J Amer Oil Chem Soc (2007) 84:281–288
DOI 10.1007/s11746-006-1027-z

ORIGINAL PAPER

Barley Protein Isolate: Thermal, Functional, Rheological, and Surface Properties

Abdellatif Mohamed · Mila P. Hojilla-Evangelista · Steve C. Peterson · Girma Biresaw

Received: 5 September 2006 / Accepted: 28 November 2006 / Published online: 20 January 2007

Abstract Barley protein isolate (BPI) was extracted in 0.015 N NaOH in a 10:1 ratio solvent:flour and was precipitated by adjusting the pH to 4.5 and freeze-dried. The thermal properties of BPI were determined using modulated differential scanning calorimetry (MDSC). BPI with 4% moisture content exhibited a glass transition (T_g) with 140 °C onset, 153 °C middle, and 165 °C end temperatures and a ΔC_p of 0.454 J/g per °C. The high moisture content sample (50%) showed a T_g at 89, 91, or 94 °C and 0.067 ΔC_p. Acetylation had no apparent effect on the foaming and emulsifying properties of protein from barley flour but exhibited the least-stable foam among BPI samples. Foaming capacities of both barley protein isolates were ~12% less than that of acid-precipitated soy protein isolate reported in the literature. Acetylated BPI showed the highest surface hydrophobicity compared to the other samples. The surface-tension test confirmed that unmodified and modified BPI possessed surface activity. BPI phosphorous oxycloride-crosslinked was the most effective in lowering the surface tension of aqueous NaCl, while the crosslinked BPI was the least effective. The G' value of BPI suspension was greater than G'' at all frequencies from 0.1 to 100 rad/s. The strain value at which linear behavior ceased and nonlinear behavior began ranged from 3 to 10%.

Keywords Barley · Proteins · Crosslinking · Acetylation · Rheology · Extraction · Foaming · DSC · Glass transition · Surface tension

Names are necessary to report factually on available data; however, the United States Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

A. Mohamed (✉) · S. C. Peterson · G. Biresaw
Cereal Products and Food Science Research,
National Center for Agricultural Utilization Research,
Agricultural Research Service,
United States Department of Agriculture,
1815 N. University Street, Peoria, IL 61604, USA
e-mail: mohameda@ncaur.usda.gov

M. P. Hojilla-Evangelista
Plant Polymer Research,
National Center for Agricultural Utilization Research,
Agricultural Research Service,
United States Department of Agriculture,
1815 N. University Street, Peoria, IL 61604, USA

Introduction

Barley is the fourth most important cereal in the word after wheat, rice and corn. Storage proteins are present in all grains. They form the filler between starch granules in the endosperm and provide a nitrogen source during seed germination. These proteins are known as prolamines because they are generally rich in proline and glutamine amino acids. Hordein is the largest protein fraction (40–50%) of barley storage proteins. Hordein is soluble in aqueous alcohol and consists of four fractions designated as D, C, B, and A based on their mobilities in reversed-phase high-performance liquid chromatography (HPLC) analysis [1]. The average protein content of barley (hordein) is reported between 11 and 16%. The protein content of barley flour can be altered by air classification.

Robin and Pomeranz [2] reported a 2.5% average increase in protein content at 65% milling extraction. Pomeranz analyzed the high protein fraction for their

amino acid composition and reported low lysine (Lys), arginine (Arg), aspartic acid (Asx), threonine, glysine, alanine, valine (Val), methionine, and isolucine, while the same fraction was high in glutamic acid, proline, and cysteine [3]. The large shift in protein content with air classification suggests new uses for barley flour such as high-protein products and the low-protein content fraction for malting and brewing. Barley prolamine with high and equal electrophoretic mobility were low in SH groups, and contain amino acid composition similar to that of wheat gliadins. The molecular weights of barley proteins range between 43 and 44 kDa [4]. In the same publication, Ewart [4] also pointed out the similarities between hordein and wheat gliadins with two obvious differences: hordein contained more Pro, Lys, and Val, but less Asx, and the ratio of polar to nonpolar amino acid residues was very low [4, 5].

Most protein extraction procedures involve the original solvents of Osborn [6], where water extracts albumins, salt solubilizes globulins, 70% ethanol extracts prolamine (gliadins), and acidic or basic solutions solubilizes glutenin. These fractions are interrelated due to the presence of protein fraction contaminants in their electrophoretic bands [7]. Different extraction enhancers such as 2-mercaptoethanol and 0.5% sodium dodecyl sulfate (SDS) were used to extract up to 94% of barley proteins [8–10].

