Colloids and Surfaces B: Biointerfaces 151 (2017) 295–303

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Foam fractionation as a tool to study the air-water interface structure-function relationship of wheat gluten hydrolysates

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ARTICLE INFO

Article history:
Received 11 October 2016
Received in revised form 2 December 2016
Accepted 20 December 2016
Available online 22 December 2016

Keywords:
Wheat gluten
Hydrolysate
Foam fractionation
Hydrophobicity
Air-water interfacial properties

ABSTRACT

Enzymatic hydrolysis of wheat gluten protein improves its solubility and produces hydrolysates with foaming properties which may find applications in food products. First, we here investigated whether foam-liquid fractionation can concentrate wheat gluten peptides with foaming properties. Foam and liquid fractions had high and very low foam stability (FS), respectively. In addition, foam fractions were able to decrease surface tension more pronouncedly than un-fractionated samples and liquid fractions, suggesting they are able to arrange themselves more efficiently at an interface. As a second objective, foam fractionation served as a tool to study the structural properties of the peptides, causing these differences in air-water interfacial behavior. Zeta potential and surface hydrophobicity measurements did not fully explain these differences but suggested that hydrophobic interactions at the air-water interface are more important than electrostatic interactions. RP-HPLC showed a large overlap between foam and liquid fractions. However, a small fraction of very hydrophobic peptides with relatively high average molecular mass was clearly enriched in the foam fraction. These peptides were also more concentrated in un-fractionated DH 2 hydrolysates, which had high FS, than in DH 6 hydrolysates, which had low FS. These peptides most likely play a key role in stabilizing the air-water interface.

1. Introduction

Commercial wheat gluten is the co-product of the industrial starch isolation. It mainly consists of wheat storage proteins [1]. It is predominantly used in bakery and animal feed applications, but there is also a clear industrial interest in alternative valorization routes [2,3]. One of the main obstacles for a wide application of gluten proteins in food is their low solubility in aqueous media [4]. Enzymatic hydrolysis not only strongly improves their solubility but also induces foaming properties [5].

In a food and beverage context, foams are important in e.g. meringues, beer and whipped dairy products. They consist of a gaseous phase dispersed in a liquid, usually in the form of closely packed air bubbles in an aqueous phase. While foams, which consist of many air-water (A-W) interfaces, are thermodynamically unstable, they can be stabilized by surface-active compounds [6,7]. Because of their amphiphilic nature, proteins and peptides have some affinity for A-W interfaces. They can adsorb to interfaces, thereby lowering the surface tension but also sterically preventing gas bubbles to approach and eventually merge with other gas bubbles [6,8]. After adsorption at the interface, proteins tend to interact and form a visco-elastic film which stabilizes the foam.

Several authors have discussed the link between foaming and structural properties of wheat gluten hydrolysates. A recurring observation is that hydrolysates with a relatively low degree of hydrolysis (DH), which represents the percentage of peptide bonds cleaved (see below), lead to better foam stability than hydrolysates with a high DH [9–13]. Evidently, a higher DH implies a lower average molecular mass (MM). The importance of a high MM has also been illustrated by the improvement of foaming properties upon transglutaminase treatment [14,15]. Additionally, fractionation with membrane technology has shown that peptide fractions with high average MM have better foaming properties than fractions with lower MM. It is important to note that in different studies [16–19] the peptides in the high MM fractions were also the more hydrophobic ones. The relevance of hydrophobicity for foaming of peptides has also been suggested in a previous study from our group [13]. In this context, it is necessary to keep in mind

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http://dx.doi.org/10.1016/j.colsurfb.2016.12.031
0927-7765/

that protein hydrolysates contain a very heterogeneous mixture of peptides, of which it is not always clear to what extent certain peptides contribute to a hydrolysate's overall functionality. Selectively enriching gluten hydrolysates in peptides which are more efficient in stabilizing A-W interfaces will help to gain insight in the mechanism whereby they stabilize foam. Ways to do so include separation with membranes, as already mentioned above, or by chromatography. However, these techniques are often labor-intensive and time-consuming. In addition, they are not selective for peptide fractions with a higher affinity for A-W interfaces. In contrast, foam fractionation processes usually consist of a fairly simple setup [20–22]. They are mostly used industrially to isolate proteins from more complex waste-streams [21,23], such as e.g. from whey [22]. However, they can also be used to separate peptides or proteins with high affinity for an A-W interface from a mixture of peptides or proteins with a lower affinity for such interface. This concept has been used to selectively enrich either α-amylase or lysozyme from a mixture of both by foam fractionation at different pH values [24], and to enrich specific bio-active peptides from complex protein hydrolysates [25,26]. Thus far, the potential of foam fractionation to alter the functional properties of protein hydrolysates has not been investigated.

