsorbed layer of GH constituents through hydrophobic and electrostatic interactions. By doing so, they would form an additional secondary protein layer below the A-W interface, which could reduce gas bubble coalescence. However, in such a mechanism, the gas permeability is apparently not affected, because the disproportionation in the mixed GH + EW protein solutions was very close to that of the pure GH solutions (Figure 8). Furthermore, interfaces stabilized by mixed GH + EW solutions did not have higher surface shear viscosity than GH stabilized interfaces (see Section 3.4), suggesting that steric or electrostatic effects, rather than an increased surface viscosity, are at the basis of the additional measure of FS provided by the EW proteins in the protein solutions containing both GH and EW.

4. CONCLUSIONS

563 The impact of partial substitution of EW proteins by various GHs on the foaming and A-W
564 interfacial properties of the mixtures was investigated. It was established that, in general, the GH
565 constituents had the ability to form high amounts of foam and to diffuse to and adsorb at an A-
566 W interface rapidly, while EW proteins provided foams and bubbles with high stability once they
567 have formed. They also formed strong viscoelastic protein films at the A-W interface.
568
569 Despite differences in FS between the GH solutions, there were no such differences when GHs
570 were mixed with EW proteins. Thus, it did not matter which GH sample was used to replace part
571 of the EW proteins. Replacing one sixth of EW proteins by GHs drastically increased FC. The
572 resulting systems also had high FS. When half of the EW proteins were replaced by GHs, a similar
573 trend was observed. This suggests that both protein types were present at the interface and
574 contributed to foam formation and stabilization. The separate contributions of bubble
575 disproportionation and coalescence to bubble destabilization were assessed. Bubbles formed in
576 GH + EW protein solutions shrank at rates comparable to those in GH solutions alone. However,
577 the former bubbles were more resistant to coalescence than the latter. Thus, the improved FS of
578 GH + EW protein solutions seemed to originate from an elevated resistance to coalescence rather
579 than to disproportionation.
580
581 Maximum bubble pressure measurements revealed that the rates of diffusion to and adsorption
582 at the A-W interface in the mixtures were very similar to those of the GHs. Thus, GHs dominated
583 the early stages of protein adsorption at the A-W interface. Surface dilatational elasticity and
584 surface shear viscosity measurements showed that, in the later stages of adsorption, the A-W

interface was still dominated by the presence of GHs. Thus, GHs reached the interface more rapidly, adsorbed at it, and apparently could not easily be displaced by the EW proteins. Nonetheless, the presence of EW proteins in the mixed GH + EW protein solutions led to higher FS values. We hypothesize that this is caused by formation of a secondary protein layer of EW proteins below the A-W interface. This layer, which is probably sustained by electrostatic and hydrophobic interactions with the adsorbed layer of GHs, seemingly provides increased resistance to bubble coalescence, probably by electrostatically or sterically hindering gas bubbles from approaching each other. Future research to further study such mixed protein films and the interactions leading to their formation would shed light on this hypothesis.

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FIGURE CAPTIONS

Figure 1: Illustration of a typical bubble disproportionation experiment. Air bubbles are trapped under a planar air-water (A-W) interface and shrink over time until they are no longer detectable in the used set-up (< 10 µm). Their bubble radius is plotted over time to obtain a shrinkage rate curve.

Figure 2: Illustration of a typical bubble coalescence experiment. Air bubbles are trapped under a planar air-water (A-W) interface (left figure), subjected to a controlled pressure drop, during which the bubbles expand and some of them coalesce (middle figure), and finally returned to their original state. The fraction of bubbles which coalesced in the process is a measure for the ability of the protein film to stabilize air bubbles against coalescence.

Figure 3: Foam volume over time of whipped solutions of tryptic (T) and peptic (P) gluten hydrolysates (GHs) with degrees of hydrolysis (DH) of 2 and 6 (0.050% or 0.150% $w_{protein}$/v), egg white (EW) proteins (0.300% $w_{protein}$/v) and mixed solutions consisting of 0.050% gluten hydrolysate + 0.250% EW protein or of 0.150% GH + 0.150% EW protein.