Transglutaminase (TG) is an enzyme that catalyzes protein crosslinking (XL) through inter- or intramolecular ε(γ-glutamyl)lysine isopeptidic bond formation. These bonds cause the polymerization of proteins [11, 12]. Unlike phosphorous oxycloride, TG-catalyzed reactions have been used extensively to modify the functional properties of various food proteins, including beef actomyosin, oat globulin, pea legumin, and oat, rice, and maize prolamine [13–18]. The effects of TG XL on the physico-chemical properties of milk proteins have been investigated [19]. These studies have focused on the modification of both whey and casein fractions of milk protein. TG has also been used to crosslink milk proteins with other proteins to form films and other biopolymers [19]. A mutant form of C hordein with a cysteine residues inserted at the N and C terminals had more elastic protein contributing to wheat-flour dough strength [20].

Proteins isolates or concentrates are important ingredients in the food industry. Solubility and emulsifying properties such as emulsion stability or capacity are important for their use in different applications [21]. The objectives of this work were to prepare BPI and determine the effect of isolation, acetylation and crosslinking methods on the protein solubility, structure, thermal, rheological and surface properties.

Materials and Methods

Materials and Protein Isolation

Barley flour was obtained from Honeyville Grains, Honeyville, Salt Lake City, UT. Barley flour was defatted with hexane in a 4:1 ratio. The defatted barley flour was dispersed by adding 50 mL of 0.015 N NaOH to 5 g of barley flour (10:1) at room temperature. The slurry was stirred using a magnetic stirrer in room temperature for 1 h and was then centrifuged at 4,000*g* for 10 min at 10 °C. The precipitate was dissolved again in 0.015 N NaOH stirred for 1 h and centrifuged at 4,000*g* for 10 min. The combined supernatant from each extraction was acidified with 2 N HCl to pH 4.5. The supernatant was centrifuged at 10,000*g* for 20 min. The precipitate was BPI.

Moisture

The moisture contents of the flour and the isolated BPI were determined using a thermogravimetric method by heating a pre-weighed sample for 1 h at 150 °C. All runs were carried out in triplicate.

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to examine possible aggregate formation during BPI isolation. SDS-PAGE was performed according to Laemmli [22], as described by Khan et al. [23], using 11.8% acrylamide and 0.1% bis-acrylamide for the separating gel. The stacking gel was prepared with 4.5% acrylamide and 0.1% bis-acrylamide. Precipitate samples were analyzed as native or reduced with DTT to test if proteins formed aggregates involving disulfide bonds.

Differential Scanning Calorimetry (DSC)

Precipitate samples were analyzed using a TA Instruments, modulated MDSC™ 2920 (TA Instruments, New Castle, DE). Sets of low (4.0%) moisture content and high (50%) moisture samples were analyzed. For each run, a 50 mg sample was sealed in a stainless-steel pan and heated to 250 °C at 3 °C per min, with 24 cm^3 per min nitrogen flow rate.

Surface Hydrophobicity

Surface hydrophobicity was determined by a method based on Kato and Nakai [24] using a Perkin Elmer LS50B Luminescence Spectrometer. BPI (200 mg) was

dispersed in 15 mL of 0.01 M phosphate buffer (pH 8.0) and stirred for 3 h at 35 °C. The suspension was centrifuged at 4,000*g* at 10 °C for 20 min and the supernatant was recentrifuged again for 20 min. One milliliter of the supernatant was added to 4.0 mL Biuret reagent, mixed immediately, left to stand for 30 min, and the UV absorbance was read at 540 nm (1.0 mL pH 8.0 buffer used as the blank). The standard curve dilutions were 1:2, 1:5, 1:8, and 1:10 using 0.01 M phosphate buffer, pH 8.0. The first scan was treated as the excitation wavelength. The initial emission wavelength was 484 nm, and the instrument was set to scan 200–470 nm. The peak excitation wavelength was used to optimize the selection of the emission scan parameters. The instrument was scanned from about 20 nm above the excitation peak (~480 nm) to 600 nm. The results were verified by recording the peak emission wavelength with the maximum fluorescence intensity (FI), which was found to be 470 nm. Averages of two blank runs were performed (scans were repeated until agreement was <2 nm). The final FI value was obtained by subtracting the FI of the blank from the FI of the sample.

