# 2S Albumin from Buckwheat (*Fagopyrum esculentum* Moench) Seeds

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Sucrose density gradient centrifugation showed that ~30% of total buckwheat proteins migrated with a 2S sedimentation coefficient. The main part of that fraction, polypeptides in the range of molecular mass from 8 to 16 kDa, were water soluble and represented albumins. SDS−PAGE analysis in nonreducing and reducing conditions showed that these polypeptides were not linked by disulfide bonds. The albumins make 25% of total salt soluble proteins, but that content is dramatically reduced under S-deficiency conditions. Determination of amino acid composition showed high methionine (9.2%) and lysine (5.6%) contents. That characteristic offers the possibility of transfer of the genes for individual albumin polypeptides to legumes and cereals limited in those essential amino acids to improve their nutritional quality.

**Keywords:** *Fagopyrum esculentum Moench; buckwheat; seed storage protein; seed albumins; S deficiency*

## INTRODUCTION

Seed storage proteins of buckwheat (*Fagopyrum esculentum*) have been scarcely characterized, although they determine the high nutritional quality of the buckwheat seed (Pomeranz and Robins, 1972). These proteins have a very well balanced composition of amino acids, and according to net protein utilization (NPU) they are classified close to the proteins of animal sources. Of main interest to investigators have been the activity and regulation of proteinases, peptidases, and hydrolytic enzymes involved in the degradation of these proteins in the process of germination (Dunaevsky and Belozersky, 1989, 1993).

Our previous paper focused on buckwheat storage globulins that account for 70% of total seed proteins. They consist of two protein fractions: major, 13S legumin-like; and minor, 8S vicilin-like globulins (Radović et al., 1996). The contribution of glutelins is minor (4%), whereas prolamins are completely absent (Radović, 1998).

According to previously published data, in addition to globulins, a significant portion of buckwheat storage proteins display an albumin fraction. The size of that fraction relative to total proteins in a common buckwheat ranges from 18% (Javornik et al., 1981) to 32% (Zhang et al., 1998), depending on cultivar. It was shown that the proportion of nitrogen isolated as albumins is ~12% of total (Javornik et al., 1981). Albumins with a storage function were found in many dicotyledonous plants and are usually composed of disulfide-linked low molecular weight (lmw) polypeptides with a sedimentation coefficient of 2S (Shewry, 1995). The common feature of 2S albumins is their high contents of glutamine and also the essential amino acids cysteine and methionine, usually limited in legume seed (Ericson et al., 1986). Much of the recent interest in 2S albumins has focused on their exploitation for improving the nutritional quality of plants through genetic engineering.

In this paper we analyzed the polypeptide and amino acid compositions of the albumin fraction of buckwheat seed, as a possible protein fraction that could be used to transfer their genes to other plant species having limited potential due to their essential amino acid composition. In addition, the influence of sulfur deficiency on the composition of storage proteins was investigated.

## MATERIALS AND METHODS

**Plant Material.** Buckwheat (*F. esculentum* Moench cv. Darja) was field-grown at the Botanical Garden of The University of Belgrade. The mature seeds were harvested and used immediately or stored in liquid nitrogen for protein extraction.

When the influence of S nutrition was examined, plants were grown in sand−perlite with nutrient solution. In control plants sulfur was supplied as 1 mM $MgSO_4$. S-deficient plants were grown in 0.05 mM $MgSO_4$ until the first flower appeared, and sulfur was omitted thereafter. The level of Mg was kept constant by varying the concentration of $MgCl_2$.

**Isolation of Protein Fractions.** Buckwheat seeds were frozen in liquid nitrogen and ground to a fine powder. The **globulin** fraction was extracted in 5−10 volumes of buffer A [0.035 M potassium phosphate, pH 7.6, 0.4 M NaCl, 1 mM phenylmethanesulfonyl fluoride (PMSF)] at room temperature for 1 h. The buckwheat **albumins** were prepared by (a) extensive dialysis against the water of total salt extract of mature buckwheat (Schraeder, 1982); **(**b**)** extraction with 4 volumes of water for 1 h followed by centrifugation at 12000*g* for 30 min; supernatant was then saturated with ammonium sulfate to 90%, and the precipitate was dissolved in deionized water and extensively dialyzed against deionized water in a cold room (Ono et al., 1978).

