

Note

Decrease in Rice Allergenic Proteins of Polished Rice Grains by Incubating with a Miso Solution

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The effect of miso on allergenic proteins in rice seeds was investigated. When polished rice grains were incubated at 37°C for 30–120 min with a 10% miso solution, but not with heat-treated miso or 1% NaCl, the amount of soluble proteins extracted from the rice grains with 1 M NaCl markedly decreased. SDS-PAGE, immunoblotting and densitometric analyses of these soluble proteins and insoluble proteins indicate that 26 kDa globulin and 14–16 kDa allergens in the grains were decreased to 15–60% during incubation with the miso solution, especially soybean-koji miso, without any large change in the content of major insoluble proteins.

Key words: rice; allergen; food allergy; miso

Cereal grains have been reported to sometimes be causative of food allergy.¹⁾ Several clinical studies have suggested that rice grains were responsible for severe atopic dermatitis in some adult patients.^{2–4)} Some allergenic proteins, including 14–16 kDa allergens and 26 kDa globulin, have been identified and characterized structurally and immunochemically.^{5–9)} Some trials for the production of hypoallergenic rice have been conducted by using of an enzymatic processing¹⁰⁾ and genetic engineering.¹¹⁾ Hypoallergenic rice produced by the enzymatic method in combination with a detergent treatment has already been clinically evaluated and supplied to patients allergic to rice grains.^{10,12,13)} Miso is well-known to possess proteolytic activity which degrades soybean proteins, including a major soybean allergen (Gly m Bd 30 K), into peptides and amino acids during fermentation.^{14,15)} In the present study, we applied miso without any heat treatment to the reduction of rice allergenicity. Incubation of polished rice grains for only 60–120 min with a soybean-koji miso solution effectively reduced the content of some allergenic proteins in the grains.

One gram of polished rice grains (*Oryza sativa* L var. *japonica* cv. Koshihikari) was incubated with 2

ml of 10% (w/v) of each of three types of miso solution, soybean-koji miso, rice-koji miso and heat-treated soybean-koji miso (Nakamo Shokuhin Corp., Nishikasugai, Japan), and with 2 ml of a 1% (w/v) NaCl solution as a negative control at 37°C for 30, 60 and 120 min. After being washed with 20 ml of a 1% NaCl solution three times, the rice grains were crushed with a glass stick in 2 ml of cold 1 M NaCl. The soluble proteins in the crushed rice were then extracted twice by an ultrasonic treatment at 60 W for 5 min and separated from the insoluble materials by centrifugation at 2,000 × *g* for 10 min and then passing through a membrane filter (5.00 μm). The filtrate was used as a 1 M NaCl extract for the subsequent analyses. Each precipitate was mixed with 2 ml of Laemmli's SDS-PAGE sample buffer, boiled for 3 min, and centrifuged as already described. The supernatant was used as insoluble proteins directly for the SDS-PAGE analysis described later. The protein concentration of the 1 M NaCl extract was determined by the method of Lowry *et al.*¹⁶⁾ SDS-PAGE (15% acrylamide gel) and immunoblotting were done according to the methods of Laemmli¹⁷⁾ and Towbin *et al.*,¹⁸⁾ respectively. Gel sheets were stained with CBB, while blotted PVDF membranes were stained with the monoclonal antibody, 25B9, specific for rice 14–16 kDa allergens, as described previously.¹⁹⁾ The staining intensity of each protein band was densitometrically analyzed by a densitograph (ATTO, Tokyo).

The soluble proteins remaining in the rice grains after being incubated with the miso solutions and 1% NaCl solution were evaluated by measuring the protein concentration of 1 M NaCl extracts (Fig. 1). No difference in protein concentration was observed among the four samples after incubating for 30 min, the values being almost identical to those of the 1 M NaCl extract of polished rice grains without any incubation (data not shown). Incubation for 60 min and 120 min with the soybean-koji miso and rice-koji

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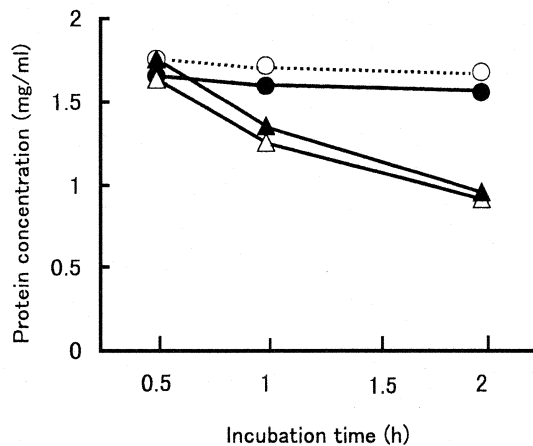


Fig. 1. Decrease of Soluble Proteins in Rice Grains during their Incubation with a Miso Solution.

