

Extraction, purification and characterization of globulin from common buckwheat (*Fagopyrum esculentum* Moench) seeds

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Abstract

Salt-soluble globulin was extracted from common buckwheat (*Fagopyrum esculentum* Moench) seeds. The protein content of buckwheat globulin (BWG) was over 90%. Anion-exchange chromatography (with stepwise salt gradient elution) was used to obtain two fractions of BWG, corresponding to the basic and acidic polypeptides, respectively, at a ratio of approximately 1:2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that the acidic and basic polypeptides were linked by disulfide bonds. The basic polypeptide has an estimated molecular weight of 23–25 kDa, an isoelectric point in slightly alkaline region (pH 8–9), and showed a high degree of homology with other legumin-like proteins. Disulfide and sulfhydryl contents in BWG were estimated as 36.4 and 3.20 $\mu\text{M/g}$ of protein, respectively. BWG exhibited beneficial functional properties such as high solubility, emulsifying activity and emulsion stability, while the foaming properties were relatively poor. BWG had lower water holding capacity and comparable fat binding capacity when compared to a commercial soy protein product.

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1. Introduction

Cereal grains and legume seeds have been used as the main source of dietary proteins for human nutrition, but storage proteins in both crops are deficient to varying degrees in several essential amino acids. The major amino acid deficiency in legume seed proteins is the sulfur-containing amino acids, especially methionine, while cereal proteins are deficient in lysine (Shotwell & Larkins, 1989). Amongst the existing crops, common buckwheat (*Fagopyrum esculentum* Moench), an under-utilized pseudocereal, assumes special importance because of the short growth span and high nutritive value of its grains (Pomeranz & Robbins, 1972; Rout & Chrungoo, 1996). Buckwheat proteins have a high biological value due to well-balanced amino acid composition and are rich in lysine and arginine

(Pomeranz & Robbins, 1972). The salt-soluble globulin represents the major Osborne fraction of buckwheat seed proteins (Belozersky, 1975) and has been classified as a legumin-like storage protein (Derbyshire, Wright, & Boulter, 1976). Buckwheat globulin (BWG) can be a potential source of nutritive food ingredient for human consumption. In order to develop dietary proteins for utilization as ingredients in the food industry, it is necessary to determine the physicochemical and functional properties of the proteins, since the ultimate success for utilization depends largely upon desirable functional characteristics (Aluko & Yada, 1995; Nwanekezi, Alawuba, & Owuamanam, 1994).

In this study, globulin was extracted from buckwheat flour and its chemical composition was determined. BWG was fractionated by anion-exchange chromatography, and both BWG and the isolated fractions were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis to determine the molecular weights of their constituent polypeptides. Some physicochemical properties of BWG

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and the isolated fractions were assessed, and the N-terminal sequence of one BWG fraction, constituting the basic polypeptide, were determined and compared with other major plant storage proteins to assess their sequence homology. Some functional properties of BWG including solubility, water and fat binding capacity, and emulsifying and foaming properties were determined, and compared to a commercial soy protein product in order to evaluate its potential as a food ingredient.

2. Materials and methods

2.1. Extraction of buckwheat globulin

Buckwheat flour was obtained from Nikkoku Flour Milling Co. Ltd. (Japan) and defatted by Soxhlet extraction with hexane. BWG was extracted from the defatted buckwheat flour using 0.5 M NaCl solution at a ratio of 1:10 (w/v) according to the Osborne fractionation scheme (Osborne & Mendel, 1914). The mixture was stirred for 60 min at room temperature and then centrifuged at 15,000g for 20 min. The supernatant, containing globulin and albumin co-extracted by NaCl, was dialyzed against distilled water for 48 h at 4 °C, using dialysis membrane tubing (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) with molecular weight cutoff (MWCO) of 12–14 kDa. During dialysis, distilled water was changed 4 times daily to maintain osmotic pressure. The supernatant containing the water-soluble albumin was discarded. The precipitated salt-soluble globulin was recovered by centrifugation at 15,000g for 20 min at 4 °C. The residue obtained was BWG and it was then freeze-dried, ground to a fine powder and was kept in an airtight container at 4 °C.

2.2. Chemical composition of buckwheat flour and globulin

The crude protein content of buckwheat flour was determined by the micro-Kjeldahl method (Kjeltec, 1986) using a nitrogen-to-protein conversion factor of 5.53 (Tkachuk, 1969). The crude fat and ash content were measured according to the Association of Official Analytical Chemists standard procedure (AOAC, 1984), while the total carbohydrate content (%) in the BWG samples was calculated by difference.

