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Foaming characteristics of oat protein and modification by partial hydrolysis

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Abstract

Foaming ability of oat protein isolate (OPI) was analysed at pH 4 and 7. Foaming properties were influenced by partial hydrolysis with trypsin (OPT) and alcalase (OPA). The viscoelasticity of the protein film, the interactions between the protein molecules, and the network forming within the protein film were analysed by interfacial rheology. At pH 7, foams made of OPI and OPT were found to be stable with OPI having the fastest foaming ability. At pH 4, the foaming properties of OPI were found to be poor due to limited solubility. The specific cleavage behaviour of trypsin improved the foaming properties, especially at pH 4, resulting in a homogenous foam structure, a fast foaming ability and a highly viscoelastic interfacial film. The formation of a thick steric protein layer at pH 7 and the formation of strong hydrophobic interactions at pH 4 were found to be the dominating foam stabilisation mechanisms. In conclusion, oat protein ingredients were developed with targeted functional properties.

Keywords

Foaming ability; Oat protein isolate; Enzymatic hydrolysis; Interfacial shear rheology; Dilatational rheology

Introduction

Oat (*Avena sativa* L.) has the potential to be a sustainable protein source with valuable nutrient properties. Its techno-functionality i.e. emulsification [1] and foaming [2–4] properties have previously been investigated. However, molecular characteristics such as high molecular weight protein profile and low solubility in acidic pH limit its use as functional ingredient in dispersed food systems such as foams and emulsions [3, 5]. Physical, chemical or enzymatic treatments have demonstrated the potential to modify the structure of oat protein improving solubility, emulsion and/or foaming properties [6]. For example, acetylation and succinylation of oat protein isolate (OPI) was shown to improve foaming properties and the emulsion activity index [1], while deamidation of OPI also improved the emulsion activity index along with the solubility [7]. Enzymatic cross-linking of OPI with transglutaminase resulted in higher surface tension compared to the non-transglutaminase-treated control [8]. The most common modification of oat protein was enzymatic hydrolysis with tryptic hydrolysis of oat bran protein [5] and oat protein concentrate [9, 10] found to improve the protein solubility, water-holding capacities, and emulsifying and foaming activities. The impact of hydrolysis on protein structure can be summarised as a reduction of tertiary structure and molecular mass and an increase in the exposure of hydrophobic side chains which are often hidden in a hydrophobic core of the molecule [11].

Many studies have addressed the foaming properties of hydrolysed plant proteins on the basis of foaming capacity and foam stability, for example rice bran meal [12], bean (*Phaseolus vulgaris* L.) protein concentrates [13], rapeseed protein isolate [14], amaranth protein [15], *Lupinus angustifolius* protein [16], soy protein isolate [17] and pumpkin oil cake protein [18]. Yet, only a few studies have focused on the characterisation of the interfacial film of hydrolysed plant proteins and the effect of proteolysis on dynamic surface pressure measurements, in connection to foaming capacity and stability [19–23]. Interfacial rheology is a valuable tool for measuring the structural properties of proteins at interfaces. It is subdivided into dilatational rheology and interfacial shear rheology with dilatational rheology detecting three-dimensional deformation upon change of surface area, while interfacial shear rheology measures the effect of applied shear in plane. Thus, to the best of our knowledge, there has been no in-depth characterisation of oat protein and/or oat protein hydrolysate foams that applied dilatational and interfacial shear rheology to understand the foaming ability and stability of this highly structured protein.

The aim of this study was to develop a systematic understanding of the foaming characteristics of oat protein isolate and to evaluate the impact of enzymatic hydrolysis on both the foaming properties and interfacial rheology. To this purpose, foaming properties were characterised and compared for oat protein isolate and two different oat protein hydrolysates (prepared with alcalase and trypsin, respectively). It was hypothesised that, depending on the applied enzyme, enzymatic hydrolysis would influence the molecular weight distribution, which in turn would affect the solubility of oat protein. It was also hypothesised that the different molecular weight profiles would alter the viscoelastic characteristics of the protein films, as well as the interactions of proteins in the interfacial film and their network forming ability, in turn influencing both the foaming properties and stability of the foams.

Materials and methods

Materials

Oat protein concentrate (OPC) was obtained according to the Kaukovirta-Norja, Myllymäki, Aro, Hietaniemi and Pihlava [24] patent for dry fractionation of oat grits with lipid removal by supercritical CO_2 extraction prior to milling and air classification. Oat protein isolate (OPI) was produced by alkaline extraction at pH 9.2 from OPC containing approximately 43% protein (Nx6.25), 33% starch, 3.5% fat, 3.5% ash, 11% moisture and 6% total dietary fibre. For this purpose, the oat protein concentrate was diluted 1:6 in distilled water and the pH was adjusted to 9.2 with 1 M NaOH. The mixture was stirred for 1.5 hours at room temperature and centrifuged at 5,000 g for 20 min at 20 °C. The supernatant was lyophilised. OPI contained approximately 83% protein, 1.5% starch, 6.5% fat, 2% ash, 3% moisture and 4% total dietary fibre.