Figure 4: Lag times, as a measure for early stage diffusion of proteins to the interface, of solutions of tryptic (T) and peptic (P) gluten hydrolysates (GHs) with degrees of hydrolysis (DH) of 2 and 6 (0.050% or 0.150% $w_{protein}$/v), egg white (EW) proteins (0.300% $w_{protein}$/v) and mixed solutions consisting of 0.050% $w_{protein}$/v GH + 0.250% $w_{protein}$/v EW protein or of 0.150% $w_{protein}$/v GH + 0.150% $w_{protein}$/v EW protein. Capital letters represent significant ($P < 0.05$) differences between

an EW protein solution, a given GH solution and a solution containing the mixture of both. Lowercase letters represent significant differences between the different GHs or GH + EW protein mixtures.

Figure 5: $|S_{ST-t}|$ values, as a measure for the continuous early stage adsorption and rearrangement of proteins at the interface, of solutions of tryptic (T) and peptic (P) gluten hydrolysates (GHs) with degrees of hydrolysis (DH) of 2 and 6 (0.050% or 0.150% $w_{protein}$/v), egg white proteins (EW) (0.300% $w_{protein}$/v) and mixed solutions consisting of 0.050% $w_{protein}$/v GH + 0.250% $w_{protein}$/v EW protein or of 0.150% $w_{protein}$/v GH + 0.150% $w_{protein}$/v EW protein. Capital letters represent significant ($P < 0.05$) differences between an EW protein solution, a given GH solution and a solution containing the mixture of both. Lowercase letters represent significant differences between the different GHs or GH + EW protein mixtures.

Figure 6: Surface dilatational elastic moduli E', as a measure for the coherence and elasticity of adsorbed protein films at the interface, of solutions of tryptic (T) and peptic (P) gluten hydrolysates (GHs) with degrees of hydrolysis (DH) of 2 and 6 (0.300% $w_{protein}$/v), egg white (EW) proteins (0.300% $w_{protein}$/v) and mixed solutions consisting of 0.050% $w_{protein}$/v GH + 0.250% $w_{protein}$/v EW protein or of 0.150% $w_{protein}$/v GH + 0.150% $w_{protein}$/v EW protein. Capital letters represent significant ($P < 0.05$) differences between an EW protein solution, a given GH solution and a solution containing the mixture of both. Lowercase letters represent significant differences between the different GHs or GH + EW protein mixtures.

Figure 7: Surface shear viscosity of a 0.300% $w_{protein}$/v egg white (EW) protein solution, solutions of 0.300% $w_{protein}$/v tryptic and peptic gluten hydrolysates (GHs) with degrees of hydrolysis (DH) of 2 and 6, and mixed solutions consisting of 0.150% $w_{protein}$/v GH + 0.150% $w_{protein}$/v EW protein.

Figure 8: Shrinkage time of air bubbles, stabilized by a 0.300% $w_{protein}$/v egg white (EW) protein solution, 0.300% $w_{protein}$/v tryptic (T) and peptic (P) gluten hydrolysates (GHs) with degrees of hydrolysis (DH) of 2 and 6 and mixed solutions consisting of 0.150% $w_{protein}$/v GH + 0.150% $w_{protein}$/v EW protein as a function of their initial bubble radius.

Figure 9: Fraction of air bubbles, stabilized by a 0.300% $w_{protein}$/v egg white (EW) protein solution, 0.300% $w_{protein}$/v tryptic (T) and peptic (P) gluten hydrolysates (GHs) with degrees of hydrolysis (DH) of 2 and 6 and mixed solutions consisting of 0.150% $w_{protein}$/v GH + 0.150% $w_{protein}$/v EW protein, that coalesced after a controlled pressure drop. n indicates the number of air bubbles assessed to calculate the fraction of coalesced bubbles.

