



Analysis of the distribution of rice allergens in brown rice grains and of the allergenicity of products containing rice bran



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ABSTRACT

To understand the allergenicity of rice bran, the distribution of rice allergens in brown rice grains was analysed and the allergenicity of cosmetics and health foods containing rice bran determined. RAG2 and a 19-kDa globulin were localized in polished rice, while a 52-kDa globulin was localized in rice bran. The 52-kDa globulin was also identified as the most likely causative allergen of rice bran allergy. Several products containing intact rice bran were found to contain the 52-kDa globulin. Our study provides the first data regarding cosmetics and health foods containing potential rice bran allergens. Western blot analysis using a rice-bran-allergic patient's plasma showed that 52-kDa globulin was detected as an IgE-binding protein of rice bran and some rice bran-containing cosmetics and health foods. Our results indicate that patients with rice bran allergy need to be careful about using products containing intact rice bran as a constituent.

1. Introduction

Rice is the staple food for over a half of the world's population, containing carbohydrate, oil and protein as energy sources. On the other hand, the number of allergenic substances detected in various foods has been increasing in recent years, and cases of patients who are allergic to rice have also been reported from both Asian and Western countries. The clinical symptoms of rice allergy include atopic dermatitis (Ikezawa et al., 1992; Uchio, Miyakawa, Ikezawa, & Ohno, 1998), eczema (Hoffman, 1975; Shibasaki, Suzuki, Nemoto, & Kuroume, 1979), and food protein-induced enterocolitis syndrome (Mehrer, Kakakios, & Kemp, 2009; Sicherer, Eigenmann, & Sampson, 1998). Several rice proteins have already been identified as rice allergens, the causative agents of rice allergy. These rice allergens include the non-specific lipid transfer protein-1 (nsLTP1) (Enrique et al., 2005), the α -amylase/trypsin-inhibitor RAG2 (Matsuda, Sugiyama, Nakamura, & Torii, 1988), an α -globulin (19-kDa globulin) (Limas et al., 1990; Urisu et al., 1991), the 33-kDa glyoxalase I (Gly I) (Usui et al., 2001), a 52-kDa and a 63-kDa globulin (Satoh, Nakamura, Komatsu, Oshima, &

Teshima, 2011), and the granule-bound starch synthetase (GBSSI) (Ikezawa et al., 1999).

Rice bran contains some beneficial components, such as oil, vitamins and minerals (Revilla et al., 2009). It also has dietary fibre, including cellulose and hemicellulose, which are known to have functional properties (Abdul-Hamid & Luan, 2000; Liu et al., 2015; Qiu et al., 2016; Qiu, Yadav, & Yin, 2017). Thus, rice bran has been used as an ingredient of cosmetics and health food supplements. On the other hand, the first case of occupational contact urticaria resulting from the domestic handling of rice bran in the form of rice bran pickles, has been reported (Inomata, Morita, Sawaki, & Aihara, 2012). The authors of that case report identified an approximately 50-kDa IgE-binding protein as the causative agent in this case, by western blotting of rice bran extracts using serum from the patient with the rice bran allergy. This case report shows that it is necessary to understand the allergenicity of whole brown rice and rice bran, as well as that of polished rice, and to consider sensitization by percutaneous exposure to rice.

Some proteins in foods are known to be responsible for various types of food allergies. Although the pathogenesis of food allergy from

Abbreviations: 1D, one-dimensional; 2D, two-dimensional; CBB, coomassie brilliant blue; DTT, dithiothreitol; HRP, horseradish peroxidase; PBS, phosphate-buffered saline, pH 7.2; PBS-T, PBS containing 0.05% Tween 20; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

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percutaneous sensitization is yet to be fully elucidated, there are several reports that some patients who were primarily sensitized to hydrolyzed wheat proteins (HWPs) in cosmetics experienced immediate food allergic reactions after ingesting foods containing HWPs (Laurière et al., 2006) or ingesting wheat food products (Fukutomi et al., 2011).

In this study, we focussed on the rice allergens in rice bran to assess the allergenicity of rice bran. In the initial step, as there are no reports yet of detailed investigation of proteins derived from rice (brown rice, rice bran, and polished rice) using the three kinds of purification methods, we attempted to identify the rice allergens in brown rice, rice bran and polished rice and analyzed the distribution of rice allergens in rice grains. Next, to clarify the protein that needed to be targeted, products containing rice bran were examined; protein extraction and detection of rice allergens were carried out for cosmetics and health foods containing rice bran, and their allergenicity evaluated. Finally, the IgE-binding proteins containing the causative allergens in the patient with rice bran allergy were identified.

