

Studies on distribution of protein and allergen in graded flours prepared from whole buckwheat grains

Naofumi Morita ^{a,*}, Tomoko Maeda ^{a,b}, Reimei Sai ^a, Kazuyoshi Miyake ^a,
Hiroyuki Yoshioka ^a, Atsuo Urisu ^c, Taiji Adachi ^{a,d}

^a Laboratory of Food Chemistry, Graduate School of Life and Environmental Sciences, Osaka Prefecture University,
Gakuen-cho 1-1, Sakai, Osaka 599-8531, Japan

^b Department of Life and Health Sciences, Hyogo University of Teacher Education, 942-1, Shimokume, Yashiro, Katou-gun, Hyogo 673-1494, Japan

^c Department of Pediatrics, School of Medicine, Fujita Health University, Toyoake, Aichi 470-1192, Japan

^d Venture Business Laboratory, Center for Advanced Science and Innovation, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Received 15 December 2005; accepted 26 February 2006

Abstract

Buckwheat (*Fagopyrum esculentum* Moench) is one of the traditional crops and has become a renewed target of interest or a popular crop as a healthy foodstuff, because it is a good source of cereal protein which is rich with essential amino acids. However, what is critical to our health is that buckwheat contains proteins which cause a hypersensitive reaction (allergy). In this study, to decrease the allergenic activity of buckwheat products, some graded flours were prepared from various portions of buckwheat grain, and its protein distribution and characteristics of each graded flour fractions were determined. The protein and ash contents of all buckwheat fractions increased in the order from the inner fraction to the outer fraction. From SDS–PAGE patterns, the concentrations of 15, 22, 35, 39 and 50 kDa proteins of the inner fractions were lower, and there were clear differences in the protein compositions between the inner and outer buckwheat flour fractions. Detection of allergenic proteins by IgE-immunoblotting showed that sera pooled from 9 patients reacted with proteins of 15 and 22 kDa bands, named Fag e 2 and Fag e 1, respectively. IgE binding activities of proteins, Fag e 1 or Fag e 2 of the inner fractions were weaker than those of the outer fractions. Therefore, the inner fractions without major allergic proteins could be separated from whole buckwheat grains, and also expected to be applied to manufacturing processed foods with low allergen. Furthermore, the graded-milling procedure could be used to provide less-allergenic buckwheat flours.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Allergen; Buckwheat; *Fagopyrum esculentum*; SDS–PAGE; IgE

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is one of the traditional crops cultivated in central and east Europe and Asia, and now becomes a part of the renewed interest as an alternative crop for organic cultivation or as healthy food. In Japan, the milled buckwheat has been traditionally used for making ‘soba’ noodle, and recently the buckwheat flours have been blended to cookie or pasta

ingredients (Maeda, Miyake, Tahara, & Morita, 2004b, 2004c) and some functional properties have been studied and used for other types of flour products (Miyake, Morita, Handoyo, Maeda, & Morita, 2004b, 2004c). Since the germ exists in the central portion of buckwheat grain differing from wheat grains (Kim, Maeda, & Morita, 2005; Maeda, Kim, & Morita, 2004a; Maeda, Maeda, & Morita, 2001; Maeda & Morita, 2000, 2001, 2003; Maeda, Ohkura, & Morita, 1999), buckwheat is generally milled from the central part after removal of germ, and then some fractions are prepared as buckwheat flours from groats without seed coat. And the ratio or constituent of the fractions is not always constant, hence various buckwheat flours can be

* Corresponding author. Tel.: +81 72 2549459; fax: +81 72 2549921.
E-mail addresses: morita@biochem.osakafu-u.ac.jp (N. Morita),
mtomoko@life.hyogo-u.ac.jp (T. Maeda).

prepared causing different qualities of final products. Therefore, flour qualities of buckwheat have to be adjusted to suit the final products, but regular milling method is neither developed nor established.

On the other hand, buckwheat can grow up in a short period with moderate need for fertilizer and the seeds contain 15–17% protein and are rich in essential amino acids, such as lysine, methionine and tryptophan (Eggum, 1980). In addition, the seeds include rutin, flavonoid and glycoside, which perform as an antagonist to capillary fragility related to hemorrhagic diseases or hypertension and also display antioxidant activity (Griffith, Couch, & Linda-uer, 1994). However, a critical problem to our health is that buckwheat seeds contain proteins, which cause a hypersensitive reaction (allergy). Buckwheat allergy takes place in Japan and some Asian countries. Especially in Japan, five foodstuffs such as egg, milk, wheat, buckwheat and peanut are designated as specific staples to cause the allergic reaction and therefore the indications were made obligatory for foods including these foodstuffs as their ingredients by the Ministry of Health and Welfare in Japan on 2001. Japanese people have been eating buckwheat as one of major foods, and their eating habits of buckwheat may trigger the onset of allergy reaction. So, the patients suffering from buckwheat allergy are mostly adult and they know the good taste and favorable texture. Therefore, such patients need buckwheat flour with low- or non-allergenicity to enjoy the good tastes of buckwheat.

In addition, people in Europe, North America and Japan as well as some Asian countries have developed serious buckwheat allergy or allergic symptom (Hirao et al., 2005; Park et al., 2000; Wang et al., 2004). As a result, the proteins would limit the utilization of buckwheat as general food ingredients. Buckwheat allergy is taking place in Japan and especially in some parts of Asia that is an immunoglobulin (IgE)-mediated hypersensitive response causing serious symptoms with anaphylactic shock. Identification and characterization of the major allergens of buckwheat have been studied by Park et al. (2000). In addition, the purification and characterization of allergenic protein of 24-kDa obtained from buckwheat (*Fagopyrum tartaricum*) have been reported by Wang et al. (2004). Electrophoretic immunoblotting study confirmed that IgE-antibody binding to the seed storage proteins of buckwheat shows various patterns in different patients (Urisu et al., 1994). Especially, proteins of 17, 50 and 100 kDa (Yanagihara, 1980), 8–9 kDa (Yano, Nakamura, Hayakawa, & Torii, 1989), 24 kDa (Urisu et al., 1994), 22, 36, 39–40 and 70–72 kDa (Nair & Adachi, 1999), 14 and 18 kDa (Yoshimasu, Zhang, Hayakawa, & Mine, 2000), and 16 kDa (Tanaka et al., 2002) have been found to bind to IgE. Furthermore, the 22 kDa protein displayed binding activity with almost all sera from different patients, and the protein is considered to be the major allergenic protein (Urisu et al., 1994). The allergen, named Fag e 1, and its corresponding cDNA were previously isolated (Nair & Adachi, 1999). In addition, Yoshioka, Ohmoto, Uris,

