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Fractionation and characterization of tartary buckwheat flour proteins

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Abstract

Protein fractions (albumin, globulin, prolamin and glutelin) were extracted from defatted tartary buckwheat flour. Albumin was the predominant protein fraction (43.8%) followed by glutelin (14.6%), prolamin (10.5%), and globulin (7.82%). Albumin was relatively rich in histidine, threonine, valine, phenylalanine, isoleucine, leucine and lysine. Globulin had high levels of methionine and lysine. Prolamin was high in histidine, threonine, valine, isoleucine, and leucine. Glutelin was rich in histidine, threonine, valine, isoleucine, and leucine. SDS–PAGE analysis, under non-reductive and reductive conditions, showed that disulfide bonds existed in the four fractions. Non-reduced albumin showed major bands at 64, 57, 41, and 38 kDa, and globulin at 57, 28, 23, 19 and 15 kDa. Reduced albumin and globulin shared two common bands at 41 and 38 kDa. Reduced prolamin showed major bands at 29, 26, 17 and 15 kDa. In vitro pepsin digestibility of the four fractions (from high to low) was: albumin > globulin > prolamin and glutelin.

Keywords: Tartary buckwheat; Protein fractions; SDS–PAGE; In vitro digestibility

1. Introduction

The genus *Fagopyrum* has about 15 species distributed in different parts of the world (Tahir & Farooq, 1988). Among these species, only two types of buckwheat are used as food around the world: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) (Bonafaccia, Gambelli, Fabjan, & Kreft, 2003). Buckwheat (*F. esculentum* and *F. tataricum*) is a dicotyledonous crop of the Polygonaceae family. The buckwheat embryo traverses the starchy endosperm in a triangular seed enclosed by pericarp (hull) of the mature achene (fruit) (Steadman et al., 2000).

Protein fractionation, according to the Osborne classification for buckwheat flour, has been reported (Imai & Shibata, 1978; Pomeranz, 1983; Tahir & Farooq, 1985; Wei, Hu, Zhang, & Quyang, 2003). Even though there is no general agreement, most researchers find that buckwheat protein is mainly composed of albumin and globulin. Seed storage proteins of common buckwheat have been characterized by several researchers (Fujino, Funatsuki, Inada, Shimono, & Kikuta, 2001; Radovic, Maksimovic, Brkljacic, Varkonji-Gasic, & Savic, 1999; Radovic, Maksimovic, & Varkonji-Gasic, 1996; Skerritt, 1986). In common buckwheat, salt-soluble globulin is represented mainly by the 13S 280 kDa component (Javornik, Eggum, & Kreft, 1981). The protein consists of three fractions, with molecular masses between 43–68, 57–58 and 26–36 kDa (Radovic et al., 1996). 2 S Albumin from common buckwheat was identified by sucrose density gradient centrifugation and it is composed

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of polypeptides in the range of molecular mass from 8 to 16 kDa (Radovic et al., 1999). Recently, the physiological properties of common buckwheat protein have also been studied. In rat feeding experiments, studies have proved that common buckwheat protein has hypocholesterolemic (Kayashita, Shimaoka, Nakajoh, Yamazaki, & Kato, 1997) and anticonstipation activity (Kayashita, Shimaoka, Yamazaki, & Kato, 1995), and shows suppression of mammary carcinogenesis (Kayashita, Shimaoka, Nakajoh, Kishida, & Kato, 1999) and colon carcinogenesis (Liu et al., 2001).

Compared with common buckwheat protein, tartaty buckwheat protein and its Osborne fractions, albumin, globulin, prolamin and glutelin, have been scarcely researched. More research is needed to increase our knowledge of these protein types. Tartary buckwheat is commonly taken as a diet in eastern Asian countries (Kawakmi, Kayahara, & Ujihara, 1995). In China, tartary buckwheat is mainly grown in some mountainous regions, such as Liang Shan Yi Autonomous region in Sichuan province and Jing Zhou in Gui Zhou province (Li & Zhang, 2001).

The objectives of this study were: (a) determination of the amounts of albumin, globulin, prolamin and glutelin in tartary buckwheat flour; (b) amino acid composition analysis; (c) protein electrophoresis analysis in reduced and non-reduced SDS–PAGE; (d) in vitro pepsin digestibility of these fractions.