Enzymatic hydrolysis of OPI

The OPI was hydrolysed using two different enzymes – trypsin (from bovine pancreas, Cat. no.: T8003, EC: 3.4.21.4, 10,000 BAEE units/mg protein) and alcalase (from *Bacillus licheniformis*, Cat. no.: P4860, EC: 3.4.21.62, 2,59 AU/g protein). Both enzymes were purchased from Sigma Aldrich Co. LLC (Taufkirchen, Germany). Hydrolysis was performed in a 10% OPI suspension under constant stirring at 45 °C and pH 8 in an automated titrator (Titrando 902; Deutsche Methrom GmbH & Co. KG, Filderstadt, Germany). 4 g of a 5% trypsin solution (prepared in 1 mM HCL) were added, the enzyme to substrate ratio was 1:150 during

tryptic hydrolysis. In case of alcalase, 6 g of a 10% alcalase solution (prepared in distilled water) were added and the enzyme to substrate ratio was 1:50 during alcalase hydrolysis. The pH was adjusted to pH 8 according to the pH-stat method by Adler-Nissen [25]. The degree of hydrolysis (DH) was set by calculation according to the following equation:

$$DH = \frac{V_B \cdot N}{\alpha \cdot M_P \cdot h_{tot}} \cdot 100$$

To ensure the desired DH, the amount V_B of NaOH with the normality N, the degree of dissociation α, the mass of protein M_P and a h_{tot}-value of 7.31 meqv/g protein [5] were inserted into the equation. As soon as the desired DH of 3% was reached, the enzymes were inactivated by heat treatment (75 °C, 30 min).

Chemical characterisation of oat protein isolate and hydrolysates

The Mettler Toledo Infrared Dryer LP16 (Mettler-Toledo GmbH, Giessen, Germany) was used to determine the dry mass content (%) in 0.1-0.2 g sample at 105 °C. Nitrogen contents of OPI and its hydrolysates were measured by the combustion or Dumas method with a Dumatherm® (C. Gerhardt GmbH & Co. KG, Königswinter, Germany) using a conversion factor of 6.25 for the calculation of the protein content. The starch content was determined with the enzymatic Megazyme kit K-TSTA 09/14 (Megazyme Inc., Chicago, Illinois, USA). The ash content was determined from 1 g sample in a muffle furnace at 540 °C. The fat content was determined by extraction in a Soxhlet apparatus with petrol ether. The amount of extracted fat was determined gravimetrically.

Determination of molecular weight distribution with high performance size exclusion chromatography (HPSEC)

HPSEC was performed at room temperature using the HPLC ÄKTAbasicTM 10 system (Amersham Biosciences, Uppsala, Sweden), consisting of a separation unit (pump P-900, UV-monitor UV-900 operating at 280 nm, UV-flow cell (10 mm), injection valve INV-907, mixer M-925 and flow restrictor FR-904) and a personal computer running UNICORN™ control system version 5.01 (Amersham Biosciences, Uppsala, Sweden). The chromatographic column used was Superdex 200 Increase 10/300 GL (GE healthcare GmbH, Solingen, Germany). 0.2 M NaCl; 0.2 M sodium phosphate buffer of pH 7 or 0.2 M NaCl; 0.2 M sodium citrate buffer of pH 4 were used as the mobile phase with a flow rate of 0.5 ml/min. Prior to HPSEC, all buffers were filtered and degassed with mobile phase conditioner M-3522 (Bio-Rad Munich, Germany). The appropriate amount of OPI, OPA and OPT to make a 10 mg/ml protein solution was dispersed in the mobile phase, stirred for 90 min and left overnight in the refrigerator at 6 °C. Prior to chromatography, the sample was equilibrated to room temperature and centrifuged at 10,000 g and 10 °C for 10 min in order to separate undissolved matter. The molecular weights of the peaks in the chromatograms of the samples were calculated using the following gel filtration calibration kits with low and high molecular weight standards (GE healthcare GmbH, Solingen, Germany): feritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (44 kDA), carbonic anhydrase (29 kDa), ribonuclease (13.7 kDa), aprotinin (6.5 kDa) and somatostatin (1.6 kDa) purchased from Sigma Aldrich Co. LLC (Taufkirchen, Germany).

Determination of the isoelectric point

Zeta potential of OPI, OPT and OPA suspensions (1% w/v) at pH 7, 6, 4 and 3 was measured using a Zetasizer nano ZS (Malvern Instruments, Malvern, UK). The change of zeta potential was plotted against pH. The isoelectric point is the calculated pH at which the zeta potential is zero.

Preparation of protein solutions and analysis of soluble protein concentration

The protein solutions for the determination of the protein solubility, foaming properties, dilatational rheology and interfacial shear rheology were prepared as follows: OPI-, OPA- and OPT-powders were dispersed in distilled water by magnetic stirring for at least 1.5 hours at concentrations of 0.14% (w/v) on a powder sample base in 100 ml distilled water. pH was adjusted to 4 or 7, respectively, with 0.1 M HCl or 0.1 M NaOH. The suspensions were centrifuged at 10,000 g for 10 min at 10 °C, and the pH of the supernatant was adjusted again. For analysis of the soluble protein concentration, the protein content of the supernatant was determined using the Kjeldahl method.

Protein solubility

The protein solubility [%] was calculated as:

$$Protein\ solublity\ [\%] = \frac{Soluble\ protein\ concentration\ in\ supernatant\ [\%]}{0.14\ g \cdot \frac{Sample\ protein\ content\ [\%]}{100}} \cdot 100$$

Foaming properties

The foaming properties were investigated with a dynamic foam analyser DFA 100 (Krüss GmbH, Hamburg, Germany) according to Böttcher and Drusch [26]. A volume of 50 ml protein solution was poured in a glass column with a diameter of 40 mm. Pressurised air was introduced through a porous glass frit with a pore size of 10-16 µm (Duran Group purchased from Carl Roth GmbH, Karlsruhe). Foaming was stopped at a total height of 180 mm (sum of liquid and foam). The foaming process and the foam collapse were observed for a total of 30 min. The height of foam, height of liquid and brightness profile of the foam were recorded using the transmissibility of the applied LED light with two frames per second. In addition, every 10 min a photograph of the foam was taken with a NIKON D3100 camera. All experiments were carried out in triplicate.