2. Materials and methods

2.1. Rice samples and protein extracts

Mature brown seeds of a japonica rice cultivar (*Oryza sativa* cv. Koshihikari) that is available commercially were used for the experiments. Rice bran and polished rice were made from the brown rice by milling the brown rice at a rice dealer's. Four grams of brown rice, rice bran or polished rice were pulverized twice with a Multi Beads Shocker (Yasui Kikai Corp., Osaka, Japan) at 2,000 rpm for 30 s. A part of each of the powdered samples (0.1 g) was suspended in 1 ml of 1 × Laemmli Sample Buffer, prepared by dilution of 2 × Laemmli Sample Buffer (#161-0747, Bio-Rad Laboratories, Hercules, CA, USA) with distilled water, or in 1 ml of 1 M NaCl with protease inhibitors (Halt Protease Inhibitor Cocktail, #87785, Thermo Fisher Scientific Inc., MA, USA), by rotating overnight at 4 °C with a Rotator (RT-50, TAITEC Corp., Saitama, Japan) for extracting the rice total proteins and salt-soluble proteins, respectively. The protein solutions extracted with 1 × Laemmli Sample Buffer or 1 M NaCl from each of the rice samples were then centrifuged at 20,000 × g for 30 min at 4 °C and the supernatants were collected as the total proteins and salt-soluble proteins, respectively. The protein concentrations in the total protein and salt-soluble protein fractions were determined with a 660 nm Protein Assay Reagent Kit (#22660, Thermo Fisher Scientific Inc., MA, USA) in the presence or absence, respectively, of the Ionic Detergent Compatibility Reagent (IDCR, #22663, Thermo Fisher Scientific Inc., MA, USA). The protein solutions were stored in aliquots at −80 °C until further use.

2.2. Health foods and cosmetics containing rice bran and protein extracts

Health foods and cosmetics that are commercially available were used for these experiments (Table 1), in which 2 ml of liquid, paste and foam samples, 0.1 g of powdered soap, and 0.8 g of powdered tablets, and 0.8 g of rice bran powder were suspended in 2 ml, 5 ml, 4 ml and 4 ml, respectively, of phosphate-buffered saline (PBS). Samples were suspended overnight at room temperature (soap) or at 4 °C (others) by constant mixing by rotation with a Rotator (RT-50, TAITEC Corp., Saitama, Japan) for extracting the water-soluble proteins. After centrifugation of the samples at 15,000 × g for 30 min at room temperature (soap) or at 4 °C (others), the collected supernatants were filtered via a disposable syringe filter unit (DISMIC-25CS, 0.45 µm hydrophilic cellulose acetate membrane, ADVANTEC, Tokyo, Japan). The filtered supernatants were then concentrated by filtration through an ultrafiltration membrane (Amicon Ultra-4, 3,000 MWCO, Merck Millipore Corp., Darmstadt, Germany) and collected as protein fractions containing the allergens. The protein concentrations in the water-soluble protein fractions were determined with a 660 nm Protein Assay Reagent Kit (Thermo Fisher Scientific Inc., MA, USA). The protein solutions were

then stored in aliquots at −80 °C until further use.

2.3. Patient plasma

The plasma of an 11-year-old Japanese female patient with a history of allergic symptoms to rice and rice bran, was used for this study. Informed consent for use of the plasma sample was obtained from the patient. Our study was conducted with the approval of the Ethical Review Committee of Fujita Health University, National Institute of Health Sciences, and Food Research Institute, National Agriculture and Food Research Organization.

2.4. SDS-PAGE and CBB staining

For the electrophoresis, 3 µg of the protein fractions extracted from brown rice, rice bran and polished rice were separated by SDS-PAGE on a 10–17.5% SDS-polyacrylamide gradient gel (#NTH-5X5HP, DRC Co., Ltd., Tokyo, Japan) under reducing conditions. For the protein extracts obtained from the cosmetics and health foods, except for sample nos. 5 and 15, 5 µg of the extracted proteins were separated by SDS-PAGE. For sample no. 5 or 15, 1.76 or 1.33 µg of the protein extracts were used for SDS-PAGE. Coomassie brilliant blue (CBB) staining (Quick CBB, #99-50101, Wako Co., Ltd., Tokyo, Japan) was used to detect the protein bands separated electrophoretically on the polyacrylamide gels. The stained gels were scanned with a SAYACA imager (DRC Co., Ltd., Tokyo, Japan) and stored as TIFF image files. The scanned images were processed with Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, CA, USA).

