



Original article

Buckwheat (*Fagopyrum esculentum* Moench) low molecular weight seed proteins are restricted to the embryo and are not detectable in the endosperm

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Abstract

Buckwheat (*Fagopyrum esculentum* Moench) proteins are nutritionally important because of their high and balanced content of essential amino acids making their biological value much higher than that of cereal proteins. We analyzed extracts of buckwheat endosperm and embryo proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). On electropherograms of endosperm proteins, six intense bands were detected. Two most intense bands were in the range of molecular weights (M.W.s) from 50 to 60 kDa. Protein of 57 kDa has been shown not to cross-react against antibodies raised against proteins of M.W. ranging between 23 and 25 kDa. There are no reports about the allergenicity of other endosperm proteins. On the electropherogram of buckwheat endosperm no low M.W. proteins could be detected. In this study we have demonstrated the tissue specific presence of proteins of different size classes of the endosperm and embryo tissues.

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Keywords: Allergenic proteins; Common buckwheat; Endosperm; *Fagopyrum esculentum*; Low molecular seed proteins; SDS-PAGE electrophoresis

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) proteins are nutritionally important because of their high and balanced content of essential amino acids [1,2] making their biological value [3] much higher than that of cereal proteins [4]. Proteins are, along with starch, the main endogenous factors responsible for the textural characteristics of buckwheat products [5,6]. Correlations have been reported between protein content and hardness, cohesiveness, adhesiveness, springiness and chewiness, as evaluated on buckwheat dough prepared from the endosperm fractions [5,6]. All textural characteristics, except adhesiveness, correlated negatively with protein content. Buckwheat is an undemanding, low-input

plant [7–9]. The major (13S globulin) and minor classes of storage protein constitute about 33 and 6.5% of total seed proteins, respectively [10].

Buckwheat proteins have been reported to prevent gallstone formation more strongly than soy protein isolates, retard mammary carcinogenesis by lowering serum estradiol, and to suppress colon carcinogenesis by reducing cell proliferation [11–13]. These are probably connected with the limited digestibility of buckwheat proteins [3,14,15]. The high content of proteins in certain buckwheat grain milling fractions suggests a potential application of these materials for special dietary products [16]. Tannin–protein complexes can be potent radical cation scavengers in the gastrointestinal tract and may act as a radical sink [17,18]. Buckwheat seed proteins can contain selenium [19,20], a trace element essential in human nutrition. As buckwheat does not contain gluten proteins, it is a common supplement for patients with celiac disease [21,22]. Buckwheat genes and proteins are irreplaceable as molecular markers in evolutionary studies including study of the evolutionary fate of specific types of proteins [23]. Although buckwheat allergy is not very common, buckwheat flour has been

Abbreviations: IgE, immunoglobulin E; M.W., molecular weight; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TEMED, tetramethylethylenediamine; Tris–HCl, Tris (hydroxymethyl) amino-methane hydrochloride.

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regarded as a very potent allergen [24,25]. Few people, especially those with multiple allergies, exhibit buckwheat allergy [22]. Generally, allergic disorders are caused by eating buckwheat food or by using pillows filled with buckwheat husks [22].