To further characterise the different foams, the brightness profile generated by the foaming device was analysed. After 800 s, the brightness distribution was measured in an area of 1 px width using Adobe Photoshop CS6 Extended. The brightness distribution ranges from 0-255 (black to white). In this study, data points between 1 and 253 were used to eliminate the background colour. From these data, the median (BD_m) as well as the width (BD_w) between d10 (10% of the brightness values are beneath this value) and d90 were determined. A black foam is compact/dense and a grey-white one has already collapsed. The BD_m of the box plot diagram of the brightness distribution describes the average bubble size (small bubble size = dense foam), thus high values indicate a less dense foam (= looser foam). A large BD_w indicates a non-homogeneous bubble size distribution (foam structure) and, therefore, the occurrence of an unstable foam is highly probable.

For a comprehensive foam characterisation, foaming ability was evaluated by foaming speed (K_f [mm/min]), which represents the speed of adsorption at and stabilisation of the newly formed interfacial area, and the comparative characteristics of the foam properties after 800 s were analysed in the box plot diagram of the brightness distribution (Box plot of BD_800s).

Dilatational rheology

The dilatational rheology was performed with an automated drop tensiometer OCA20 (Dataphysics Instruments GmbH, Filderstadt, Germany) with an oscillating drop generator unit ODG20 (Dataphysics Instruments GmbH, Filderstadt, Germany). The oscillation was performed as sinusoidal disturbance on a drop system with 15 µl ± 0.25 µl in its equilibrium (30 min drop ripening). The measurement was executed with a constant angular frequency of 0.1 Hz and a rising dA/A of around 1% to 20% within 8 steps, where one step consists of 10 periods with 10 s intervals between every measurement. For every step, 300 images were taken. The results are expressed by the following equation.

$$E^* = E' + iE'', \quad (1)$$

where E* is the complex surface dilatational modulus, E' the real part as elastic proportion, and iE" the imaginary part as the viscous proportion of the protein film [27]. The loss factor tanφ represents the relation of the viscous and elastic part. Values close to zero represent highly elastic interfacial films.

Interfacial shear rheology

Interfacial shear rheology was investigated using the Rheometer Physica MCR301 (Anton Paar GmbH, Ostfildern, Germany) with an interfacial rheology system cell (IRS) and a bicone tool. The cell has a diameter of 80 mm, and the bicone tool has a diameter of 68.25 mm and an angle of 5.16 °. The cell was filled with 120 ml of protein solution and bubbles were removed before each measurement. Sodium azide was added at a concentration of 0.02% in order to prevent spoilage. During the time-dependent measurement, the proteins form a viscoelastic film at the interface which can be examined when harmonic sinusoidal deformation (strain) is applied. The film formation was monitored for 17 hours with a deformation of 10^{-3} and frequency of 0.1 Hz at 25 °C. Afterwards, a frequency sweep with a deformation of 10^{-5} and frequencies from 1 Hz to 0.015 Hz with 15 measuring-points and an amplitude sweep with a frequency of 0.1 Hz and deformations from 10^{-4} to 1 with 31 measuring-points were performed in order to characterise the film. The amplitude sweep was crucial to ensure a time dependent measurement in the linear viscoelastic area. The results were expressed with the help of Eq. (2) [28, 29]:

$$G^*(\omega) = G'(\omega) + iG''(\omega), \quad (2)$$

with ω straining frequency, G' the elastic modulus (storage modulus) and G" the viscous modulus (loss modulus).

Statistical analysis

Statistical significance was analysed by the two-way analysis of variance (ANOVA) using SPSS Statistics Version 25 (SPSS Inc., Chicago, USA). The influence of the two factors, protein type (OPI, OPT or OPA) and pH-value (4 or 7), on the dependant variables, soluble protein concentration/protein solubility, foaming speed, as well as the results of the dilatational

rheology (E', E'') was assessed ($p < 0.05$). Post-hoc tests were done by Tukey's test ($p < 0.05$). Hydrolysis was carried out in a single batch. All experiments (soluble protein concentration, foaming, dilatational rheology) were at least done in triplicate.

Results

Chemical composition and isoelectric point

The chemical composition of the oat protein isolate (OPI), the tryptic hydrolysate (OPT) and alcalase treated oat protein (OPA) powder is displayed in Table 1. Differences in the chemical compositions of the OPI-, OPA- and OPT-powders can be explained by the different powder preparation procedures. For example, the increase in ash content from 3.2% for OPI to 3.9% for the hydrolysates was due to the addition of NaOH during hydrolysis with the pH-stat method.

Table 1 displays the isoelectric points of OPI, OPT and OPA determined via electrophoretic mobility as well as the resulting absolute values of ζ-potential at pH 4. The isoelectric points were in the order OPA > OPI > OPT and consequently OPT had the lowest absolute value of ζ-potential at pH 4. Generally, the isoelectric point of a protein is related to the proportion of acidic and basic amino acids in the protein. As OPI and its hydrolysates were a mixture of different polypeptides and small peptide fractions (Fig. 1), the isoelectric points determined via electrophoretic laser scattering corresponded to these mixtures.

Table 1: Chemical composition of OPI, OPT and OPA in [%], isoelectric point [-] and absolute value of ζ-potential at pH 4 [mV] ± standard deviation

	OPI	OPA	OPT
Protein	82.6 ± 0.2	80.0 ± 1.9	77.4 ± 0.5
Starch	1.3 ± 0.04	1.2 ± 0.03	1.1 ± 0.03
Moisture	2.0 ± 0	4.1 ± 0.1	5.3 ± 1.2
Ash	3.2 ± 0	3.9 ± 0	3.9 ± 0
Fat	6.3 ± 0.2	5.9 ± 0.2	6.4 ± 0.2
Dietary fibre*	4.6*	4.9*	5.9*
Isoelectric point	4.9 ± 0.04	5.1 ± 0.1	4.6 ± 0.02
ζ-potential at pH 4	7.4 ±0.9	5.3 ± 0.8	1.5 ±0.5