2.3. Structural and physicochemical properties of buckwheat globulin

2.3.1. Isolation of basic and acidic polypeptides from buckwheat globulin by anion-exchange chromatography

BWG was reduced and alkylated with 5% β -mercaptoethanol (β -ME) in 6 M guanidine hydrochloride according to Crestfield, Moore, and Stein (1963) to separate the disulfide-linked acidic and basic polypeptides. The alkylated protein sample was subjected to anion-exchange chromatography using a fast protein liquid chromatography

(FPLC) system (Pharmacia Biotech, Piscataway, NJ, USA) in a DEAE–Sephacrose column (Pharmacia Biotech, Piscataway, NJ, USA). The column size was 2.5 cm $\times$ 50 cm, holding 100 mL of resin. The column was equilibrated with 20 mM Tris–HCl buffer at pH 7 containing 6 M urea at 4 °C. Protein dispersion (17 mg/mL) was prepared in the same buffer and stirred for 1 h at room temperature. The solution was then centrifuged at 15,000g for 30 min at 4 °C. The supernatant was filtered through a 0.2 μ m filter (Puradisc 25 AS, Whatman Inc., Clifton, NJ, USA) and then applied to the column. The flow rate was 1.5 mL/min and the eluant was monitored at 280 nm by UV-absorbance. A stepwise salt gradient from 0 to 0.5 M NaCl in the same buffer was applied and 5-mL fractions were collected. Fractions were then pooled, dialyzed against distilled water for 48 h at 4 °C, and freeze-dried. The recovery of protein from the column was determined by measuring the protein contents of the pooled fractions according to the method of Lowry, Rosenbrough, Farr, and Randall (1951).

2.3.2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

BWG and its fractions isolated from ion-exchange chromatography were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) using a Pharmacia Phast System (Pharmacia Biotech, Piscataway, NJ, USA) and 12.5% homogenous gels according to the method of Laemmli (1970) with and without addition of β -mercaptoethanol (β -ME). Molecular weights of individual protein bands were determined using a Phoretix densitometric image analysis system (Phoretix International, Newcastle Upon Tyne, UK) with standard protein markers.

2.3.3. Isoelectric focusing

Isoelectric focusing (IEF) was performed using PhastGel IEF 3–9 media in a Phast System Separation-Control Unit (Pharmacia Biotech, Piscataway, NJ, USA). The gel was pre-focused at 2.5 mA for 10 min at 15 °C to form the pH gradient. Aliquots of samples (4 μ L) containing 20 μ g proteins were applied to the gel at the cathodal position. Focusing was carried out at 2.5 mA for 30 min at 15 °C. After focusing, the gel was fixed twice by 20% trichloroacetic acid (TCA) for 15 min and then washed twice in the destaining solution for 15 min. The gel was stained with 0.02% Phast-Gel Blue R in the destaining solution with 0.1% cupric sulfate (w/v) and then destained. The pI markers were from a Broad Range pI Calibration Kit (Amersham Pharmacia Biotech Limited, Buckinghamshire, UK). The isoelectric points of the protein bands were determined using the Phoretix 1D Standard Gel Analysis program, version 3.01 (Phoretix International, Newcastle Upon Tyne, UK).

2.3.4. Analysis of sulfhydryl and disulfide contents

The sulfhydryl (SH) and disulfide (SS) contents of BWG and the isolated fractions were determined by the method

of Beveridge, Toma, and Nakai (1974). Protein samples (75 mg) were dissolved in 10 mL of Tris–Gly buffer (0.086 M Tris, 0.09 M glycine, 0.04 M EDTA, pH 8.0) containing 8 M urea. The solution was gently stirred overnight until a homogenous dispersion was achieved. For SH content determination, 4 mL of the Tris–Gly buffer was added to 1 mL of protein solution. Then 0.05 mL of Ellman's reagent (5,5'-dithio-bis-2-nitrobenzoic acid in Tris–Gly buffer, 4 mg/mL) was added, and absorbance was measured at 412 nm after 5 min. For total SH content [SH + reduced SS] analysis, 0.05 mL of β -ME and 4 mL of Tris–Gly buffer were added to 1 mL of the protein solution. The mixture was incubated for 1 h at room temperature. After an additional hour of incubation with 10 mL of 12% TCA, the mixtures were centrifuged at 5000g for 10 min. The precipitate was twice re-suspended in 5 mL of 12% TCA and centrifuged to remove β -ME. The precipitate was dissolved in 10 mL of Tris–Gly buffer. Then 0.04 mL of Ellman's reagent was added to 4 mL of this protein solution, and the absorbance was measured at 412 nm after 5 min. Sample and reagent blanks were prepared for each determination. The calculation was as follows:

$$\mu\text{M SH/g} = 73.53 \times A_{412} \times D/C,$$

where A_{412} is the absorbance at 412 nm, C is the sample concentration (mg/mL), D is the dilution factor: 5 and 10 are used for SH and total SH (SH + reduced SS) content analysis, respectively, 73.53 is derived from $10^6/(1.36 \times 10^4)$; 1.36×10^4 is the molar absorptivity (Ellman, 1959) and 10^6 is for the conversion from molar basis to $\mu\text{M/mL}$ basis and from mg solids to g solids. Half of the value after subtracting the SH value from the total SH value was defined as the SS content.