Acetylation

Two acetylation procedures were used. Procedure A was as follows. 2 g BPI was dissolved in 120 mL sodium acetate solution (55 g sodium acetate/120 mL distilled water). The mixture was placed in an ice bath, stirred, add 2.4 mL acetic anhydride was added to the protein solution [25]. The pH was stabilized with a pH-stat attached using 1 N NaOH. The protein solution was dialyzed against distilled water for 24 h and freeze-dried.

Procedure B was as follows. 2 L of 30 mM phosphate buffer, pH 7.6, were prepared by dissolving 1.288 g sodium phosphate monobasic, monohydrate and 7.190 g sodium phosphate dibasic, anhydrous in 2 L distilled water. To the 2 L phosphate buffer, 30 g of BPI was added, mixed, placed in an ice bath, and stirred. Slowly, 45 mL acetic anhydride was added to the protein solution, where the pH was stabilized at 7.6 using 1 N NaOH. The acetylated protein solution was dialyzed against distilled water for 24 h and freeze-dried.

Crosslinking and Solubility

BPI was crosslinked using 0.1, 0.2, and 0.3% phosphorus oxycloride at pH 10. Phosphorus oxycloride was added to the barley protein isolate suspension (1 g in 30 mL distilled water). The crosslinking agent was added slowly while the suspension was stirred for 2 h. The crosslinked BPI was freeze-dried. A portion of the crosslinked material was used to determine the effect of crosslinking on the BPI solubility as an indicator of the degree of crosslinking.

Solubility was determined by dispersing 1 g of the BPI samples in 30 mL 0.015 N NaOH. Samples were stirred at 30 °C for 1 h and centrifuged at 3,000*g* for 15 min at 4 °C. The supernatant was then analyzed for protein content using a LECO CHN-2000 instrument (Feuerwerkerstr, 39 Ch-3602 Thun, Switzerland).

Emulsifying and Foaming Properties

The emulsification activity index (EAI, in m^2/g) and emulsion stability index (ESI, in min) were determined by following the method of Wu et al. [26]. Homogenization was done on mixtures of 2 mL corn oil and 6 mL sample solutions (1 mg protein per mL, pH adjusted to 7.0) by using a handheld homogenizer (Model 1000, Omni International, Waterbury, CT) operated at the high setting (20,000 rpm) for 1 min. Aliquots (50 μL) were pipetted out from the bottom of the homogenized mixture at 0 and 10 min, and then diluted with 5 mL of 1% SDS solution. UV absorbance was read at 500 nm. EAI and ESI values were calculated by using the equations of Wu et al. [26].

The oaming capacity and stability of the samples (10 mg protein per mL) were determined at the pH where the protein solubility was greatest by adapting the procedure described by Myers et al. [27]. Twenty milliliters of the sample protein solution (10 mg protein per mL), adjusted to pH 7.0, was pipetted into a 250-mL graduated column with a coarse fritted disk at the bottom. Air was introduced into the column from the bottom at a flow rate of 100 mL/min and 20 psi. The foaming time was started at the first appearance of air bubbles. The foam capacity was the volume (mL) of foam produced in 1 min. Foam stability was expressed as the percentage of foam remaining after standing for 15 min.

Surface Tension

Surface-tension measurements were conducted as a function of protein concentration. Samples were extracted in 0.5 M NaCl solution by suspending 0.35 g of BPI in a liter of NaCl solution. The suspension was stirred for 2 h and centrifuged at 300*g* for 20 min. The supernatant was used for the surface-tension analysis. Surface tension was measured using the axisymmetric drop-shape analysis method [28] on an FTA 200 automated goniometer (First Ten Angstroms, Portsmouth, VA 23704). The BPI solution was placed in a

10 mL disposable syringe, equipped with a 17 gauge (1.499 mm OD) blunt disposable needle (Becton Dickinson & Co., Franklin Lakes, NJ). The syringe was locked into place on the instrument and surface-tension measurement was conducted in the automatic trigger mode. In this mode, the solution was pumped at the desired rate until a pendant drop with the specified volume was formed. This was then followed by an automatic acquisition of drop images for the specified duration, analysis of the images, and display of surface tension versus time data (spreadsheet and plot).