**Preparation of the 31000*g* Supernatant and Sucrose Gradient Centrifugation.** Buckwheat seeds were ground in liquid nitrogen and extracted for 1.5 h, at 20 °C in 10 volumes (w/v) of buffer containing 0.035 M potassium phosphate, pH 7.6, 0.5 M NaCl, and 0.02% $NaN_3$. The extract was centrifuged

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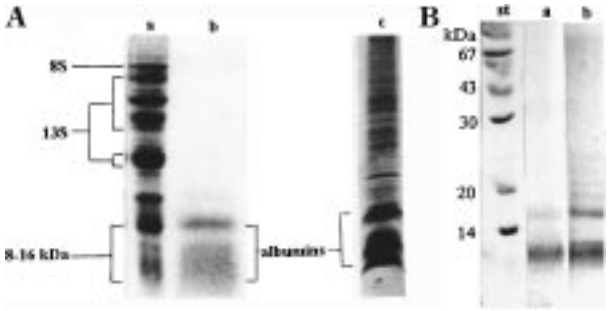


**Figure 1.** (A) SDS−PAGE of buckwheat seed proteins: (a) total salt soluble, 80 $\mu$g; (b) albumins extracted by dialysis of total salt soluble proteins against water, 25 $\mu$g, separated on 12.5% gel in reducing conditions; (c) the same fraction as (b) separated on 16.5% high-resolution gel system, 80 $\mu$g. 13S globulin consists of two groups of polypeptides: 32−43 and 23−25 kDa; 8S globulin consists of 57 kDa polypeptide. (B) SDS−PAGE of buckwheat seed albumins: (a) in reducing conditions, 20 $\mu$g; (b) in nonreducing conditions, 20 $\mu$g. Albumins were prepared by ammonium sulfate saturation of water extract.

for 15 min at 31000*g* (Sorvall, RC-5B, SS-34 rotor). The 31000*g* supernatant was layered onto a 10−30% sucrose gradient and centrifuged for 40 h at 20 °C (Beckman, L5-65, SW28 rotor). Fractions of 1.0−1.2 mL were taken and proteins photometrically monitored at 280 and 260 nm. The sedimentation coefficient of protein fractions was estimated by a comparison with soybean and pea proteins with known values of sedimentation coefficients (Spielmann et al., 1982).

**Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE).** Protein samples were analyzed by electrophoresis in 12−15% SDS−polyacrylamide slab gels (Laemmli, 1970). Separation of lmw peptides was performed on a high-resolution gel system (16.5% separating gel, a 10% spacer gel, and a stacking gel). The gels were stained with Coomassie brilliant blue.

**Determination of Amino Acid Composition.** The amino acid composition of albumins (obtained by ammonium sulfate precipitation) was determined with an automatic Beckman amino acid analyzer. Samples were hydrolyzed in vacuo for 20 h in the presence of 6 M HCl, at 110 °C, as reported earlier (Raina and Datta, 1992; Rout et al., 1997; Zhang et al., 1998).

## RESULTS

**Isolation of Buckwheat Albumins.** An SDS−PAGE separation of albumins obtained by dialysis of total salt soluble proteins against water showed that this fraction contains a few polypeptides with a molecular mass close to 16 kDa and a group with a molecular mass range from 8 to 12 kDa (Figure 1A). The abundance of albumins in salt soluble proteins was ∼25%. A similar set of polypeptides was obtained when albumins were isolated by a 90% saturation of water extract with ammonium sulfate. The polypeptide pattern of albumin fraction was the same regardless of the presence or abscence of reducing agent (Figure 1B).

The separation of total salt soluble proteins on a sucrose gradient showed (Figure 2) the existence of three distinct fractions—13S, 8S (already characterized; Radović et al., 1996), and 2S, which comprise 30% of the total. A comparison of albumins, obtained as described, with polypeptides belonging to the 2S fraction showed that albumin polypeptides form the main part of that fraction.