2.3.5. Net titratable charge

pH-Titration of BWG was carried out on 1% (w/v) protein solutions in 0.1 M NaCl according to Ma, Oomah, and Holme (1986). The pH of the protein solution was adjusted to 10.0, and 0.10 N HCl was added in small increments until an arbitrary end point of pH 3.0 was reached. Net titratable charge was determined as mmol H^+ /g protein.

2.3.6. N-terminal amino acid sequence analysis

Fractionated basic polypeptide was subjected to SDS–PAGE and transferred to a 0.2 μm polyvinylidene difluoride (PVDF) membrane in a transfer buffer (0.039 M glycine, 0.048 M Tris, 0.037% SDS, 20% methanol, at pH 8.3) at a constant voltage of 100 V for 1.5 h. After Coomassie blue staining and destaining, the protein band (23 kDa) was cut out and subjected to partial N-terminal amino acid sequencing by a G-1000 Protein Sequencer (Hewlett Packard, Waldbronn, Germany). Multiple sequence alignment was done by ClustalW 1.8 Multiple Sequence Alignment program (European Bioinformatics Institute, Cambridge, UK). Shading of the multiple alignment files was done by Boxshade 3.21 for comparing similarity with the basic poly-

peptides from other legumin-type proteins. The amino acid sequences from other legume were retrieved from the Swiss-Prot Protein Knowledgebase, release 41.22.

2.4. Functional properties of buckwheat globulin

Protein solubility was determined according to the procedure described by Morr et al. (1985) using 1% protein dispersions prepared in distilled water adjusted to pH 3–11 with 2 N NaOH or HCl.

Water hydration capacity (WHC) of BWG was determined by the method of Quinn and Paton (1979). Five grams of protein sample was weighed into a preweighed 50 mL centrifuge tube. Distilled water was added in small increments under continuous stirring until a paste was formed. The tube was then centrifuged at 2000g for 10 min. The supernatant was discarded and the tube was re-weighed. WHC was calculated as weight difference per gram of dry sample.

Fat binding capacity (FBC) of BWG was determined according to Sosulski and Jones (1976). Four grams of protein sample was dispersed in 24 mL corn oil and mixed. The mixture was vortexed at 10 min intervals twice for 30 s, held for 30 min, and then centrifuged at 1600g for 30 min. The clear oil obtained after centrifugation was measured its volume and decanted. By subtracting the volume of decanted clear oil from the total oil added, fat absorption was calculated as mL oil absorbed per gram of protein sample.

Emulsifying activity index (EAI) and emulsifying stability index (ESI) of BWG solutions (0.5% in 0.01 M phosphate buffer, pH 7.0) were determined by the turbidimetric method (Pearce & Kinsella, 1978). 4.5 mL of protein solution and 1.5 mL corn oil were homogenized for 1 min with an Omni Mixer homogenizer equipped with a micro-attachment at a speed of 8000 rpm. The emulsion thus prepared was diluted with 0.1% SDS and turbidity was measured at 500 nm. EAI was calculated and expressed in m^2/g . ESI was defined as the time in minutes for A_{500} to decrease to 75% of the value at zero-time.

Both foaming capacity (foamability) and foam stability of BWG samples (1%) were estimated by the procedure of Yasumatsu et al. (1972). Protein sample (1%) was added to 20 mL distilled water and a paste was formed after thoroughly mixing. The mixture was shaken vigorously for 1 min with same amplitude and frequency. Foamability was determined as the percentage of foam volume at time 0 by the initial volume. Foam stability at 30 and 60 min was expressed as the percentage of foam volume at 30 and 60 min respectively by foam volume at 0 min.

3. Results and discussion

3.1. Chemical composition of buckwheat flour and globulin

The chemical composition of buckwheat flour was as follow (% on dry weight basis): carbohydrate, 84.6; protein,

10.7; lipid, 2.91; and ash, 1.83. Carbohydrate was the major chemical constituent in buckwheat seeds, while the crude lipid and ash contents were relatively low. The yield of BWG extracted from defatted buckwheat flour was about 4%. The protein content of BWG extracted from defatted buckwheat flour was 93.9%, indicating a highly homogeneous protein sample together with 5.49% carbohydrate and 0.607% ash content. In this study, a commonly used nitrogen-to-protein conversion factor of 5.7 for wheat or 6.25 for most foods was replaced by 5.53.