FIGURE 1

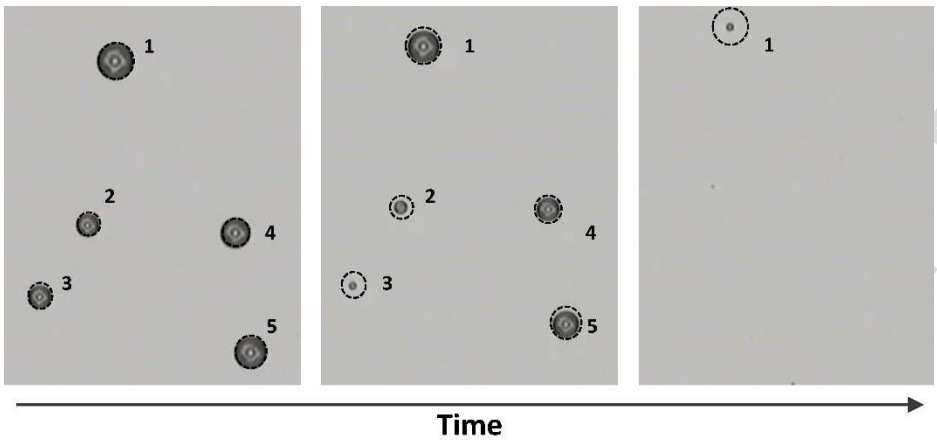


FIGURE 2

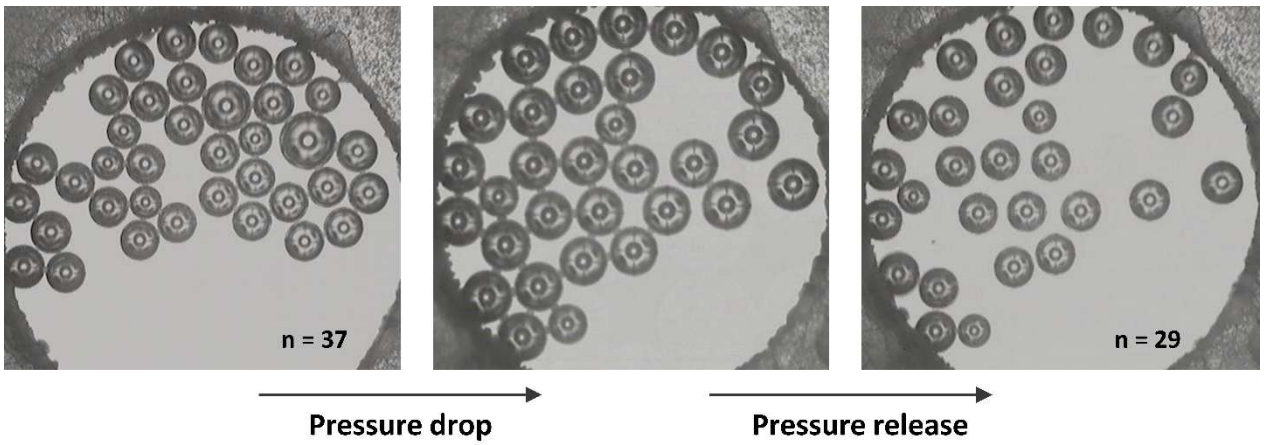


FIGURE 3

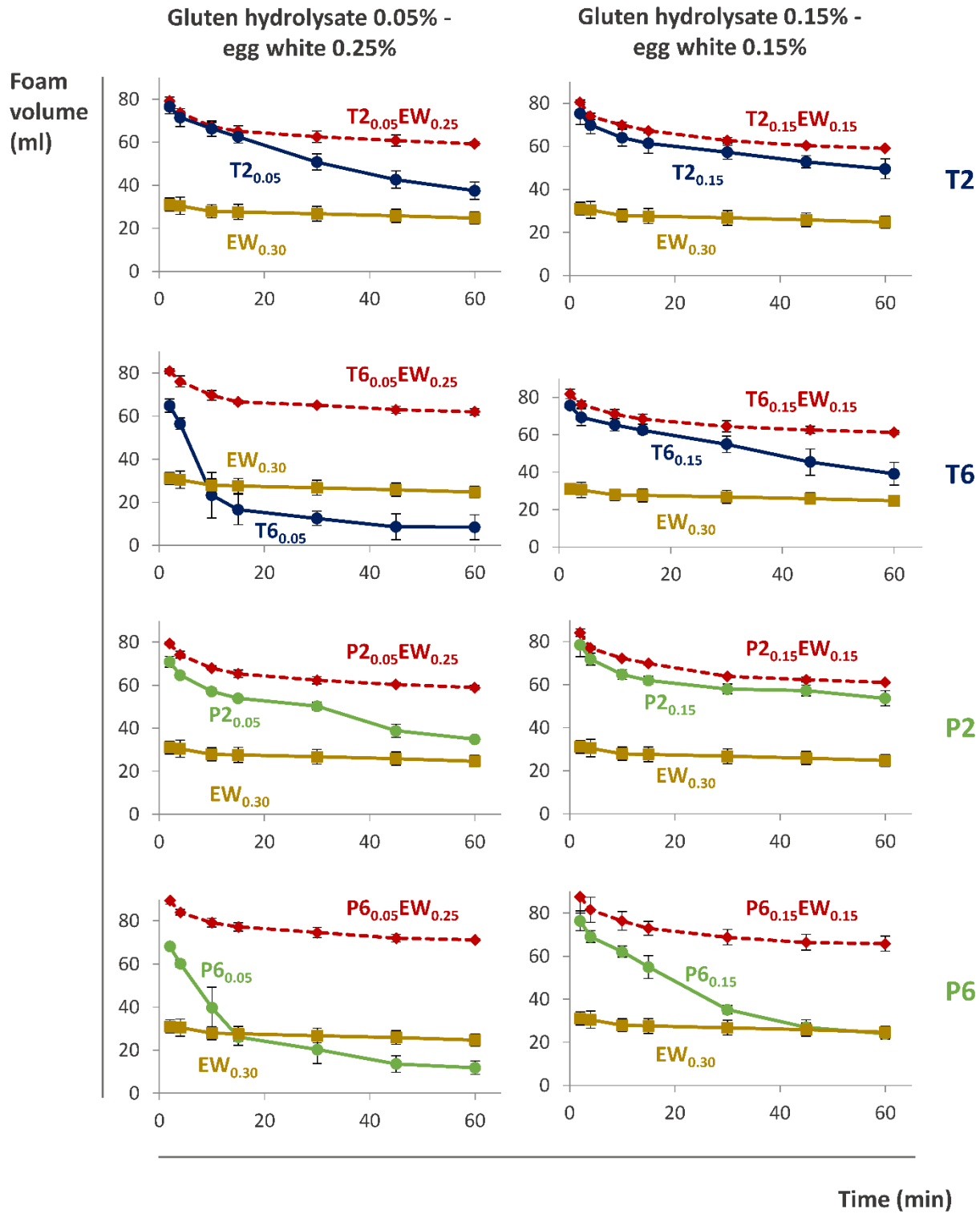

Gluten hydrolysate 0.05% - egg white 0.25%
Gluten hydrolysate 0.15% - egg white 0.15%
Foam volume (ml)
T2
T6
P2
P6
T2$_{0.05}$EW$_{0.25}$
T2$_{0.05}$
EW$_{0.30}$
T2$_{0.15}$EW$_{0.15}$
T2$_{0.15}$
T6$_{0.05}$EW$_{0.25}$
T6$_{0.05}$
T6$_{0.15}$EW$_{0.15}$
T6$_{0.15}$
P2$_{0.05}$EW$_{0.25}$
P2$_{0.05}$
P2$_{0.15}$EW$_{0.15}$
P2$_{0.15}$
P6$_{0.05}$EW$_{0.25}$
P6$_{0.05}$
P6$_{0.15}$EW$_{0.15}$
P6$_{0.15}$
0
20
40
60
80
Time (min)