The data from each run were automatically saved as both a spreadsheet and a movie. The spreadsheet contained the time and surface tension for each image and the movie contained each of the drop images as well as calibration information. In all cases, the surface tension decreased with time and leveled off to a constant equilibrium value after a long period. The average equilibrium surface tension values of 3–5 repeat runs were used to characterize the surface tension of the aqueous BPI solutions. The automatic trigger mode was programmed to pump a total volume of 5–18 μL of solution at 1 μL/s, and acquire images at a rate of 0.067 s per image, with a post-trigger period multiplier of 1.25 between images, which allowed a total of 35 images to be captured during a total acquisition period of 789 s.

Rheology

A TA Instruments ARES Series V controlled-strain rheometer (New Castle, DE) running Orchestrator version 7.0.8.23 software was used to obtain rheological measurements. The geometry used was a stainless-steel 50-mm-diameter parallel-plate fixture. A circulating water bath was used to maintain the temperature at 25.0 ± 0.1 °C for all tests. For the rheological tests, samples contained 30% (w/w) solids which were added to water with gentle stirring, and then the sample was subjected to a Thinky ARE-250 (Tokyo, Japan) for 15 min (10 min mix, 5 min de-aerating) in order to further homogenize the sample.

Results and Discussion

SDS-PAGE

This method was used here to determine the extent of BPI aggregate formation during preparation, which is common when storage proteins are isolated using the acid precipitation procedure [29]. The formation of protein aggregates was obvious on the SDS-PAGE gel (Fig. 1) as indicated by the presence of high-molecular-weight (high-MW) protein molecules at the top of the gel compared to the barley flour control. Some high-MW protein molecules passed through the stacking gel into the separating gel, but stayed closer to the top of the gel (Fig. 1). The SDS-PAGE gel showed only the reduced form of the protein.

Differential Scanning Calorimetry

Protein isolation yield was 70%, while the protein content of the BPI was 90.5% dry basis. The modulated DSC testing of a low-moisture (4%) BPI sample exhibited a T_g at 140.7, 153.3, and 165.8 °C for the onset, middle, and end temperatures, respectively, with 0.413 J/g per °C ΔC_p (Fig. 2). The 50% moisture content sample had a glass transition at 123.2, 136, and 149.1 °C for the onset, middle, and end temperatures, respectively, with ΔC_p of 0.1169 J/g per °C. When measuring the glass transition, we measured the molecular mobility of the material. A drop in the T_g temperature was expected, because molecules were more mobile at higher moisture contents [29]. The sample was subjected to three heating and cooling

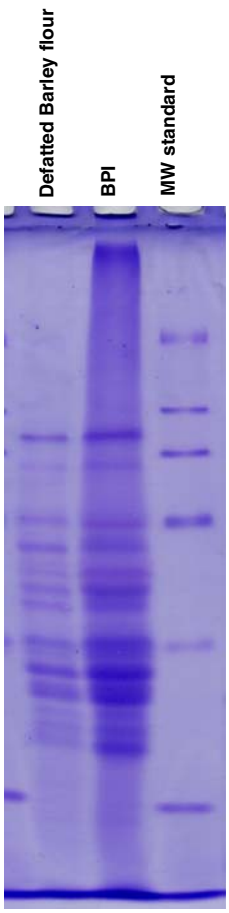

Fig. 1 SDS-PAGE profile of reduced native barley flour and BPI using phosphate buffer

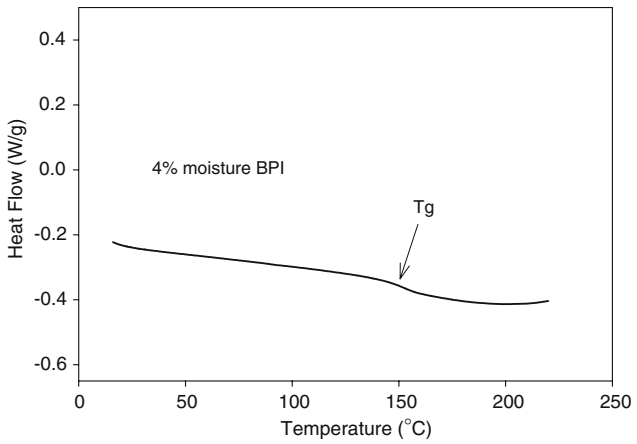

Fig. 2 DSC thermogram of BPI at 4% moisture content showing the glass transition (T_g)

cycles, in order to determine structural changes in the BPI. Cycling decreased the T_g temperatures but not the ΔC_p value. This indicated that BPI irreversibly denatured during the isolation process. That was in agreement with the SDS-PAGE analysis, where the profile indicated substantial aggregation (Fig. 1).