**Amino Acid Composition.** The amino acid composition of albumins compared with that of total salt soluble proteins and of the basic subunit of 13S globulin is shown in Table 1. The albumins of buckwheat have a high nitrogen content (glutamine plus asparagine plus arginine), which is a common characteristic of all storage proteins. It is noticeable that albumins are not deficient in any essential amino acid. On the contrary, exceptionally high contents of lysine (5.6%) and methionine (9.2%) were found. A high lysine content is not an exclusive characteristic of the albumin fraction but of buckwheat seed proteins in general, whereas the high content of methionine highlights albumins in salt soluble proteins.

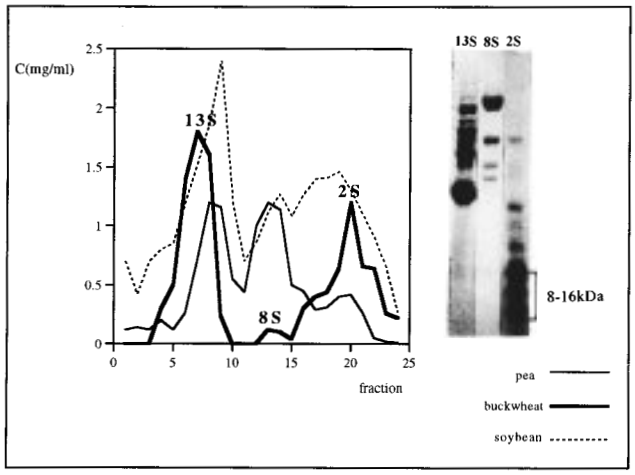


**Figure 2.** Comparative sucrose density gradient (10−30%) of seed salt soluble proteins from buckwheat, pea, and soybean (left). Fractions of buckwheat gradient were collected and peak fractions analyzed on SDS−PAGE under reducing conditions (right).

**Table 1. Amino Acid Composition of Buckwheat Seed Proteins**

| amino acid | total salt soluble proteins,[a] % | 13S basic subunit,[b] % | albumins, % |
|---|---|---|---|
| Asp | 9.01 | 12.30 (Asx) | 6.44 |
| Thr | 3.72 | 2.73 | 3.17 |
| Ser | 4.73 | 5.11 | 4.56 |
| Glu | 16.58 | 25.70 (Glx) | 11.19 |
| Pro | 3.71 | 2.57 | 2.22 |
| Gly | 5.60 | 10.40 | 3.03 |
| Ala | 4.38 | 4.06 | 2.27 |
| Val | 4.36 | 3.00 | 2.31 |
| Met | 2.04 | 1.57 | 9.21 |
| Ile | 3.50 | 3.02 | 2.08 |
| Leu | 6.43 | 6.17 | 5.62 |
| Tyr | 2.80 | 2.13 | 5.65 |
| Phe | 4.38 | 1.93 | 2.04 |
| Lys | 5.70 | 6.93 | 5.60 |
| His | 2.36 | 2.40 | 1.62 |
| Arg | 8.97 | 5.93 | 5.11 |
| Cys | | 1.03 | 0.70 |

[a] Data from Javornik et al. (1981). [b] Data from Rout et al. (1997); all presented data are obtained using the method described under Materials and Methods; cysteine was determined as cystine; Asx-Asn+Asp; Glx-Gln+Glu.

**Influence of Sulfur Nutrition on Protein Composition.** Because we found that albumins are methionine-rich, we examined the influence of sulfur deficiency on the polypeptide composition of buckwheat seed. The protein composition of seeds from plants grown at a suboptimal level of sulfur supply was modified (Figure 3). The changes are especially notable for the albumin fraction and the basic subunit polypeptides of 13S globulin. Both albumin polypeptide groups, the 8−12 and 16 kDa, were dramatically reduced. The level of 13S basic polypeptides was reduced to 50% of the control values.

