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**Buckwheat (*Fagopyrum esculentum*)
Allergenic Seed Proteins are
Restricted to the Embryo and are not
found in the Endosperm.**

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INTRODUCTION

- * 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.
- * The high content of proteins in certain buckwheat grain milling fractions suggests a potential application of these materials for special dietary products.
- * Buckwheat does not contain gluten proteins.



- * Although buckwheat allergy is not very common, buckwheat flour has been regarded as a very potent allergen. Few people, especially those with multiple allergies, exhibit buckwheat allergy.
- * Allergic disorders are caused by eating buckwheat food or by using pillows filled with buckwheat husks.



Slovenian traditional buckwheat dishes (Photo: I. Kreft). Pillows contained buckwheat pericarps (Photo: I. Kreft).

- * The allergy resulting from ingestion is caused by the storage proteins in the grains.
- * Buckwheat allergy is clearly connected with the abundant legumin-type buckwheat grain proteins. It 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.
- * The proteins of **24, 19, 16 and 9 kDa** are strong candidates for major allergens.
- * The **BW24KD** allergen has been proposed to be **Fag e 1**. Maruyama-Funatsuki et al. (*Fagopyrum*, 2004) found some differences in the BW24KD protein content between different cultivars and within cultivars of buckwheat, although no plants were found to lack BW24KD.
- * The possibility of components of buckwheat grain other than protein acting as allergens has not been investigated. We have therefore sought a part of the buckwheat grain that does not contain buckwheat allergy causing proteins.

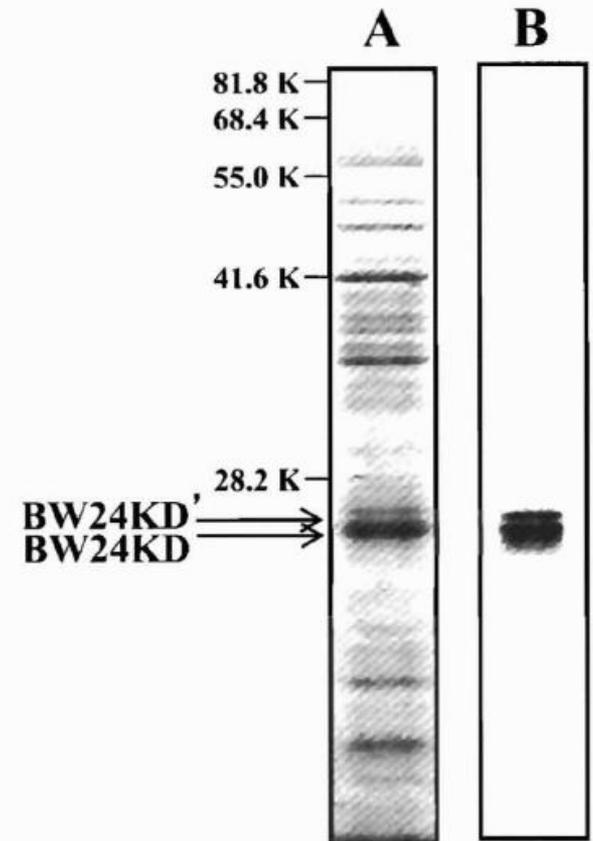


Fig. 1. Immunoblot analysis of seed proteins of Kitawasesoba using an antibody against BW24KD.

MATERIAL AND METHODS

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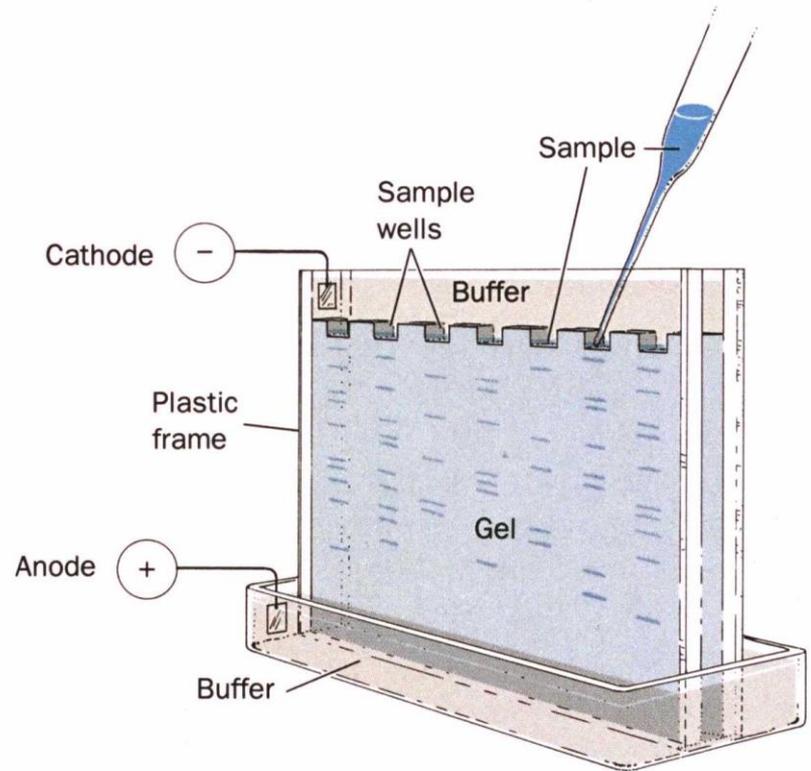
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- * **SDS-PAGE electrophoresis:** Buckwheat seed proteins extracted from endosperm and embryo were analysed by SDS-PAGE electrophoresis. Proteins were prepared as described by Laemmli (*Nature*, 1970).
- * Vertical electrophoresis was carried out at 40 mA for 1 hour, and about 3 hours at 60 mA. Proteins were stained for 24 hours with 0.05% Coomassie Brilliant Blue R-250, and destained in deionized water. For standard we used wide range molecular marker SigmaMarker™ M 4038 (M.W. 6,500-205,000)