* Dietary fibre is the calculated difference to 100% based on the chemical components of OPC

Molecular weight distribution

The major protein component of oat globulin is 12S globulin, a hexamer with a molecular weight of 322 kDa consisting of 6 dimers each with a molecular weight of 54 kDa [30]. Consequently, in HPSEC chromatograms at pH 7 (Fig. 1 a), a large peak at 10.9 ml with a molecular weight of 315 kDa represented the hexameric form of the globulin (Fig. 1 a). For the hydrolysates, this peak shifted to a lower molecular weight due to the enzymatic hydrolysis. A large peak representing protein structures smaller than 10 kDa was also visible in OPI with this peak increasing in size for the hydrolysates. At pH 7, the distribution was complex. Thus, in Fig.1 c the percentage of each molecular weight class determined by HPSEC at pH 7 are shown in detail. The hexameric structure (fraction >300 kDa) was degraded during treatment with alcalase or trypsin. However, tryptic hydrolysis resulted in an increase in the 100 to 300 kDa fraction and the < 10 kDa fraction; thus, protein structures with higher molecular weight were still present pointing to the trypsin hydrolysis being highly specific. In contrast, a strong

degradation, as reflected by the increase of the smaller 10-50 kDa and < 10 kDa fractions, was observed in Fig. 1 c for the alcalase hydrolysed oat protein.

Oat globulins showed a limited solubility under acidic conditions. Consequently, OPI revealed protein particles with hexameric structures at pH 7 (Fig. 1 a and c) whereas 100% of the protein structures were smaller than 20 kDa at pH 4 due to protein precipitation (Fig. 1 b and Fig. 1 d). The size of soluble protein particles in OPI and its hydrolysates at pH 4 was in the order OPT > OPA > OPI (Fig. 1 b). From the relative size distribution in Fig. 1 d it can be seen that the fraction 5-20 kDa was highest for OPT (approx. 92%) followed by OPI and OPA with approx. 89.5%.

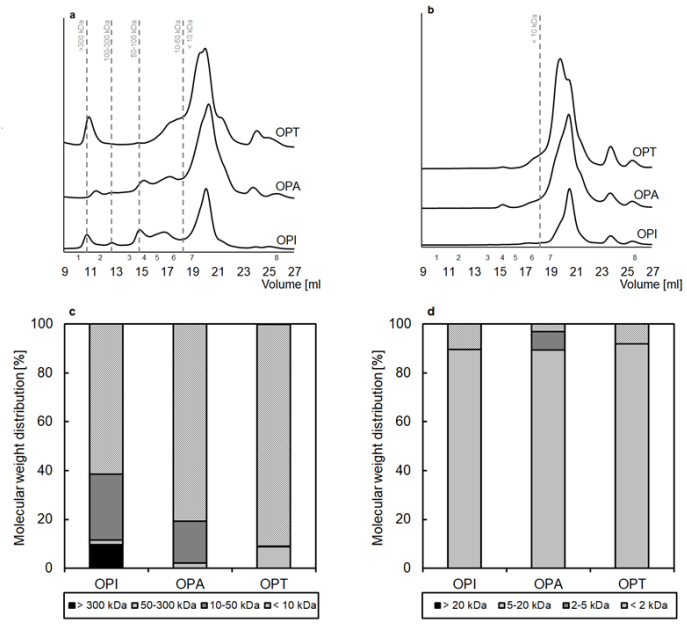

Fig. 1: HPSEC chromatogram of OPI-, OPA- and OPT-solutions at pH 7 (a) and pH 4 (b) (Elution positions of the standard proteins are indicated by small numbers, 1: ferritin 440 kDa, 2: aldolase 158 kDa, 3: conalbumin 75 kDa, 4: ovalbumin 44 kDa, 5: carbonic anhydrase 29 kDa, 6: ribonuclease 13.7 kDa, 7: aprotinin 6.5 kDa, 8: somatostatin 1.6 kDa), molecular weight distribution analysed by high performance size exclusion at pH 7 (c) and pH 4 (d) (the relative size distribution is the relevant integrated peak area as percentage of the overall chromatogram peak area). Values are means of three replicates

Analysis of the soluble protein concentration and protein solubility

Figure 2 shows the concentration of soluble protein in the supernatant after centrifugation. At pH 7 the soluble protein concentration of OPI was 0.08 ± 0.007% and was not significantly influenced by hydrolysis (OPT 0.08 ± 0.01% and OPA 0.07 ±0.004%). In contrast, at pH 4 the hydrolysis significantly increased the soluble protein concentration from 0.02 ± 0.002% (OPI) to 0.06 ± 0.006% (OPA) and 0.07 ± 0.008% (OPT), respectively. There was a significant influence of the pH-value on the soluble protein content of OPI, but not on OPT and OPA.

The protein solubility is displayed in Table 2. At pH 7 the protein solubility of OPI was 73.3 ± 6.7% and was not significantly influenced by hydrolysis (OPT 76.4 ± 11.6% and OPA 66.0 ± 4.3%). However, at pH 4 hydrolysis significantly increased the protein solubility from 17.6 ± 2.2% to 50.3 ± 5.3% (OPA) and 63.3 ± 7.9% (OPT). The pH-value significantly influenced the protein solubility of OPI, but not the one of OPA and OPT.

