

Effects of concentration and partial hydrolysis on functional properties of hull-less barley protein concentrates

E. Şirin and E. Yalçın*

Bolu Abant İzzet Baysal University, Faculty of Engineering, Department of Food Engineering, Gölköy Campus, 14030 Bolu, Turkey; yalcin_e@ibu.edu.tr

Received: 27 July 2018 / Accepted: 25 November 2018

© 2019 Wageningen Academic Publishers

RESEARCH ARTICLE

Abstract

Effects of protein concentration and partial hydrolysis on protein solubility (PS), foaming properties and water holding and oil absorption capacities of hull-less barley protein concentrate (HBPC) were investigated in this research. Defatted hull-less barley flour (dHBF) was used for the production of HBPCs which were prepared using different extraction methods. HBPC-1 was extracted from dHBF using 0.15 M NaCl at pH 9 according to flour to solvent ratio of 1:10 and an isoelectric precipitation was applied at pH 5. HBPC-2 was extracted from dHBF using 0.06 M NaOH at pH 11.2 with flour to solvent ratio of 1:20 and an isoelectric precipitation was applied at pH 5.4. HBPC-1 and HBPC-2 were applied to partial-enzyme hydrolysis with subtilisin A for producing barley protein hydrolysates. SDS-PAGE images of dHBF, HBPCs and hydrolysates were also studied to monitor the extracted and hydrolysed proteins. PS properties of HBPCs were not significantly influenced by increasing protein concentration, while the pH of the medium significantly influenced this property. The PS properties of hydrolysates at pH 6 increased as a result of the partial hydrolysis. The soluble proteins of HBPC-1 exhibited better foaming ability at all protein concentrations and pH environments. In addition, the soluble proteins of the HBPC-2 presented a better foam stability. Foaming properties were influenced by partial hydrolysis. The partial-enzyme hydrolysis did not improve the foaming properties of concentrates.

Keywords: hull-less barley, protein concentrate, partial hydrolysis, functional properties, gel electrophoresis

1. Introduction

Barley (*Hordeum vulgare* L.) is a cool climate grain which is an important agricultural crop utilised for different purposes in most of the countries. The most of the cultivated barley is hulled barley. Hull-less barley has a naked caryopsis gene and so the kernels are not hulled. There is a growing interest in barley for production of functional foods due to its condensed bioactive compounds (Celus *et al.*, 2006). Hull-less barley has higher crude protein, starch and micro-nutrients (Newman and Newman, 2008; Yalçın, 2010). Barley is an abundant and cheap protein source among cereals. The total protein content changes from 8 to 13% (w/w) depending on the variety (Zhao *et al.*, 2010).

Barley proteins can be classified according to Osborne fractionation, which generally considers solubility

properties in aqueous solutions (Osborne, 1924). They are water soluble albumins (3-5% of the total protein), dilute salt soluble globulins (10-20% of the total protein), alcohol soluble prolamins (35-55%, mainly hordeins) and diluted acid/base soluble glutelins (35-45% of the total protein) (Celus *et al.*, 2006; Yalçın, 2010). Barley hordeins are divided into five groups based on their electrophoretic mobilities and amino acid compositions: the B-hordeins (70-80%), C-hordeins (10-20%), D- and γ -hordeins (less than 5% of the total hordein fraction), and finally A-hordeins (low molecular weight salt soluble hydrophobic polypeptides) (Celus *et al.*, 2006; Wang *et al.*, 2010).

Proteins play an important role as macromolecular surfactants in foam and emulsion-type food products in which the phase separation phenomenon is particularly an important concern. The rate of phase separation can

be controlled by reducing the interfacial tension between the continuous phase (water) and the dispersed phase (air in foam or oil in emulsion). This is achieved by adding an amphiphilic molecule (like protein) that acts as surfactant by adsorbing to and positioning itself at the interface (Damodaran, 2005). In addition, traditional food proteins (caseins, egg and meat proteins), which have excellent functional properties suitable for several applications in the food industry, are mixtures of several protein components. An interaction of these components in the interfacial layer impacts their ability as surfactants to stabilise dispersed systems (Damodaran, 2005). Non-traditional plant proteins, such as cereal and legume proteins are not as functional as animal proteins in terms of acting surface-active components at interfaces. Functional properties and biological functions of food proteins were extensively discussed with their mechanisms in the literature (Damodaran, 1997, 2005; Foegeding and Davis, 2011; Zayas, 1997). Among the functional properties of food proteins, protein solubility and foaming properties have significant role in processed products, such as whipped toppings, ice cream, spreads, cakes, mousses, marshmallows and soft drinks and beverages.

The functional properties of proteins can be improved through physical, chemical, enzymatic, and genetic modifications (Damodaran, 2005; Howell, 1996). Enzymatic modification is a promising approach to improve the functional properties of proteins. The most practical enzymatic modifications are the hydrolysis and polymerisation reactions. Enzyme hydrolysis of proteins is carried out with proteases, such as pepsin, trypsin, chymotrypsin, and other nonspecific proteases (Damodaran, 2005). Generally, enzyme hydrolysis of protein structure can be explained briefly as a reduction of tertiary structure and molecular mass and an increase in the exposure of hydrophobic side chains which are often buried in a hydrophobic core of the molecule (Brückner-Gühmann *et al.*, 2018). Partial enzyme hydrolysis is employed for accomplishing the degree of hydrolysis less than 5% with the resulting of large peptides (Howell, 1996). Degree of hydrolysis is the percentage of the total number of peptide bonds cleaved or hydrolysed with protease. It was reported that partial enzyme hydrolysis of food proteins may improve solubility, foaming ability and emulsifying activity, conversely highly hydrolysed proteins weaken the stability of emulsions and foams (Damodaran, 2005).