2.5. Western blotting

2.5.1. Western blotting with rice allergen-specific animal antibodies

For western blotting using rice allergen-specific polyclonal antibodies, 3 µg of the extracted proteins from brown rice, rice bran and polished rice were separated by SDS-PAGE on a 10–17.5% SDS-polyacrylamide gradient gel (#NXV-3X8HP, DRC Co., Ltd., Tokyo, Japan) under reducing conditions, and then transferred onto polyvinylidene difluoride (PVDF) membranes (#162-0177, Bio-Rad Laboratories, Hercules, CA, USA). For the protein extracts obtained from the cosmetics and health foods, except for sample nos. 5 and 15, 5 µg of the extracted proteins were separated by SDS-PAGE and transferred onto PVDF membranes, as described above. For sample no. 5 or 15, 1.76 or 1.33 µg of the protein extracts were used for the western blotting. The membranes were blocked with 0.5% casein-PBS for 2 h at room temperature and then incubated for 1 h at room temperature with each of the antibodies (anti-RAG2; anti-glyoxalase I; anti-19 kDa globulin; anti-52 kDa globulin; 1:5,000 dilution; dilution with 0.1% casein-PBS). After washing three times with PBS containing 0.05% Tween 20 (PBS-T), the membranes were incubated with horseradish-peroxidase (HRP)-linked anti-rabbit IgG [1:10,000 dilution; dilution with 0.1% casein-PBS; Amersham™ ECL™ Rabbit IgG, HRP-linked F(ab')₂ fragment (from donkey), #NA9340, GE Healthcare UK Ltd., Little Chalfont, England] for 1 h at room temperature. After washing again with PBS-T three times, the bound secondary antibodies were detected using Immunostar LD (#290-69904, Wako Co., Ltd., Tokyo, Japan), in accordance with the manufacturer's protocol. The signal intensities of the bands were detected using SAYACA imager (DRC Co., Ltd., Tokyo, Japan).

2.5.2. Western blotting using the plasma from the patient with rice allergy

For the western blot analysis using the plasma obtained from the patient with rice allergy, the same volume of the extracted proteins from the rice samples or from the cosmetics and health foods used in the western blotting using rice allergen-specific antibodies were separated by SDS-PAGE and transferred onto PVDF membranes as described above. The membranes were blocked with 0.5% casein-PBS for 2 h at room temperature and then incubated for 30 min at room temperature

Table 1
List of health foods and cosmetics used in this study and summary of the results of the western blot analysis of these products using rice allergen-specific antibodies.