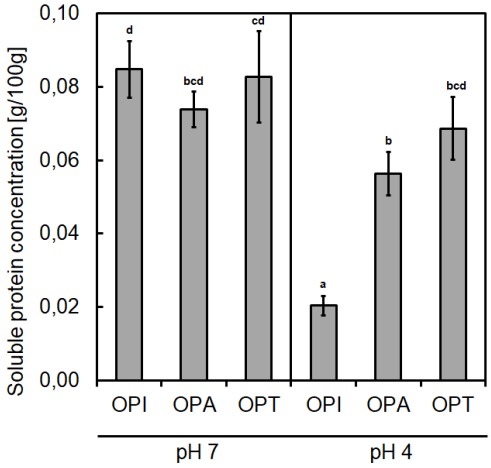

Fig. 2: Soluble protein concentration [%] at 0.14 g OPI, OPT and OPA weighed in 100 ml suspension at pH 7 and pH 4. The values are means ± standard deviation of four replicates. Different letters indicate significant differences between the samples ($p < 0.05$)

Table 2: Protein solubility [%].

	OPI	OPA	OPT
pH 7	73.3 ± 6.7^{bcd}	66.0 ± 4.3^{bcd}	76.4 ± 11.6^{cd}
pH 4	17.6 ± 2.2^{a}	50.3 ± 5.3^{bcd}	63.3 ± 7.9^{bcd}

Different letters indicate significant differences between the samples ($p < 0.05$)

Foaming ability

Figure 3 displays the foaming speed of OPI, OPT and OPA at pH 7 and pH 4. The ranking for fastest foam development at pH 7 was OPI (136.4 ± 1.3 mm min^{-1}) > OPT (118.4 ± 0.3 mm min^{-1}) > OPA (108.8 ± 0.6 mm min^{-1}), and at pH 4 it was OPT (114.6 ± 0.6 mm min^{-1}) > OPA (96.5 ± 3.5 mm min^{-1}) > OPI (82.7 ± 2.4 mm min^{-1}). Statistical analysis revealed significant differences between all samples except between the tryptic hydrolysate (OPT) at pH 4 and 7.

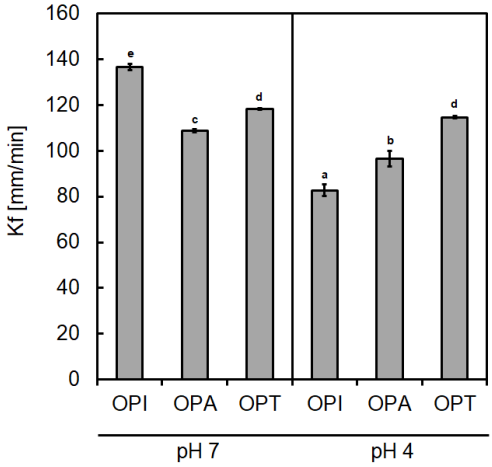

Fig. 3: Foaming speed (Kf) of OPI-, OPA- and OPT-solutions at pH 7 and pH 4. Values are means of three replicates ± standard deviation. Different letters indicate significant differences between the samples ($p < 0.05$)

The foam properties after 800 s, obtained with the dynamic foam analyser, are displayed in Fig. 4. OPT at pH 4, OPT at pH 7, OPA pH 4 and OPI at pH 7 had the most homogenous foam

structures with the lowest BD_m and the densest foams with a small BD_w. OPI at pH 4 and OPA at pH 7 formed non-homogeneous foam structures and less dense foams with high BD_m and a large BD_w.

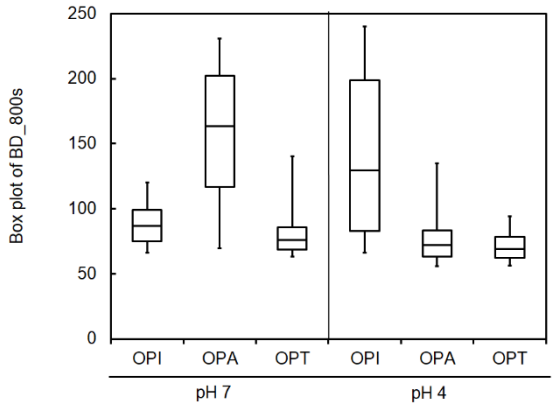

Fig. 4: Boxplot diagram of brightness distribution (BD) of 0.14% (w/v) OPI-, OPA- and OPT-solutions at pH 7 and pH 4 after 800 s observation time. Values are means of three replicates ± standard deviation

Dilatational rheology

For OPI and OPA, the interfacial dilatational modulus |E*| decreased with increasing pH (Fig. 5) while no statistically significant difference was obtained between |E*| of OPT at pH 7 and pH 4. The highest values of |E*| were shown for OPT at pH 4 (37.9 ± 1.9 mN m^{-1}), followed by OPI at pH 7 (35.9 ± 2.0 mN m^{-1}) and OPT at pH 7 (32.6 ± 0.4 mN m^{-1}). However, no significant difference of |E*| was found between these samples. The significantly lowest interfacial dilatational modulus was measured for OPI at pH 4 (10.9 ± 3.4 mN m^{-1}). Even though the viscoelasticity indicated by |E*| decreased with decreasing pH for OPI, the ratio between the elastic and viscous part remained constant (no significant difference between the loss factor tanφ in Fig. 5). Whereas, tanφ increased with decreasing pH for the hydrolysates indicating an influence on the ratio of the viscous and elastic part. For all samples, the elastic modulus E' was higher than the viscous modulus E'', indicating the more elastic properties of the protein film (data not shown).