The allergy resulting from ingestion is caused by the low molecular weight (M.W.) storage proteins of buckwheat grains [26–29]. Buckwheat allergy is clearly connected with the abundant legumin-type buckwheat grain proteins with M.W.s ranging from 18 to 29 kDa [30–32]. Buckwheat allergy is an immunoglobulin E (IgE)-mediated hypersensitive response capable of causing anaphylactic shock. Electrophoretic immunoblotting confirmed that IgE-antibodies from different patients give different patterns on binding to the seed storage proteins of common buckwheat [28]. Proteins of 17, 50 and 100 kDa [26], 8–10 kDa, with strong IgE-binding activity [27,28], 24 kDa (BW24KD) [29,31], 22, 36, 39–40 and 70–72 kDa [30], 14 and 18 kDa [25] and 16 kDa [33] have all been found to bind IgE. The proteins of 24, 19, 16 and 9 kDa are strong candidates for major allergens, and the 19-kDa allergen was relatively specific for buckwheat allergic patients [34]. The 24-kDa allergen has been proposed to be Fag e 1 [29,35], which is the beta subunit of a 13 S globulin found in buckwheat seeds. The globulin has an approximate mass of 62 kDa, and the subunits are linked by disulfide bonds and separated under reducing conditions [36]. The 57 kDa polypeptide was not recognized by sera from allergic patients [29,30]. The 8S globulin has been shown to be a trimer, composed of subunits of M.W. 57–58 kDa, a structure common for all vicilin-like storage proteins [10]. We have sought a part of the buckwheat grain where low M.W. proteins cannot be detected.

2. Results and discussion

Buckwheat seed proteins extracted from endosperm and embryo were analyzed by SDS-PAGE electrophoresis. Six intense bands were detected from buckwheat endosperm extracts (Fig. 1). Two most intense bands were in the range of M.W.s from 50 to 60 kDa. These proteins have been shown not to be associated with allergy [29,30]. In contrast, no low weight protein bands were detected in the range from 6.5 to 29 kDa on the electropherogram of buckwheat endosperm extracts. The most active proteins causing buckwheat allergy have been clearly connected with the abundant legumin-type buckwheat grain proteins, with M.W.s ranging from 18 to 29 kDa [31,32,36,37]. These protein bands are shown here to be associated exclusively with buckwheat embryo.

Milisavljević et al. [38] showed that antibodies made against 23–25 kDa polypeptides did not cross-react with the 57 kDa polypeptide, which is a subunit of 8S globulin (the main buckwheat storage protein) and is one of the six intense bands that we detected on the electropherogram of buckwheat endosperm protein extract. This finding is in agreement with the reports of Urisu et al. [29] and Nair and Adachi

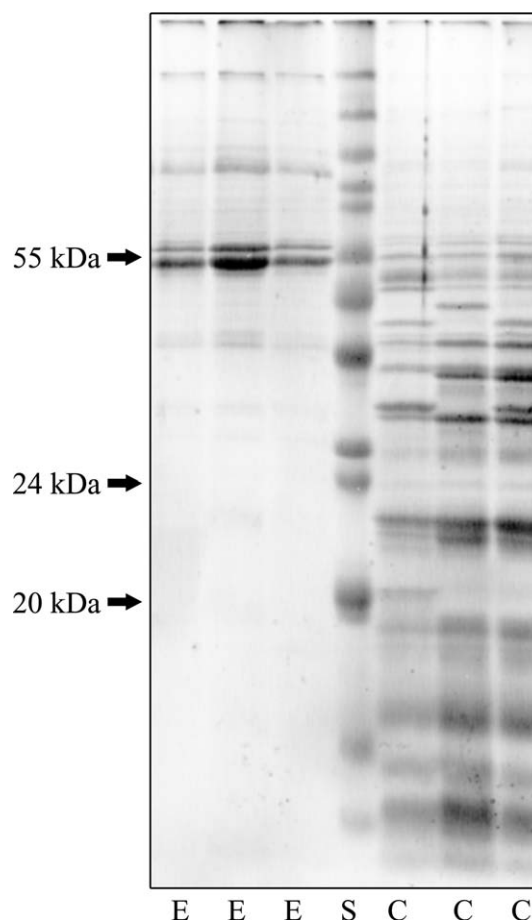


Fig. 1. Electropherogram of common buckwheat endosperm (E) and embryo (C); S-Wide range molecular markers. The most intensive protein bands on endosperm electropherograms range from 36 to 205 kDa. No protein bands on endosperm electropherograms were detected in the range from 6.5 to 36 kDa.

[30] that the 57 kDa polypeptide was not recognized by sera from allergic patients.