Sample No.	Health foods/ Cosmetics	Product form	Purpose of use	Manufacturer	Total number of ingredients	Rice bran	Defatted rice bran	Fermented rice bran	Rice bran extract	Koji mold	RAG2	Glyoxalase I	19 kDa globulin	52 kDa globulin
1	Health foods	Tablet	Enzyme supplement	A	5	✓			✓	N.D.	N.D.	N.D.	N.D.	N.D.
2		Tablet	Enzyme supplement	A	6	✓			✓	N.D.	N.D.	N.D.	N.D.	N.D.
3		Tablet	Enzyme supplement	A	6	✓			✓	N.D.	N.D.	N.D.	N.D.	N.D.
4		Paste	Powder for making pickles	B	5	✓			✓	++	N.D.	+	N.D.	N.D.
5		Liquid	Health beverage containing enzyme and plants	C	15	✓			✓	N.D.	N.D.	+	N.D.	N.D.
6		Tablet	Supplement for decrease of lactic acid	D	12		✓			N.D.	N.D.	++	N.D.	N.D.
7		Tablet	Supplement for improvement of eyesight	D	12		✓			N.D.	N.D.	+	N.D.	N.D.
8		Tablet	Supplement for improvement of memory	D	8		✓			+	N.D.	+++	N.D.	N.D.
9		Tablet	Supplement for energy supply	D	11		✓			N.D.	N.D.	N.D.	++	++
10		Tablet	Nutritive supplement foods	E	3	✓				N.D.	N.D.	++	N.D.	N.D.
11		Tablet	Nutritive supplement foods	E	2	✓				++	N.D.	+++	++	++
12		Tablet	Supplement for energy supply	F	6		✓			+	N.D.	++	++	++
13		Tablet	Supplement with peptides that contain glutamine	F	6		✓			N.D.	N.D.	+	N.D.	N.D.
14		Tablet	Enzyme supplement	G	11		✓			N.D.	N.D.	N.D.	N.D.	N.D.
15		Liquid	Health beverage containing enzyme and plants	H	5	✓				N.D.	N.D.	+	N.D.	N.D.
16		Powder	Supplement containing rice bran and GABA	I	2	✓				+	N.D.	++	N.D.	N.D.
17		Tablet	Supplement containing rice bran	J	6	✓				+	N.D.	N.D.	N.D.	N.D.
18	Cosmetics	Soap	Body and face wash	K	12	✓				N.D.	N.D.	N.D.	N.D.	N.D.
19		A cloth bag filled with rice bran powder	Body and face scrub	L	12	✓				N.D.	N.D.	+	N.D.	N.D.
20		A cloth bag filled with rice bran powder	Preventing spots and dullness	L	14	✓				N.D.	+	N.D.	N.D.	N.D.
21		A cloth bag filled with rice bran powder	Skin pore-tightening and moisturizing agent	L	14	✓				N.D.	+	N.D.	N.D.	N.D.
22		Soap	Body and face wash	M	17				✓	N.D.	N.D.	N.D.	N.D.	N.D.
23		Foam	Face wash	N	14			✓		N.D.	++	N.D.	N.D.	N.D.
24		A cloth bag filled with rice bran powder	Body and face wash	P	1	✓				+++	+++	+++	+++	+++
25		Paste	Face pack	N	4			✓		N.D.	+	N.D.	N.D.	N.D.

and then overnight at 4 °C with the plasma collected from the patient with rice allergy (1:10 dilution, diluted with 0.1% casein-PBS). After washing three times with PBS-T, the membranes were incubated with HRP-linked anti-human IgE [1:500 dilution; dilution with 0.1% casein-PBS; Horseradish peroxidase-conjugated IgG fraction of polyclonal goat antiserum directed against human IgE, Fc-specific, GAHu/IgE(Fc)/PO, Nordic MUBio, Susteren, Netherlands] for 90 min at room temperature. After washing again with PBS-T three times, the bound secondary antibodies were detected using Immunostar LD (#290-69904, Wako Co., Ltd., Tokyo, Japan) in accordance with the manufacturer's protocol. The signal intensities of the bands were detected using SAYACA imager (DRC Co., Ltd., Tokyo, Japan) and stored as TIFF image files (Adobe Systems, San Jose, CA, USA).

3. Results and discussion

3.1. Distribution of rice allergens in rice grains

In order to determine the distribution of the protein components in rice grains, the proteins were extracted from brown rice, rice bran and polished rice. The extracted rice proteins were subjected to SDS-PAGE, and the gel was stained with CBB for detecting the protein bands. The results showed that the protein fractions extracted with 1 × Laemmli sample buffer also contained most of the protein fractions extracted with 1 M NaCl (Fig. 1A), which contain most of the known rice allergens (Satoh et al., 2011; Urisu et al., 1991). This result indicates that extraction with 1 × Laemmli sample buffer is a better method for extraction of total rice proteins than that with 1 M NaCl.

The distribution of rice allergens in rice grains was then examined by western blot analysis using rice allergen-specific antibodies. The salt-soluble proteins extracted with 1 M NaCl were transferred onto PVDF membranes, and the membranes were then reacted with the rice allergen-specific antibodies. The results of the western blot analysis revealed that while RAG2 (Fig. 1B) and the 19-kDa globulin (Fig. 1D) were scarcely detectable in rice bran, they were detected in the brown rice and polished rice; on the other hand, glyoxalase I was detected in all the samples (Fig. 1C). Rice glyoxalase I shows amino acid sequence similarity with bacterial glyoxalase I, which is a ubiquitous enzyme in various organisms, and detected in various plant tissues (Usui et al., 2001). In a previous study, RAG2 and the 19-kDa globulin were demonstrated to be localized in the rice endosperm (Limas et al., 1990; Matsuda et al., 1988). On the other hand, the 52-kDa globulin was strongly detected in rice bran and brown rice, but only weakly detected in polished rice (Fig. 1E). A previous study identified the 52-kDa globulin in the aleurone layer (Kurokawa et al., 2014). The aleurone layer is one of the four layers of rice bran (Lloyd, Siebenmorgen, & Beers, 2000). These findings suggest that RAG2 and the 19-kDa globulin are mainly localized in the inner parts of the rice grains, while the 52-kDa globulin is mainly localized in the outer parts of the rice grains, that is, in the rice bran.