The protein solubility properties of barley protein isolate (BPI) were studied as a function of pH and NaCl concentration either in native and hydrolysed forms in our previous study (Yalçın and Çelik, 2007). In addition, the foaming properties of BPI and its hydrolysates were investigated as a function of pH and protein concentration (Yalçın *et al.*, 2008). The protein solubility and emulsifying

properties of barley protein concentrate were studied as a function of pH, NaCl concentration and protein concentration (Bilgi and Çelik, 2004). It was also reported that the functional properties of hordeins were significantly improved at a 2.4–4.7% deamidation degree (Zhao *et al.*, 2010). The structure, solubility and emulsifying properties of the deamidated barley glutelins were also investigated by Zhao *et al.* (2011). In addition, Zhang *et al.* (2015) indicated that more flexible and high molecular weight barley proteins could be produced with deamidation.

The objective of this study was to investigate the effects of protein concentration and partial enzyme hydrolysis on protein solubility, foaming properties and water holding and oil absorption capacities of hull-less barley protein concentrates (HBPC) and hydrolysates. This research has also emphasis on using hull-less barley as a source of protein concentrate for the food industry.

2. Materials and methods

Hull-less barley (advanced line) used in this study was obtained from the Field Crops Improvement Center, Ankara, Turkey. Hull-less barley grains was milled into straight-grade to fine flour in a laboratory Buhler AG roller mill (Uzwil, Switzerland) after tempering. Hull-less barley flour (HBF) was stored at 4 °C until using.

Defatting process

Defatting of HBF was accomplished by extraction of crude oil with hexane for 3 h at room temperature with continuous mixing. After precipitation of flour, crude oil-hexane mixture was carefully removed and the precipitated flour was air-dried for 48 h at room temperature. Defatted HBF (dHBF) was stored at 4 °C until using.

Preparation of hull-less barley protein concentrates

HBPC-1 was prepared according to the methods of Aluko *et al.* (2001) and Chandi and Sogi (2007) with some modifications by extracting from dHBF with dilute salt solution (0.15 M NaCl) at pH 9 according to flour to solvent ratio 1:10. The slurry was mixed for 2 h on the magnetic stirrer (MR Hei-Standard, Heidolph, Germany) at room temperature. Then, the slurry was centrifuged (Nüve, NF 1200 Model, Ankara, Turkey) at 9,000×g for 15 minutes and the supernatant was collected, then the pH of the supernatant was adjusted to 5 and mixed for 30 minutes at room temperature, then centrifugation applied at 9,000×g for 20 minutes. The precipitate (protein concentrate) was collected and solubilised in distilled water (dH₂O) for neutralisation. Then, the protein concentrate (HBPC-1) was freeze-dried (Alpha 1-2 LDplus model, Christ, Osterode am Harz, Germany) and stored at 4 °C until using.

HBPC-2 was produced according to the method of Wu *et al.* (1979) and as modified by Bilgi and Çelik (2004). Defatted-HBF and 0.06 M NaOH with flour to solvent ratio of 1:20 were stirred for 25 min at room temperature. During mixing, pH of the slurry was adjusted to 11.2. Then, the slurry was centrifuged at 3,300×g for 15 minutes and supernatant was removed and its pH was adjusted to 5.4 to precipitate proteins. After mixing for 15 minutes, the blend was centrifuged at 3,300×g for 15 min, the precipitate (protein concentrate) was collected and solubilised in dH₂O for neutralisation, then the concentrate was freeze-dried. After lyophilisation of the concentrates, protein contents were determined using micro-Kjeldahl method on dry weight basis (dwb). The protein content was defined as the percentage of protein in the lyophilised powder, while extraction efficiency was described as the percentage of the amount of lyophilised concentrate relative to the amount of dHBF.

Partial hydrolysis of protein concentrates

Partial hydrolysis of HBPCs was basically carried out according to the method of Adler-Nissen (1986). The pH-stat technique was applied by using the Mettler-Toledo T50 (Switzerland) model automatic titrator in order to produce the hydrolysates at desired degree of hydrolysis (DH, %). HBPC-1 and HBPC-2 were hydrolysed with subtilisin A (Type VIII, 12 Units/mg solid enzyme, Sigma-Aldrich P5380, St. Louis, MO, USA) enzyme produced from *Bacillus licheniformis*. Subtilisin A is a serine endoproteinase with a broad specificity. In this research, the optimum hydrolysis conditions used were pH 8 and temperature of 50 °C. The ratio of enzyme to substrate was 1:300 (w/w). When the desired DH (%) was calculated according to Yalçın *et al.* (2008) which was based on Adler-Nissen (1986), the hydrolysis reaction was terminated by heating in a boiling water bath for 5 minutes. The hydrolysates were then freeze-dried and kept at 4 °C. In this research, the barley protein hydrolysates (BPHs) having 2% and 5% DH were produced and the hydrolysates of HBPC-1 were defined as BPH-1 and BPH-2, respectively, while the hydrolysates of HBPC-2 were defined as BPH-3 and BPH-4, respectively.

Chemical analysis

Moisture and protein (Nx6.25) contents were determined according to AACCI Approved Methods of Analysis No. 44-15.02 and 46-12.01, respectively (AACCI, 2010). Mean values and corresponding standard deviations of the results were reported.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of dHBF, HBPC-1, HBPC-2 and hydrolysates was performed based on the original procedure of Laemmli (1970) and also according to the method of Keskin *et al.* (2018). Protein extraction was done according to the method of Keskin *et al.* (2018). The acrylamide concentrations of resolving and stacking gels were 12.5 and 5%, respectively. The gels were stained overnight with Coomassie Brilliant Blue G-250 (Merck, Darmstadt, Germany). Apparent molecular weights were estimated using the wide range protein marker (Sigma-Aldrich S8445).