FIGURE 4

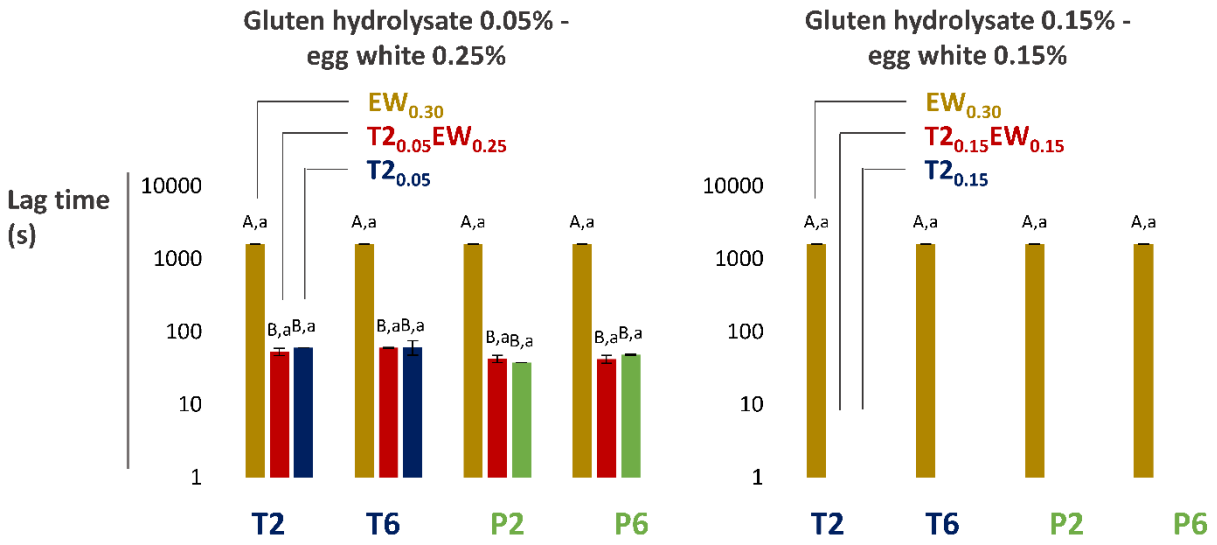


FIGURE 5

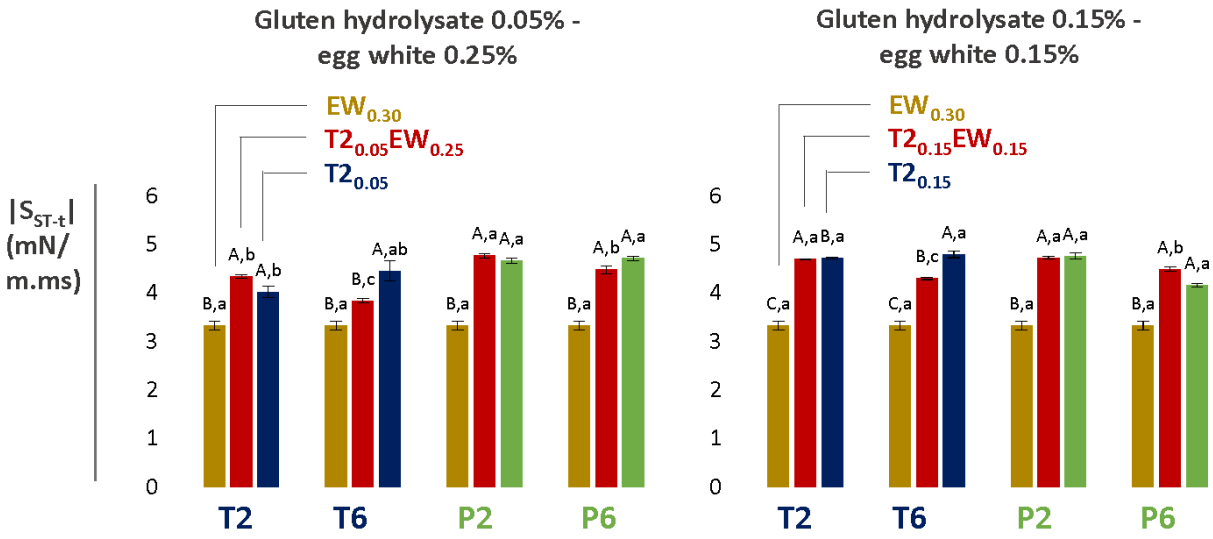


FIGURE 6

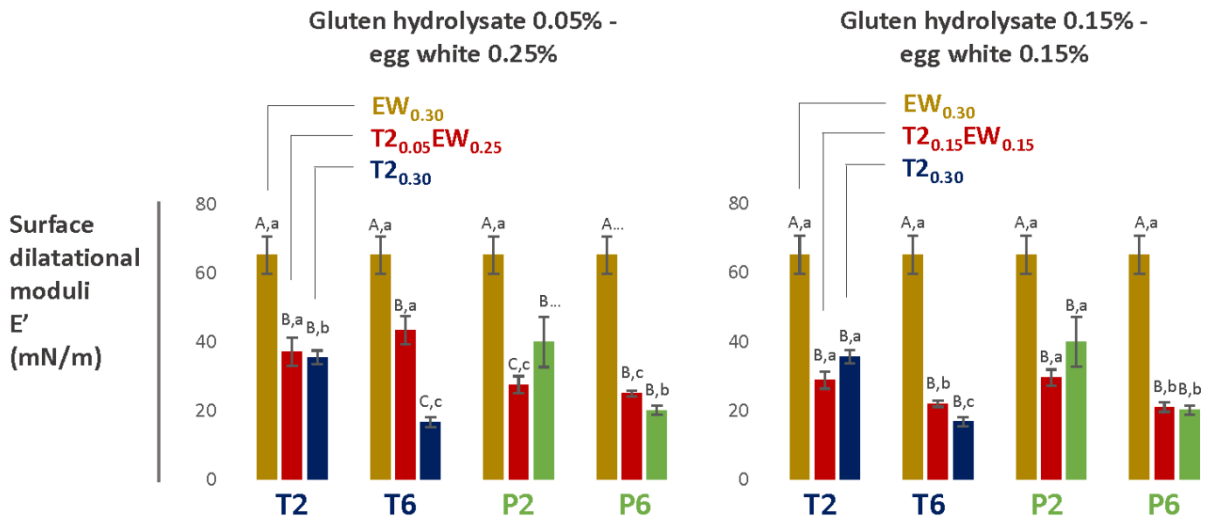


FIGURE 7