Mine, and Adachi (2004a) and Yoshioka et al. (2004b) have reported on cDNA isolations and epitope analysis of buckwheat allergenic proteins of 22 and 15 kDa, named Fag e 1 and Fag e 2, respectively. From these previous results, if the distribution of proteins with allergenic activity is clearly characterized depending on the part of buckwheat grain, low- or non-allergen buckwheat foods will be possible to be obtained using the specific buckwheat fractions. In this study, to decrease the allergenic activity of buckwheat products, some graded flours were obtained from various portions of buckwheat grain, and the protein distribution and characteristics of each graded flour fraction were determined. Our results are expected to be applied to the development of milling procedure of buckwheat grains to provide non- or low-allergenic buckwheat products. Since the purpose of the present study is not absolute quantification but relative quantification of the allergenic proteins in buckwheat flours, the focus was basically to determine the distribution of the only proteins obtained from the portions of whole grains. Furthermore, the distributions of allergenic proteins in the finely graded fractions from the whole buckwheat grain have not been reported yet and the present study is considered to be the first of its kind to try to determine their distributions.

2. Materials and methods

2.1. Grain, flour and chemicals

The buckwheat grain used was Mancan cultivar which had been imported from China and was supplied by Miyake Flour Milling Co., Ltd. (Osaka, Japan). The milling method for buckwheat flours used in the present study was almost similar to that for wheat flour. Firstly, the husk was removed from whole buckwheat grains by dehulling apparatus with disks, and the remained groats with endosperm and bran were milled to buckwheat flours of 17 fractions. After the groats was cut into 7–8 pieces, the cut germ is removed together with broken flours of 3–10% weight of the original grain because the germ existed in the central portion of the grain and also harder than that of wheat grain. In addition, there are some differences in the characteristics between the wheat and buckwheat grains. The weight of bran in buckwheat is much lighter than that in wheat, and there is no difference in the weight between endosperm only and groats including bran. In addition, as the proteins of buckwheat are concentrated in the bran part of the outermost layer, they are tightly attached to the bran during milling and it is quite difficult to separate the proteins from the bran. Furthermore, the endosperm of buckwheat is softer than that of soft-type wheat. Therefore, the purification to be conducted for the separation of bran from endosperm by the specific gravity in the wheat flour milling process is not used for the milling processes of buckwheat flours. As for the rolling system, brake or smooth rolls of 6 or 9–10 and 3 or 5 are used for wheat and buckwheat millings, respectively. In addition, since

the germ and bran are sometimes attached during buckwheat milling, the gather rolls with rougher tooth than brake rolls are required to be added to the buckwheat milling process.

To obtain the 17 fractions in the present study, 2 tons of buckwheat grains were milled per 1 h and the yields of each fraction are shown in Table 1. Generally, if the volume of buckwheat grains on the milling process becomes larger, the numbers of fractions to be obtained also increase accordingly. For the case of portion of each fraction, the inner fraction (FS-1) corresponds to the middle part of the endosperm between the germ and bran which is indicated by arrows in Fig. 1. Major fractions are shown in Fig. 2, and in the order from FS group to the final F3

group, the part for fractioning becomes closer to the bran portion of the outermost layer as shown in Fig. 2. Likewise, the buckwheat was gradually milled and the 17 fractions were obtained and stepwisely named as shown in Fig. 3. Namely, the fraction number 1 in Table 1 means the central part of the endosperm between the germ and bran as shown in Fig. 1, and the parts of other fractions approaching to the outer layer of the bran according to the increase in the fraction numbers are shown in Table 1 and Fig. 3.

The whole flour was prepared by recombination of thus prepared 17 fractions at their same ratio as they are originally composed of the grain on the weight basis as shown in Table 1. Other chemicals were of analytical grade without purification.

2.2. Protein, ash and moisture contents

The protein, ash and moisture contents of flours derived from all buckwheat fractions were determined by the procedures of AACC methods (AACC 44-15A 2000; AACC 46-11A 2000; AACC 08-01 2000), respectively. As to the moisture content, buckwheat flours were stored in a refrigerator at 4 °C and the flours were equilibrated to RT before analyses. The moisture contents were determined from the loss in the weight of the sample after heating at 130 °C for 3 h in an oven.

2.3. Extraction of water-soluble protein from buckwheat fractions

Water-soluble proteins including allergenic proteins were extracted according to the method as reported by Wang et al. (2004) with some modifications. Ten milliliters of sterilized water was added to three grams of each fraction, mixed vigorously for 30 min and stood for 10 min at room temperature (RT). The suspension was centrifuged at 1250g for 10 min at RT, and the supernatant was collected and dialyzed to remove salts, and the inner solution was freeze-dried. The recovered water-soluble proteins

Table 1
Summary of characteristics of graded buckwheat flours

Fraction number	Fraction name	Water (%)	Yield (%)
1 (the innermost fraction)	FS-1 ^a	14.8	17.56
2	FS-2	14.7	3.19
3	F1-1	14.9	11.41
4	FS-3	14.3	9.92
5	F1-2	14.0	9.13
6	F1-3	14.0	9.13
7	FS-4	13.9	0.57
8	FS-5	13.9	0.57
9	F2-1	11.1	3.42
10	F2-2	12.5	1.71
11	F2-3	13.4	9.18
12	F3-1	12.7	1.05
13	F2-4	11.2	1.31
14	F3-2	11.1	3.59
15	F3-3	10.6	4.56
16	F3-4	10.1	4.56
17 (the outermost fraction)	F3-5	10.0	9.13

Whole buckwheat grain was gradually milled to obtain the 17 fractions. FS-1 with fraction no. 1 means flours obtained from the central part of endosperm between the germ and bran as shown in Fig. 1. Other fractions were approaching to the outer layer of bran according to the increase in the fraction numbers.