Determination of protein solubility properties

Protein solubility (PS, %) properties were investigated according to the method of Yalçın and Çelik (2007). PS properties of HBPC-1 and HBPC-2 were investigated in dH₂O at concentrations of 0.25, 0.5 and 1.0% (w/v) based on protein content (bpc). PS properties of the BPH-1, BPH-2, BPH-3 and BPH-4 hydrolysates were determined in dH₂O at the concentration of 0.25% (w/v) bpc. PS properties were studied as a function of pH (2-10) of the medium. The amount of soluble protein in the clear filtrate was determined in UV/VIS spectrophotometer (Shimadzu, 1700 Model, Kyoto, Japan) by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard. PS (%) was expressed as a percentage of total protein concentration. All analyses were performed as triplicate and the mean values and corresponding standard deviations were reported in related figures. After determination of PS (%), the remaining clear filtrate including soluble proteins at relevant pH was immediately used for testing foaming properties.

Determination of foaming properties

Foaming properties (FP) of the soluble proteins of HBPC-1, HBPC-2 and hydrolysates were determined according to the method of Yalçın *et al.* (2008). FP of HBPC-1 and HBPC-2 were investigated in dH₂O at concentrations of 0.25, 0.5 and 1.0% (w/v) bpc. FP of the hydrolysates was determined in dH₂O at concentration of 0.25% (w/v) bpc. FP was studied as a function of pH (2-10) of the medium. A 10 ml clear filtrate including soluble proteins was sparged with dry air (Rocker 420 Oil-less Compressor, Rocker Scientific Co. Ltd., New Taipei City, Taiwan) at a constant flow rate of 0.5 l/min adjusted with air flowmeter (Cole Palmer Air Flowmeter, Vernon Hills, IL, USA) for 10 s at room temperature. Immediately after turning off the air, foam height was measured using the calibrated markings. Foaming ability of the samples was described as foam volume (FV, cm³). Foam stability is expressed as foam half-life (FHL, min). FP was performed as quadruple

determinations and the mean values and corresponding standard deviations were reported in figures.

Determination of water holding and oil absorption capacities

Water holding capacity (WHC) and oil absorption capacity (OAC) of HBPCs and hydrolysates were measured according to the method of Ahmedna *et al.* (1999) with slight modifications. WHC (grams of water per gram of sample) and OAC (grams of oil per gram of sample) were calculated according to Ahmedna *et al.* (1999). The experiments were carried out as triplicate determinations and the mean values and corresponding standard deviations were reported.

Statistical analysis

Data were analysed by using one-way analysis of variance (ANOVA) in SPSS (Version 20.0 for Windows) statistical programme. When significant ($P < 0.05$) differences were found, the Duncan test was used to determine the differences among means.

3. Results and discussion

Extraction efficiency, moisture and protein contents of HBPCs and hydrolysates were shown in Table 1. HBPC-1 was extracted in dilute salt (0.15 M NaCl) solution at mild alkaline pH (pH 9) and any reducing agent was not used. It was expected that albumins and globulins were soluble in dilute salt solution. However, the low extraction efficiency of HBPC-1 may be explained that there are some compounds like phytates, phenolics and fibres, which bind to proteins, are making proteins difficult to separate from barley flour. In addition, the extraction solvent could not disrupt bonds between protein and other compounds in dHBF (Wang *et al.* 2010).

Besides, high extraction efficiency of HBPC-2 can be explained with high alkaline (pH 11.2) condition that helps to break down the hydrogen bonds and causes dissociation of hydrogen from carboxyl and sulfhydryl groups. An increased negative charge on protein molecules also caused improved protein solubility in the extraction solvent (Wang *et al.*, 2010).

The protein contents of hydrolysates were found very close to native forms. BPH-1 and BPH-3 hydrolysates having 2% DH were produced at the hydrolysis times of 7.0 and 10.5 minutes, respectively, while BPH-2 and BPH-4 hydrolysates having 5% DH were produced at the hydrolysis times of 20.0 and 90.2 minutes, respectively (the graph of DH (%) vs time (min) was not shown).

SDS-PAGE patterns of protein concentrates and hydrolysates

SDS-PAGE patterns of dHBF, HBPC-1 and HBPC-2 and hydrolysates having 2% (BPH-1 and BPH-3) and 5% (BPH-2 and BPH-4) degree of hydrolysis were investigated in unreduced (Lane 1-7) and reduced (Lane 8-14) conditions (Figure 1). In this electrophoregram, barley proteins were defined according to Yalçın (2010). In reduced condition, all proteins of dHBF could enter into the gel due to breaking of disulphide bonds with 2- β -mercaptoethanol (Lane 8). In unreduced condition, there were also significant differences between both concentrates (Lane 2 and 5). HBPC-1 included intense protein bands around 60 kDa (C-hordeins), 30 and 38 kDa (γ - and B-hordeins), 20-23 kDa and 10 kDa (albumins and globulins; Lane 2). Conversely, HBPC-2 had intense protein bands between 28-70 kDa (γ - and B-hordeins and C-hordeins; Lane 5). In unreduced condition, the hydrolysates of HBPC-1 (BPH-1 and BPH-2; Lane 3 and 4, respectively) did not have any protein bands above 23 kDa, they had mostly albumins and globulins. The hydrolysates of HBPC-2 (BPH-3 and BPH-4; Lane 6 and

Table 1. Extraction efficiency, moisture and protein contents of hull-less barley protein concentrates (HBPCs) and barley protein hydrolysates (BPHs).

Sample	Extraction efficiency (%) ¹	Moisture (%) ²	Protein (%) ^{2,3}
HBPC-1	1.22±0.015	5.4±0.21a	70.6±0.14c
BPH-1 (%2 DH)	-	5.1±0.19ab	68.7±0.14d
BPH-2 (%5 DH)	-	5.2±0.10ab	69.4±0.42d
HBPC-2	9.33±0.250	4.0±0.19c	90.7±0.18a
BPH-3 (%2 DH)	-	5.0±0.00b	90.7±0.11a
BPH-4 (%5 DH)	-	5.0±0.00ab	89.3±0.38b

¹ The percentages of the ratio of the weight of lyophilised powder to weight of dHBF used in the extraction. Calculated from three repetitions (n=3).

² Values followed by the different letter in the same column are significantly different ($P < 0.05$).

³ On dry weight basis (dwb), Mean \pm sd, n=3.