The protein concentration of each 1 M NaCl extract was determined by the method of Lowry *et al.* for rice grains incubated with a 1% NaCl solution (○), heat-treated soybean-koji miso solution (●), soybean-koji miso solution (△), and rice-koji miso solution (▲) for 30, 60 and 120 min.

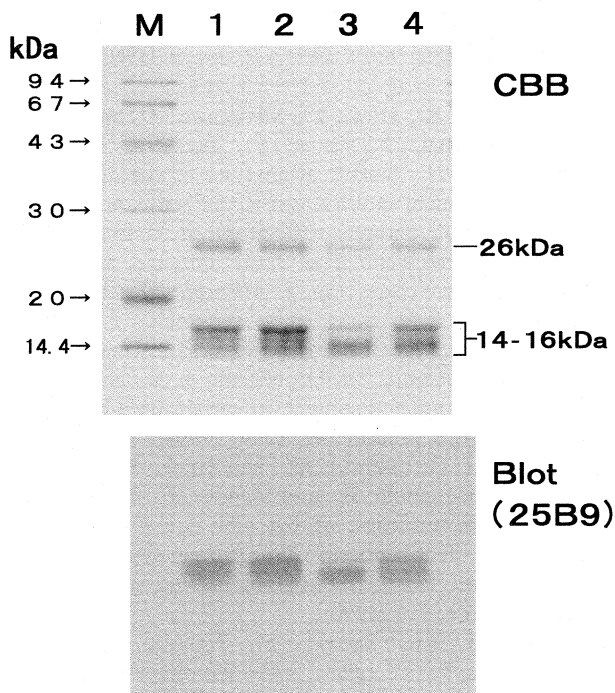


Fig. 2. SDS-PAGE and Immunoblotting Analyses of the Soluble Proteins in Rice Grains Incubated with Miso Solutions for 120 min.

Polished rice grains were incubated with solutions of heat-treated soybean-koji miso (lane 2), soybean-koji miso (lane 3), rice-koji miso (lane 4), and a 1% NaCl solution (lane 1), and the soluble proteins were extracted from these rice grains with 1 M NaCl and subjected to the analyses. The gel sheet was stained with CBB (upper panel) and the blotted membrane was immunostained with monoclonal antibody 25B9 specific to the 14–16 kDa allergen (lower panel). A molecular mass standard (M) was also applied to the same gel.

miso solutions reduced the protein concentration to about 75% and 55%, respectively, whereas incuba-

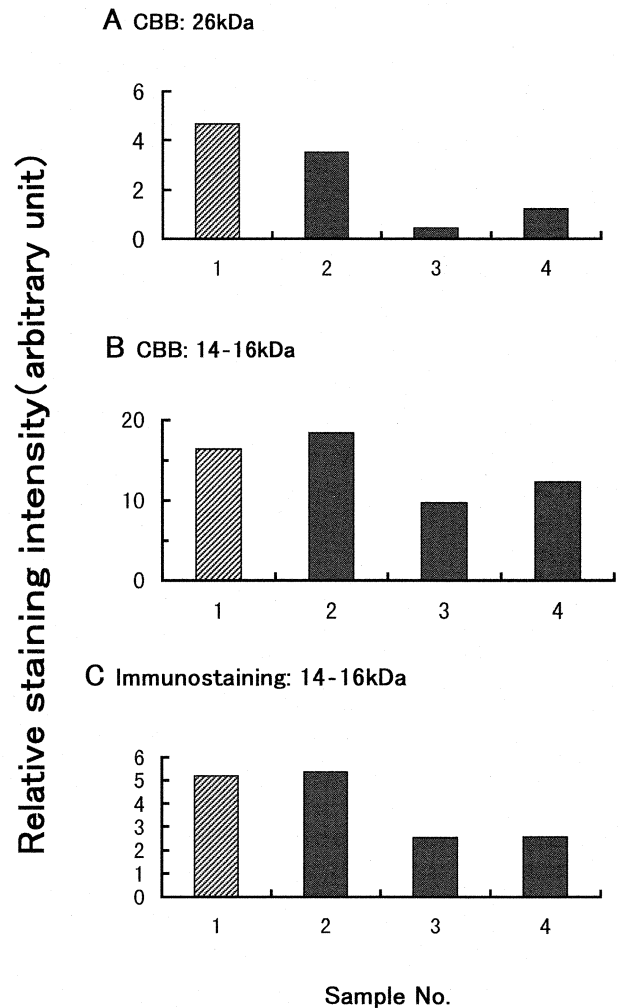


Fig. 3. Relative Staining Intensity of the 26 kDa and 14–16 kDa Bands Detected by SDS-PAGE and Immunoblotting.