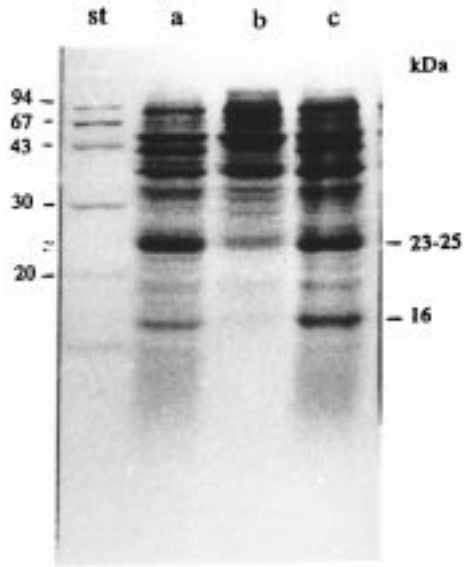


**Figure 3.** SDS−PAGE of total salt soluble proteins from S-deficient buckwheat seed: (a) field-grown buckwheat; (b) buckwheat grown on sand−perlite in the absence of sulfur; (c) buckwheat grown on sand−perlite in the presence of 1 mM $MgSO_4$. Fifty micrograms of proteins was loaded on each lane.

## DISCUSSION

As shown, the albumin fraction can be added to the inventory of buckwheat seed storage proteins, represented by 25% of the total seed proteins. According to its sedimentation coefficient, the fraction consisted mainly of low molecular mass albumin polypeptides (8−16 kDa), and this fraction of buckwheat proteins could be classified under the 2S albumins that are widely distributed in dicotyledonous seeds (Youle and Huang, 1981). There were no changes in protein pattern when samples were treated with 2-mercaptoethanol, suggesting that there were no inter- and intramolecular disulfide bonds. This finding contradicted the results of Ono et al. (1978), who described the albumin fraction as proteins containing disulfide bond polypeptide groups: one of molecular mass 13 kDa and the other of molecular mass 8kDa, which were cleaved after a reduction to smaller polypeptides (7−8 kDa). According to our results, buckwheat albumins are single-chain polypeptides, a characteric unusual for dicotyledonous 2S proteins but typical of sunflower albumins (Kortt and Caldwell, 1990).

Buckwheat seed albumins have another peculiarity among dicotyledonous 2S albumins. Their content of cysteine (measured as cystine) is unusually low, but it seems that an extremely high content of methionine (9.2%) is sufficient for the function of albumins as a source of sulfur for germinating seedlings. With regard to the limitations of the method used for the determination of amino acid composition, the estimated value for methionine, although very high, is probably minimal. A high incorporation of $^{35}$S-methionine in albumin polypeptides, noticed in our in vivo labeling experiments (data not shown), additionally confirms our finding (Maksimović, 1997). Under sulfur deficiency, the albumin fraction was dramatically reduced (Figure 3), a result that correlated well with the behavior of other methionine-rich proteins (Chandler et al., 1984). A high methionine content has also been shown for 2S albumins of Brazil nut (Altenbach et al., 1987, 1992) and sunflower (Kortt and Caldwell, 1990). Besides methionine, the high content of lysine is also of great importance (5.6%).

Both sets of data confirming the high methionine and high lysine contents could be considered when discussing the possibility of using genes for buckwheat albumins for transfer to cereals and/or legumes, major components of human diet, to improve their nutritional quality. It is well-known that lysine is the first nutritionally limiting essential amino acid in most cereals and that there are only a few lysine-rich seed storage proteins identified so far. A high lysine content has been also found for the basic subunit of 13S buckwheat globulin (Rout et al., 1997). However, that polypeptide is a part of the large hexameric globulin whose structure and biosynthetic pathway is too complex for the appropriate expression and storage in a heterologous system (Muntz et al., 1993). Individual albumin polypeptides seem to be more suited to that purpose. The content of methionine, on the other hand, is limited in legumes. High-methionine albumins from buckwheat could be associated with the family of methionine-rich proteins (MRPs) and thereafter may be useful as a vehicle for improving the nutrititional value of legume proteins. In our further experiments we are planning to fractionate buckwheat albumins, to distinguish individual polypeptides, and to determine the specificity of each as a possible vehicle appropriate for the aforementioned purposes.

## ABBREVIATIONS USED

SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis, MW, molecular weight; lmw, low molecular weight; NPU, net protein utilization; PMSF, phenylmethanesulfonyl fluoride; MRP, methionine-rich proteins.

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