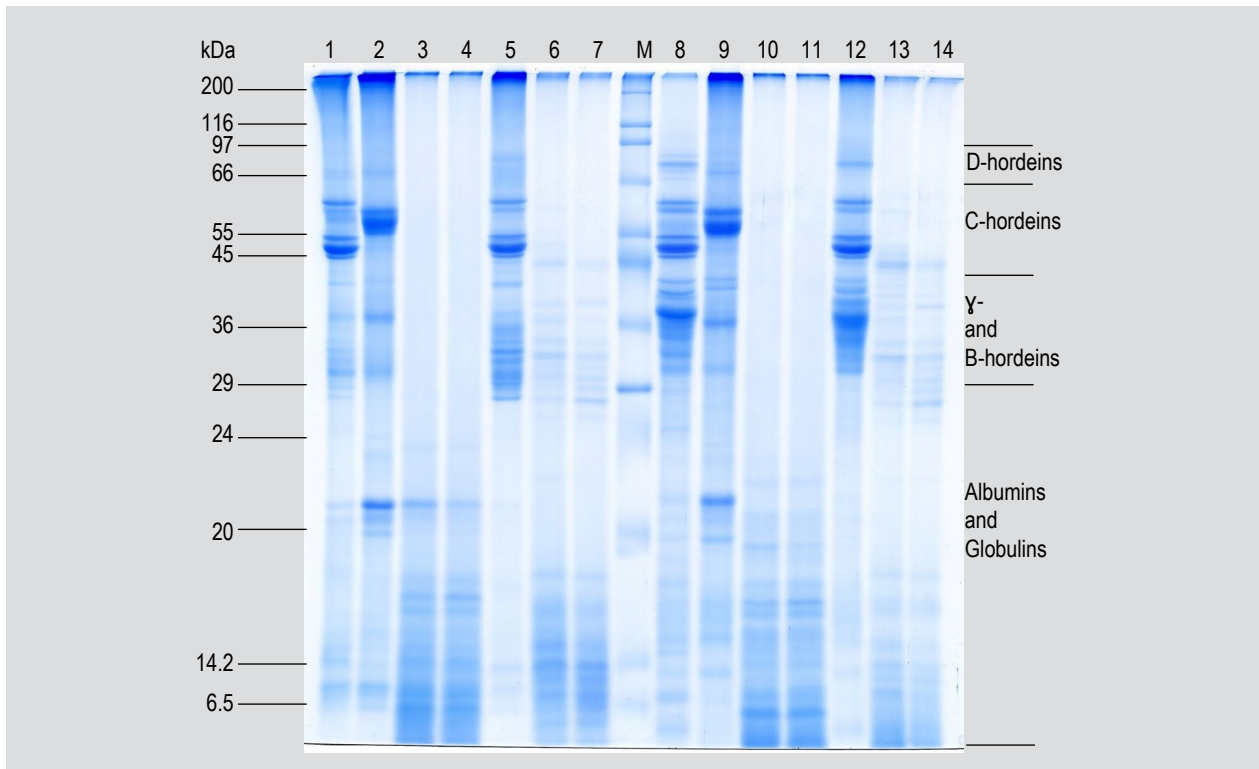


Figure 1. SDS-PAGE patterns of dHBF, HBPC-1, HBPC-2 and hydrolysates having 2% (BPH-1 and BPH-3, respectively) and 5% (BPH-2 and BPH-4, respectively) degree of hydrolysis in unreduced (1: dHBF, 2: HBPC-1, 3: BPH-1, 4: BPH-2, 5: HBPC-2, 6: BPH-3, 7: BPH-4) and reduced (8: dHBF, 9: HBPC-1, 10: BPH-1, 11: BPH-2, 12: HBPC-2, 13: BPH-3, 14: BPH-4) conditions. M: Wide-range protein marker. HBPC = hull-less barley protein concentrate; dHBF = defatted hull-less barley flour; BPH = barley protein hydrolysates.

7, respectively) had protein bands between 28-44 kDa (γ - and B-hordeins region) and also below 20 kDa (albumins and globulins region) in unreduced condition (Figure 1).

In reduced condition, HBPC-1 included fewer protein bands than HBPC-2 (Lane 9 and 12, respectively). HBPC-1 had distinct protein bands around 60 kDa region (C-hordeins), also had a few bands between 30 and 40 kDa region (γ - and B-hordeins), as well as had a distinct protein band at 23 kDa which was in albumins and globulins region. Conversely, HBPC-2 included D-hordeins, C-hordeins, γ - and B-hordeins of original raw material (dHBF). Briefly, alkali extracted HBPC-2 included both hordein and glutelin fractions from original flour, but not albumin and globulin proteins. In reduced condition, the hydrolysates of HBPC-1 (Lane 10 and 11) and HBPC-2 (Lane 13 and 14) had similar protein band patterns like their unreduced forms (Figure 1).

Protein solubility properties

Effect of protein concentration on PS (%) behaviours of HBPC-1 and HBPC-2 was shown in Figure 2. The PS results of each concentrate were analysed on SPSS statistical programme (IBM SPSS, New York, NY, USA), however only standard deviations calculated from triplicate analysis were presented. It was observed that there were significant

differences on PS behaviours of HBPC-1 and HBPC-2. The PS properties of HBPC-1 were higher at all protein concentrations and pH points than that of HBPC-2. The highest PS values were obtained with HBPC-1 at 0.5% (w/v) concentration at all pH points ($P < 0.05$), especially at pH 2, HBPC-1 had the highest PS (80.7%) value at that concentration. The PS value (76.7%) obtained at 0.25% (w/v)