3.2. Structural and physicochemical properties of buckwheat globulin

3.2.1. Isolation of basic and acidic polypeptides from buckwheat globulin by anion-exchange chromatography

BWG was fractionated into two fractions by anion-exchange chromatogram using stepwise salt (NaCl) elution (Fig. 1). Unbound protein was eluted as peak A (fraction A) when the protein sample was introduced to the column in buffer without NaCl. Based on the chromatographic profile, fraction A and B represented 34% and 66% of the total eluted protein, respectively, indicating a ratio of approximately 1:2. The total recovery of protein from the column was about 72%. There are several techniques commonly used for the isolation of legumins and vicilins from storage proteins such as ammonium sulphate fractionation, gel filtration, ion-exchange chromatography and isoelectric precipitation (Casey, Domoney, & Elis, 1986). In the present study, anion-exchange chromatography was used as it provides high protein recovery and good separation. Also, highly homogeneous basic polypeptides (fraction A) can be isolated in a single chromatographic step and no further purification step was needed.

3.2.2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Fig. 2 shows the SDS-PAGE patterns of BWG and the two chromatographic fractions. Under non-reducing conditions (no β -ME) (lane 2), BWG was resolved into a large number of bands with molecular weights (M_w) ranging from about 17 to 65 kDa. Three major protein bands having M_w of 50, 56, and 65 kDa, respectively, and several minor bands (34–36 kDa) were observed. The addition of β -ME led to dissociation of the high M_w components in BWG into polypeptides with smaller M_w (lane 3), suggesting the presence of inter-molecular disulfide bonds. Among the resolved bands, the 23 and 35 kDa species represent the major components in reduced BWG. The SDS-PAGE patterns of both fractions A and B were similar either in the absence (data not shown) or presence of reducing agent (lanes 4 and 5, respectively), suggesting that they do not contain polypeptides linked by disulfide bonds.

In the presence of β -ME, fraction A (lane 4) shows a single band with M_w between 23 and 25 kDa, identical with the major band in lane 3. The SDS-PAGE profile of fraction B (lane 5) shows a similar pattern to reduced BWG (lane 3), except that there was a pronounced reduction in the intensity of the 23–25 kDa band. This demonstrated an almost complete isolation of the fraction A protein component by anion-exchange chromatography. Three major bands with M_w of 47, 41 and 35 kDa, respectively, and one minor band at 31 kDa, were observed in fraction B (lane 5).

The SDS-PAGE data demonstrate that BWG is composed of a large number of polypeptides differing in molecular weight and relative concentration, suggesting that it exists as a hetero-oligomer composed of a non-equimolar ratio of various monomers. The relative ease of disruption

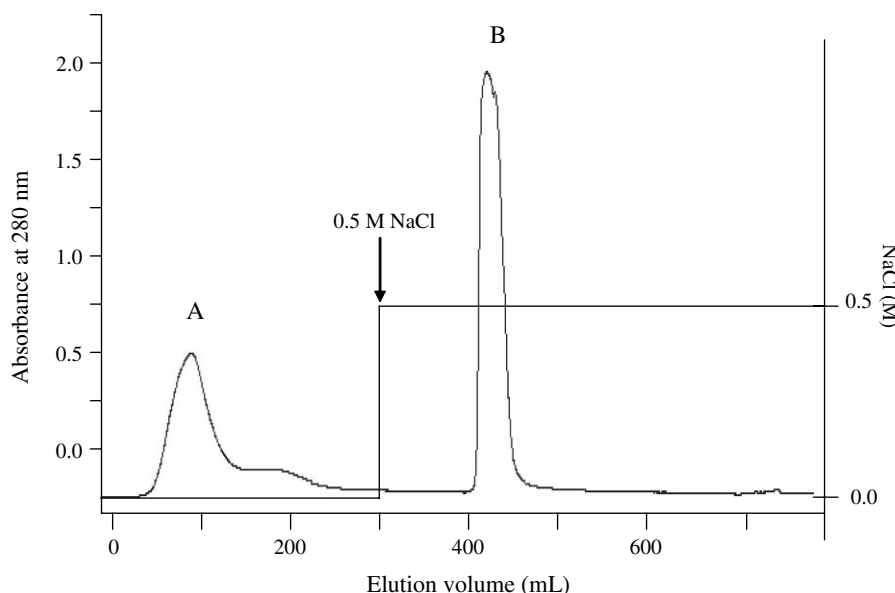


Fig. 1. Anion-exchange chromatographic fractionation (in DEAE-Sephacrose column with 20 mM Tris-HCl buffer containing 6 M urea, pH 7, at 4 °C) of buckwheat globulin by stepwise elution with 0.5 M NaCl.

