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Functional and Film-forming Properties of Fractionated Barley Proteins

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Abstract Barley proteins are expected to have unique functional properties due to their high content of alcohol soluble protein, hordein. Since the barley proteins obtained by conventional isoelectric precipitation method cannot represent hordein fraction, barley proteins were fractionated to albumin, globulin, glutelin, and hordein with respect to extraction solvents. Functional properties and film-forming properties of solubility-fractionated barley proteins were investigated to explore their potential for human food ingredient and industrial usage. The 100 g of total barley protein comprised 5 g albumin, 23 g globulin, 45 g glutelin, and 27 g hordein. Water-binding capacities of barley protein isolates ranged from 140-183 mL water/100 g solid. Hordein showed the highest oil absorption capacity (136 mL oil/100 g), and glutelin showed the highest gelation property among the fractionated proteins. In general, the barley protein fractions formed brittle and weak films as indicated by low tensile strength (TS) and percent elongation at break (E) values. The salt-soluble globulin fraction produced film with the lowest TS value. Although films made from glutelin and hordein were dark-colored and had lower E values, they could be used as excellent barriers against water transmission.

Keywords: barley protein, protein fraction, edible film, hordein, functional property

Introduction

Barley production accounts for 12% of worldwide grain production and is the 4th major cereal produced in the world after wheat, rice, and corn (1). Barley had been used for human consumption in Korea as an alternative to rice to compensate for rice production shortages. Most of the barley produced in western countries is used to make malt to produce beer and for animal feed and very little barley is used for human consumption. The taste and appearance of barley, along with its poor baking qualities, have limited its use in human foods. However, there has been growing research interest in recent years for the utilization of barley ingredients in a wide range of food applications such as β-glucan and dietary fiber in health foods.

The chemical composition of barley is influenced by genotypic and environmental factors, and the composition values usually fall between 60-64% starch, 8-15% protein, and 2-3% lipid (2). Barley protein can be classified as albumin, glutelin, globulin, and prolamin according to the media in which it is solubilized: water extracts albumin, salt solubilizes globulin, alkali solution solubilizes glutelin, and aqueous ethanol extracts prolamin (3).

The protein represents 8-12% of the barley endosperm and the major proteins in barley endosperm are the hordeins, the alcohol-soluble prolamins. Hordeins comprise 30-50% of the total grain proteins and the amount is reported to be proportional to the nitrogen content (4). Hordeins are complexes of subunit proteins. Hordeins consist of 2 major subunits, sulfur-rich B hordein and sulfur-poor C hordein, and 2 minor subunits, sulfur-rich γ-hordein and sulfur-poor D hordein (5).

Research interests about barley protein have been limited to the chemical composition, protein quality, and amino acid composition of barley, which are important factors in animal feed. To evaluate the potential of barley proteins for human food ingredients and industrial usages, extensive information about their functional properties are needed. However, only limited studies have been reported about the functional properties of barley proteins. For example, Yalçin and Çelik (6) reported the effect of pH on the solubility properties of hordein fraction. Bilgi and Çelik (7) and Mohamed *et al.* (8) reported solubility, emulsion capacity, and foaming property of barley proteins prepared by conventional isoelectric precipitation method. But these functional properties of isoelectric precipitated proteins could not be used for evaluating the potential of proteins for industrial application since many of hordein fractions had been removed during the protein preparation procedure. Water absorption, oil absorption, and gelation property of fractionated barley protein have not been reported to date.

In addition to the above functional properties, grain storage proteins have exhibited excellent film-forming properties. The formation of edible films by wheat gluten, rice protein, and sorghum protein could be attributed to the film-forming properties of the grain proteins. Edible films from plant proteins have been used as selective barriers against gases, vapors, and solute movement in heterogeneous foods or between food and its environment to extend shelf life and improve food quality (9). Among the plant proteins, soy protein films have been used to provide carrier materials for incorporating antimicrobials (10,11) and antioxidants (12). Corn zein (CZ) has been explored for the antioxidative packaging materials for extending shelf life of cooked turkey (13) and precooked pork patties (14).