^a FS-1 included fractions of FS-1-1 and FS-1-2.

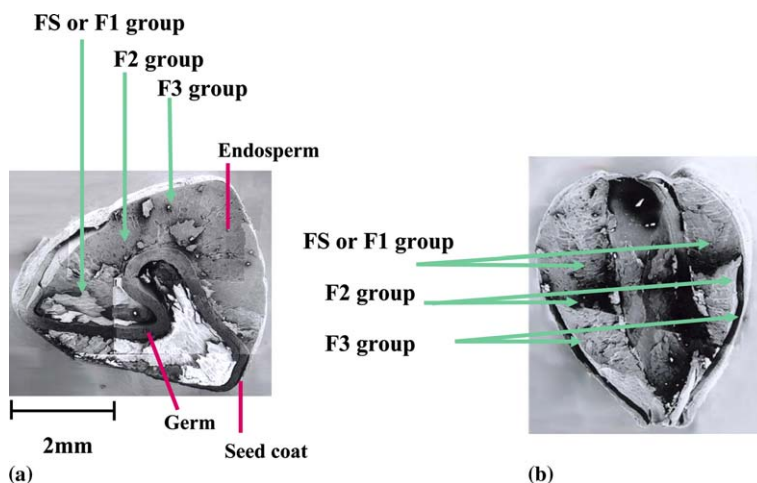


Fig. 1. (a) Cross-sectional and (b) vertical-sectional views of buckwheat grain and the corresponding portions of various milled buckwheat flours.

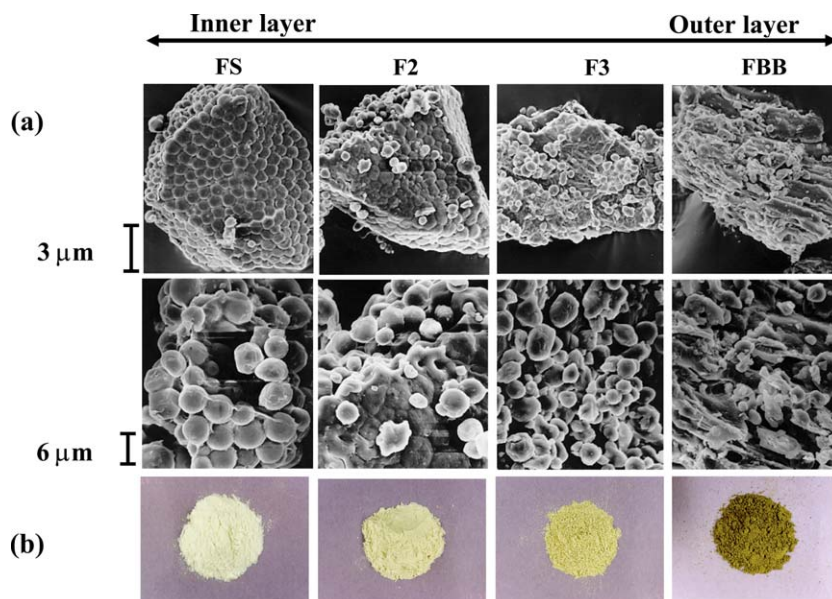


Fig. 2. (a) SEM images and (b) appearances of various milled buckwheat flours. FS ($\times 1000$, 2500); F2 ($\times 1000$, 2500); F3 ($\times 1000$, 2500); FBB ($\times 1000$, 2500). FBB is husk flour.

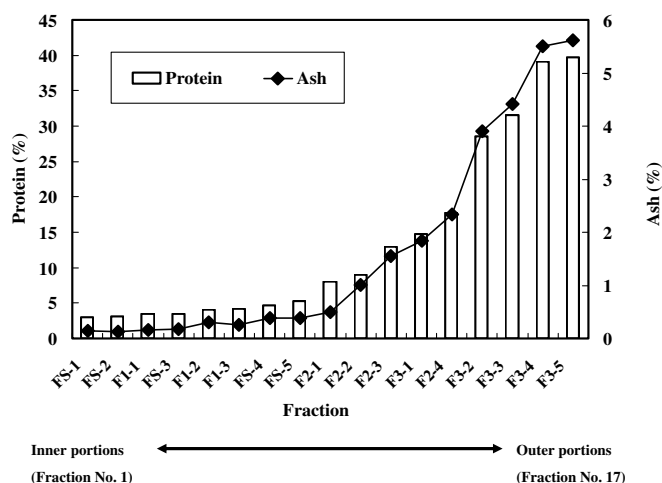


Fig. 3. Distributions of protein and ash contents in various buckwheat flour fractions. Whole buckwheat grain was gradually milled to obtain the 17 fractions. FS-1 (fraction no. 1) was obtained from the central part of endosperm between the germ and bran as shown in Fig. 1, and the other fractions were the same and their parts were approaching to the outer layer of bran according to the increase in the fraction numbers as shown in Table 1.

were different depending on the fractions and they ranged 20–110 mg. The prepared dry sample was used for the following experiments.