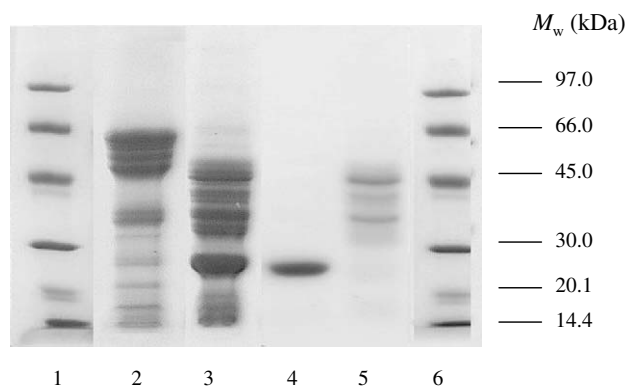


Fig. 2. SDS-PAGE patterns of buckwheat globulin and the two fractions from anion-exchange chromatography. Lanes 1 and 6, low molecular weight markers; lanes 2 and 3, buckwheat globulin; lane 4, fraction A; lane 5; fraction B. Lane 2, without β -mercaptoethanol; lanes 3, 4, and 5, with β -mercaptoethanol.

of the oligomeric structure of BWG in the absence of β -ME indicates that the major attractive forces between the disulfide-linked monomers are non-covalent in nature, such as hydrogen bonding and hydrophobic and electrostatic interactions. These observations are consistent with previous findings which show that BWG is a hexamer with non-identical monomers that interact non-covalently, and each monomer is composed of disulfide-linked large acidic and small basic polypeptides with M_w ranging from 32 to 57.5 and 23 to 25.8 kDa, respectively (Dunaevsky & Belozersky, 1989; Maksimović, Varkonji-Gasić, Radović, & Savić, 1996; Marcone, Kakuda, & Yada, 1998; Rout & Chrungoo, 1996). The major BWG protein bands (M_w 50–65 kDa) observed under non-reducing condition (lane 2) may represent the non-identical monomers, and hence the BWG hexamer has a deduced M_w in a range of 300–390 kDa from six monomers (M_w 50–65 kDa). The deduced M_w of the native BWG hexamer is in agreement with the M_w estimated by laser light scattering (342 kDa) (Choi & Ma, 2006) and gel filtration chromatography (335 kDa) calibrated with protein standards (Marcone et al., 1998). The value is also within the range of M_w for over 20 11S globulins isolated from dicotyledonous and monocotyledonous plants, typically in a narrow range of 300–370 kDa (Marcone et al., 1998). Recently, several genomic clones corresponding to 13S buckwheat globulin have been characterized. The acidic and basic polypeptides were found to contain 293 and 191 amino acids, respectively, and the calculated M_w of 13S buckwheat globulin was 342,258 Da (Fujino, Funatsuki, Inada, Shimo, & Kikuta, 2001; Nair & Adachi, 2002; Nair, Ohmoto, Woo, & Adachi, 1999; Samardzic, Milisavljevic, & Brkljacic, 2004).

Our data also demonstrate similarity in the molecular size distribution of polypeptides from BWG and from 11S globulins of monocotyledonous and dicotyledonous plants, falling within two very narrow M_w ranges of 20–27 and 30–39 kDa (Marcone et al., 1998). In a previous study on selected plant globulins, it was found that these two regions correspond to the basic and acidic polypep-

tides, respectively (Derbyshire et al., 1976). Hence, the components from fraction A and B should represent respectively the basic and acidic polypeptides of BWG. The estimated M_w of the protein band in fraction A (23–25 kDa) is close to that (26 kDa) of the basic polypeptide isolated from buckwheat globulin (Rout, Chrungoo, & Rao, 1997). Due to the heterogeneity of fraction B, further structural analysis was conducted only on fraction A.