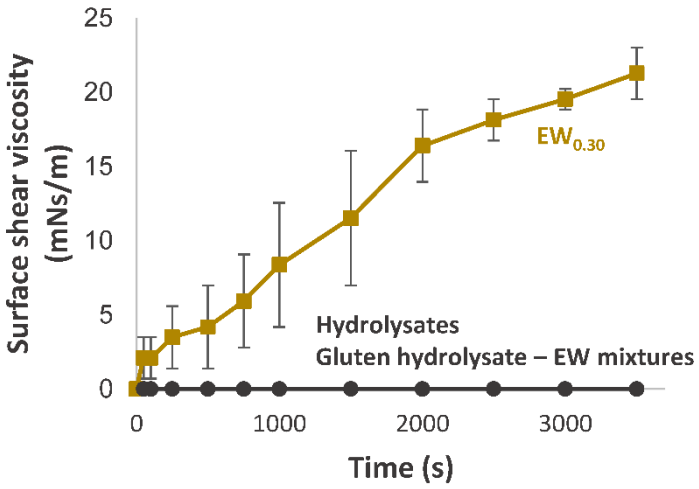


FIGURE 8

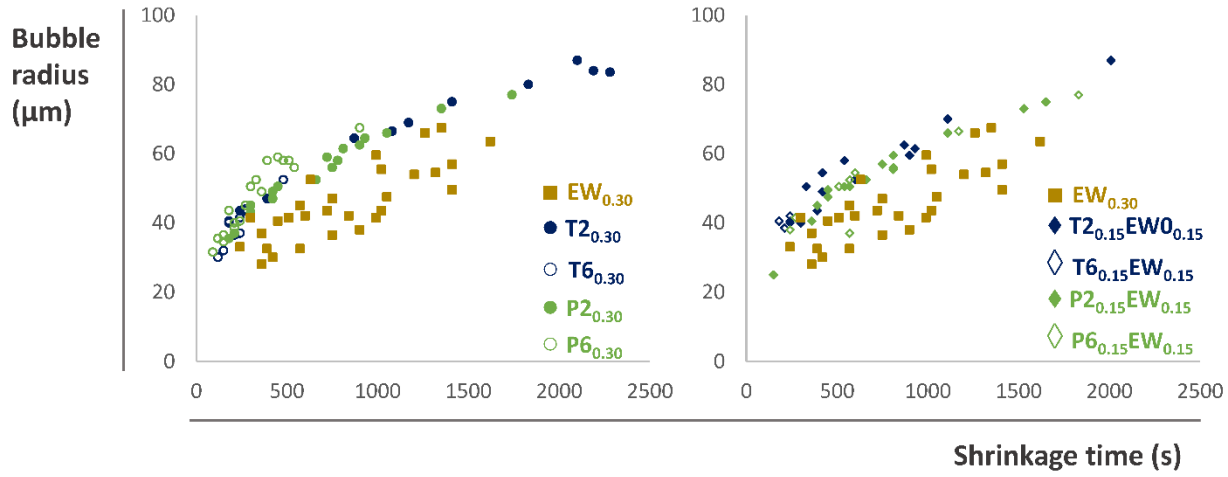
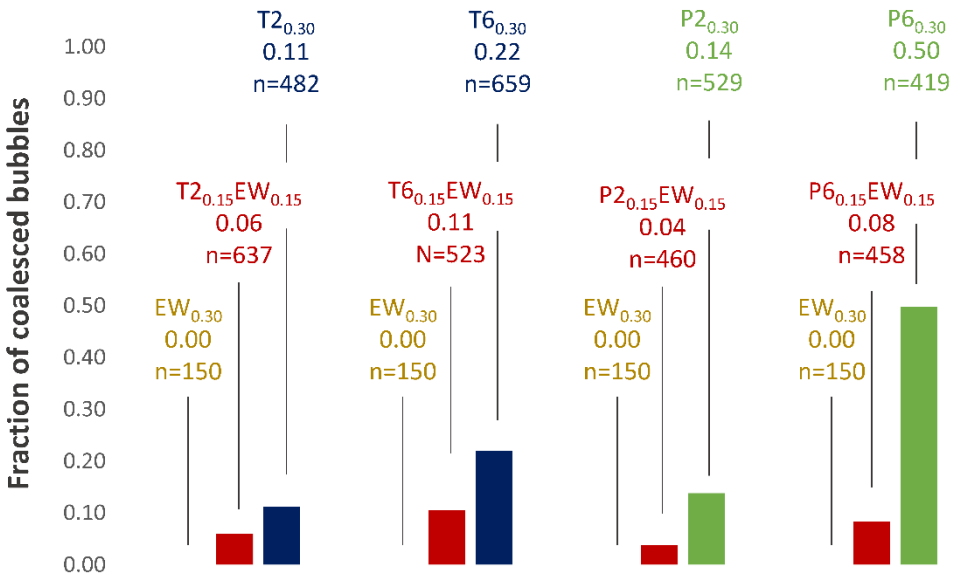
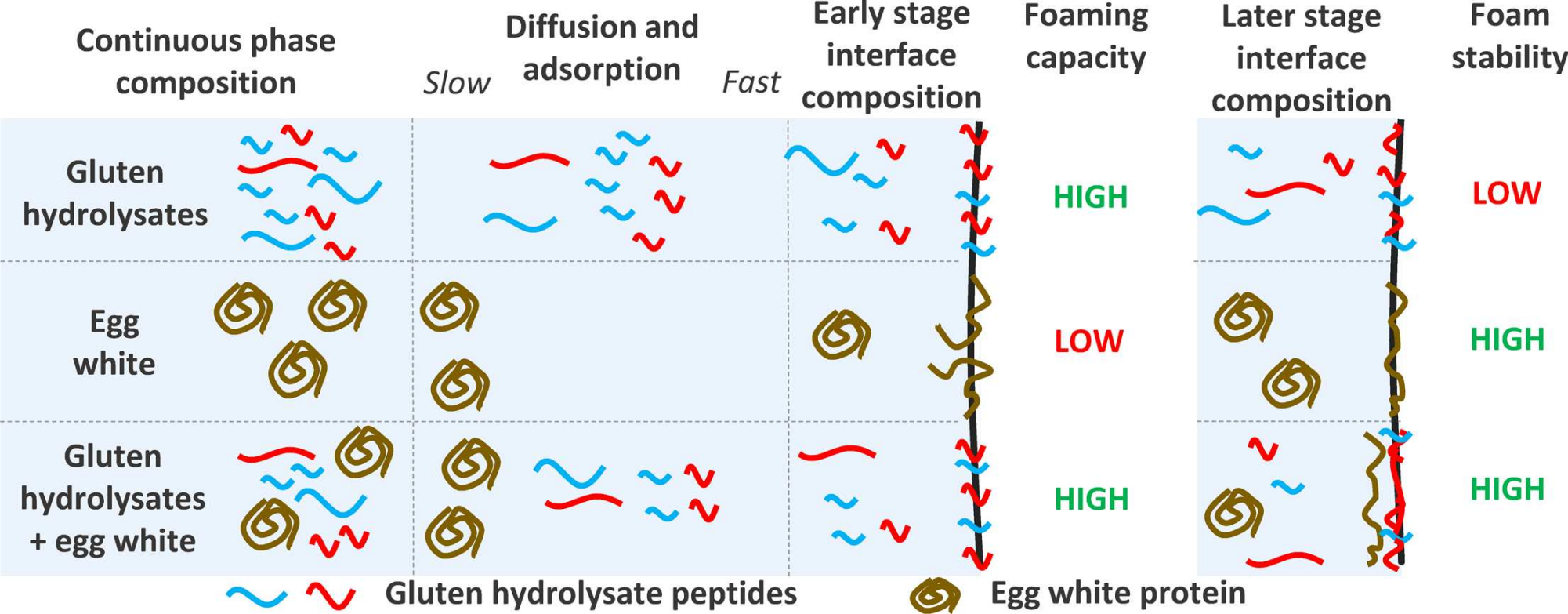


FIGURE 9




Continuous phase composition
Diffusion and adsorption
Slow
Fast
Early stage interface composition
Foaming capacity
Later stage interface composition
Foam stability
Gluten hydrolysates
HIGH
LOW
Egg white
LOW
HIGH
Gluten hydrolysates + egg white
HIGH
HIGH
Gluten hydrolysate peptides
Egg white protein