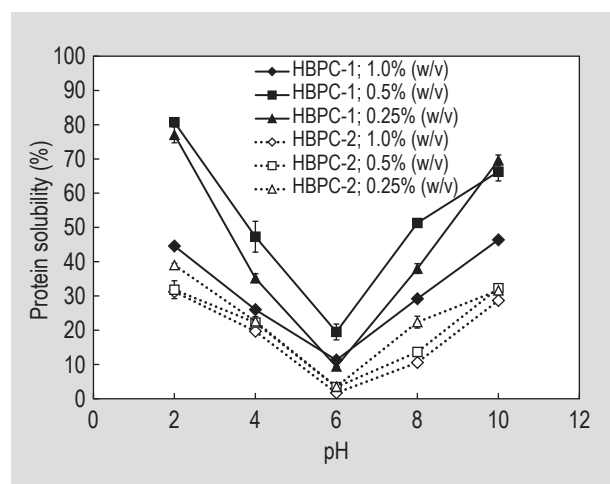


Figure 2. Effect of protein concentration on protein solubility properties of the concentrates at different pH points.

concentration and pH 2 followed ($P < 0.05$). PS decreased with an increasing of protein concentration at all pHs. Besides, the PS of HBPC-2 was not influenced from an increasing of protein concentration at all pHs. The highest PS obtained with HBPC-2 was observed at 0.25% protein concentration at pH 2 (39.0%, $P < 0.05$).

The lowest PS values were obtained at pH 6 for both concentrates. It was thought that isoelectric point of the concentrates might be around pH 6. Wu *et al.* (1979) also reported that the isoelectric point of barley proteins was close to pH 6. Similar findings were also stated in previous works (Ahmedna *et al.*, 1999; Bilgi and Çelik, 2004; Yalçın and Çelik, 2007).

HBPC-1 exhibited better PS property because it was mainly consisted of water soluble albumin and globulin proteins. The low PS behaviours of HBPC-2 can be explained with partial protein denaturation during alkaline extraction. In addition, Harris and Angal (1989) and Cepeda *et al.* (1998) previously reported that an increasing of protein concentration may decrease the protein solubility due to protein aggregation.

Effect of partial hydrolysis on PS behaviours of BPH-1, BPH-2, BPH-3 and BPH-4 hydrolysates was presented in Figure 3. The PS results of hydrolysates were also analysed on SPSS statistical programme and the standard deviations calculated from triplicate analysis were shown. The PS values of BPH-1 and BPH-2 hydrolysates were generally higher at all pHs (except for pH 6) than that of the BPH-3 and BPH-4 hydrolysates. The highest PS values among the hydrolysates were generally obtained with the BPH-2 (having 5% DH) at all pHs, except for pH 6. Instead, the PS value of BPH-4 (51.8%) was higher than that of BPH-2 (47.5%) at pH 6. In addition, at pH 2 and pH 10, PS values of BPH-2 were 70.7% and 62.7% ($P < 0.05$), respectively. At

pH 4, pH 6 and pH 8, PS values of BPH-1 and BPH-2 were higher than that of their native form (HBPC-1, Figure 2 and 3). Briefly, an increasing of DH values caused an increase on PS values of BPH-2 and BPH-4 hydrolysates. At all pHs, PS values of BPH-3 and BPH-4 were also higher than that of their native form (HBPC-2, Figure 2 and 3).

Although the lowest solubility value of concentrates was generally observed at pH 6, PS value of all hydrolysates increased an average of 45% at that pH (Figure 3). The improvement of PS property of hydrolysates at all pHs could be explained with decreasing of the size of protein molecule, increasing of the polar groups on molecule during hydrolysis or increase in the interactions between water and hydrophilic groups and resulting in the changing of the ratio of hydrophilic to hydrophobic regions on protein molecule (Brückner-Gühmann *et al.*, 2018; Kong *et al.*, 2007; Ruiz-Henestrosa *et al.*, 2009; Wang *et al.*, 2008; Yalçın and Çelik, 2007).

Foaming properties of the protein concentrates

Effect of protein concentration on FV of HBPC-1 and HBPC-2 was shown in Figure 4. The amount of soluble proteins of concentrates obtained at relevant pH corresponded to PS results as given in Figure 2. The soluble proteins of HBPC-1 had higher FVs at all protein concentrations and pH points than that of the HBPC-2. The soluble proteins of HBPC-1 had the highest FV at 0.5% (w/v) concentration and pH 4 (110.4 cm³). When the concentration of HBPC-1 was increased to 1.0% (w/v), the lowest FV values were obtained at this concentration, such as 76.1 cm³ at pH 8 and 78.5 cm³ at pH 10. Besides, at pH 6, the highest FV was obtained at 1.0% (w/v) concentration (98.1 cm³) with the soluble proteins of HBPC-1. The FV values of HBPC-2 were not strongly dependent on protein concentration and pH of the medium. The highest and lowest FV values of HBPC-2 were

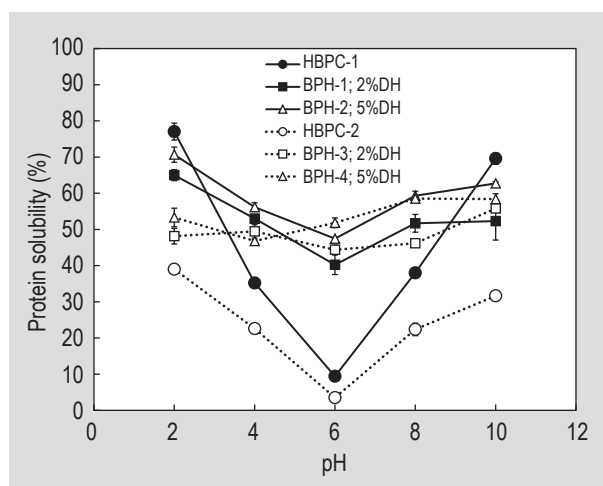


Figure 3. Protein solubility properties of hydrolysates and their native form at 0.25% (w/v) concentration and different pH points.

