

Production of Hypoallergenic Rice by Enzymatic Decomposition of Constituent Proteins

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ABSTRACT

A method is proposed to produce hypoallergenic rice usable for patients with rice allergy. Newly harvested rice grains were dipped in carbonate (pH 9) containing glycerin monooleate and Actinase and then the mixture was exposed to a reduced pressure for degassing. The degassed mixture was incubated at 37°C for 24 hr to hydrolyze proteins. The process produced rice grains from which the major allergenic globulin was decomposed. The product was evaluated by the radioallergosorbent test (RAST), with the result that its RAST value suggested negative allergenicity in most cases. The product was clinically administered to 7 patients with atopic dermatitis and no allergic reaction was observed in 6 of the 7.

INTRODUCTION

FOOD ALLERGY is an immunological response resulting specifically from intake of a responsible food. Hen's egg, cow's milk, soybean, wheat and rice are generally known as allergens for sensitive persons. Atopic dermatitis is a type of allergic skin disease and, in Japan, where rice is consumed as a staple foodstuff, many examples of rice-associated atopic dermatitis are reported (Yamada et al., 1985; Miyakawa et al., 1988). Although a topical steroid therapy has been widely tried, the only curative means is elimination of the intake of rice and its products. Most of the patients with rice-associated atopic dermatitis are frequently sensitive to wheat (Yamada et al., 1985), corn (Hoffman, 1975) and barley (Goodwin and Rawcliffe, 1983), so that they are usually forced to eat millet instead.

Shibasaki et al. (1979) first reported that a high degree of allergenicity was found in a globulin fraction of the rice seed protein. Matsuda et al. (1988) isolated an allergenic protein of 16kd from the globulin fraction. However, no attempt has been made to eliminate the responsible protein to obtain hypoallergenic rice products. The objectives of this study were to: (1) develop a rice product which was practically needed in Japan as a safe staple food for patients with atopic dermatitis, (2) produce such a product, and (3) characterize its quality.

MATERIALS & METHODS

Materials

Polished rice (*Oryza sativa* L. *japonica*, cultivar Koshihikari), newly harvested in Miyazaki prefecture in 1988 was used in one case. In another case, commercially available *japonica*-type polished rice was used.

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Enzymes

Actinase AS (for food use) was obtained from Kaken Kagaku (Japan). Reagent grade preparations of papain, α -chymotrypsin, trypsin, pepsin and pancreatin were obtained from Sigma Chemical Co.

Proteins

The newly harvested rice was pulverized and sifted from a 48-mesh sieve. The rice powder (100g) was treated with water (1L) for 4 hr at room temperature and centrifuged at 5000xg for 20 min to obtain a supernatant. The supernatant was dialyzed against running water for 2 days and the non-diffusible fraction lyophilized to obtain a freeze-dried powder as a water-soluble fraction. Similar procedures were carried out to obtain salt-soluble, ethanol-soluble and acetic acid-soluble fractions by treating the water insoluble fraction with 2M NaCl, 60% ethanol and 1M acetic acid (1L each), respectively. The resulting insoluble residue after a series of these extractions was also dialyzed and lyophilized.

Degree of hydrolysis (DH)

The salt-soluble fraction (1g on a dry matter basis) was dispersed in 100 mL of 1M carbonate (pH 9) and used as a substrate solution. When pepsin was used, the fraction was dispersed in water and then adjusted at pH 1.5 with 1N HCl. The substrate solution (2 mL) was hydrolyzed with each of the enzymes at an enzyme-substrate ratio of 1/100 at 37°C for 24 hr. When papain was used, L-cysteine was dissolved in the substrate solution at 10mM. The hydrolysate was mixed with 2 mL of 20% trichloroacetic acid (TCA), allowed to stand for 30 min and then filtered to obtain a 10% TCA-soluble fraction. Each of the 10% TCA-soluble fractions and the substrates were analyzed for total nitrogen content by the micro-Kjeldahl method (Clark, 1943). DH value was expressed as a percentage of 10% TCA-soluble nitrogen vs substrate nitrogen.