3.2. Allergenicity of health foods and cosmetics containing rice bran

Because of its own functionally effective features, rice bran is often used as an ingredient in cosmetics and health foods. Some of the products, containing rice bran as a constituent, examined in this study are shown in Table 1. Rice bran is included in the products in different forms, such as intact rice bran, defatted rice bran, fermented rice bran and rice bran extract. The protein fractions derived from rice bran and other ingredients were extracted from these products, and separated by SDS-PAGE for comparing the electrophoretic patterns of the proteins. The gel stained with CBB revealed that some of the protein extracts yielded clear bands, while others showed smeared patterns after electrophoresis (Fig. 2A). These differences could probably be attributed to different rates of protein degradation. For example, the results indicate a number of clear bands for sample no. 17, but a smeared pattern for

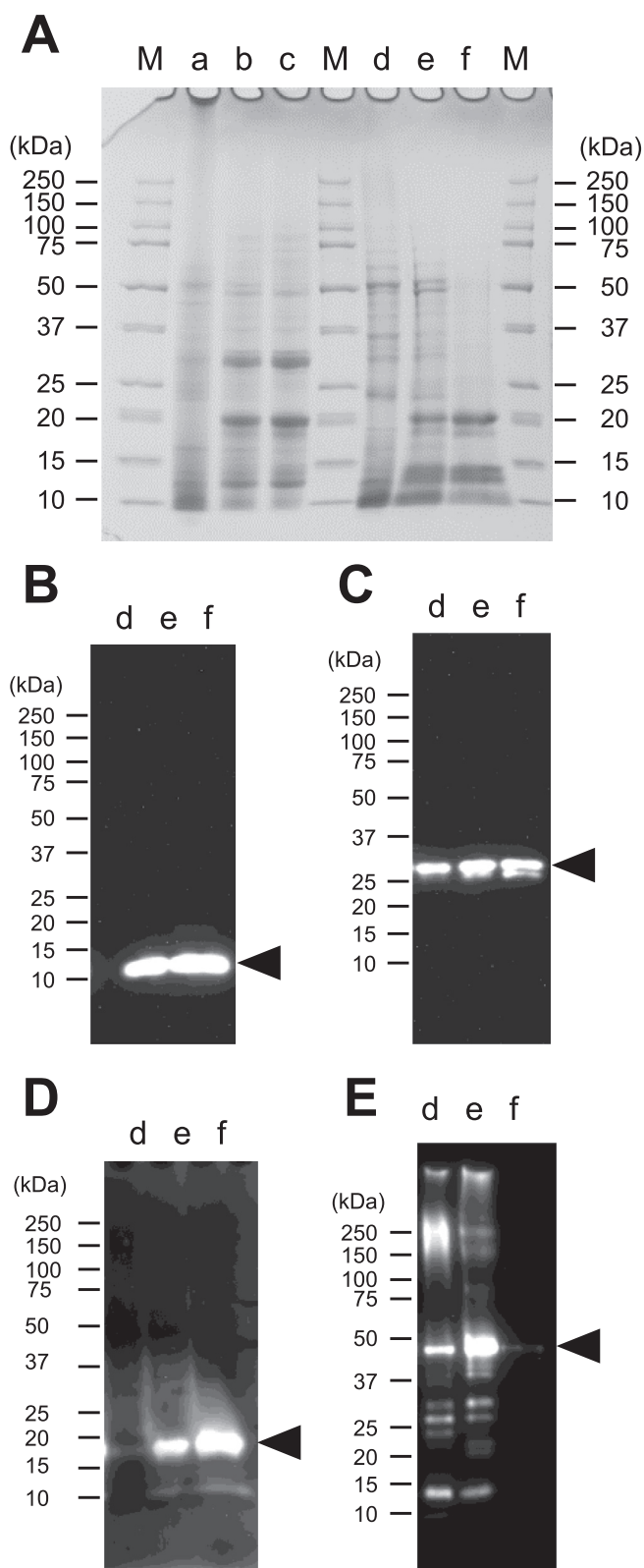


Fig. 1. SDS-PAGE of the protein fractions extracted from rice bran, brown rice and polished rice and western blot analyses using rice allergen-specific antibodies. A: The SDS-PAGE gel stained with CBB. The extracted protein fractions with 1 × Laemmli Sample Buffer (lanes a-c) or 1 M NaCl (lanes d-f) from rice bran (lanes a and d), brown rice (lanes b and e) and polished rice (lanes c and f) were separated by SDS-PAGE, followed by staining of the gel with CBB. M: Protein size marker. B-E: Western blot analysis for the rice allergens using rice allergen-specific antibodies: (B) RAG2; (C) glyoxalase I; (D) the 19-kDa globulin; (E) the 52-kDa globulin. Arrowheads indicate the positions of the rice allergens.