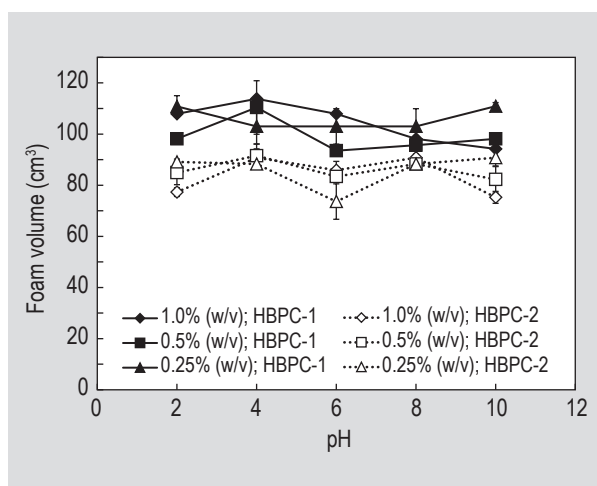


Figure 4. Effect of protein concentration on foam volumes of protein concentrates at different pH points.

obtained with 0.5% and 0.25% (w/v) concentrations at pH 4 (91.6 cm³) and pH 6 (73.6 cm³), respectively (Figure 4).

Effect of protein concentration on FHL of HBPC-1 and HBPC-2 was shown in Figure 5. At all concentrations, the FHL values of HBPC-2 were generally higher than that of the values of HBPC-1 at studied pH range. The FHL values of HBPC-2 were dependent on protein concentration and pH of the medium. At pH 4 and pH 8, the highest FHL value was determined as 30 min with the soluble proteins of HBPC-2 at 1.0% (w/v) concentration. The FHL values achieved at pH 4 (23.8 min) and pH 8 (20.0 min) at 0.5% (w/v) concentration with same concentrate followed that value. At pH 4 and 10, FHL values of the soluble proteins of HBPC-1 were dependent on protein concentration. At around isoelectric point (pH 6), the highest FHL value (8.1 min) was obtained at 0.5% (w/v) concentration with the soluble proteins of HBPC-1 (Figure 5).

The higher foam volume at an extreme pH values occurred because of an increased protein solubility and flexibility at the interface. Due to repulsive net charges, soluble proteins can diffuse to the air-water interface more rapidly and can encapsulate the air bubbles and can enhance foam volume (Adebowale and Lawal, 2004; Wang *et al.*, 2010; Yalçın *et al.*, 2008). In addition, decreasing of foaming properties of food proteins at isoelectric region was also displayed in previous studies (Bilgi and Çelik, 2004; Mwasaru *et al.*, 2000; Ragab *et al.*, 2004; Yalçın *et al.*, 2008). They reported that at around isoelectric region, the protein solubility and protein adsorption to the interface are very low because of that foaming properties decrease.

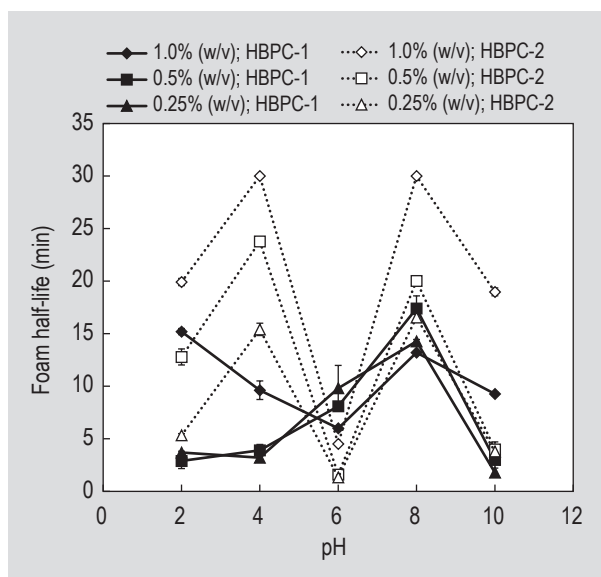


Figure 5. Effect of protein concentration on foam half-life of protein concentrates at different pH points.

Foaming properties of the hydrolysates

Effect of partial hydrolysis on FV of the soluble proteins of the hydrolysates and their native forms prepared at 0.25% (w/v) concentration were presented as a function of pH (2-10) in Figure 6. The amount of soluble proteins of hydrolysates and their native forms obtained at relevant pH corresponded to PS results given in Figure 3. Partial hydrolysis did not properly improve the foaming properties of concentrates. Besides, the BPH-1 and BPH-2 hydrolysates exhibited higher foaming properties than that of the BPH-3 and BPH-4 hydrolysates. However, FV values of BPH-1 and BPH-2 hydrolysates were not higher than that of their native form (HBPC-1) at all pH points (Figure 6). In addition, the soluble proteins of BPH-2 exhibited higher FV values at pH 2 (88.3 cm³), pH 4 (85.9 cm³) and pH 10 (93.7 cm³) than that of the BPH-1. Among the hydrolysates, the highest FV was obtained with the soluble proteins of BPH-3 at pH 10 (95.7 cm³), FV of the soluble proteins of BPH-2 obtained at pH 10 followed (93.7 cm³) that value. These values were also higher than FV value of the soluble proteins of HBPC-2 found at same pH (Figure 6). Among the hydrolysates, the lowest FV was observed with the soluble proteins of BPH-3 at pH 4 (71.1 cm³). It was observed that the isoelectric point region of the hydrolysates generally changed to pH 4 as also reported by Yalcin and Celik (2007) previously. Besides, the FV values of the soluble proteins of all hydrolysates considerably increased at pH 6 (Figure 6).

