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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