In this work, a fairly simple foam fractionation was carried out to investigate its potential for concentrating peptides with improved foaming and interfacial properties. The peptide composition, structural properties and A-W interfacial properties of these fractions were assessed to gain insight in the mechanism of foam stabilization by wheat gluten peptides.

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 [27] 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.

2.2. Enzymatic hydrolysis

A 6.0% ($w_{protein}$/v) wheat gluten aqueous dispersion in 0.03% (w/v) NaCl was incubated with trypsin or pepsin at pH-stat conditions. For each enzyme, hydrolysis was performed until DH 2 and DH 6. Tryptic hydrolysis was at 50 °C, pH 8.0 and using an enzyme to substrate ratio of 1:480 (DH 2) or 1:20 (DH 6) on protein mass basis. Peptic hydrolysis was performed at 37 °C, pH 3.5 and using an enzyme to substrate ratio of 1:1200 (DH 2) or 1:300 (DH 6) on protein mass basis. When the desired DH (see Section 2.3) was reached, the pH was adjusted to 6.0 with 0.5 M NaOH and proteolysis was stopped by heating the protein suspension for 15 min at 95 °C. The hydrolysates were then centrifuged (10 min, 12,100*g*) at room temperature and supernatants were filtered and freeze-dried. Tryptic DH 2 or DH 6 hydrolysates are further referred to as T2 and T6, respectively, and those of peptic DH 2 or DH 6 hydrolysates as P2 and P6, respectively.

2.3. Determination of degree of hydrolysis

DH is the percentage of the number of peptide bonds hydrolysed (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 the pH constant during hydrolysis, using the formula:

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

X is the consumption (ml) of acid or base needed to keep the pH constant during hydrolysis and M_x the molarity of the acid or base. The term α is a measure for the degree of dissociation of α-NH_3^+ (neutral or alkaline conditions) or α-COOH (acidic conditions) groups. Under the conditions used, in tryptic hydrolysis α is 0.89 [28], whereas in peptic hydrolysis it is 0.29 [29]. M_p is the mass of protein used, h are the hydrolysis equivalents [milli-equivalents (meq/g) protein] and h_{tot} is the theoretical number of peptide bonds per unit protein. For gluten protein, h_{tot} is 8.3 meq/g protein [28,30].

2.4. Foam fractionation

Aliquots (50 ml) of protein solutions [0.15% (w_{prot}/v)] of T2, T6, P2 and P6 were temperature equilibrated in graduated glass cylinders (internal diameter 60.0 mm) in a water bath at 20 °C. A standardized stirring test based on Caessens et al. [31] was performed. The protein solutions were stirred for 70 s using a rotating propeller (outer diameter 45.0 mm, thickness 0.4 mm) at 2000 rpm. After stirring, the propeller was immediately removed and the glass cylinder was sealed with a plastic paraffin film. After 15 min, the foam and liquid phases were freeze-dried separately. This yielded eight samples, with an extra letter in their code (F for foam or L for liquid fraction).

2.5. Analysis of foaming properties

Foaming properties of protein solutions [0.05% (w_{prot}/v)] of T2, T6, P2 and P6 and of their respective foam or liquid fractions were determined with the standardized stirring procedure described above (Section 2.4). The foaming capacity (FC) was defined as the foam volume 120 s after the start of stirring. The foam volume was also measured 4, 10, 15, 30, 45 and 60 min after the start of stirring. Foam volumes were calculated based on foam height and cylinder diameter, and expressed in ml. The decrease of foam volume over time was an indication for the foam stability (FS) of a given sample.

2.6. Analysis of zeta potential

Protein solutions of T2, P2, T6 and P6 [0.15% ($w_{protein}$/v)] in deionized water and of their respective foam and liquid fractions were placed in a disposable capillary zeta cell (Malvern Instruments, Malvern, United Kingdom) to determine zeta potential in a Zetasizer Nano ZS (Malvern) based on laser Doppler micro-electrophoresis.

2.7. Analysis of protein surface hydrophobicity

The protein surface hydrophobicity of solutions of T2, T6, P2 and P6 and of their respective foam and liquid fractions was determined with 1-anilino-8-naphtalene sulfonic acid (ANS) as fluorescent probe. Samples containing between 0.18 and 0.90 mg protein/ml deionized water were prepared. Aliquots (200.0 μl) of these samples were transferred to a 96-well plate, and 10.0 μl 8 mM ANS in deionized water was added. The fluorescence intensity of the protein samples was measured with a Synergy Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA), using 390 and 480 nm as excitation and emission wavelengths, respectively. The relative fluorescence intensity was then calculated as the difference in intensity of the protein-ANS mixture and the control sample (ANS in water), divided by the intensity of the control sample. The slope of the plot of relative fluorescence intensity as a function of