2.4. SDS-PAGE

The powder samples of water soluble extracts as described above were dissolved to the sterilized water, stood for 30 min at RT and centrifuged at 25,000g for 1 min. The obtained supernatant (10 μ l) was electrophoresed by the procedure of Laemmli (1970). Proteins were sep-

arated with 15% SDS-PAGE gel for 150 min at 30 mA and 150 V. A mixture of myosin (MW 205 kDa), β -galactosidase (MW 116 kDa), phosphorylase (MW 97 kDa), transferrin (MW 80 kDa), BSA (MW 66 kDa), glutamate dehydrogenase (MW 55 kDa), ovalbumin (MW 45 kDa), carbonic anhydrase (MW 30 kDa), trypsin inhibitor (MW 21 kDa), lysozyme (MW 14 kDa) and aprotinin (MW 6.5 kDa) was used as the molecular-weight marker (Molecular Probes Inc., Eugene, OR, USA). The gel was stained with Commassie brilliant blue R-250 (Millipore Co. Headquarters Billerica, MA, USA) and the molecular-weight distribution of protein was identified. The concentration of protein from the each fraction was not the same, and therefore the concentrations of proteins used were adjusted to the level enough to make clear bands of the fractions.

2.5. Detection of allergenic proteins by IgE-immunoblotting

After separation by SDS-PAGE as described above, the part of the gel containing molecular-weight marker was electroblotted onto a polyvinylidene difluoride (PVDF, Millipore Co. Headquarters Billerica, MA, USA) hydrophobic membrane (Immobilon-P Membrane, 0.45 μ m, 26.5 cm $\times$ 3.75 m roll) for 4 h at 500 mA and 60 V according to the procedure reported by Yoshioka et al. (2004a, 2004b). The sera used for IgE-immunoblotting were from 9 buckwheat-allergic patients and indicated positive score in radioallergo sorbent test (RAST). The characteristics of all patients are shown in Table 2. To be more exact, binding of IgE to allergenic protein is different depending on each patient, but investigation by polling is still necessary to compare and detect the low-allergenic buckwheat flours for the patients with various allergy reactions and symptoms. Furthermore, since the sera of patients

Table 2

Summary of characteristics of patients with buckwheat allergy for IgE-immunoblotting

No.	Age	Sex	BW binding (%)	Mj binding (%)
1	24	Male	15.58	5.03
2	11	Male	12.93	5.18
3	10	Female	7.77	2.67
4	11	Male	5.35	1.32
5	23	Female	4.49	1.39
6	4	Female	2.56	0.11
7	35	Male	1.44	0.54
8	33	Male	0.47	0.06
9	10	Female	20.72	2.17

BW binding: IgE binding to total buckwheat seed protein.

Mj binding: IgE binding to major buckwheat allergenic protein.

indicated positive scores for RAST in the present study described above, the serum of negative control for buckwheat allergy was considered to be not necessary for the IgE-immunoblotting.

The membrane was incubated with sera from 9 patients hypersensitive to buckwheat for 3 h at RT. IgE binding proteins of Fag e 1 and Fag e 2 were detected by monoclonal alkaline phosphatase-conjugated anti-human IgE (Sigma, 1:1000 diluted) as the secondary antibody. The one % of polyvinylpyrrolidone (PVP, Nacalai tesque, Kyoto, Japan) was used to block the blot, and the bound antibodies were detected with the chemiluminescent substrate CDP-Star (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

3. Results and discussion

3.1. Protein, ash and moisture contents

The protein and ash contents of all buckwheat fractions increased in the order from the inner fraction to the outer fraction as shown in Fig. 3. The increasing ratio was of quite stepwise and the clear differences in the distribution of protein and ash were observed depending on the positions in whole buckwheat grain. Moreover, moisture con-

tents in the fractions are shown in Table 2. Buckwheat flours of the outer fractions contained relatively lower moisture contents, as compared with those of the inner fractions. As to the rutin that improves the physiological activity in humans, the amounts in the buckwheat flours of the outer fractions were distinctly larger than those in the inner fractions (data not shown). The amount of lipid increased in the order from the inner to the outer fractions, but that of carbohydrate decreased. In addition, amino acids, maltose values and damaged starch of the outer fractions were abundant, as compared with the inner fractions except for the husk of flour (Miyake, Maeda, & Morita, 2004a).

3.2. SDS-PAGE

As shown in Fig. 4, the electrophoretic patterns of various fractions indicated that most fractions contained the various molecular weight proteins depending on the position in buckwheat grain. To compare the distribution of protein bands among all fractions, the strength of each band was calculated as area count by the image analysis using Scion Image (NIH, Bethesda, Maryland, USA) as shown in Fig. 5. Since the concentration of protein from each fraction was not the same as described above, the concentrations of proteins used were adjusted to make clear bands of the fractions.

As for the distribution of protein bands in Fig. 5, the concentration ratio of 50 or 15 kDa protein to total concentrations for the inner fractions was lower than the outer fractions. In addition, concentration of 10 kDa protein that has been studied by Matsumoto et al. (2004) also increased for the outer fractions, as compared with the inner fractions. Especially, FS-1 (FS-1-1 and FS-1-2), FS-2, FS-3 and F1-3 fractions did not contain these proteins at all. In contrast, the 22 kDa protein was distributed more widely in the inner fractions than the outer fractions. Regarding to the proteins with 35 and 39 kDa, the distribution was different depending on the position in the buckwheat grain although there was no common tendency

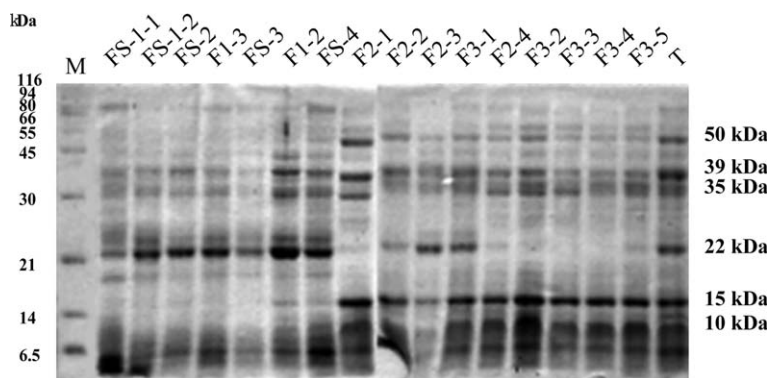


Fig. 4. SDS-PAGE of water-soluble protein extracted from various buckwheat flour fractions. FS-1 was composed of FS-1-1 and -1-2 of two fractions obtained from only former and latter stages of the milling, respectively. Abbreviations are the same as in Fig. 3, except that M and T mean the molecular-weight marker and total flours recombined with 17 fractions at the same ratio as original buckwheat grain, respectively.