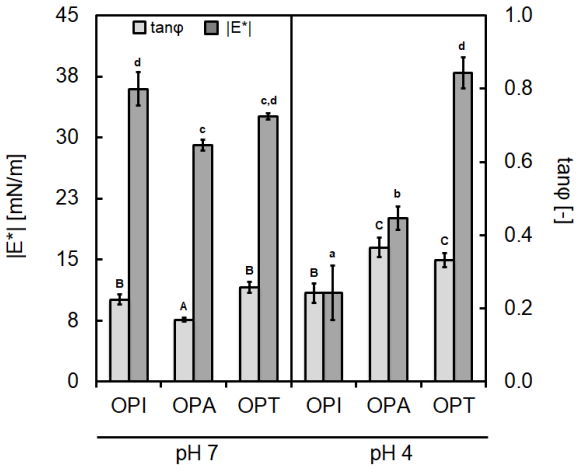

Fig. 5: Dilatational rheology – Interfacial dilatational modulus |E| and tanφ at 10% dA/A (area oscillation) and 0.1 Hz of 0.14% (w/v) OPI-, OPA- and OPT-solutions at pH 7 and pH 4, after 30 min drop film ripening. Values are means of three replicates ± standard deviation. Different letters (capital letters: tanφ, small letters: |E*|) indicate significant differences between the samples ($p < 0.05$)*

Interfacial shear rheology

The time-dependant film formation could be examined by application of small deformation and frequency. After 30 min, the complex interfacial shear modulus |G*| started at a lower level for samples at pH 4 (0.15 mN m^{-1} for OPI and OPT, 0.14 mN m^{-1} OPA) compared to pH 7 (0.20 mN m^{-1} for OPI, 0.16 mN m^{-1} OPT and 0.17 mN m^{-1} for OPA). Nevertheless, only for OPI and OPT at pH 4 did the interfacial shear modulus |G*| increase over time which means that for these two samples strong intermolecular interactions between the proteins at the interface developed over time (Fig. 6). Furthermore, the amplitude sweep of OPI and OPT at pH 4 after 17 h of film formation revealed that stronger interactions developed between the protein molecules in OPT (higher level of G') than those in OPI. OPT withstood a higher deformation because the intersection point of G' and G'', which equals the yield point, occurred at higher deformation than for OPI at pH 4 (Fig. 6).

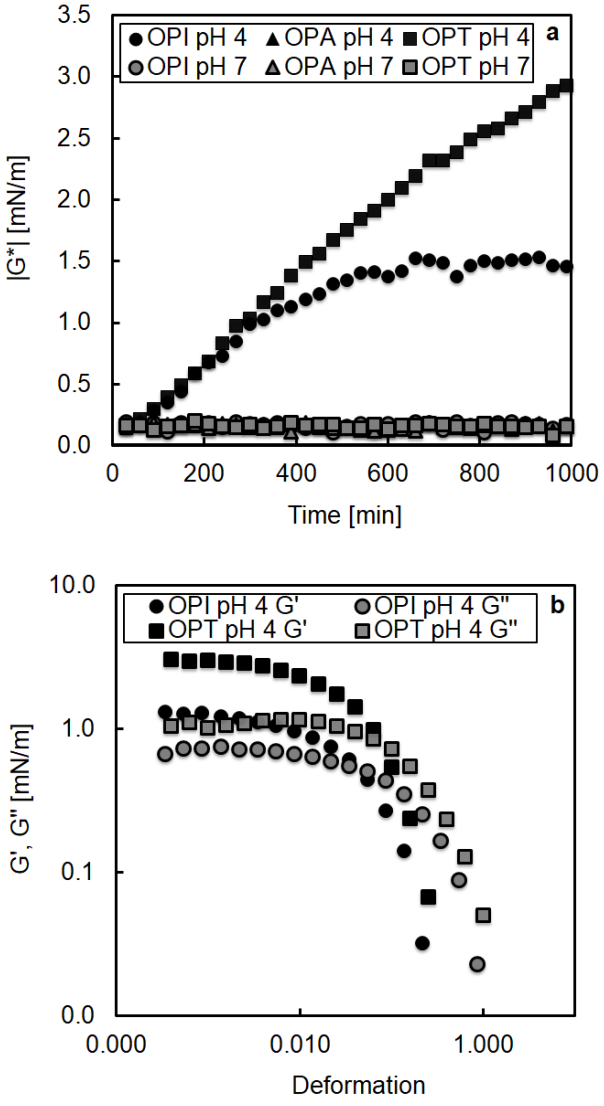

Fig. 6: Shear time sweeps of OPI, OPA and OPT at pH 7 and pH 4 with addition of 0.02% sodium azide, deformation: 10^{-4}, frequency 0.1 Hz, 25 °C (a), shear amplitude sweeps of OPI and OPT at pH 4 with addition of 0.02% sodium azide at 0.1 Hz frequency and deformation from 10^{-4} to 1; film age: 17 hours, Temperature: 25 °C (b)

In summary, at pH 4 the protein content of OPI differed significantly from all other samples, while there were no significant differences between most other samples. Foaming speed was highest for OPI at pH 7, OPT at pH 7 and OPT at pH 4 (Fig. 3) and consequently the interfacial films of these samples revealed the highest values of viscoelasticity (Fig. 5). As a result, the most homogenous and densest foam structures were formed (Fig. 4). However, films of OPI at pH 4, OPA at pH 4 and pH 7 had the slowest foaming speed (Fig. 3) and the lowest values of viscoelasticity (Fig. 5) and resulted in less homogeneous and less dense foam structures (Fig. 4). At pH 4, OPI was not able to form a stable foam due to the lower amount of soluble protein (Fig. 2) and lower viscoelasticity of the resultant protein film (Fig. 5).

Discussion

Molecular characteristics and protein solubility

The increase in protein solubility found in oat protein concentrate (OPI in Table 2) at neutral and especially alkaline pH was also reported by Nivala, Mäkinen, Kruus, Nordlund, & Ercili-Cura [31], Konak et al. [3], Ma and Harwalkar [4], Loponen et al. [32] and Guan et al. [5]. The increase in solubility at alkaline pH was attributed to an increase in protein net charge. Consequently, the protein partially unfolded due to intramolecular side-chain charge repulsion, which caused the breaking of hydrogen bonds and disruption of hydrophobic interactions [33].