3.2.3. Isoelectric focusing

BWG and the fractionated basic polypeptides (fraction A) were analyzed by IEF under reducing condition (Fig. 3). BWG exhibited several bands focused mainly in the acidic region (pH 5.85–6.95), probably corresponding to the acidic polypeptides (fraction B) (lane 2). For the basic polypeptides (lane 3), two bands were focused in the alkaline region (pH 7.88 and 8.23), with a major band focused at pH 8.23. The data indicate that the BWG basic polypeptides have isoelectric points at a slightly alkaline pH region. In previous studies, the isoelectric point of a buckwheat protein product prepared by alkaline extraction and isoelectric precipitation at pH 4.5 was found to be around pH 4 (Tomotake, Shimaoka, Kayashita, Nakajoh, & Kato, 2002). Differences in isoelectric points between the larger and the smaller polypeptides of the 13S globulin from buckwheat were observed. The larger polypeptides were focused in the acidic region of the gel, while a part of the small polypeptide group was focused in the region of about pH 6.8. Due to the pH range limit (4.3–7.7), a complete set of basic polypeptides was not detected by IEF in that study (Radović, Maksimović, & Varkonji-Gasić, 1996). However, the values observed from Fig. 3 are similar

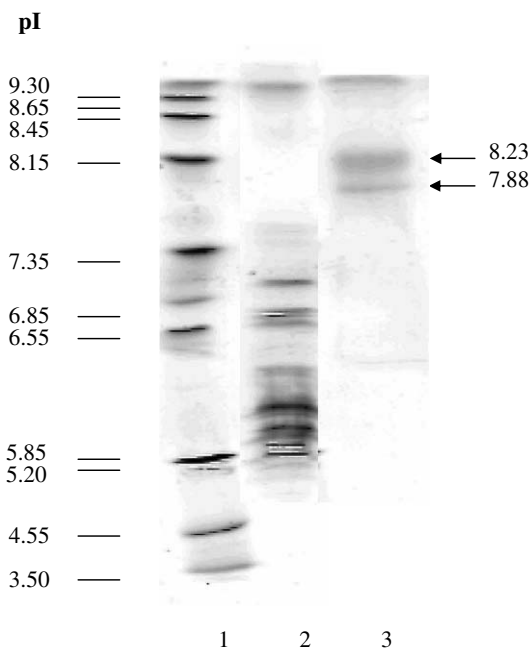


Fig. 3. Isoelectric focusing profiles of buckwheat globulin and its basic polypeptides fractionated by anion-exchange chromatography. Lane 1, broad pI marker; lane 2, buckwheat globulin; lane 3, basic polypeptides.

to the ranges reported for oat globulin by Peterson and Brinegar (1982), which were approximately pH 5.9–7.2 for the acidic and pH 8.7–9.2 for the basic polypeptides.

3.2.4. Analysis of sulfhydryl and disulfide contents

Table 1 shows the SH and SS contents of BWG and the isolated polypeptide fractions. BWG has a SS content of 36.4 $\mu\text{M/g}$ protein and a SH content of 3.2 $\mu\text{M/g}$ protein. This suggests that most cysteine residues exist as disulfide linkages rather than as free SH groups. This data is compatible with the observation from reducing and non-reducing SDS-PAGE patterns (Fig. 2) that some of the protein components were linked by disulfide bonds. Both the basic and acidic polypeptides have much lower free SH and SS contents than BWG, suggesting that most of the disulfide bonds exist in the form of inter-molecular disulfide linkages between the basic and acidic polypeptides. Free SH groups and SS bonds have significant influence on the functional properties of food proteins, and can play an important role in the formation of relatively rigid structures in protein conformation (Shimada & Cheftel, 1989). The blocking of free SH groups or the prevention of SH-SS interchange reaction favoring the formation of BWG macroaggregate has been demonstrated by laser light scattering in our recent study (Choi & Ma, 2006).

3.2.5. Net titratable charge

The net titratable charge of BWG (2.20 mmol H^+ /g protein) was higher than that (1.33 mmol H^+ /g protein) of Supro 610, a commercial soy protein isolate. The results indicate that higher quantity of free carboxyl groups are present in BWG than in Supro 610, and more acid is therefore needed to decrease the pH from 10 to 3. The higher net titratable charge of BWG may be related to higher hydrolysis capacity. More amide groups were hydrolyzed to free carboxyl groups which were then converted to carboxylate ions during neutralization to pH 7, leading to a higher net negative charge. It has been reported that the net titratable charge increases as the degree of hydrolysis increases (Ma & Khanzada, 1987; Vojdani & Whitaker, 1994).

3.2.6. N-terminal amino acid sequence analysis of basic polypeptide

The sequence of 15 N-terminal amino acid residues of the BWG basic polypeptide (basicBWG) and the alignment

of the sequence with corresponding regions of basic polypeptides from other legume-type proteins (Bharali & Chungoo, 2003) are presented in Fig. 4. BWG basic polypeptide (basicBWG) showed a marked homology (73.3%) with FA02 and BW24KD with 11 of 15 residues being identical.