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The film-forming properties of grain proteins are closely related to the functional properties of proteins (15). For example, CZ, an alcohol-soluble prolamin fraction, has exhibited unique film properties with high water vapor barrier property and heat sealability (16,17). Hordeins are insoluble in water, and the proline and glutamic acid composition of hordein is completely different from that of alkali-soluble glutelins. Therefore, different functional properties are expected from the protein fractions obtained using different extraction solvents.

Information is lacking on the functional properties and the related film-forming properties of different types of proteins in barley. In the present study, barley protein fractions were prepared by different extraction methods and the functional properties of the barley protein fractions were determined. The mechanical and barrier properties of edible films made from fractionated barley proteins were measured and compared with corn zein films and soybean protein isolate films.

Materials and Methods

Materials Hull-less barley (*Hordeum vulgare* L., naked type) was milled with a Bühler laboratory mill (Bühler Bros., Inc., Uzwil, Switzerland) and passed through a 140 mesh screen. Commercial soy protein isolates (SPI, Supro® 610; Protein Technologies International Inc., Ieper, Belgium) and corn zein (CZ, Grade F-4,000; Freeman Industries, Tuckahoe, NY, USA) were used. The protein concentrations of commercial protein products were 90.0% (d.b.) for SPI and 90.5% (d.b.) for corn zein. Other reagents, including glycerol, were analytical reagent grade.

Isolation of barley protein fractions Barley powders were defatted with hexane and suspended in 15 volumes of distilled water. The soluble protein fraction was extracted from the barley suspension by mixing at room temperature. The suspension was centrifuged and the albumin proteins were isolated by adjusting the supernatant pH to the minimum solubility pH. The barley precipitate was suspended in 15 volumes of 0.5 M NaCl. The suspension was centrifuged and the salt-soluble proteins (globulin) were obtained by precipitation at minimum solubility pH. The alkali-soluble glutelin proteins were extracted by suspending the albumin and globulin-free precipitate in 0.1 M NaOH at room temperature. The suspensions were centrifuged and the glutelin fractions were isolated by isoelectric precipitation. The remaining precipitate was freeze-dried and the alcohol-soluble protein fraction (hordein) was extracted with 8:2 (ethanol:water) aqueous alcohol. The protein-alcohol solution was concentrated and freeze-dried (Fig. 1).

Measurement of minimum solubility pH Protein-solvent suspensions from the extraction procedures were centrifuged at 25°C (3,000×g). The supernatant pHs were adjusted to 3.0-10.0 using 0.1 M HCl or 0.1 M NaOH, and solution turbidities at 320 nm were measured using a spectrophotometer. The solution pH with the highest turbidity was the minimum solubility pH.

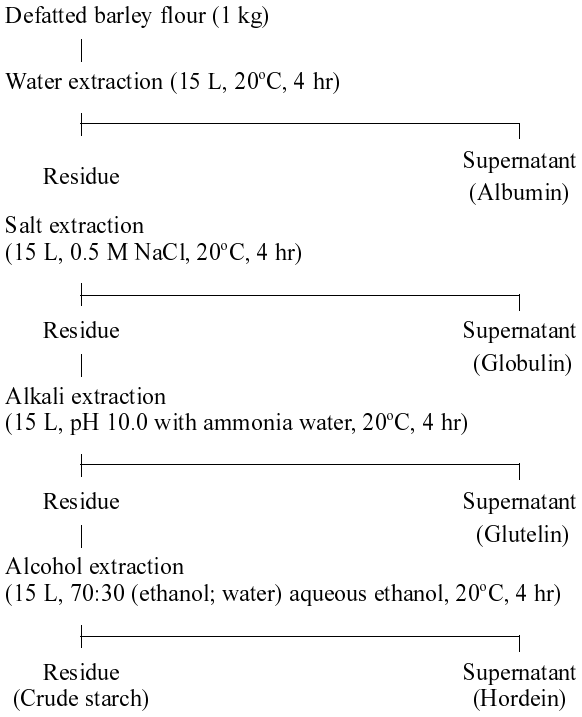

Fig. 1. Flow diagram for extraction of barley proteins.