Immunoassay

The protein fractions from rice powder, the enzymatic hydrolysates of globulins and the enzymatically treated products from rice grains and rice powder were analyzed for their immunological reactivity. Filter paper (5 mm in diameter, Advantec No. 6) was activated with cyanogen bromide (BrCN) (Ceska et al., 1972). Each of the test samples (5 mg on a dry matter basis) was dispersed in 0.1M carbonate (pH 8.4, 1 mL) and then coupled with the CNBr-activated paper disc at 4°C for 72 hr. The allergen coupling disc was submitted to radioallergosorbent test (RAST) according to the method of Ceska et al. (1972). Outpatients with rice-associated atopic dermatitis at the Yokohama City University Hospital were asked to donate blood for serum testing. RAST value was expressed by Phadebas RAST unit/mL (PRU/mL).

Surfactants and their functionalities

Sugar esters, monoglycerides and sorbitan monoesters for food uses were obtained from Kao Co. (Japan). Each surfactant in water at 10ppm was poured into a glass bottle, and the headspace over the surfactant solution was evacuated to 20 mmHg with mild shaking to observe whether foams formed. The water-containing surfactant at 10 ppm was also determined for surface tension at 20°C with a du Noüy's tensiometer. On the other hand the salt-soluble fraction was hydrolyzed with Actinase at pH 9 in the presence of the surfactants at 100 ppm each under conditions similar to those adopted for the determi-

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nation of DH values. A similar procedure was carried out in the absence of any surfactant. Percent inhibition was expressed by

$$\left(1 - \frac{\text{DH in the presence of surfactant}}{\text{DH in the absence of surfactant}}\right) \times 100$$

Degassing

Rice grains (1 kg) were dipped in 1M carbonate (pH 9, 2L) containing a surfactant and an enzyme, and the mixture was degassed under reduced pressure (20 mmHg) at room temperature for 30 min.

Electrophoresis

Each sample of newly harvested rice and the final product obtained through a series of treatments was pulverized with a mill. The powder (1g) was treated twice with 2M NaCl (5 mL each) to obtain an extract. The residue was treated with 0.1N NaOH (5 mL) to obtain another extract. The extracts were precipitated with trichloroacetic acid (4% in final), and the precipitates were submitted to electrophoresis. SDS-14% polyacrylamide gel electrophoresis was carried out according to the procedure of Weber and Osborn (1969). Each of the salt-extracts from the product (83 mg) and the intact rice (0.83mg) was loaded on one lane of the gel. Each of the alkali-extracts from the product (0.83 mg) and the intact rice (0.83mg) was also loaded similarly. A mixture of rabbit muscle phosphorylase b (MW, 97k), bovine serum albumin (MW, 67k), ovalbumin (MW, 43k), bovine carbonic anhydrase (MW, 31k), soybean trypsin inhibitor (MW, 22k) and hen's egg lysozyme (MW, 14k) was used as molecular weight markers. Coomassie brilliant blue R-250 (Sigma Chemical Co.) was used as the staining reagent.

Clinical examination

Seven outpatients with rice-associated atopic dermatitis at the Yokohama City University Hospital were fed a diet without rice and rice products for 1 month or longer and then with a diet of hypoallergenic rice for 2 weeks or shorter, because in these human subjects symptoms of atopic dermatitis appear within 2 weeks if they eat ordinary rice foods. The rest of this hypoallergenic rice was not limited in quantity or in quality, and the outpatients ate their own foods *ad libitum*. During the feeding period they were clinically examined for symptoms of atopic dermatitis.

RESULTS & DISCUSSION

IN THE PRESENT STUDY, the class of rice seed proteins with allergenicity was defined. For this, sera taken from ten rice-associated atopic patients were used. The salt-soluble fraction known to contain the 16kd globulin (Matsuda et al., 1988) was immunologically positive to the sera of 9 of the 10 patients (Table 1). This result suggested that the decomposition of the globulin fraction was effective in producing hypoallergenic rice usable in most cases. Accordingly, we tried to establish a process for eliminating globulin-type allergens. Throughout this work we checked the allergenicity of the products by RAST.