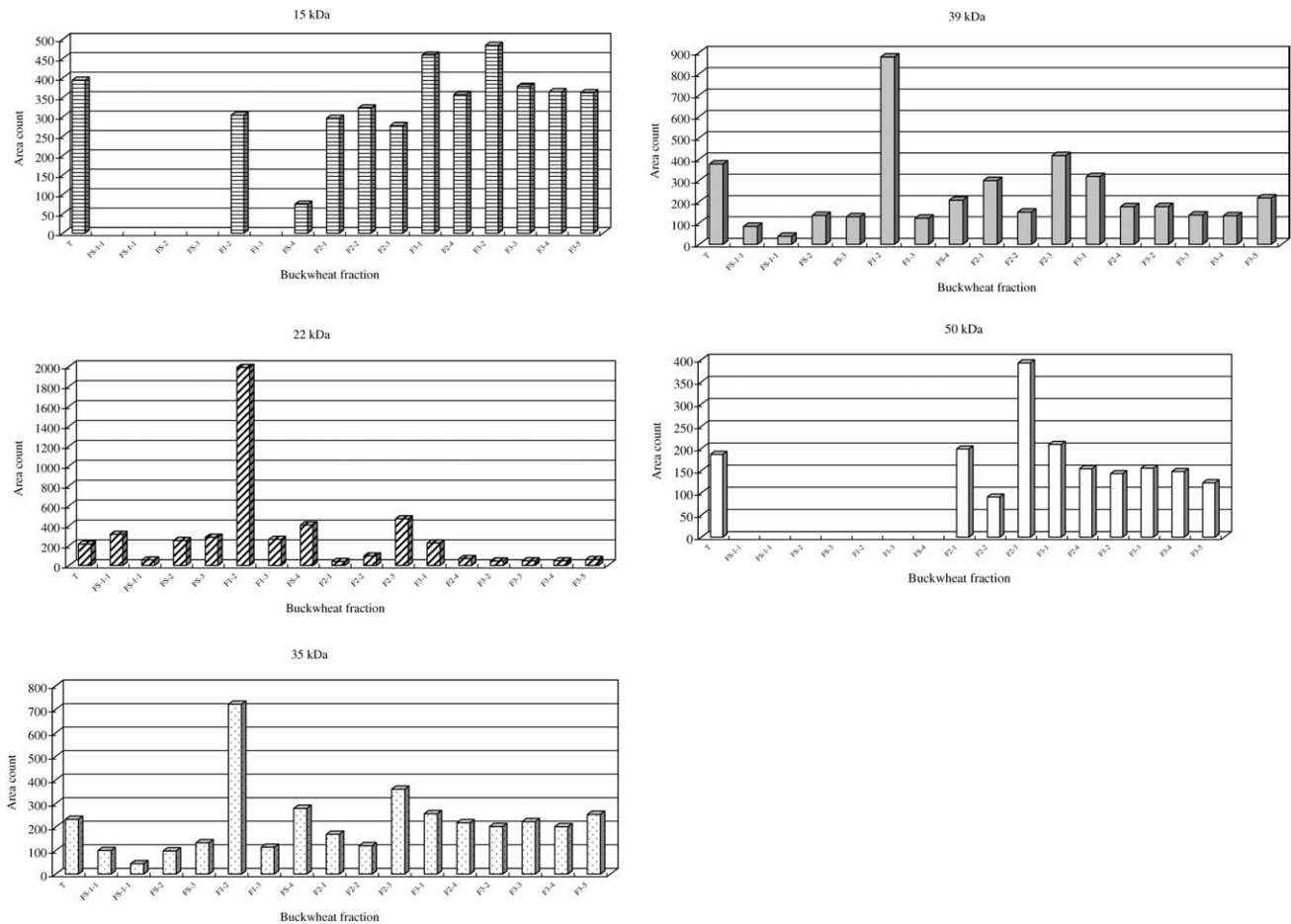


Fig. 5. Distribution of various molecular-weight proteins of water-soluble extracts obtained from various buckwheat flour fractions. Strength of band was calculated as area counts by image analysis with the same protein concentration. Abbreviations are the same as in Fig. 3.

among samples. The disappearance of 15 and 50 kDa proteins was clearly observed for the inner position flours. In addition, the FS-1, FS-2 and FS-3 of the innermost fraction also decreased the concentrations of other protein bands (35 and 39 kDa) as compared with other fractions (Fig. 5). Therefore, there were clear differences in the protein compositions that would be responsible for buckwheat allergy between buckwheat flours derived from the inner and outer fractions.

3.3. Detection of allergenic proteins by IgE-immunoblotting

The immunoblots showed that sera pooled from 9 patients reacted with proteins of 15 and 22 kDa bands (Fig. 6). These molecular-weight proteins were identical with the major buckwheat allergens that were responsible for food-induced allergic reactions, Fag e 2 and Fag e 1, respectively (Nair & Adachi, 1999; Yoshimasu et al., 2000; Yoshioka et al., 2004a, 2004b). Actually, the IgE binding strength with Fag e 2 and Fag e 1 was different among the buckwheat fractions. Since the concentration of protein in each fraction for the IgE-immunoblotting was different, the binding strength was compared after

adjusting to make the same level of protein concentration as shown in Fig. 7. Fag e 2 of the inner fraction expressed weaker IgE binding reaction than that of outer fractions. The Fag e 1 of the FS-1 also showed poorer IgE binding expression than some of other fractions; however Fag e 1 of F2-1 or F3-2 of the middle or outer fractions did not express the IgE binding reactions. These results might be due to the small amount of 22 kDa protein in F2-1 or F3-2 as shown in Figs. 4 and 5. Furthermore, the whole (total) flours reconstructed with all fractions as described above contained larger amounts of 15 kDa protein than 22 kDa as shown in Fig. 5, and then the IgE binding expression was also stronger for the Fag e 2. The amounts of Fag e 1 and Fag e 2 in the graded buckwheat flours were changed depending on the fractions as shown in Figs. 4 and 5. On the other hand, protein of 10 kDa that has been reported by Matsumoto et al. (2004) also reacted with pooled sera of 9 patients. And its IgE expression was also stronger for the outer fractions than for the inner fractions. However, the total amounts of protein were also quite different for each fraction as shown in Fig. 3. Therefore, the inner fractions are sure to include relatively smaller amount of allergenic proteins than other fractions, resulting in the