Enzymatic hydrolysis resulted in different molecular weight profiles. While in OPI the hexameric structure was still present at pH 7 (Fig. 1 a and c), it was degraded by enzymatic action in OPT and OPA. Trypsin hydrolysis was highly specific: it is known to cleave peptides from the C-terminal end between lysine and arginine which must, however, not be followed by proline [34]. Thus, the acidic polypeptide of oat globulin was found to be less susceptible to tryptic action, and the basic polypeptide in the interior region of the protein remained intact [5, 35, 36]. In contrast, alcalase was a mixture of different proteases [37] with a broad specificity for several peptide bonds. Svendsen and Breddam [38] isolated a glutamic acid specific endopeptidase from a commercial alcalase mixture. Glutamic acid is one major amino acid that is especially accessible in the α-subunit of the 12S oat globulin [35, 39–41]. Therefore, the use of alcalase completely degraded the α-subunit of the 12S oat globulin [36], and the dimer-association was disintegrated. Nieto-Nieto, Wang, Ozimek, and Chen [36] confirmed the differential influence of tryptic hydrolysis (DH = 5.3%) and alcalase hydrolysis (DH = 5.8%) on the molecular weight pattern of OPI (analysed by HPSEC and SDS-PAGE).

As a result of enzymatic action, at pH 4, the hydrolysates (OPA and OPT) had a significantly higher protein solubility compared to the unmodified protein OPI (Table 2). Several studies have shown an improved solubility of plant proteins due to enzymatic hydrolysis over a wide pH range but especially around the isoelectric point [19, 42–46]. In addition to the exposure of more polar groups, the accessibility of hydrophobic regions was increased by hydrolysis [47]. For tryptic hydrolysates, a shift of the isoelectric point from 4.9 to 4.6 took place (Table 1). The increasing number of carboxyl groups in the course of hydrolysis and the resulting shift of the isoelectric point to more acidic conditions was also reported for a tryptic collagen hydrolysate [48]. Ma and Wood [10] also observed, similar to this study, that the solubility of oat protein hydrolysates (1 % tryptic oat hydrolysate solutions, DH not given) was significantly increased at acidic pH with a slight but not significant decrease at alkaline pH. The behaviour under alkaline conditions was related to the increased availability of hydrophobic patches upon hydrolysis, thus, facilitating protein-protein interactions which led to precipitation [49, 50].

Foaming ability and mechanisms of stabilisation at pH 7

Foams are thermodynamically unstable dispersed systems with a continuous liquid phase and a dispersed gas phase. Surface-active components, for example proteins, can help to form and maintain the foam structure. The foaming process in the presence of proteins is essentially divided into consecutive steps: the initial movement of protein molecules to the interface followed by the adsorption of the protein molecules where conformational changes and protein network formation then result in development of a viscoelastic film [51–56]. Intermolecular interactions, such as hydrogen bonding, hydrophobic interactions, covalent bonding (intermolecular disulfide bonds) and electrostatic interactions between the proteins at the interface play an important role during film formation [57, 58]. It was stated by Dickinson [59] that a good foam or emulsion stabilising biopolymeric material should be able to form a charged stabilising layer and/or a thick steric layer (i.e. a high molecular weight of 10^4 – 10^6 Da is needed). Both, electrostatic and steric stabilisation are important colloidal stabilisation mechanisms in emulsions and foams [59].

Under neutral conditions, no significant difference was found for the soluble protein concentration of OPI and its hydrolysates (Fig. 2); but due to enzymatic action, the molecular weight decreased in the order OPI > OPT > OPA (Fig. 1 a and c). It is evident that the native globulin structure present in unmodified oat protein with protein fractions > 300 kDa accelerated and promoted the formation of a protein film with an adequate thickness. The size of 12 S globulin was reported to be between 11 nm [8] and 12 nm [41]. Ercili-Cura et al. [8] analysed AFM images of oat protein films at pH 7.2 and observed a protein film composed of large clusters. Considering the structure of a 12 S globulin hexamer as an oblate cylinder with a height of 8.5 nm [60], it is evident that a nearly native structure would form thick and stable films and a size reduction by hydrolysis would reduce the thickness of the interfacial layer. Consequently, OPI at pH 7 formed a strong viscoelastic film (|E*| in Fig. 5) with dominating elastic behaviour (tanφ<1). OPI also had the highest speed of adsorption at and greatest stabilisation of the newly formed interface (significantly higher foaming speed in Fig. 3) as well as having more homogenous and denser foam structures (Fig. 4) compared to its hydrolysates. A higher value of K_f indicates that less time is needed to reach a foam height of 180 mm or, in other words, more foam can be generated in a shorter time. Compared to OPI, OPT had a significantly lower foaming speed under neutral conditions but no significant difference of |E*| and tanφ indicating that the nature of the film, its viscoelasticity and ratio of elastic to viscous parts, was similar but the reduction in protein particle size dominated the film properties. In contrast, OPA, with the greatest loss in high molecular weight protein structure (Fig 1), was significantly slowest at adsorption and stabilisation of the newly formed bubble interface at pH 7 (Fig. 3). The increased amount of low molecular weight peptides (Fig. 1 a and b) with reduced hydrophilic and hydrophobic regions have previously been shown to reduce functionality [61]. In a study by Singh and Dalgleish [62], the small peptides were found unable to form strong intermolecular interactions at the interface resulting in insufficient packing and lower viscoelasticity. Consequently, at pH 7, OPA had the lowest value of |E*| compared to OPI and OPT (Fig. 5).