At the first step the most suitable enzyme to hydrolyze globulins was screened. The globulin fraction that was highly decomposed with Actinase gave a RAST value that suggested negative allergenicity. Papain was the second effective enzyme, while the mammalian digestive enzymes, pepsin, trypsin, α -chymotrypsin and pancreatin, were not effective (Table 2). Using Actinase and papain, we prepared enzymatically treated rice grains and rice powder. As shown in Table 3, an Actinase-treated rice powder became negative in allergenicity, whereas in the case of rice grains the same treatment was ineffective in lessening the RAST value sufficiently.

Considering differences in effectiveness of Actinase treatment between rice powder and rice grains, we added a surfactant treatment which would support the permeation of protease into rice grains through their cell walls and membranes. We also added a degassing process. The combination of the sur-

Table 1—RAST values of proteins fractionated from rice

Serum	Protein				Residue
	Water-soluble	Salt-soluble	Ethanol-soluble	Acetic acid-soluble	
	RAST value (PRU/mL)				
1	2.5	3.8	1.3	0.9	0.6
2	1.0	0.6	<0.34	<0.34	<0.34
3	<0.34	0.5	0.7	<0.34	<0.34
4	<0.34	0.5	<0.34	<0.34	0.5
5	<0.34	0.6	<0.34	1.1	<0.34
6	1.6	3.6	<0.34	0.7	<0.34
7	<0.34	0.4	<0.34	0.5	<0.34
8	0.4	0.6	<0.34	<0.34	<0.34
9	<0.34	<0.34	<0.34	0.8	<0.34
10	1.4	1.7	1.4	2.4	1.3

Table 2—Hydrolysis of the globulin fraction by proteolytic enzymes and RAST values of the resulting hydrolysates

Enzyme	Reaction pH	Degree of hydrolysis (%)	RAST value* (PRU/mL)
Actinase AS	9	94.3	<0.34
Papain	9	86.2	1.6
α -Chymotrypsin	9	72.4	3.4
Trypsin	9	70.1	3.6
Pepsin	1.5	58.9	4.2
Pancreatin	9	53.5	4.8
None ^b	9	0.0	3.8

* Serum No. 1 (Table 1) was used for RAST.

^b Control.

Table 3—RAST values of rice grains and powder treated with proteases

Material	Treatment		
	Actinase	Papain	None ^b
	RAST value* (PRU/mL)		
Rice grains	1.1	3.9	5.1
Rice powder	<0.34	1.6	5.1

* Serum No. 1 (Table 1) was used for RAST.

^b Control.

Table 4—Properties of surfactants

Surfactant	Foam formation ^a	Surface tension ^a at 20°C (dyn/cm ²)	Actinase inhibition ^b (%)
Sugar ester			
Stearate	+	57	49
Palmitate	+	56	52
Oleate	+	58	52
Myristate	+	57	54
Laurate	+	57	59
Monoglyceride			
Stearate	+	66	38
Oleate	—	52	39
Caprate	—	54	15
Sorbitan monoester			
Stearate	+	67	36
Palmitate	+	65	35
Oleate	+	52	20
Laurate	+	51	12

* Surfactants were used at 10 ppm each. +, stable foam formed and —, no stable foam formed during degassing at 20 mmHg.

^b Surfactants were used at 100 ppm each.

factant treatment and the degassing process was effective in giving RAST values indicating no allergenicity.

Next, we selected the most suitable surfactant. Three properties are required for the surfactant: no foam formation, effectively lowering the surface tension for a permeation of the dissolved enzyme, and the lowest inhibition of Actinase action. Considering these requirements, we selected glycerin mono-oleate as the most suitable surfactant (Table 4). Although glycerin monocaprate might also be satisfactory, we did not use this surfactant, because it sometimes gave off a fatty acid flavor (probably by hydrolysis of the ester linkages).

Desirable conditions were examined with respect to the kind of solvent, surfactant concentration and enzyme/rice ratio for

Table 5—Effect of enzymatic reaction conditions on RAST values of the products produced from rice grains

Material rice grains	Solvent	Surfactant conc (ppm)	With or without degassing	Enzyme/rice ratio	RAST value* (PRU/mL)
Newly harvested	Water	0	Without	1/100	1.6
	Water	20	Without	1/100	0.8
	Water	0	With	1/100	0.7
	Water	20	With	1/100	0.7
	1M carbonate	0	Without	1/100	1.1
	1M carbonate	20	Without	1/100	0.4
	1M carbonate	0	With	1/100	0.6
	1M carbonate	10	With	1/100	0.4
	1M carbonate	20	With	1/100	<0.34
	1M carbonate	20	With	3/1000	0.7
	1M carbonate	20	With	1/1000	1.3
	1M carbonate	10	With	3/1000	0.9
	1M carbonate	10	With	1/1000	1.3
	1M carbonate	20	With	0	5.1
Commercially available	1M carbonate	20	With	1/100	0.9

* Serum No. 1 (Table 1) was used for RAST.