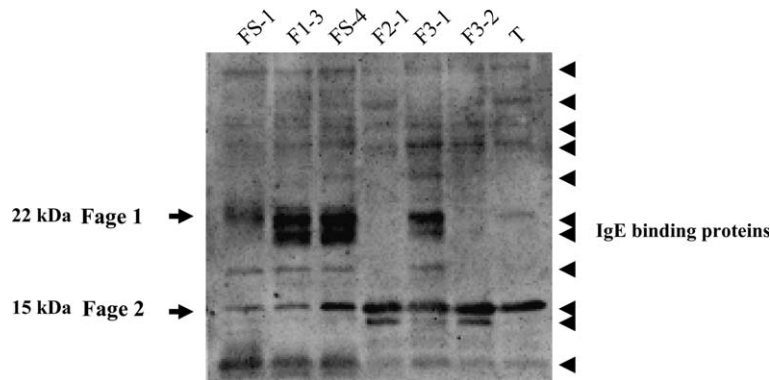


Fig. 6. IgE-immunoblotting of water-soluble protein extracted from various flour fractions using the sera from 9 patients hypersensitive to buckwheat. Abbreviations are the same as in Fig. 3. Sera pooled from 9 patients were used as shown in Table 2.

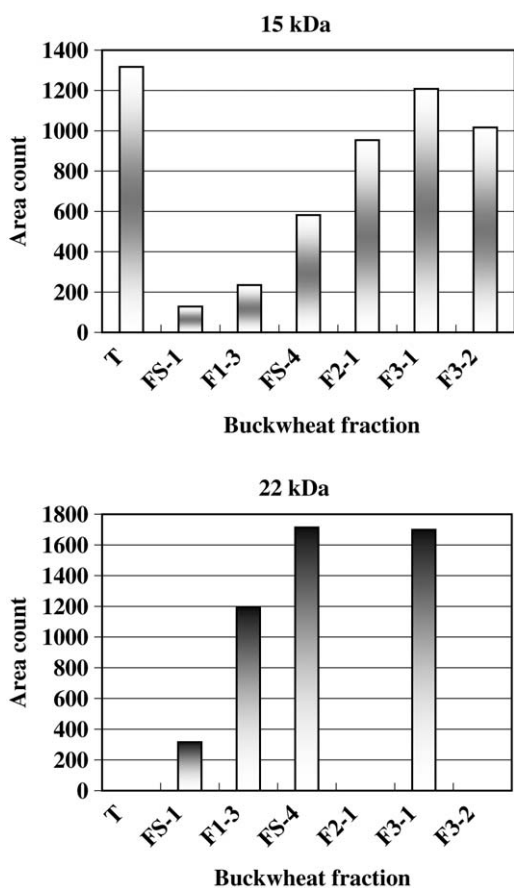


Fig. 7. Distribution of IgE binding proteins of various buckwheat flour fractions by image analysis. Strength of band was calculated as area counts by image analysis with the same protein concentration. Abbreviations are the same as in Fig. 3.

weaker or poorer IgE binding expression. As a result, the inner fractions with low-molecular-weight allergic proteins were mostly removed; therefore, its application to processed foods with low allergen would be expected.

Buckwheat has become more popular in many countries as a new healthy foodstuff (Fujimura, Minami, Watanabe, & Tadera, 2003; Gabrovska et al., 2002; Kreft, Strukelj, Gaberscik, & Kreft, 2002; Rapala, Ostrowska,

Bednarczyk, Dulinski, & Kozik, 2003), but the occurrence of buckwheat allergy is also increasing in Asia and Europe (Kim et al., 2003; Nakamura & Yamaguchi, 1974; Wieslander et al., 2000), so authors have taken it up as the focus of this study. Many cases of buckwheat allergic subjects have been reported, and buckwheat might cause occupational asthma in noodle makers, bakers and packing workers (Davidson, Passero, & Settiban, 1992; De Maat-Bleeker & Stapel, 1998; Göthe, Wieslander, Ancker, & Forsbeck, 1983; Nakamura & Yamaguchi, 1974; Park & Nahm, 1996; Schumacher, Schmid, & Wüthrich, 1993; Takahashi, Ichikawa, Aihara, & Yokota, 1998; Wüthrich & Trojan, 1995). Thus, the development of hypoallergenic buckwheat would supply important pseudo-cereal foods to the allergic persons. As to the protein in buckwheat, recently the cDNAs coding for 15 and 22 kDa proteins with IgE-binding activity has been isolated from developed autogamous strain of common buckwheat (Yoshioka et al., 2004a, 2004b). Since the transgenic techniques have the potential of modifying, decreasing or even removing allergenic substances in rice and soybean (Herman, Helm, Jung, & Kinney, 2003; Tada et al., 1996), the similar approaches are also expected to develop transgenic hypoallergenic crop of common buckwheat. From the cloning and immunological viewpoints, the characteristics of allergenic protein in buckwheat seeds have been studied (Nair & Adachi, 1999; Urisu et al., 1994; Yano et al., 1989; Yoshioka et al., 2004a, 2004b). Nevertheless, few studies on a practical application of hypoallergenic buckwheat flours to processed foods have been published. In addition, productions of hypoallergenic buckwheat foods would be required, as much as possible, without using transgenic techniques. Therefore, the present 17 fractions of buckwheat gradually milled from the innermost to the outermost grains were used for various processed foods depending on the distribution of buckwheat protein and the potency of allergen. Especially, the inner fractions that contained smaller amount of proteins with relatively lower allergenic properties than other fractions, could be applied for hypoallergenic buckwheat products with suitable qualities. Thus, the present

graded milling method of buckwheat was found to be effective on the preparation of hypoallergenic buckwheat flours, resulting in resolution of buckwheat allergy.