Quantification of isolated protein fraction and their protein content The nitrogen content of barley protein was analyzed using the semi-micro Kjeldahl method. A conversion factor of 6.25 was used to calculate the protein content (18). The percentage of the fractionated protein in barley flour, recovery of protein fraction, and protein content of isolated protein fraction (protein content) were calculated as reported in Eq. 1, 2, and 3, respectively.

$$\text{Protein fraction in barley flour (\%)} = \frac{\text{Amount of protein extracted in the supernatant (g)}}{\text{Amount of defatted barley flour (g)}} \times 100 \quad (1)$$

$$\text{Recovery of protein fraction (\%)} = \frac{\text{Amount of protein in isolated protein fraction (g)}}{\text{Amount of protein extracted in the supernatant (g)}} \times 100 \quad (2)$$

$$\text{Protein content of isolated protein fraction (\%)} = \frac{\text{Amount of protein in isolated protein fraction (g)}}{\text{Amount of isolated protein fraction (\%)}} \times 100 \quad (3)$$

Functional properties of isolated protein fractions Water absorption was determined as described by Bae and Rhee (19). A 0.5 g sample was dispersed in 10 mL of distilled water. The contents were mixed for 30 sec using a glass rod and, after 30 min, centrifuged at 1,000×g for 30 min. The supernatant was carefully decanted; the weight of water absorbed in the sample was then measured. The

water absorption capacity of the protein isolate was expressed as the percentage increase of the sample weight. Oil absorption of the protein sample was determined as described by Beuchat with modification (20). Corn oil (10 mL) was added to 0.5 g protein sample in a centrifuge tube and the mixture was homogenized for 30 sec at 8,000 rpm using a homogenizer (T-25; IKA Labotechnik, Staufen, Germany). The protein dispersion was centrifuged at 990 ×g for 30 min after holding for 3 hr at room temperature. The free oil, separated after centrifugation, was pipetted off and oil absorption was calculated based on the weight of oil absorbed in the sample.

Gelation properties were investigated using the method described by Lawal (21). Barley albumin suspensions of 2-20% were prepared in distilled water, and glutelin suspensions of 2-20% were prepared in 0.1 M sodium borate buffer (pH 10). The globulin suspensions were prepared in 0.5 M NaCl solution. The 5 mL of dispersion from each preparation was transferred into a test tube and heated in a boiling water bath for 30 min. Then the test tubes were rapidly cooled to 15°C in a bath of cold water, and were cooled further at 4°C for 2 hr. The lowest gelation concentration was taken as the concentration at which the sample did not fall or slip from the inverted tube.

Preparation of protein film Three different solvent systems were applied to make films according to the characteristics of the protein sample. To make barley glutelin or SPI films, solutions were prepared by dissolving 2.0 g of glycerol and 4.0 g of barley glutelin or soy protein sample in 100 mL of distilled water. For globulin film, sample and glycerol were dissolved in 0.5 M NaCl. The film solutions were homogenized (T-25; IKA Labotechnik) at 10,000 rpm for 2 min. The pH of each solution was adjusted to 10.0 with 1.0 M ammonia water (28 g of ammonia/100 g of ammonia water). For barley hordein and CZ films, aqueous ethanol with 80:20 of ethanol:water and ethanol with 95% purity were used as the solvent systems, respectively, to dissolve 2 g of glycerol and 4 g of protein.

The solutions were heated to 90°C on a hot plate magnetic stirrer for 10 min and kept at room temperature for 5 min to allow bubbling to dissipate prior to pouring. All of the solutions in the beakers were poured onto 25×25 cm Teflon™ film-coated glass plates and dried overnight at room temperature. Three individual films were prepared for each sample.