2. Materials and methods

2.1. Materials

Tartary buckwheat flour was obtained from the milling factory for minor crops in Liang Shan region in Sichuan province. Flour was defatted for 24 h with *n*-hexane with continuous stirring, air-dried at room temperature, and stored at 4 °C until used. The electrophoretic chemicals were purchased from Sigma Chemical Co. (St. Louis, USA). Molecular weight markers were purchased from Shanghai Institute of Biochemistry (Shanghai, China). Pepsin was purchased from Deyang Biochemical Company (Deyang, China). All other chemicals used were of analytical grade.

2.2. Protein fractionation

2.2.1. Albumin

Defatted tartary buckwheat flour (100 g) was stirred for 1 h with 1,000 ml of distilled water at room temperature. The suspension was centrifuged at 10,000*g* at 4 °C for 20 min in a refrigerating centrifuge (Hitachi Koki Co. Ltd., Japan). Then, the precipitate was reextracted for 30 min by the same method. Combined supernatants were freeze-dried.

2.2.2. Globulin

The residue resulting from albumin extraction was extracted for 1 h with 1000 ml of 1 M NaCl at room temperature. The suspension was centrifuged at 10,000*g* at 4 °C for 20 min.The extraction step was repeated for 30 min. Combined supernatants were dialyzed extensively against distilled water and freeze-dried.

2.2.3. Prolamin

After globulin was extracted, the residue was extracted for 1 h with 1000 ml of 55% (v/v) 1-propanol at room temperature. The suspension was centrifuged at 10,000*g* at 4 °C for 20 min and the supernatant was decanted. The extraction step was repeated for 30 min. Combined supernatants were concentrated and freeze-dried.

2.2.4. Glutelin

The residue was extracted for 1 h with 1000 ml of 0.05 M NaOH at room temperature. The suspension was centrifuged at 10,000*g* at 4 °C for 20 min and the supernatant was decanted. The extraction step was repeated for 30 min. Combined supernatants were precipitated by adding TCA to a final concentration of 10%. The suspension was centrifuged at 10,000*g* at 4 °C for 20 min and the pellet was dissolved in water, dialyzed extensively against distilled water and freeze-dried.

The protein content of the fractions was determined by the Kjeldahl method ($N \times 6.25$).

2.3. Amino acid analysis

The amino acid composition of the fractions was determined with an automatic amino acid analyzer (Agilent 1100, USA). The samples were hydrolyzed with 6 M HCl for 24 h at 110 °C in a sealed tube. Tryptophan was not determined.

2.4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out using the discontinuous system (10% separating/4% stacking gel) described by Laemmli (1970) with and without reduction of the protein by 2-ME. The following buffer system was used: pH 8.8, Tris–HCl, 0.1% (w/v) SDS for the separating gel; pH 6.7, Tris–HCl, 0.1% (w/v) SDS for the stacking gel; 0.025 M Tris–HCl, 0.192 M glycine, and 0.1% (w/v) SDS (pH 8.3) for the running buffer, and pH 6.7 Tris–HCl, 20% (v/v) glycerol, 1%(w/v) SDS, and 0.05% bromophenol bule as sample buffer. Reduction of disulfide bonds was performed by adding 2-ME (5% v/v) and heating at 100 °C for 3 min. All samples (reduced and non-reduced) were centrifuged at 4000*g* for 10 min, and the supernatants were used to load the

gels. Electrophoresis was conducted at a constant current of 20 mA for about 3 h. The gels were stained in Coomassie brilliant blue R-250. Molecular weights of protein subunits were calculated using the following markers: phosphorylase (97,400), bovine serum albumin (66,200), rabbit actin (43,000), bovine carbonic anhydrase (31,000), trypsin inhibitor (20,100), and hen egg white lysozyme (14,400).

2.5. In vitro pepsin digestibility

In vitro pepsin digestibility was determined according to the method of Rick and Fritsch (1974), but with a slight modification. A 0.2-g sample was placed in a 100-ml Erlenmeyer flask containing 20 ml, pH 1.0, HCl, 20 mg of pepsin (1:3000, from porcine stomach mucosa) was added, and the flask was incubated in a shaking water bath at 37 °C. After 3 h, a 5ml aliquot of pepsin hydrolysate was added to 5 ml of 20% trichloroacetic acid and mixed. The solution was allowed to stand for 10 min, and centrifuged at 4000*g* for 20 min. The supernatant was collected. A blank was prepared by the same method, using 20 mg of pepsin without substrate.