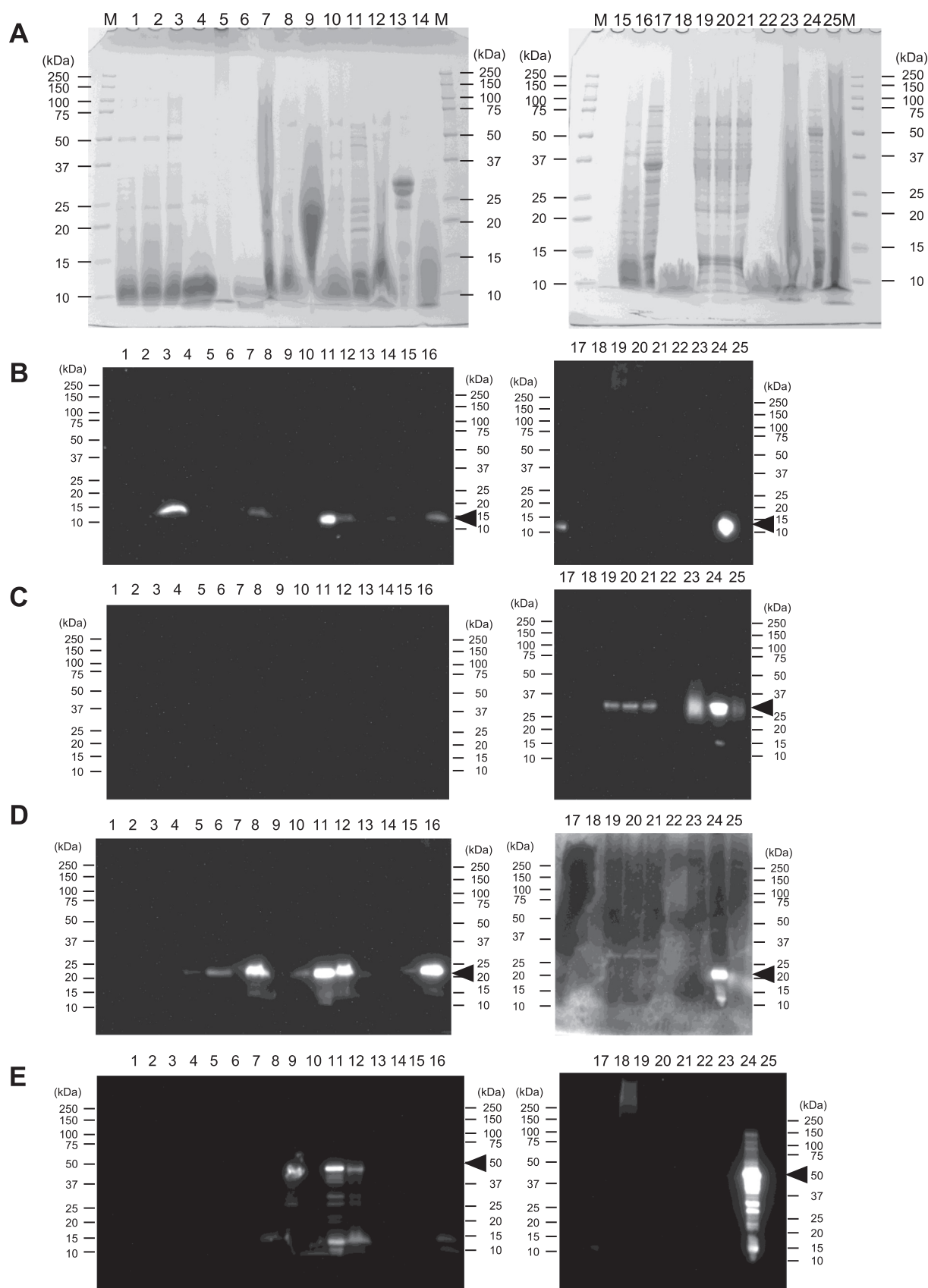


Fig. 2. SDS-PAGE of protein fractions extracted from the products containing rice bran and western blot analysis using rice allergen-specific antibodies. A: The SDS-PAGE gel stained with CBB. The extracted protein fractions with $1 \times$ PBS from health foods and cosmetics containing rice bran as an ingredient were separated by SDS-PAGE, followed by staining of the gel with CBB. Lane numbers 1–25 indicate the sample numbers shown in Table 1. M: Protein size marker. B–E: Western blot analysis for the rice allergens using antibodies specific for RAG2 (B), glyoxalase I (C), the 19-kDa globulin (D) and the 52-kDa globulin (E). Arrowheads indicate the positions of the rice allergens.

sample no. 9, although the product forms of both products were similar. This suggests that the pattern of the protein bands are not dependent on the product form. On the other hand, the products that contained defatted rice bran (sample nos. 6, 7, 8, 9, 12 and 14), fermented rice bran (sample nos. 23 and 25) or rice bran extract (sample no. 22) showed smeared patterns, even though 11 of the 15 products (sample nos. 1, 2, 3, 10, 11, 16, 17, 19, 20, 21 and 24) that contained intact rice bran showed clear bands. Among them, sample nos. 1, 2, 3, 10, 11, 16, 17 and 24 contained intact rice bran as the principal ingredient. This result indicates that the form and the weight of the rice bran used as an ingredient in products may affect to the patterns of the protein bands.

For detecting the rice allergens in the products containing rice bran as a constituent, the extracted protein fractions from these products containing proteins derived from rice bran and other ingredients were analyzed by western blotting using antibodies to rice allergens, that is, anti-RAG2, anti-glyoxalase I, anti-19-kDa globulin and anti-52-kDa globulin antibodies. The results are shown in Fig. 2 and summarized in Table 1. The results indicate that some of the protein extracts reacted with the anti-RAG2 (Fig. 2B), anti-glyoxalase I (Fig. 2C), anti-19-kDa