Film thickness Film thickness was measured with a hand-held micrometer (Teclock, Nagano, Japan). Measurements to test mechanical properties were taken at 5 different locations on the film samples. To test water barrier properties, measurements were made at 7 different locations. The mean thickness was used to calculate the mechanical and barrier properties of the films.

Measurement of mechanical properties Ten specimens (150×25 mm) were cut from 3 individual films and conditioned at 25°C and 50% RH for 48 hr in an environmental chamber (Sang Woo Co., Seoul, Korea).

Texture analyzer (TA-XT2; Stable Micro Systems, Surrey, England) was used to measure tensile strength (TS, MPa) and percent elongation at break (E, %) according to ASTM Standard Method D 882-01 (22). Initial grip separation was set at 100 mm and cross-head speed was set at 50 mm/min.

Measurement of water vapor permeability Water vapor permeability (WVP) was calculated from Eq. 4:

$$WVP = WVTR(L/\Delta p) \quad (4)$$

where WVTR is the water vapor transmission rate in ng/m^2·sec of films measured at 25°C and 50% RH, L is the mean thickness of film specimens in m, and Δp is the actual difference in partial water vapor pressure between the 2 sides of film specimens in Pa.

The WVTR was determined gravimetrically using a modification of the ASTM Standard Method E 96-00 (23). The actual RH values of the film inside the cups and the film WVP values were calculated with the correction method reported by McHugh *et al.* (24). The mean of the initial and final stagnant air gap height was used in the calculation.

Color measurement Color values of soy protein films were measured with a Chroma meter (CR-200; Minolta Camera Co., Osaka, Japan). Film specimens were placed on the surface of a white standard plate (calibration plate CR-A43; L=95.91, a=0.05, and b=1.27) and Hunter L, a, and b color values were measured. From the Hunter color values, total color difference (ΔE) was calculated as:

$$\Delta E = \sqrt{(L-L')^2+(a-a')^2+(b-b')^2} \quad (5)$$

where L, a, and b values are Hunter color values of standard low density polyethylene (LDPE) film (L=95.79, a= –0.05, and b=1.73).

Statistical analysis Measurements were replicated 3 times for each type of film, with individually prepared films as the replicated experimental units. Statistics on a completely randomized design were performed by analysis of variance (ANOVA) with SAS (Release 9.13, SAS Institute Inc., Cary, NC, USA) software. Duncan's multiple range test was used to detect differences among film property mean values ($p<0.05$).

Results and Discussion

Extraction of barley protein Crude protein content of defatted barley flour was 10.3%. The total isolated barley protein comprised 4.9% albumin, 23.3% globulin, 45.6% glutelin, and 26.2% hordein (Table 1). Generally, the amount of albumin is relatively low (3 to 5% of the total protein) and considerable quantities of globulins (10 to 20% of the total protein) are found in the barley endosperm. The main components of barley protein are hordein (35 to 45%) and glutelin (35 to 45%) (25). The distribution of protein between the different solubility fractions reportedly depends on the variety and the agronomic conditions (26,27).

The minimum solubility pH of the fractionated protein isolates were pH 4.3 for albumin, pH 3.4 for globulin, and pH 5.0 for glutelin. Hordein, on the other hand, was solubilized in aqueous alcohol with 50:50 to 80:20 of

Table 1. Protein fraction in barley flour, recovery of the protein fraction, protein content of isolated protein fraction, and minimum solubility pH of barley protein fractions

Protein fraction	Protein fraction in barley flour (%)	Recovery of the protein fraction (%)	Protein content of fractionated protein (%)	Minimum solubility pH
Crude protein	10.3			
Albumin	0.5	81	85	4.3
Globulin	2.4	91	87	3.4
Glutelin	4.7	92	91	5.0
Hordein	2.7	87	92	-

Table 2. Functional properties of fractionated barley proteins

Protein fraction	Water absorption (mL/100 g solid)	Oil absorption (mL/100 g solid)	Gelation property (g/100 g)
Albumin	183.0±8.5[a]	112.3±2.9[c]	12.7±0.6[a]
Globulin	140.3±12.9[b]	125.7±2.5[b]	8.7±0.6[b]
Glutelin	156.7±3.2[b]	126.5±2.8[b]	8.2±0.3[b]
Hordein	145.7±5.9[b]	136.0±3.6[a]	-
SPI[1)]	161.1	122.2	7.0

[1)]Soy protein isolates (19).

ethanol:water (v/v) but did not dissolve in water.