In vitro pepsin digestibility was calculated by the following equation:

$$\text{In vitro digestibility} = \frac{(N \text{ content in the supernatant} - N \text{ content in the blank})}{N \text{ content in the sample}} \times 100\%$$

3. Results and discussion

3.1. Distribution of protein fractions

Table 1 shows the Kjeldahl nitrogen and the protein content of fractions from defatted tartary buckwheat flour. Albumin was the predominant protein fraction (43.8%) followed by glutelin (14.6%), prolamin (10.5%), and globulin (7.82%). This is in disagreement with earlier reports (Imai & Shibata, 1978; Pomeranz, 1983; Tahir & Farooq, 1985; Wei et al., 2003). Imai and Shibata (1978) reported 40–77% albumin and globulin, 0.7–2.0% prolamin, and 23–59% glutelin and residual protein for commercial buckwheat flour. Pomeranz (1983) reported that buckwheat protein was composed of about 80% albumin and globulin. Tahir and Farooq (1985) found that the proportions of albumin plus globulin, prolamin, glutelin, and residual protein were 38–44%, 2–5%, 21–29% and 28–37%, respectively, for 4 buckwheat species. Wei et al. (2003) reported that proportions of albumin, globulin, prolamin, and glutelin were 16.8–30.3%, 4.96–21.6%, 3.08–7.01%, and 11.5–16.0%, respectively, for four buckwheat species. Fractionation of plant proteins on the basis of solubilities in different solvents is only an estimation of the protein composition. Different extraction methods and various species may lead to greatly different results. Table 1 also shows the nitrogen content of the protein fractions. The globulin content was the lowest (7.82%) but its protein content was the highest (92.9%).

Table 1
Distribution and purity of protein fractions of defatted tartary buckwheat flour

Fraction	*N* extracted, %total *N*	Protein (*N* × 6.25), %
Albumin	43.8	41.8
Globulin	7.82	92.9
Prolamin	10.5	30.8
Glutelin	14.6	75.5

3.2. Amino acid composition of the protein fractions

The amino acid composition of the protein fractions is shown in Table 2. In terms of essential amino acids, albumin was relatively rich in histidine, threonine, valine, phenylalanine, isoleucine, leucine and lysine; globulin had high levels of methionine and lysine; prolamine was high in histidine, threonine, valine, isoleucine, and leucine; and glutelin was rich in histidine, threonine, valine, isoleucine and leucine. In a word, the four fractions had adequate amounts of essential amino acids for child and adult diets except that several essential amino acids in globulin were slightly inadequate. Among non-essential amino acids, the levels of glutamic acid and aspartic acid were adequate in all fractions, and prolamin had the highest arginine content of the four fractions.

3.3. Electrophoretic characterization

Defatted tartary buckwheat flour and the protein fractions were analyzed by SDS–PAGE with and without reduction of the protein by 2-ME (Fig. 1). As observed in Fig. 1A, albumin, globulin and defatted tartary flour shared some common electrophoretic bands at 64, 57, 41, and 38 kDa. Under non-reductive electrophoretic conditions, albumin also showed a doublet-like band at 52–51 kDa and some minor bands at 22–14 kDa. Globulin also showed major bands at 34, 28, 26, 23, 21, 19 and 15 kDa. Prolamin showed two minor bands at 20 and 14 kDa and two major bands

Table 2
Amino acid compositions of protein fractions g/100 g protein

Amino acid	Albumin	Globulin	Prolamin	Glutelin	FAO/WHO ref pattern	
					Child	Adult
Asp	10.5	9.72	7.07	9.97		
Glu	20.2	30.3	11.6	20.5		
Ser	5.45	3.87	4.36	5.01		
His	2.51	1.99	3.87	2.67	1.9	1.6
Gly	6.04	4.40	6.43	5.69		
Thr	3.69	2.91	5.54	3.61	3.4	0.9
Ala	5.58	8.31	3.11	5.73		
Arg	8.76	4.58	14.67	8.15		
Tyr	2.73	2.84	4.63	3.36		
Cys	2.99	2.52	3.41	2.48		
Val	4.78	3.37	5.88	5.31	3.5	1.3
Met	1.60	5.18	1.63	1.81	2.5	1.7
Phe	5.28	3.92	4.56	4.89	6.3	1.9
Ile	3.74	2.42	4.29	4.14	2.8	1.3
Leu	6.18	4.49	9.06	6.64	6.6	1.9
Lys	5.90	5.31	4.30	5.29	5.8	1.6
Pro	3.97	3.85	5.54	4.67		