Surface Hydrophobicity

High surface hydrophobicity (S_o) value indicates high solubility, minimal aggregation, and possibly exposure of hydrophobic regions that are otherwise buried inside globular proteins due to denaturation. The S_o values of BPI changed significantly because of isolation, acetylation, and crosslinking. The acetylated BPI, using procedure A, showed the highest S_o value (61.5) while barley flour exhibited the lowest (26.8) among all the samples. Acetylation procedure B showed a much higher S_o (90.8), indicating a higher degree of modification. The product of procedure B was used for the remaining tests. Generally, a high S_o is considered a contributing factor to higher protein foaming capacity but is also correlated with bitter taste [28, 30]. The unmodified acid-precipitated proteins displayed S_o values between those of barley flour and the acetylated sample (44.5). The doubling of the S_o value relative to the flour was indicative of the exposure of the hydrophobic regions because of acid denaturation of the globular proteins, while the proteins present in the flour are native with limited dispersability due to aggregation. The chemical crosslinking had S_o values lower than the acid-precipitated samples (40.0), indicating that crosslinking caused the loss of a small number of the exposed hydrophobic regions on the surface of the protein. Crosslinking can lower protein dispersability, thus causing hydrophobic regions to approach each other resulting at a lower S_o value. Ahn et al. [31] reported a reduced S_o value after enzymatic crosslinking of wheat, barley, and soy proteins.

Crosslinking and Solubility

Phosphorus oxycloride is known to form a phosphate bridge or isopeptide linkage, resulting in a higher MW and lower water solubility [32]. The addition of 0.1, 0.2, and 0.3% phosphorus oxycloride gradually reduced the solubility of the BPI in water. Higher levels of phosphorus oxycloride used in the crosslinking reaction of the BPI was observed by a linear decrease of solubility (Fig. 3). Generally, crosslinking has also been shown to reduce S_o (Table 1).

Emulsifying and Foaming Properties

The emulsifying activity index (EAI) of protein from barley-flour samples produced substantial and very

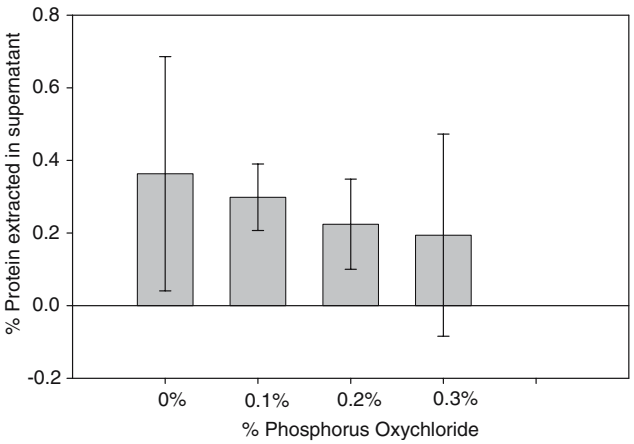

Fig. 3 BPI % solubility as a function of % phosphorous oxycloride

Table 1 Surface hydrophobicity

Sample	S_o[a]
Defatted barley flour	26.8 ± 4.6
BPI	44.5 ± 3.8
Acetylated method	
A	61.5 ± 4.5
B	90.8 ± 6.4
Crosslinked BPI[b]	
0.1 (%)	41.3 ± 7.8
0.2 (%)	47.1 ± 3.0
0.3 (%)	41.1 ± 3.1

[a] Surface hydrophobicity

[b] Percentage of phosphorus oxycloride used

Table 2 Foaming and emulsifying properties of barley protein

Sample	Foaming capacity (mL)	Foam stability (% foam left)	EAI (m^2/g protein)	ESI (min)
Flour, defatted	117 ± 3 a	70.5 ± 1.1 ab	70.6 ± 2.1 b	14.2 ± 0.7 a
Flour, acetylated	107 ± 12 a	81.4 ± 1.3 a	67.0 ± 2.1 c	13.7 ± 0.4 ab
BPI, acid-precipitated	116 ± 13 a	65.8 ± 12.4 b	83.1 ± 1.2 a	13.5 ± 0.2 ab
BPI, acetylated	114 ± 2 a	36.8 ± 3.2 c	82.8 ± 0.3 a	12.8 ± 0.5 b