Using alignment that permits maximum homology, basicBWG exhibited the highest level of identity (53.3%) with legumin beta chain from *Taxus baccata*. It also shows a high degree of homology with other legumin-like proteins varying from 46.7% (Oat, Vicia, Pisum, 11Sglb sesame, 11Sglb coffee, Prunin_almond) to 40% (Glycinin, Ricinus, Rice, Magnolia, 11Sglb quinoa and 11Sglb sunflower) and 33.3% (Rape and 11Sglb cucumber). Homology at this level suggests that all 11S globulins including BWG may have been derived from a common ancestral gene. The sequence data show that Gly-Leu-Glu and the globally conserved position of P7' cysteine were in the conserved N-terminal domain. This indicates the significance in secondary structure formation, and the formation of an inter-chain disulfide linkage between the acidic and basic chains (Hager, Jensen, Gilroy, & Richardson, 1992). The results further confirm that BWG

Vicia	1	GLEETICSLKIRENI
Pisum	1	GLEETICSAKIRENI
11Sglb quinoa	1	GLEETICSAKIRENI
11Sglb sunflower	1	GVEETICSMKFKVNI
Rape	1	GLEETICSMRTHKNI
Magnolia	1	GLEETICQSKLTNTI
Bwleg	1	GIDENVCTMKLRNI
Glycinin	1	GVEENICTLKLHNTI
11Sglb coffee	1	GLEETLCTVKLSNI
11Sglb sesame	1	GLEETLCTAKLRNI
11Sglb cucumber	1	GLEETICTLRLKQNI
Rice	1	GLDETCTLRVFQNI
Ricinus	1	GLEETFCIRLRHNTI
Prunin_almond	1	GLEETFCISLRKNTI
Oat	1	GLEENFCDLEAEENI
FA02	1	GLECAFONLKFQNV
BW24KD	1	GLECAFONLKFQNV
FA18	1	GVEQGFONLKFRRNF
basicBWG	1	GLECAFONLKFQNV
Taxus	1	GLEQLFONMLRLHNA

Fig. 4. Alignment of 15 N-terminal amino acid sequence of the basic polypeptide of buckwheat globulin (basicBWG) with the basic polypeptides from other legumin-type proteins. Identical amino acid residues are in black and similar amino acid residues are in grey. The amino acid sequences from other legume were retrieved from the Swiss-Prot Protein Knowledgebase, release 41.22 as follows: Vicia (*Vicia faba* Legumin type B, P16078), Pisum (*Pisum sativum* Legumin K, P05693), 11Sglb quinoa (*Chenopodium quinoa* 11S globulin, AAS67036), 11Sglb sunflower (*Helianthus annuus* helianthinin G3, P19084), Rape (*Brassica napus* cruciferin, J05233), Magnolia (*Magnolia salicifolia* Legumin beta chain, S68819), Bwleg (common buckwheat 13S basic chain, P83004), Glycinin (*soybean Glycine max*, BAA74953), 11Sglb coffee (*Coffea arabica* 11S storage globulin, AAC61983), 11Ssesame (*Sesame indicum* 11S globulin, AAK15087), 11Sglb cucumber (*Cucurbita maxima* 11S globulin beta subunit precursor, P13744), Rice (*Oryza sativa* glutelintype I, X05662), Ricinus (*Ricinus communis* (castor bean), AAF73008), Prunin_almond (*Prunus dulcis*, CAA55009), Oat (basic subunit of oat globulin), FA02 (*F. esculentum* leg1, O23878), BW24KD (major allergen from common buckwheat, AAF34635), FA18 (*F. esculentum* leg2, O23880), Taxus (*Taxus baccata* Legumin beta chain, S68815).

Table 1

Sulfhydryl and disulfide contents of buckwheat globulin and the fractionated basic and acidic polypeptides^a

	SH content ^b ($\mu\text{M/g}$ protein)	SS content ^c ($\mu\text{M/g}$ protein)
BWG	3.20 $\pm$ 0.07	36.4 $\pm$ 2.49
Basic polypeptide	0.446 $\pm$ 0.04	3.87 $\pm$ 1.21
Acidic polypeptide	0.762 $\pm$ 0.19	3.93 $\pm$ 0.09

^a Average of duplicate determinations $\pm$ standard deviation.

^b Sulfhydryl content.

^c Disulfide content.

is a legumin-like protein. The amino acid sequence of the basic subunit of legumin-type protein from grains of common buckwheat (Bwleg, Swissprot Accession No. P83004) has previously been described (Bharali & Chrungoo, 2003). However, when compared with the basicBWG in this investigation, the percentage of homology with basic polypeptides from other buckwheat globulins was found to vary between 33.3% for Bwleg (13S globulin basic chain common buckwheat) and 60% for FA18 (legumin2 from common buckwheat). Variations in homology of basic polypeptides from buckwheat globulin may be attributed to different common buckwheat species, and different methods of extraction and purification.