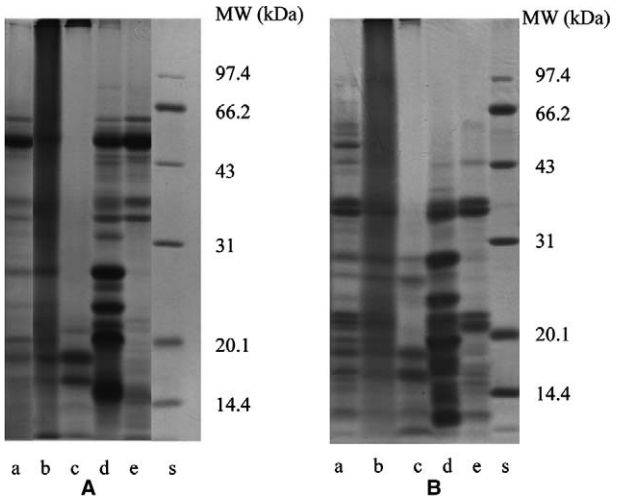

Fig. 1. SDS–PAGE under non-reductive conditions (A) and under reductive conditions (B): (a) defatted tartary flour; (b) glutelin; (c) prolamin; (d) globulin; (e) albumin; (s) molecular weight marker.

at 17 and 15 kDa. Glutelin only showed some minor bands with a poor definition because glutelin contained protein components which did not enter the gel, and we also found that lyophilized glutelin had lower solubility in gel sample buffer.

Under reductive electrophoretic conditions, albumin, globulin and defatted tartary flour also shared two common electrophoretic bands at 41 and 38 kDa which were about in the same region without 2-ME. In other words, they remained after reduction. Outstanding changes were noticed after reduction of albumin and globulin and the common bands at 64 and 57 kDa were reduced to lower molecular weight bands. Albumin also exhibited a major doublet at 21–20 kDa and a minor set of bands at 20–13 kDa. This is in disagreement with an earlier report (Radovic et al., 1999) that the common buckwheat water soluble fraction gave bands in the range 8–16 kDa and had no disulfide bonds. Globulin exhibited two major bands, at 29 and 24 kDa, and five dense bands at 20–13 kDa. After reduction, prolamin showed two more bands at 29 and 26 kDa which might come from the high molecular weight proteins that did not enter the gel. In addition, prolamin also showed two major bands at 17 and 15 kDa which remained after reduction. This is in agreement with the results of Skerritt (1986), who reported that the common buckwheat alcohol-soluble fraction had a lower molecular range (28–10 kDa). Glutelin also had more bands. In spite of the reduction effect, some proteins in prolamin and glutelin were unable to enter the gel. Defatted tartary flour showed many bands at 66–12 kDa.

Table 3
In vitro pepsin digestibilities of defatted tartary buckwheat flour protein fractions and other food proteins

Substrate protein	In vitro pepsin digestibility (%)
Albumin (defatted tartary buckwheat flour)	81.2
Globulin (defatted tartary buckwheat flour)	79.6
Prolamin (defatted tartary buckwheat flour)	67.0
Glutelin (defatted tartary buckwheat flour)	58.1
Isolated protein (wheat germ)	89.1
Isolated protein (soybean)	85.5

3.4. In vitro pepsin digestibility

Table 3 shows in vitro pepsin digestibility of defatted tartary buckwheat flour protein fractions and other food proteins. Protein digestibility may be affected by two types of factors: exogenous factors (protease inhibitors, phytic acid, polyphenols and tannin) and endogenous factors (protein structure) (Duodu, Taylor, Belton, & Hamaker, 2003). The digestibilities of defatted tartary buckwheat flour protein fractions (from high to low) were: albumin > globulin > prolamin and glutelin. Compared with isolated protein of wheat germ and soybean, tartary buckwheat flour fractions had relatively lower digestibility. In addition to some antinutrients, the lower digestibility of these fractions might be affected by structural properties but the mechanism influencing this needs to be studied further.

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