globulin (Fig. 2D) and anti-52-kDa globulin antibodies (Fig. 2E), suggesting that the RAG2, glyoxalase I, 19-kDa globulin and 52-kDa globulin exist in some of the health foods and cosmetics that contain intact rice bran, defatted rice bran or fermented rice bran as an ingredient. The 52-kDa globulin which is mainly localized in rice bran was detected in some of the products, namely, sample nos. 9, 11, 12 and 24. It is possible that some of the health foods and cosmetics that reacted with both anti-19-kDa and anti-52-kDa globulin antibodies, namely, sample nos. 11, 12 and 24, could contain not only rice bran, but also polished rice, as raw materials (Table 1). On the other hand, sample nos. 1, 2, 3, 14, 18 and 22 did not react with any of the rice allergen-specific antibodies used in this study. Koji mold included as an ingredient in sample nos. 1, 2 and 3 could affect the detection of the 52-kDa globulin in the product. The finding that some health foods and cosmetics with rice bran contain these rice allergens, indicates that patients with rice allergy need to pay attention while considering using these products.

3.3. Detection of IgE-binding activity in plasma collected from the patient with rice bran allergy

We encountered a case of hypersensitivity to rice and rice bran in an 11-year-old female patient. In order to confirm the causative agents of this allergy induced by rice and rice bran, we performed a western blot analysis using plasma from this patient with rice and rice bran allergy. The results of western blotting using the plasma obtained from the patient with rice bran allergy and the proteins extracted from brown rice, rice bran and polished rice revealed that the IgE from the patient's

plasma bound to the proteins having molecular weights of approximately 19-kDa and 52-kDa in the rice grains (Fig. 3A). This suggests that the most likely causative proteins of the allergy in this patient were the 19-kDa protein in brown rice and polished rice (Fig. 3A, lane e and f, respectively) and 52-kDa proteins in rice bran (Fig. 3A, lane d).