Values are mean ± SD based on three replications. Means within a column followed by the same letter are not significantly different ($P > 0.05$)

stable foams (Table 2). High EAI values indicated very good emulsifying capacities, 4–7 times greater than the EAI values for defatted and low-fat soy flours reported by Heywood et al. [33]. However, the emulsions had poor stabilities (ESI values were similar to those that Heywood et al. observed for their low-fat soy-flour samples). Acetylation had no apparent effect on the foaming and emulsifying properties of BPI because protein-denaturing conditions are essential for high acetylation levels, to compensate for the steric effects of other adjuncts residues.

The BPIs had similar values for foaming capacity, emulsifying capacity (EAI) and emulsion stability. Acetylated BPI produced markedly less-stable foam than the acid-precipitated BPI, and was in fact the least-stable foam among those produced by barley flour and isolates tested. The foaming capacities of both BPI were ~12% less than that of acid-precipitated soy protein isolate. Foams produced by the acid-precipitated and acetylated barley protein isolates were also considerably less stable than foam produced by acid-precipitated soy-protein isolate (30 and 60% less foam left after standing 15 min, respectively). The EAI values of BPI were almost 50% greater than that of acid-precipitated soy protein isolate; ESI values were similar to lupin acid-precipitated proteins [29]. When compared with acid-precipitated lupin protein isolate, both BPIs had better foaming capacities and notably greater foam stabilities and EAI values, but the emulsions they formed were less stable than those of acid precipitate (AP) lupin protein isolates (ESI of 23 min) [29].

Surface Tension

The surface tension of BPI and modified BPI extracted with aq 0.5 M NaCl were measured as a function of concentration in aq NaCl solution. The modified BPIs were: acetylated (Ac-BPI), crosslinked (XL-BPI), and acetylated and crosslinked (Ac/XL-BPI). These four materials displayed a decreasing degree of solubility in aq NaCl solution in the order: XL-BPI > Ac/XL-BPI > Ac-BPI > BPI. Thus, crosslinking alone resulted in a 50-fold increase in the solubility of BPI, whereas acetylation alone resulted in a 10-fold increase in the solubility of BPI. The combined effect of crosslinking and acetylation on solubility of BPI was 30-fold.

All four materials reduced the surface tension of aqueous NaCl, indicating that they have surface-active properties. The equilibrium surface tension decreased with increasing concentration of BPI and modified BPI (Fig. 4) and reached a steady-state, constant value at very high concentrations. This was clearly demonstrated for XL-BPI and Ac/XL-BPI, for which surface tensions at high concentrations were measured. The data in Fig. 4 indicates that the surface tension of aqueous NaCl decreased in the order XL-BPI > Ac/XL-BPI > Ac-BPI > BPI, which was similar to the trend in the solubility of these materials in aq NaCl. Thus, BPI, which was the least soluble, showed the lowest surface tension indicating that it is the most effective of the four protein products in lowering the surface tension of aqueous NaCl. On the other hand, XL-BPI, which was the most soluble of the four protein products, was the least effective in lowering the surface tension of aqueous NaCl.

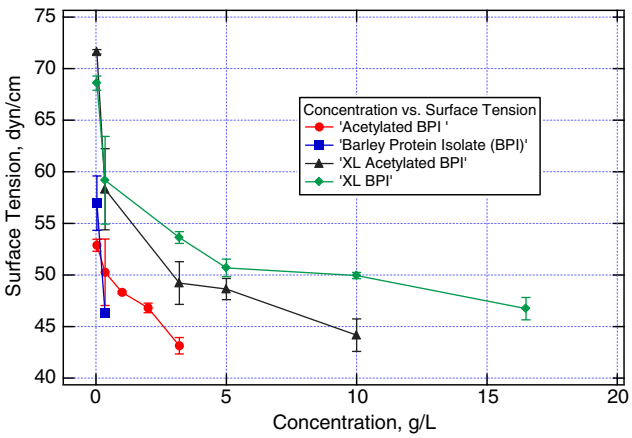

Fig. 4 Effect of BPI and modified BPI on the surface tension of aq NaCl

Rheology

Although all the samples were visibly homogeneous, consistent results in the magnitude of the shear storage modulus were difficult to obtain. Network strength of a gel-forming material can be judged from G', the shear storage modulus, which reflects the ability of a material to store elastic energy that is recoverable through elastic deformation of the bonds. Thus, when extensive crosslinking occurs in a system so that it becomes one vast network, G' increases.