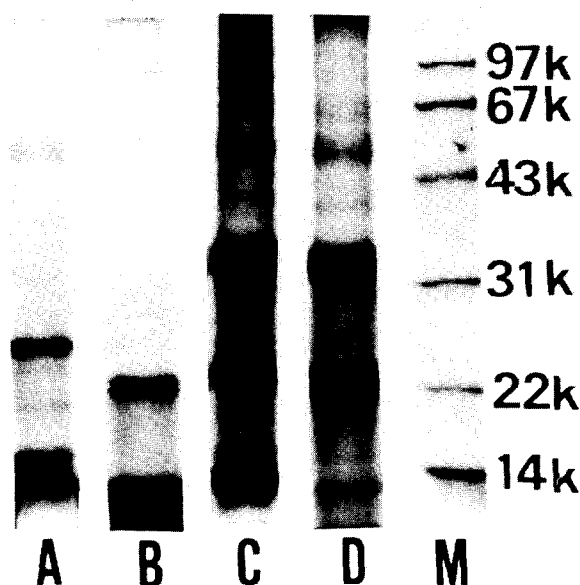


Fig. 1—SDS-PAGE of proteins fractionated from the hypoallergenic rice and its material. A, salt-soluble (globulin) fraction from material rice (0.83mg); B, salt-soluble (globulin) fraction from the hypoallergenic rice (0.83mgx100); C, alkali-soluble (glutelin) fraction from the material rice (0.83 mg); D, alkali-soluble (glutelin) fraction from the hypoallergenic rice (0.83 mg); and M, marker proteins.

the enzymatic treatment. The conditions finally selected were: 1M carbonate (pH 9) as solvent, surfactant at 20 ppm and adoption of an enzyme/rice ratio of 1/100, when newly harvested rice was used as material (Table 5).

Thus, the following process for producing a hypoallergenic rice was proposed. First, newly harvested rice grains in 1M carbonate (pH 9) containing glycerin monooleate at 20 ppm were degassed under a reduced pressure. Second, treatment of the suspension at 37°C for 24 hr with Actinase AS, 1/100 weight of rice grains, and finally, washing with water to remove water-soluble components. The hypoallergenic rice pro-

Table 6—RAST values of the final rice product as hypoallergenic rice and those of newly harvested rice as the material

Serum	RAST value (PRU/mL)	
	Hypoallergenic rice	Material rice
1	<0.34	5.1
11	<0.34	7.1
12	<0.34	3.1
13	<0.34	1.0
14	<0.34	0.7
15	<0.34	0.4
16	0.5	0.4
17	9.9	6.2

Table 7—Performance of feeding the hypoallergenic rice to seven patients with atopic dermatitis

Patient	Age	Sex	Feeding period	Symptom
A*	14	Female	2 wk	Negative
B	4	Male	2 wk	Negative
C	5	Male	1 wk	Negative
D	16	Female	2 wk	Negative
E	18	Female	1 day	Positive
F	19	Male	2 wk	Negative
G	34	Female	2 wk	Negative

* Presenting her serum (No. 1 in Table 1).

duced by this process contained a nondetectable amount of the allergenic globulin, even when the salt-extract from 100 times the weight of the rice was loaded on the gel for electrophoresis (Fig. 1B). The hypoallergenic rice, however, retained a glutelin fraction (Fig. 1D). The rice was negative in allergenicity for the sera taken from 6 patients, with two exceptions (Table 6). Six patients fed the hypoallergenic rice three times a day for 1 or 2 weeks did not show any atopic symptoms (Table 7). A seventh patient (patient E in Table 7) developed atopic dermatitis on the first day of the feeding study. These exceptions are probably due to the existence of different types of allergens, though details await further investigation.

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