4. Conclusion

For the purpose of providing the buckwheat foods with low allergen, various graded buckwheat flours were prepared from the inner portion of whole buckwheat grains with a stepwise manner. The protein and ash contents of buckwheat flour fractions increased in the order from the inner to the outer fractions. Clear differences in the protein compositions on the SDS–PAGE patterns were observed between the buckwheat flours derived from the inner and outer fractions. According to the detection of allergenic proteins by IgE-immunoblotting, sera pooled from 9 patients allergic to buckwheat reacted with proteins of 15 and 22 kDa bands, named as Fag e 2 and Fag e 1, respectively. IgE binding to Fag e 1 and Fag e 2 of the inner fractions was poorer than that of the outer fractions. Based on these results, the inner fractions without major allergic proteins could be separated from whole buckwheat grains by the present graded milling method, and the inner buckwheat flours can be used for the production of processed foods with low allergen.

References

- American Association of Cereal Chemists (2000). *Approved methods of the AACC* (10th ed.). *Method 44-15A, Method 46-11A and 08-01*. St. Paul MN: The Association.
- Davidson, A. E., Passero, M. A., & Settipan, G. A. (1992). Buckwheat induced anaphylaxis: a case report. *Annals of Allergy*, 69, 439–440.
- De Maat-Bleeker, F., & Stapel, S. O. (1998). Cross-reactivity between buckwheat and latex. *Allergy*, 53, 538–539.
- Eggum, B. O. (1980). The protein quality of buckwheat in comparison with other protein source of plant or animal origin. *Journal of Biological Chemistry*, 252, 1102–1106.
- Fujimura, M., Minami, Y., Watanabe, K., & Tadera, K. (2003). Purification, characterization, and sequencing of a novel type of antimicrobial peptides, Fa-AMP1 and Fa-AMP2, from seeds of buckwheat (*Fagopyrum esculentum* Moench). *Bioscience, Biotechnology and Biochemistry*, 67, 1636–1642.
- Gabrovská, D., Fiedlerová, V., Holasová, M., Masková, E., Smrcinová, H., Rysová, J., et al. (2002). The nutritional evaluation of underutilized cereals and buckwheat. *Food and Nutrition Bulletin*, 23, 246–249.
- Göthe, C. J., Wieslander, G., Ancker, K., & Forsbeck, M. (1983). Buckwheat allergy: health food, an inhalation health risk. *Allergy*, 38, 155–159.
- Griffith, J. Q., Couch, J. F., & Lindauer, A. (1994). Effect of rutin on increased capillary fragility in man. *Proceedings Of the Society for Experimental Biology*, 55, 228–229.
- Herman, E. M., Helm, R. M., Jung, R., & Kinney, A. J. (2003). Genetic modification removes an immunodominant allergen from soybean. *Plant Physiology*, 132, 36–43.
- Hirao, T., Imai, S., Sawada, H., Shiomi, N., Hachimura, S., & Kato, H. (2005). PCR method for detecting trace amounts of buckwheat (*Fagopyrum spp.*) in food. *Bioscience, Biotechnology and Biochemistry*, 69, 724–731.
- Kim, C. D., Lee, W. K., No, K. O., Park, S. K., Lee, M. H., Lim, S. R., et al. (2003). Anti-allergenic action of buckwheat (*Fagopyrum esculentum* Moench) grain extract. *International Immunopharmacology*, 3, 129–136.
- Kim, J. H., Maeda, T., & Morita, N. (2005). Application of polished-graded wheat grains for sourdough bread. *Cereal Chemistry*, 82, 144–151.
- Kreft, S., Strukelj, B., Gaberscik, A., & Kreft, I. (2002). Rutin in buckwheat herbs grown at different UV–B radiation levels: comparison of two UV spectrophotometric and an HPLC method. *Journal of Experimental Botany*, 53, 1801–1804.
- Laemmli, UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.
- Maeda, T., Kim, J. H., & Morita, N. (2004a). Evaluation of various baking methods for polished wheat flours. *Cereal Chemistry*, 81, 660–665.
- Maeda, T., Maeda, N., & Morita, N. (2001). Effect of polished-graded hard-type wheat flour substitution for commonly milled hard-type wheat flour on the properties of dough and bread. *Journal of Applied Glycoscience*, 48, 27–36.
- Maeda, T., Miyake, K., Tahara, M., & Morita, N. (2004b). Substitution of buckwheat for wheat flours on processing pasta and cookie. *Fagopyrum*, 21, 99–103.
- Maeda, T., Miyake, K., Tahara, M., & Morita, N. (2004c). Effect of buckwheat substitution for wheat flour on pasta and cookie. In F. Iva, D. Václav, C. Petra, H. Ivan, H. Vojtěch, & S. Zdeněk (Eds.), *Proceedings of the ninth international symposium on buckwheat; 2004 Aug 18–22* (pp. 559–563). Prague, Czech Republic: Research Institute of Crop Production, University of Agriculture.
- Maeda, T., & Morita, N. (2000). Effect of polished-graded flour substitution to commonly milled wheat flour on the properties of dough and bread. *Journal of Applied Glycoscience*, 47, 1–12.
- Maeda, T., & Morita, N. (2001). Effect of quality of hard-type polished-graded flour on breadmaking. *Journal of Applied Glycoscience*, 48, 63–70.
- Maeda, T., & Morita, N. (2003). Flour quality and pentosan prepared by polishing wheat grain on breadmaking. *Food Research International*, 36, 603–610.
- Maeda, T., Ohkura, M., & Morita, N. (1999). Characterization of wheat flour graded by polishing and its application to breadmaking. *Journal of Applied Glycoscience*, 46, 413–422.
- Matsumoto, R., Fujino, K., Nagata, Y., Hashiguchi, S., Ito, Y., Aihara, Y., et al. (2004). Molecular characterization of a 10-kD buckwheat molecule reactive to allergic patients' IgE. *Allergy*, 59, 533–538.
- Miyake, K., Maeda, T., & Morita, N. (2004a). General components and protein properties of various graded buckwheat fractions on the milling process. *The Food Industry*, 47(20), 64–70 (in Japanese).
- Miyake, K., Morita, R., Handoyo, T., Maeda, T., & Morita, N. (2004b). Characteristics of graded buckwheat flours and functional properties of germinated buckwheat. In F. Iva, D. Václav, C. Petra, H. Ivan, H. Vojtěch, & S. Zdeněk (Eds.), *Proceedings of the ninth international symposium on buckwheat; 2004 Aug 18–22* (pp. 660–664). Prague, Czech Republic: Research Institute of Crop Production, University of Agriculture.
- Miyake, K., Morita, R., Handoyo, T., Maeda, T., & Morita, N. (2004c). Characterization of graded buckwheat flours and some properties of germinated Mancan buckwheat grains. *Fagopyrum*, 21, 91–97.
- Nair, A., & Adachi, T. (1999). Immunodetection and characterization of allergenic proteins in common buckwheat (*Fagopyrum esculentum*). *Plant Biotechnology*, 16, 219–224.
- Nakamura, S., & Yamaguchi, M. (1974). Studies on buckwheat allergosis report 2: Clinical investigation on 169 cases with the buckwheat allergosis gathered from the whole country of Japan. *Allergy Immunology*, 20, 457–465.
- Park, H. S., & Nahm, D. H. (1996). Buckwheat flour hypersensitivity: an occupational asthma in a noodle maker. *Clinical and Experimental Allergy*, 26, 423–427.
- Park, J. W., Kang, D. B., Kim, C. W., Ko, S. H., Yum, H. Y., Kim, K. E., et al. (2000). Identification and characterization of the major allergens of buckwheat. *Allergy*, 55, 1035–1041.
- Rapala, K. M., Ostrowska, K., Bednarczyk, K., Dulinski, R., & Kozik, A. (2003). Polypeptide components of oligomeric legumin-like