The soluble proteins of all hydrolysates generally exhibited the highest FHL values at pH 8. Among the hydrolysates, the highest FHL value was obtained with the soluble proteins of BPH-2 at pH 8. Partial hydrolysis caused an increased on FHL values of the all hydrolysates at pH 6 compared to their native forms (data not presented). At the isoelectric point, protein-protein interaction, that is favoured, caused to stronger interfacial films at the air/water interface in

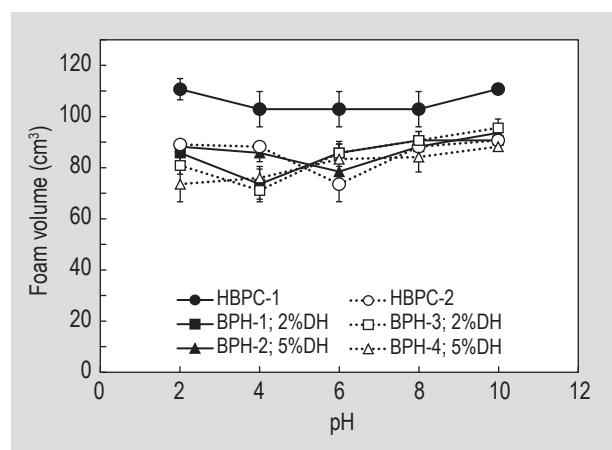


Figure 6. Effect of partial hydrolysis on foam volumes of hydrolysates and their native forms prepared at 0.25% (w/v) concentration and different pH points.

protein concentrates and hydrolysates and this stabilised the foam much tightly (Damodaran, 2005; Wang *et al.*, 2010). An increasing of DH from 2 to 5% resulted in better FHL values for the hydrolysates of HBPC-1 compared to the hydrolysates of HBPC-2. This situation may arise from the differences in composition and molecular weights of proteins and also different hydrolysis attack of the protease enzyme on protein concentrates. Brückner-Gühmann *et al.* (2018) stated that unhydrolysed protein would contribute to the formation of thick and stable films at the air/water interface and partial hydrolysis would reduce the thickness of the interfacial layer. Kapp and Bamforth (2002) also reported that limited proteolysis of barley hordein by ficin caused to an enhancement of foam stability, but the extended proteolysis was unfavourable.

Water holding capacity and oil absorption capacity properties

WHC and OAC values of the protein concentrates and hydrolysates were shown in Table 2. WHC value is a measurement of the binding of water by polar side chains of the protein, whereas OAC value is a measurement of fat-binding capacity of proteins through nonpolar side chains (Wang *et al.*, 2010). Accordingly, WHC values of HBPC-1 and HBPC-2 were found as 2.21 g H₂O/g and 2.34 g H₂O/g, respectively ($P<0.05$). After partial hydrolysis, WHC values decreased for the hydrolysates of both concentrates, thus WHC values of BPH-1 and BPH-2 were found as 1.53 g H₂O/ g and 0.95 g H₂O/ g, respectively ($P<0.05$). Besides, although WHC values of BPH-3 and BPH-4 were slightly higher than that of the values of BPH-1 and BPH-2, WHC results of all hydrolysates were significantly different ($P<0.05$). WHC values of BPH-3 and BPH-4 were also found as 1.62 g H₂O/g and 1.12 g H₂O/g, respectively ($P<0.05$).

OAC value of HBPC-1 (4.72 g oil/g) was significantly higher than that of HBPC-2 (1.86 g oil/g, $P<0.05$, Table 2). It may

be stated that HBPC-1 had more hydrophobic side chains than HBPC-2. OAC of the concentrates also decreased due to partial hydrolysis, for instance increasing DH from 2 to 5% caused a decline in OAC values of all hydrolysates, except for BPH-1. In addition, OAC value of BPH-1 was significantly higher than the other hydrolysates ($P<0.05$). Due to enzyme hydrolysis, hydrophobicity of the proteins probably decreased. It was indicated that although barley proteins (hordein, albumin, and globulin) possessed similar nonpolar amino acid profiles, WHC and OAC values were relatively low (Wang *et al.*, 2010). The same authors also reported that the OAC value of hordein was found as 2.1 g oil/g sample.

4. Conclusions

Extraction conditions played a major role on protein extraction efficiency, protein content and some physicochemical properties of HBPCs. SDS-PAGE patterns revealed that there were significant differences between protein band patterns of both concentrates. Protein solubility properties of protein concentrates were not greatly influenced by increased protein concentration, however mostly influenced from the pH of the medium. While the soluble proteins of HBPC-1 exhibited better foaming abilities at all protein concentrations and pH values, the soluble proteins of the HBPC-2 presented better foam stability values. The partial hydrolysis did not properly improve the foaming properties of concentrates. Then, increasing protein solubility at neutral pH levels due to partial hydrolysis may expand the possible use of the barley protein hydrolysates in high protein beverages and foam-type foods. These results suggest that the HBPC-1 could be recommended to be used in foam type food products. Further studies are needed to investigate some factors affecting solubility and other functional properties of hull-less barley proteins.

Table 2. Water holding capacity (WHC) and oil absorption capacity (OAC) of the hull-less barley protein concentrates (HBPCs) and barley protein hydrolysates (BPHs).

Barley sample	WHC (g H ₂ O/ g sample) ^{1,2}	OAC (g oil/ g sample) ^{1,2}
HBPC-1	2.21±0.078b	4.72±0.156a
BPH-1 (2%DH)	1.53±0.010d	2.00±0.098b
BPH-2 (5%DH)	0.95±0.009f	1.62±0.122d
HBPC-2	2.34±0.031a	1.86±0.068c
BPH-3 (2%DH)	1.62±0.037c	1.59±0.028d
BPH-4 (5%DH)	1.12±0.012e	1.54±0.145d

¹ Values followed by the different letter in the same column are significantly different ($P<0.05$).

² Mean ± sd, n=3.

Acknowledgements

Supported by a grant from Bolu Abant Izzet Baysal University Scientific Research Projects Coordination Unit, Project# BAP-2007.09.01.266.