Thus, it can be concluded that the balance between different stabilisation mechanisms will shift depending on the extent of hydrolysis as well as on the pH. Both, electrostatic interactions, mainly of hydrophobic nature, as well as steric stabilisation are discussed to be responsible for the formation of stable interfaces. Since increased protein solubility was attributed to an increased net protein charge, strong electrostatic repulsion between oat proteins at the

air/water interface was supposed to occur at pH 7. Thus, at pH 7 electrostatic repulsion was high and hydrophobic interactions low, consequently, the interfacial shear moduli |G*| representing the strength of the interactions between the protein molecules within the interfacial film [63] remained low and no interactions developed over time (Fig. 6). Nevertheless, OPI had a high foaming speed (Fig. 3) and formed a strong viscoelastic film at the air/water interface (Fig. 5) which means that formation of a thick steric layer (steric stabilisation) was the dominating stabilisation mechanism involved at pH 7.

Foaming ability and mechanisms of stabilisation at pH 4

The foaming ability of OPI under acidic conditions at pH 4 was limited due to the significantly lower solubility. Whereas, the tryptic hydrolysate had the significantly highest speed of adsorption and stabilisation of the newly formed interfacial area (Fig. 3) and formed the most homogenous and densest foam structures (Fig. 4). These improved foaming abilities for OPT were due to improved soluble protein concentration in the supernatant and protein solubility in comparison to OPI and OPA (0.07% for OPT, 0.02% for OPI in Fig. 2; 17.6% for OPI and 63.3% for OPT in Table 2) in addition to the presence of a greater proportion of the 5-20 kDa fraction (Fig. 1 d) and the reduction in isoelectric point from around 4.9 to 4.6 (Fig. 1 b and Table 1). Based on the measured value of ζ-potential at pH 4 (Table 1) the net charge of proteins present in OPT is lower compared to OPI and OPA. Thus, its affinity towards the air/water interface would increase. Gochev, Retzlaff, Exerowa, & Miller [64] proved that the globular protein β-lactoglobulin was more surface-active towards the air-water interface at its isoelectric point because of its lower net surface charge. Consequently, the viscoelasticity of the interfacial film of OPT at pH 4 was comparable to that of OPI at pH 7 (no significant difference occurred between |E*| of OPI at pH 7 and OPT at pH 4 in Fig. 5). Foaming ability strongly depended on the diffusion and adsorption of proteins to the interface, which could be facilitated by small particles with low molecular mass (high diffusion coefficient/speed) but also by the availability of hydrophobic groups (affinity to air/water-interface) [65]. Furthermore, the molecular flexibility of the proteins could be increased by controlled hydrolysis, resulting in a faster unfolding and more intermolecular interactions [66]. Additionally, the increase of hydrophobic patches due to hydrolysis could support the stability due to more distinct protein-protein interactions [20]. An increased foaming ability and stability of 1% tryptic oat hydrolysate solution was also reported by Ma [9] and Ma and Wood [10]. Thus, tryptic hydrolysis was found to be a valuable structure-tailoring tool for OPI producing protein structures with improved functionality for the use in mildly acidic food foams such as soft and airy fillings in bakery products, chocolates and pralines or a creamy froth as a topping for coffee.

It can be concluded that decreasing electrostatic repulsion at pH 4 was achievable, especially for OPT ζ-potential = 1.5 mV, Table 1), which allowed for a denser packing of and stronger association between protein particles. Nevertheless, most of the protein particles had a size <20 kDa, which means that the formation of a thick steric layer (steric stabilisation) was not the dominating stabilisation mechanism because a thick steric layer was reported to be dependent on the molecular weight (10^4 – 10^6 Da) [59]. Here, the formation of a strong viscoelastic film based on hydrophobic interactions was more prevalent and was particularly dominant in the case of OPT. Due to the shift of the isoelectric point of OPT from 4.9 to 4.6 (Table 1), stronger associations occurred in the order OPT>OPI (Fig. 6). In addition, several authors found stronger interfacial films when limited hydrolysis was applied [66–68] due to improved protein-protein or peptide-peptide interactions, which also might explain the higher

|G*| of OPT. A maximum interfacial shear viscosity near the isoelectric point was also reported by Roth, Murray, & Dickinson [69] and Izmailova [70].

However, hydrolysis with alcalase increased film viscosity at pH 4 compared to pH 7 (tanφ significantly increased in Fig. 5) with no increase in |G*| observed (Fig. 6). Here, the viscoelasticity of the film was negatively affected by greater protein degradation which prevented the formation of stronger protein-protein and peptide-peptide interactions. The small peptides present in OPA were unable to form a strongly viscoelastic, thick steric layer due to the formation of fewer intermolecular interactions between small peptides [62].

Conclusions

In this study, the potential of oat protein isolate and its hydrolysates to stabilise food foams was analysed. At pH 7, a high foaming speed and a good dispersion of gas bubbles as well as the formation of a strong viscoelastic protein film was observed for OPI. The stabilisation mechanism was dominated by the formation of a thick steric protein layer. However, its foaming properties at pH 4 were found to be limited, and enzymatic hydrolysis was used to alter the foaming properties. Tryptic hydrolysis improved the foaming properties at pH 4 and the resultant interfacial properties of the tryptic hydrolysate were comparable to those of unmodified oat protein isolate at pH 7. The stabilisation of the foam was dominated by the formation of strong hydrophobic interactions between the protein particles in the interfacial film. Alcalase hydrolysis, however, did not sufficiently improve foaming ability, stability or film properties at pH 7 or 4 because the small peptides present in OPA were unable to form a strongly viscoelastic, thick steric layer which was attributed to the greater protein degradation as well as to the formation of fewer intermolecular interactions between small peptides.

The improved foaming properties of oat protein at pH 4 after tryptic hydrolysis will broaden the applicability of oat protein as a food ingredient. Future studies should focus on the fractionation of protein size classes to better understand the foam stabilisation mechanisms.

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Compliance with ethical standards

Conflict of interest

The authors declare no conflict of interest.

Compliance with ethics requirements

This article does not contain any studies with human participants or animals.

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