The nitrogen contents of protein extraction solutions and their resulting protein powders showed that 81% of the albumin, 91% of the globulin, and 92% of the glutelin were recovered in the isolated proteins from the extraction solution. The protein contents of the lyophilized albumin, globulin, glutelin, and hordein fractions were 85, 87, 91, and 92 %, respectively (Table 1).

Functional properties of barley protein fractions Water absorption capacities (WAC) of barley protein isolates ranged from 140-183 mL water/100 g of protein isolates, and the values varied with the protein fractions obtained by using different extraction solvents. Among the solubility fractions of barley protein, albumin (183 mL water/100 g solid) showed the highest water absorption capacity. When the water binding capacities of globulin and hordein fraction of barley protein were compared with the reported WAC of SPI, WAC of globulin and hordein were lower than that of SPI (161.1 mL water/100 g solid, Table 2) (19). The factors responsible for the WAC of protein isolates are the thermodynamic properties of the system, the physico-chemical environment, and the solubility of the protein molecules (28). In the present study, the differences in water absorption between the protein fractions could be partly due to their different solubility properties.

The oil absorption capacities of barley protein isolates ranged from 112-136 mL oil/100 g protein and, among the solubility fractions of barley protein, hordein (136 mL oil/100 g) showed the highest oil absorption capacity (Table 2). The value observed here is comparable to that of lab-scale SPI (122 mL oil/100 g), as reported by Bae and Rhee (19). However, it is lower than 329 mL oil/100 g reported for commercial SPI (29). Oil absorption has been attributed to physical entrapment of oil in the protein isolates. Lin and Zayas (30) reported that the ability of protein to bind fat depends on nonpolar side chains that bind hydrocarbon chains, thereby contributing to increased oil absorption. Compared to whole barley protein, hordein had a higher proportion of proline, a non-polar amino acid (25,31). The high oil absorption capacity of hordein can be attributed to the higher hydrophobicity of the hordein fraction compared to that of the other solubility fractions.

Gelation capacities of barley protein fraction are presented in Table 2. A lower value of gelation property in Table 2 represents better gelation capacity, since the lowest gelation concentration was taken as the index of the gelation property. Therefore, glutelin exhibited the best gelation property among the solubility fractions. A protein gel is formed by the partial denaturation of individual proteins and the aggregation of protein to produce a 3-dimensional network capable of retaining significant amounts of water (21). Since the gelation mechanism of a protein is similar to the film formation mechanism (32), gelation properties seem to correlate highly with the film-forming properties of barley proteins.

Mechanical and water barrier properties of fractionated barley protein films The mechanical and water barrier properties of films from the barley protein fractions are presented in Table 3 and the film properties are compared with those of films from SPI and CZ. The fractionation of barley proteins according to their solubility not only influenced their protein functionalities but also affected film-forming properties of resulting barley protein fractions.

The TS and E of films prepared from barley protein fractions ranged from 2.1-6.4 MPa and 4.4-7.5%, respectively. Those values were significantly lower than those from SPI (8.4 MPa) and CZ (12.5 MPa) films ($p<0.05$).