Figure 5 shows the averaged strain sweep data for the barley flour and BPI samples. The reproducibility of the magnitude of G' was poor. Error bars for both the defatted barley-flour samples (empty points) and the acetylated and crosslinked BPI sample (filled down triangles) all had a fair amount of overlap. The symbols represent means of at least four runs for each sample. The rrror bars for the acetylated BPI (filled squares) were smaller than the width of the data points, however, as can be seen in Fig. 5, two different batches (from the same acetylation method) yielded two different G' values.

Acetylating defatted barley flour slightly decreased G', giving a slightly weaker gel. However, with barley protein, the process of acetylation increased G' quite significantly. When comparing acetylation with TG crosslinking [16], it appeared that crosslinking with TG had the opposite effect and reduced G', with the strongest reduction occurring in the sample that was acetylated and subsequently crosslinked [16]. This suggests that the TG crosslinking procedure does not build an extensive gel network, but perhaps forms more localized crosslinks that lead to aggregates or other smaller structures that yield a lower G' value for the entire sample. This was consistent with the reduced solubility associated with crosslinked proteins.

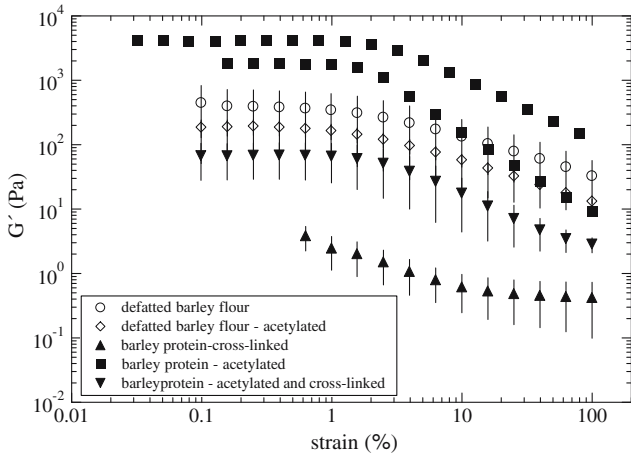

Fig. 5 Strain sweep data for 30% solids (w/w) barley flour and barley protein samples. All tests were carried out at 25 °C

Acknowledgments The authors would like to thank Jason Adkins for his technical support and Natalie Lafranzo for conducting the surface-tension measurements. The surface hydrophobicity of BPI was doubled compared to native protein in barley flour due to denaturation.

References

1. Benetrix F, Sarrafi A, Autran JC (1994) Effect of genotype and nitrogen nutrition on protein aggregates in barley. Cereal Chem 71:75–82
2. Robin GS, Pomeranz Y (1971) Composition and utilization of milled barley flour. I. Gross composition of roller-milled and air classification fractions. Cereal Chem 48(1):47–51
3. Pomeranz Y, Ward AB (1972) Composition and utilization of milled barley flour. II. Amino acid composition. Cereal Chem 49(2):240–246
4. Ewart JAD (1977) A fast moving β-gliadins from Cappelle–Desprez. J Sci Food Agric 28(12):1080–1023
5. Bernardin JE, Kasarda DD, Mecham DK (1967) Preparation and characterization of α-gliadins. J Biol Chem 242:445–450
6. Osborn TB (1907) The proteins of wheat kernel. Carnegie Institution, Washington DC
7. Ivanko S (1971) Changeability of protein fractions and their amino acid composition during maturation of barley grain. Biol Plant 13:155–162
8. Landry J, Moreaux T (1968) Heterogeneity of grains proteins: selective extraction due to amino acid composition. Bull Soc Chem Biol 52:1021–1030
9. Bietz JA, Shepherd KW, Wall JS (1975) Single kernel analysis of glutelin use in wheat genetics and breading. Cereal Chem 52:513–519
10. Shewry PR, Hill JM, Pratt HM, Legatt MM, Niflin BJ (1978) An evaluation of techniques for the extraction of hordein and dlutelin from barley seeds and a comparison of proteins composition of bomi and rios 1058. J Exp Bot 29:627–631
11. Folk JE, Cole PW (1966) Mechanism of action of guinea pig liver transglutaminase. J Biol Chem 241:5518–5525
12. Larré C, Kedzior ZM, Chenu MG, Viroben G, Gueguen J (1992) Action of transglutaminase on an 11S seed protein (pea legumin) influence of substrate conformation. J Agric Food Chem 40:1121–1128
13. Kim SH, Carpenter JA, Lanier TC, Wicker L (1993) Polymerization of beef actomicin induced by transglutaminase. J Food Sci 58(3):473–474, 491
14. Larré C, Denery-Papini S, Popineau Y, Deshayes G, Desserme C, Lefebvre J (2000) Biochemical analysis and rheological properties of gluten modified by transglutaminase. Cereal Chem 77:121–127
15. Porta R, Gentile V, Esposito C, Mariniella L, Auricchio S (1990) Cereal dietary proteins with sites for crosslinking by transglutaminase. Phytochemistry 29:2801–2804
16. Siu NC, Ma CY, Mine Y (2002a) Physicochemical and structural properties of oats globulin polymers formed by microbial Transglutaminase. J Agric Food Chem 50:2660–2665
17. Siu NC, Ma CY, Mine Y (2002b) Functional properties of oats globulin modified by calcium-independent microbial transglutaminase. J Agric Food Chem 50:2666–2672