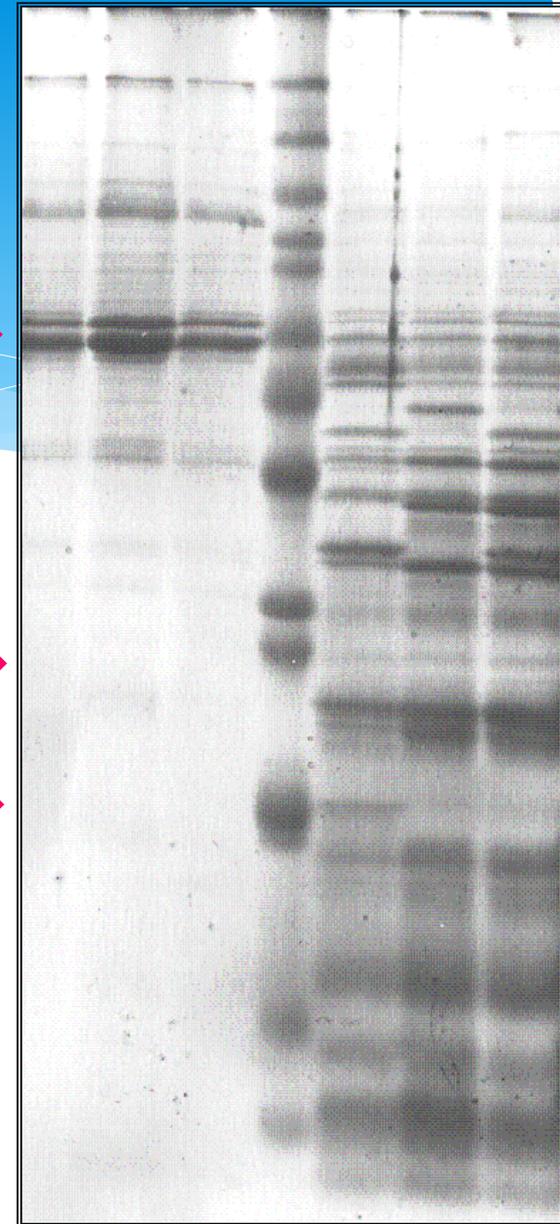
RESULTS AND DISCUSSION

- * Six intense bands were detected from buckwheat endosperm extracts. The most intense corresponded to molecular weights from 50 to 60 kDa.
- * In contrast, no protein bands were found in the range from 6.5 to 36 kDa on the electropherogram of buckwheat endosperm extracts. These protein bands are shown here to be associated exclusively with buckwheat embryo.

55 kDa →

24 kDa →

20 kDa →



E E E S C C C

Electropherogram of common buckwheat endosperm (E) and embryo (C); S-Wide range molecular marker. The most intensive protein bands on endosperm electropherograms range from 36 kDa to 205 kDa.

- * Milisavljević et al. (*J. Agric. Food Chem.*, 2004) showed that antibodies made against 23-25 kDa polypeptides did not cross-react with the 57 kDa polypeptide which is one of the 6 intense bands we detected on the electropherogram of buckwheat endosperm protein extract.
- * This finding is in agreement with the reports of Urisu et al. (*Current Advances in Buckwheat Research*, 1995) and Nair and Adachi (*Fagopyrum*, 2001) that the 57 kDa polypeptide was not recognized by sera from allergic patients.
- * Western blotting of embryo and endosperm proteins of Tartary buckwheat revealed that Fag t 2 was restricted to embryo and was not found in the endosperm (Chen et al., *Allergy*, 2011).
- * Skrabanja et al. (*Cereal Chem.*, 2004) separated different milling fractions, by a combination of sieving and repeated milling, into 23 fractions. In these fractions there were different amounts of crude protein and starch. Similarly, depending on the metabolic or physiological need of a patient, the appropriate fraction may be chosen to achieve the desired effect.

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- * 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 allergenic proteins.
 - * We have also shown that no proteins known to induce symptoms for buckwheat allergy occur in buckwheat endosperm.
 - * 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 and thus being appropriate for patients with hypersensitivity to buckwheat flour.



Thank you for your attention!