Maruyama-Funatsuki et al. [39] found some differences in the BW24KD protein content between different cultivars and within cultivars of buckwheat, although no plants were found to lack BW24KD.

Skrabanja et al. [16] separated different milling fractions, by a combination of sieving and repeated milling, into 23 fractions (seven fine flours, three coarse flours, four small semolina fractions, two large semolina fractions, six bran fractions and one husk fraction). In these fractions there were different amounts of crude protein and starch. Components responsible for technological properties may also be concentrated or regulated to obtain the desired end-use product. Similarly, depending on the metabolic or physiological need of a patient, the appropriate fraction may be chosen to achieve the desired effect [16]. The results presented here lead to the proposal that patients with hypersensitivity to buckwheat flour should use only fine flour from buckwheat endosperm, as this fraction contains no or very few low M.W. seed proteins.

In this study we have demonstrated that buckwheat seeds can be separated into husk, embryo and endosperm. No low

M.W. proteins could be detected on electropherograms of buckwheat endosperm. There appeared a tissue specific presence of proteins of different size classes of the endosperm and embryo tissues. Protein of 57 kDa has been shown not to cross-react against antibodies raised against proteins of M.W. ranging between 23 and 25 kDa [29,30,38]. There are no reports about the allergenicity of other endosperm proteins. There are no reports about the allergenicity of other endosperm proteins. It seems that endosperm cells because of their high specialization contain very low amount of low M.W. proteins, including small housekeeping proteins. With improved technology it should be possible to separate endosperm from embryo on the larger scale, which would enable products to be made from buckwheat flour that could be more appropriate for patients with hypersensitivity to buckwheat flour than buckwheat flour products generally used.

3. Conclusion

Buckwheat (*F. esculentum*) seeds can be separated into husk, embryo and endosperm. No low M.W. proteins, which are known to cause allergy, could be detected on electropherograms of buckwheat endosperm. There appeared tissue specific proteins of different size classes of the endosperm and embryo tissues. It seems that endosperm cells because of their high specialization contain very low amount of low M.W. proteins, including small housekeeping proteins. With improved technology it should be possible to separate endosperm from embryo on the larger scale, which would enable products to be made from buckwheat flour that could be more appropriate for patients with hypersensitivity to buckwheat flour than buckwheat flour products generally used.

4. Methods

4.1. Plant material

Common buckwheat (*F. esculentum* Moench) cv. Siva, was field-grown in the experimental garden of the Biotechnical faculty, University of Ljubljana.

Prior to dissection, the unhusked buckwheat seeds were soaked for 1 h in distilled water. The husk was removed from each seed under a magnifying glass for analysis; the endosperm and embryo (cotyledons) were carefully separated and placed into 1.5 ml centrifuge tubes.

Each endosperm and embryo was smashed separately with a glass rod in 300 µl of extraction buffer (0.5 M Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) (pH 6.8), 20% glycerol, 4% SDS, 3% 2-mercaptoethanol, bromophenol blue). After 2 h of extraction, the samples were incubated in a water bath at 95 °C for 5 min, centrifuged for 15 min at 10,000 rpm and 15 µl of supernatant was applied for electrophoresis.

4.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE electrophoresis)

Separation gels (15% acrylamide) (1.5 M Tris-HCl (pH 8.8), 15% acrylamide stock, 1% SDS, 1% ammonium persulfate, tetramethylethylenediamine (TEMED)) and concentration gels (1.5 M Tris-HCl (pH 6.8), 4% acrylamide stock, 1% SDS, 1% ammonium persulfate, TEMED) were prepared as described [40]. Vertical electrophoresis (Hoefer, San Francisco) was carried out at 40 mA for 1 h, and about 3 h at 60 mA. Proteins were stained for 24 h with 0.05% Coomassie Brilliant Blue R-250, 5% ethanol, and 12% TCA, and destained in deionized water. For standards we used wide range molecular markers SigmaMarker™ M 4038 (M.W. 6500–205,000).

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