18. Cao X, Mohamed A, Gordon SH, Willett JL, Sessa DJ (2003) DSC study of biodegradable poly(lactic acid) and poly(hydroxy ester ether) blends. Thermochim Acta 406:115–127
19. Yildirim M, Hettiarachchy NS (1993) Properties of film produced by crosslinking whey proteins and 11S globulins using transglutaminase. J Food Sci 63:248–252
20. Greenfield JJA, Ross-Murphy SB, Tamas L, Bekes F, Halford NG, Shewry PR (1998) Rheological properties of monomeric and polymeric forms of C-Hordein, a sulfur-poor prolamin of barley. J Cereal Sci 27:233–236
21. Wang CR, Zayas JF (1992) Emulsifying capacity and emulsion stability of soy proteins compared with corn germ flour. J Food Sci 57:726–73
22. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–686
23. Khan K, Tammiga G, Lukow O (1989) The effect of wheat flour on mixing and baking-correlations with protein fractions and high molecular weight glutenin subunit composition by gel electrophoresis. Cereal Chem 66:391–396
24. Kato A, Nakai S (1980) Hydrophobicity determined by a florescence probe method and its correlation with surface properties of proteins. Biochim Biophys Acta 13:624–628
25. Waniska RD, Shetty JK, Kinsella J (1981) Protein-stabilized emulsions: effect of modification on the emulsifying activity of BSA in model system. J Agric Food Chem 29:826–831
26. Wu WU, Hettiarachchy NS, Qi M (1998) Hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. J Am Oil Chem Soc 75:845–850
27. Myers DJ, Hojilla-Evangelista MP, Johnson LA (1994) Functional properties of protein extracted from flaked, defatted, whole corn by ethanol/alkali during sequential extraction processing. J Am Oil Chem Soc 71:1201–1204
28. Were L, Hettiarachchy NS, Kalapathy U (1997) Modified soy protein with improved foaming and water hydration properties. J Food Sci 2:821–824
29. Abdellatif Mohamed, Steven C. Peterson, Mila P. Hojilla-Evangelista, David J. Sessa, Patricia Rayas-Duarte, Girma Biresaw (2004) Effect of heat treatment and pH on the physicochemical properties of lupin protein. JAOCS 81:1153–1157
30. Babiker EE (2000) Effect of transglutaminase treatment on the functional properties of native and chymitrypsine-digested soy protein. Food Chem 70:139–145
31. Ahn HJ, Kim JH, Ng PKW (2005) Fuctional and thermal properties of wheat, barley, and soy flours and their blends treated with microbial transglutaminase. J Food Sci 70(6):380–386
32. Mathis G, Whitaker J (1984) Chemical phosphorylation of food proteins: an overview and a prospectus. J Agric Food Chem 32:699–705
33. Heywood AA, Myers DJ, Baily TB, Johnson LA (2002) Functional properties of low fat soy flour produced by an extrusion–expelling system. JAOCS 79:1249–1253