- thiamin-binding protein from buckwheat seeds characterized by partial amino acid sequencing and photoaffinity labeling. *Journal of Protein Chemistry*, 22, 167–175.
- Schumacher, F., Schmid, P., & Wüthrich, B. (1993). Zur Pizokel-Allergie: ein Beitrag über die Buckwiezenallergie. *Schweizerische Medizinische Wochenschrift*, 123, 1559–1562.
- Tada, Y., Nakase, M., Adachi, T., Nakamura, R., Shimada, H., Takahashi, M., et al. (1996). Reduction of 14–16 kDa allergenic proteins in transgenic rice plants by antisense gene. *FEBS Letter*, 391, 341–345.
- Takahashi, Y., Ichikawa, S., Aihara, Y., & Yokota, S. (1998). Buckwheat allergy in 90,000 schoolchildren in Yokohama. *Arerugi*, 47, 26–33 (in Japanese).
- Tanaka, K., Matsumoto, K., Akasawa, A., Nakajima, T., Nagasu, T., Iikura, Y., et al. (2002). Pepsin-resistant 16-kDa buckwheat protein is associated with immediate hypersensitivity reaction in patients with buckwheat allergy. *International Archives Allergy Immunology*, 129, 49–56.
- Urisu, A., Kondo, Y., Morita, Y., Wada, E., Tsuruta, M., Yasaki, T., et al. (1994). Identification of a major allergen of buckwheat seeds by immunoblotting methods. *Allergy and Clinical Immunology*, 6, 151–155.
- Wang, Z., Zhnage, Z., Zhao, Z., Wieslander, G., Norbäck, D., & Kreft, I. (2004). Purification and characterization of a 24-kD protein from tartary buckwheat seeds. *Bioscience, Biotechnology and Biochemistry*, 68, 1409–1413.
- Wieslander, G., Norbäck, D., Wang, Z. H., Zhang, Z., Mi, Y. H., & Lin, R. F. (2000). Buckwheat allergy and report on asthma and atopic disorders in Taiyuan City, Northern China. *Asian Pacific Journal of Allergy Immunology*, 18, 147–152.
- Wüthrich, B., & Trojan, A. (1995). Wheatburger anaphylaxis due to hidden buckwheat. *Clinical Experimental Allergy*, 25, 1263.
- Yanagihara, Y. (1980). Buckwheat hypersensitivity. *Kansensho* (Journal of Infectious Chemotherapy), 10, 184–188.
- Yano, M., Nakamura, R., Hayakawa, S., & Torii, S. (1989). Purification and properties of allergenic proteins in buckwheat seed. *Agricultural Biological Chemistry*, 53, 2387–2392.
- Yoshimasu, M., Zhang, J., Hayakawa, S., & Mine, Y. (2000). Electrophoretic and immunochemical characterization of allergenic proteins in buckwheat. *International Archives of Allergy and Immunology*, 123, 130–136.
- Yoshioka, H., Ohmoto, T., Uris, A., Mine, Y., & Adachi, T. (2004a). Expression and epitope analysis of the major allergenic protein Fag e 1 from buckwheat. *Journal of Plant Physiology*, 161, 761–767.
- Yoshioka, H., Yasueda, N., Ohmoto, T., Urisu, A., Mine, Y., Yamazaki, R., et al. (2004b). cDNA isolation and epitope analysis of allergenic proteins in autogamous common buckwheat. In F. Iva, D. Václav, C. Petra, H. Ivan, H. Vojtěch, & S. Zdeněk (Eds.), *Proceedings of the ninth international symposium on buckwheat; 2004 Aug 18–22* (pp. 612–620). Prague, Czech Republic: Research Institute of Crop Production, University of Agriculture.