3.3. Functional properties of buckwheat globulin

The pH-solubility curve of BWG (Fig. 5) shows a typical bell-shaped curve with minimum solubility (3.61%) at pH 5, and a maximum solubility (over 90%) at extremely acidic (pH 3) and alkaline pH (pH 11), and fairly good solubility at neutral or slightly alkaline pH. The results reveal that BWG is similar to other legume proteins in terms of solubility characteristics, making it potentially useful in food applications where a good solubility profile is needed to impart specific functional characteristics.

As shown in Table 2, the WHC of BWG was lower than that of Supro 610, a commercial soy protein isolate. WHC has been defined as the ability to physically hold water against gravity (Kinsella, 1979) and is the ability of a food structure to prevent water from being released from the three-dimensional network of the protein (Hermansson, 1986). WHC is a crucial factor in functionality as it influences the texture, color and sensory properties of products.

The FBC of BWG was higher than that of Supro 610 (Table 2). FBC has been defined as the ability of proteins to bind fat in emulsions or food systems that is important in many food formulations and may influence the texture

Table 2

Some functional properties of buckwheat globulin (BWG) and Supro 610^a

Property	BWG	Supro 610
Water holding capacity (mL/g)	1.33 ± 0.07	5.13 ± 0.03
Fat binding capacity (mL/g)	1.73 ± 0.09	1.52 ± 0.01
Emulsifying activity index (m ² /g)	88.4 ± 0.70	37.3 ± 1.10
Emulsion stability index (min)	20.1 ± 1.56	2.20 ± 0.30
Foamability (%)	75.5 ± 0.71	105 ± 5.00
Foam stability (%) (30 min)	68.9 ± 0.29	93.0 ± 3.00
Foam stability (%) (60 min)	56.3 ± 0.53	84.0 ± 4.00

^a Averages of duplicate determinations ± standard deviations.

and other food quality. It depends on several factors such as hydrophobicity, degree of denaturation, size and flexibility of protein, processing conditions, composition of additives, and temperature (Kinsella, 1979; Zayas, 1997). BWG exhibited a much higher EAI and ESI than Supro 610 at pH 7 (Table 2), suggesting a better balance of hydrophilic and hydrophobic groups in BWG. Due to the relatively high values of EAI and ESI, BWG could be used as a substitute for soy protein in emulsified foods such as meat products and mayonnaise.

Both foamability and foam stability (at 30 and 60 min) of BWG were lower than those of Supro 610 (Table 2). However, the foams produced by BWG at pH 7 were able to maintain about 70% and more than 50% of the initial volume after standing for 30 and 60 min, respectively.

4. Conclusions

Buckwheat globulin, the major storage protein fraction, was separated into two fractions by anion-exchange chromatography, corresponding to the basic and acidic polypeptides with a ratio of approximately 1:2. SDS-PAGE analysis showed that BWG polypeptides are linked by disulfide bonds. The basic polypeptide has an estimated M_w of 23–25 kDa, similar to that reported by other researchers. Partial N-terminal amino acid sequence analysis of the BWG basic polypeptide revealed a high degree of homology with other legumin-like proteins, suggesting a common ancestral origin of BWG with other legumins. BWG was found to possess some favorable functional properties such as relatively high solubility, water holding and fat binding capacity, as well as emulsifying activity and emulsion stability when compared to a widely used commercial soy protein product. Hence, BWG may have potential applications in fortified food products by virtue of its high solubility, fat binding capacity and good emulsifying properties.

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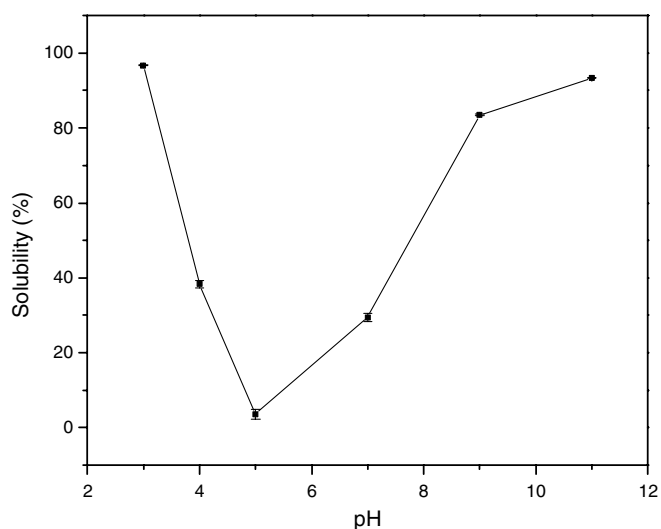


Fig. 5. Solubility profile of buckwheat globulin at various pH. Error bars represent standard deviations of duplicate determinations.

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