In general, the barley protein fractions did not form a stable network for the film structure, and resulted in brittle and weak films as indicated by the low TS and E values. There were no significant differences in E values among the protein fraction films ($p>0.05$). However, significantly different TS values were obtained with respect to the barley protein fractions ($p<0.05$). The salt-soluble globulin fraction produced film with the lowest TS value and

Table 3. The mechanical properties and water vapor permeabilities (WVP) of edible films from barley protein fractions

Sample		Thickness (μm)	Tensile strength (MPa)	Elongation (%)	WVP ($ng \cdot m/m^2 \cdot Pa \cdot sec$)	RH (%)
Barley	Globulin	55.9±4.2	$2.1^{d1)}$±0.5	4.4^{c}±1.1	4.04^{a}±0.60	61.5±1.0
	Glutelin	63.5±4.4	6.4^{c}±0.9	5.9^{c}±1.5	2.75^{b}±0.24	69.1±2.2
	Hordein	69.3±2.6	4.9^{c}±1.4	7.5^{c}±1.7	1.91^{c}±0.29	71.2±3.2
SPI		85.9±4.6	8.4^{b}±0.9	50.8^{a}±16.9	3.67^{a}±0.86	64.0±2.7
Corn zein		78.3±3.6	12.5^{a}±2.9	21.8^{b}±17.9	2.05^{c}±0.46	70.8±1.4

[1)a-d]Means with different superscripts in a column are significantly different ($p<0.05$)

Table 4. Hunter color values (L, a, and b) of edible films from barley protein fractions

Sample		Thickness (μm)	L	a	b	ΔE
Barley	Globulin	67.0±3.5	78.58±0.48	2.49±0.04	16.38±0.75	22.49±0.34
	Glutelin	65.3±2.1	64.20±0.36	9.57±0.26	30.34±0.79	43.80±0.46
	Hordein	71.0±3.9	84.30±1.38	-0.26±0.02	17.38±1.48	19.06±0.62
SPI		81.5±1.7	93.97±0.12	-1.47±0.01	10.59±0.13	9.09±0.15
Corn zein		79.0±2.3	91.74±0.42	-3.87±0.18	18.93±0.29	17.60±0.16

glutelin had highest TS value among the films from protein fractions.

The lowest TS value of globulin film might be attributed to the presence of sodium chloride in the film. Generally, a small amount of cations can bind to negatively charged polar groups in proteins to form 3-dimensional networks in film (33). However, the excess amount of sodium chloride used in the present study might adversely affect the mechanical properties of the film. The lower TS and E value of hordein film could be attributed to the presence of protein particles in the film. Upon drying the film solution, the polarity of solvent would be increased by the different rate of evaporation between the solvent constituent. The presence of hordein particles were caused by the limited solubility of hordeins in the film solution at the last stage of solvent removal.

Water barrier properties of barley protein films also were affected by protein fractions (Table 3). Among the barley protein fractions, globulin showed the highest WVP value (4.04 $ng \cdot m/m^2 \cdot Pa \cdot sec$) and the hordein fraction showed the lowest WVP value (1.91 $ng \cdot m/m^2 \cdot Pa \cdot sec$). In spite of presence of partially unsolubilized particles, the water barrier property of barley hordein film was comparable to that of CZ film. The higher water barrier properties of hordein films are believed to originate from the hydrophobic nature of the protein. Hordein produced a mechanically weak edible film with high water barrier properties.

According to the above result, the brittle and weak mechanical properties of barley protein fractions have limited their application for wrapping materials in the form of packaging films. However, the poor mechanical properties of these protein fraction films might render their application inside of the heterogeneous foods with minimizing the loss of palatability by presence of film materials in the foods.

Color of fractionated barley protein films Hunter L, a, and b color values and the total color difference (ΔE) values of barley protein films were compared (Table 4). Barley protein fractions, soy protein, and CZ developed a yellowish coloration (darkening) as evidenced by the b (yellowness) values. The SPI and CZ films were light yellow and appeared more transparent. Barley protein fractions produced dark yellow films with lower L values compared to SPI and CZ films. The barley glutelin produced dark, brownish films with low L (64.2) and high a and b values.

The results of the study showed that hordein films could be used as excellent barriers for water transmission although they were dark colored and had relatively low TS and E values compared to the SPI films and CZ films. The hordein films may serve as promising edible film materials which can be applied inside of the food and provide a barrier membranes against moisture movement between the food ingredients.

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