References

- Adebowale, K. O., and Lawal, O. S., 2004. Comparative study of the functional properties of bambarra groundnut (*Voandzeia subterranean*), jack bean (*Canavalia ensiformis*) and mucuna bean (*Mucuna pruriens*) flours. *Food Research International* 37: 355-365.
- Adler-Nissen, J., 1986. *Enzymatic hydrolysis of food proteins*, 1st edition. Elsevier Applied Science Publishers, New York, NY, USA.
- Ahmedna, M., Prinyawiwatkul, W. and Rao, R.M., 1999. Solubilized wheat protein isolate: functional properties and potential food applications. *Journal of Agricultural and Food Chemistry* 47: 1340-1345.
- Aluko, R.E., McIntosh, T. and Reaney, M., 2001. Comparative study of the emulsifying and foaming properties of defatted coriander (*Coriandrum sativum*) seed flour and protein concentrate. *Food Research International* 34: 733-738.
- American Association of Cereal Chemists International (AACCI), 2010. *Approved methods of analysis*, 11th edition. Methods No: 44-15.02 and 46-12.01. AACCI, St. Paul, MN, USA.
- Bilgi, B. and Çelik, S., 2004. Solubility and emulsifying properties of barley protein concentrate. *European Food Research and Technology* 218: 437-441.
- Brückner-Gühmann, M., Heiden-Hecht, T., Sözer, N. and Drusch, S., 2018. Foaming characteristics of oat protein and modification by partial hydrolysis. *European Food Research and Technology* 244: 2095-2106.
- Celus, I., Brijs, K. and Delcour, J.A., 2006. The effects of malting and mashing on barley protein extractability. *Journal of Cereal Science* 44: 203-211.
- Cepeda, E., Villaran, M.C. and Aranguiz, N., 1998. Functional properties of faba bean (*Vicia faba*) protein flour dried by spray drying and freeze drying. *Journal of Food Engineering* 36: 303-310.
- Chandi, G.K. and Sogi, D.S., 2007. Functional properties of rice bran protein concentrates. *Journal of Food Engineering* 79: 592-597.
- Damodaran, S., 1997. Food proteins – an overview. In: Damodaran, S. and Paraf, A. (eds) *Food proteins and their applications*, 1st edition. CRC Press, New York, NY, USA, pp. 1-24.
- Damodaran, S., 2005. Protein stabilization of emulsions and foams. *Journal of Food Science* 70: 54-66.
- Foegeding, E.A. and Davis, J.P., 2011. Food protein functionality: a comprehensive approach. *Food Hydrocolloids* 25: 1853-1864.
- Harris, E.L.V. and Angal, S., 1989. *Protein purification methods. A practical approach*, 1st edition. IRL Press at Oxford University Press, Oxford, UK.
- Howell, N., 1996. Chemical and enzymatic modifications. In: Nakai, S. and Modler, H.W. (eds.) *Food proteins: properties and characterization*. VCH Publishers Inc., New York, NY, USA, pp. 167-234.
- Kapp, G.R. and Bamforth, C.W., 2002. The foaming properties of proteins isolated from barley. *Journal of the Science of Food and Agriculture* 82: 1276-1281.
- Keskin, Ş., Yalçın, E. and Özkaya, H., 2018. Effects of storage and granary weevil infestation on gel electrophoresis and protein solubility properties of hard and soft wheat flours. *Journal of Economic Entomology* 111: 1454-1460.
- Kong, X., Zhou, H. and Qian, H., 2007. Enzymatic preparation and functional properties of wheat gluten hydrolysates. *Food Chemistry* 101: 615-620.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193: 265-275.
- Mwasaru, M.A., Muhammad, K., Bakar, J. and Che Man, Y.B., 2000. Influence of altered solvent environment on the functionality of pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. *Food Chemistry* 71: 157-165.
- Newman, R.K. and Newman, C.W., 2008. *Barley for food and health: science, technology and products*. Wiley Press, Hoboken, NJ, USA.
- Osborne, T.B., 1924. *The vegetable proteins*. Longmans Green & Co, London, UK.
- Ragab, D.D.M., Babiker, E.E. and Eltinay, A.H., 2004. Fractionation, solubility and functional properties of cowpea (*Vigna unguiculata*) proteins as affected by pH and/or salt concentration. *Food Chemistry* 84: 207-212.
- Ruiz-Henestrosa, V.P., Sanchez, C.C., Pedroche, J.J., Milla, F. and Rodriguez Patino, J.M., 2009. Improving the functional properties of soy glycinin by enzymatic treatment. adsorption and foaming characteristics. *Food Hydrocolloids* 23: 377-386.
- Wang, C., Tian, Z., Chen, L., Temelli, F., Liu, H. and Wang, Y., 2010. Functionality of barley proteins extracted and fractionated by alkaline and alcohol methods. *Cereal Chemistry* 87: 597-606.
- Wang, J.S., Zhao, M.M., Bao, Y., Hong, T. and Rosella, C.M., 2008. Preparation and characterization of modified wheat gluten by enzymatic hydrolysis-ultrafiltration. *Journal of Food Biochemistry* 32: 316-334.
- Wu, Y.V., Sexson, K.R. and Sanderson, J.E., 1979. Barley protein concentrate from high-protein, high-lysine varieties. *Journal of Food Science* 44: 1580-1583.
- Yalçın, E., 2010. Effect of partial removal of prolamins on some chemical and functional properties of barley flours. *Food Science and Biotechnology* 19: 735-742.
- Yalçın, E. and Çelik, S., 2007. Solubility properties of barley flour, barley protein isolate and hydrolysates. *Food Chemistry* 104: 1641-1647.
- Yalçın, E., Çelik, S. and Ibanoglu, E., 2008. Foaming behaviors of barley protein isolate and hydrolysates. *European Food Research and Technology* 226: 967-974.
- Zayas, J.F., 1997. *Functionality of proteins in foods*. Springer, Berlin, Heidelberg, Germany, 373 pp.
- Zhang, W., Waghmare, P.R., Chen, L., Xu, Z. and Mitra, S.K., 2015. Interfacial rheological and wetting properties of deamidated barley proteins. *Food Hydrocolloids* 43: 400-409.

Zhao, J., Tian, Z. and Chen, L., 2010. Effects of deamidation on structure and functional properties of barley hordein. *Journal of Agricultural and Food Chemistry* 58: 11448-11455.

Zhao, J., Tian, Z. and Chen, L., 2011. Effects of deamidation on aggregation and emulsifying properties of barley glutelin. *Food Chemistry* 128: 1029-1036.