The gel and membrane shown in Fig. 2 were subjected to a densitometric analysis, and the staining intensity of the 26 kDa (A) and 14–16 kDa bands (B and C for CBB-staining and immunostaining, respectively) was determined. The numbers (Nos. 1–4) of the samples are the same as those indicated in the legend to Fig. 2.

tion with the heat-treated miso and 1% NaCl solutions had almost no effect on the soluble proteins in the rice grains.

The proteins in the 1 M NaCl extracts of rice grains were analyzed by SDS-PAGE and immunoblotting. Typical staining patterns for the extracts of rice grains incubated for 120 min are shown in Fig. 2. The CBB-stained bands of 26 kDa and 16 kDa for the extracts of rice grains incubated with the soybean-koji miso and rice-koji miso appeared to be much weaker than those of the rice grains incubated with the heat-treated soybean-koji miso and 1% NaCl solutions. An immunoblotting analysis of these extracts by using monoclonal antibody 25B9, which is specific for 14–16 kDa allergens, also showed that the amount of these allergens remaining in the rice grains had been reduced by incubating with the soy-

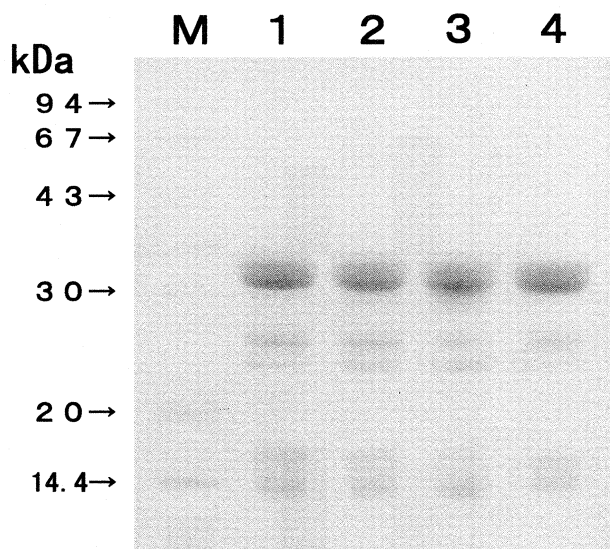


Fig. 4. SDS-PAGE Patterns of Insoluble Proteins in the Rice Grains Incubated for 120 min with the Miso Solutions.

After extracting the soluble protein with 1 M NaCl, the insoluble proteins were extracted with the SDS-PAGE sample buffer from each residual precipitate of the rice grains. The samples of each lane are from rice grains incubated with heat-treated soybean-koji miso (lane 2), soybean-koji miso (lane 3), rice-koji miso (lane 4), and 1% NaCl (lane 1).

bean-koji miso and rice-koji miso solutions. Similar electrophoretic and immunoblotting patterns were obtained for the extract incubated for 60 min (data not shown). The relative staining intensities estimated by the densitometric analysis of each band are shown in Fig. 3. The values shown in arbitrary units (Y-axis) could be compared among the four extracts (lanes 1–4) by assuming that the gel sheet and membrane had been stained uniformly. The staining intensity of the 26 kDa band for the rice grains incubated with the soybean-koji miso and rice-koji miso solutions was respectively about one tenth and one fourth that of the grains incubated with the 1% NaCl solution. The intensity of the 14–16 kDa band stained with CBB or the antibody was reduced to about a half by incubating with the soybean-koji miso or rice-koji miso, but not with the heat-treated soybean-koji miso solution.

The insoluble proteins in rice grains were extracted with the SDS-PAGE sample buffer and then analyzed by SDS-PAGE (Fig. 4). No large difference was apparent in the electrophoretic patterns among the four samples, although the staining intensity of some protein bands for the rice grains incubated with soybean-koji miso and rice-koji miso was slightly weaker than that of the other two samples. This and the foregoing results suggest that rice-grain soluble proteins, including allergenic proteins, were reduced more effectively than insoluble proteins that are the major rice-grain proteins.

This reduction of soluble proteins, including some allergens, in rice grains by incubating with miso

would probably have been due to proteolytic degradation of the proteins by the koji-derived enzymes remaining in the soybean-koji or rice-koji miso samples, because neither the heat-treated miso nor the 1% NaCl solution showed much effect on the rice proteins. Further studies are required to clarify the mechanism for the reduction of allergenic proteins in rice grains by incubating with miso. Nevertheless, the results shown in the present study suggest that miso may be useful as a functional seasoning to reduce the allergenicity of rice grains in some special dishes for allergic patients, *e.g.*, rice-based porridge, in which rice grains could be incubated with a miso solution before cooking.

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