Western blot analysis also revealed several IgE-binding proteins in some products containing rice bran as an ingredient (Fig. 3B). The protein fractions extracted from sample nos. 1, 2, 3, 10, 11, 16, 23, 24 and 25 showed IgE binding at approximately 50-kDa. Inomata et al. indicated that the approximately 50-kDa protein in rice was the causative allergen in the patient with rice bran allergy (Inomata et al., 2012). However, our results showed that the 52-kDa globulin was detected in the protein fractions extracted from sample nos. 9, 11, 12 and 24 (Fig. 2E) and was detected with plasma obtained from the patient with rice bran allergy used in this study. Additional investigation is needed to confirm the causative allergen(s) in these patients. Moreover, as mentioned above, the 19-kDa globulin is mainly localized in the inner part of the rice grain, and the 52-kDa globulin is mainly localized in the outer part of the rice grain, that is, rice bran. These findings suggest that the 52-kDa globulin is candidate for the causative agent of rice bran allergy. This result also indicates that the causative allergens in this patient with rice and rice bran allergy might have been present in the products containing rice bran as an ingredient examined in this study.

In conclusion, the distribution of rice allergens in rice grains was determined. The potential causative allergen of rice bran allergy is a 52-kDa globulin. IgE-binding proteins were also detected in the products containing rice bran as an ingredient. Electrophoresis yielded smeared patterns for some of the protein fractions extracted from the products, but clear bands for several others. Our study provides the first data on health foods and cosmetics containing potential rice bran allergens. Further investigations are required for assessing the consequences of using rice bran as an ingredient of health foods and cosmetics.

Conflicts of interest

None of the authors have any conflicts of interest to declare in relation to this manuscript.

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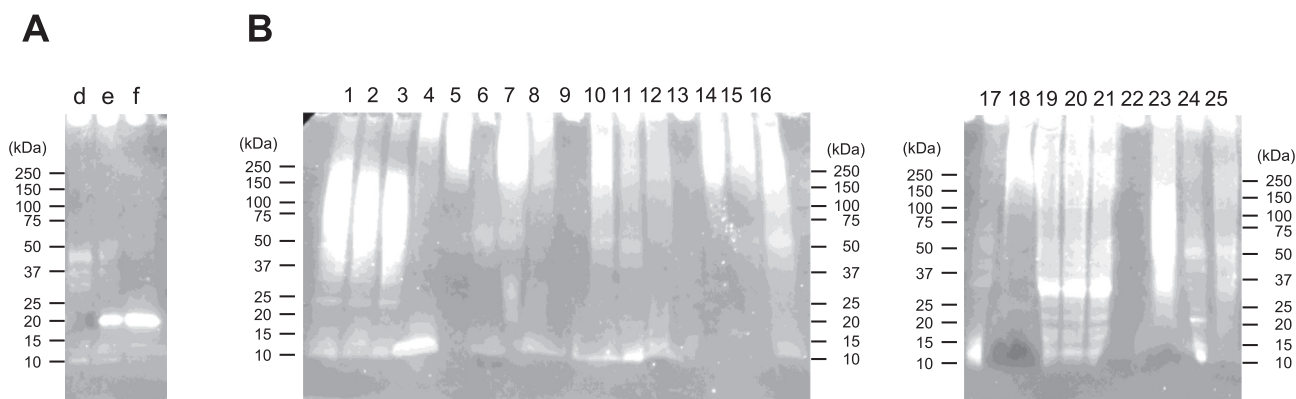


Fig. 3. Western blot analyses of the protein fractions extracted from rice grains (A) and products containing rice bran as an ingredient (B) using plasma obtained from the patient with rice bran allergy. A: The protein fractions extracted with 1 M NaCl from rice bran (lane d), brown rice (lane e) and polished rice (lane f) were separated by SDS-PAGE, followed by western blotting. B: The protein fractions extracted with $1 \times$ PBS from health foods and cosmetics containing rice bran as an ingredient were separated by SDS-PAGE, followed by western blotting. Lane number 1–25 indicate the sample numbers shown in Table 1.

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