

# Reduction of rice proteins based on their solubilities and evaluation of the low-protein rice products

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About 6% of proteins are contained in the rice seed. This study was carried out to develop low-protein rice catering to rice consumers who need to limit their intake of dietary protein as in the case of patients with kidney malfunction. In order to reduce the amount of rice proteins based on their solubility, dilute organic acid solutions were applied. By treatment with organic acid, glutelin, which is the most abundant protein in rice, as well as  $\alpha$ -globulin, an allergy-causing rice protein, has been successfully reduced. Protein quantification analysis indicated that citric acid and gluconic acid are most effective for protein reduction in rice flour. Trial food processed products made from acid-treated rice flour were shown to be almost identical to those from untreated rice. Sensory evaluation of processed food products made from acid-treated rice flour has shown no significant difference with untreated rice-based products.

**Key words:** rice, glutelin, allergenic protein, organic acid.

## INTRODUCTION

Rice is one of the most important cereals in the world especially in Asian countries. Rice is eaten as a staple diet to a large segment of the world population, and a base for a variety of food products, including snacks. Polished rice contains about 6% protein and glutelin accounts for about 60% of the total endosperm protein in rice while the alcohol-soluble prolamin accounts for 20%; the allergenic proteins globulin and albumin each account for 5-10% each<sup>1, 2)</sup> (Fig. 1). Glutelin is a seed storage protein soluble in dilute acid and alkali and composed of two subunits, namely the 22-23 kDa basic subunit and the 37-39 kDa acidic subunit<sup>3)</sup>. Albumins in rice seed have  $\alpha$ -amylase inhibitory functions and have molecular weights ranging from 14 to 16 kDa<sup>4)</sup>. Studies have shown that these albumins are potentially allergenic for patients with rice allergy<sup>5, 6)</sup>. A major component of globulin called  $\alpha$ -globulin is also one of the major allergenic proteins in rice<sup>7)</sup>.

In Japan, rice is a staple food and one of the most important food sources of starch. However, intake of rice is sometimes restricted to those who should limit intake of dietary protein such as patients with kidney failure. It is because rice is most always present in every meal, and is a main ingredient in many traditional sweets and in

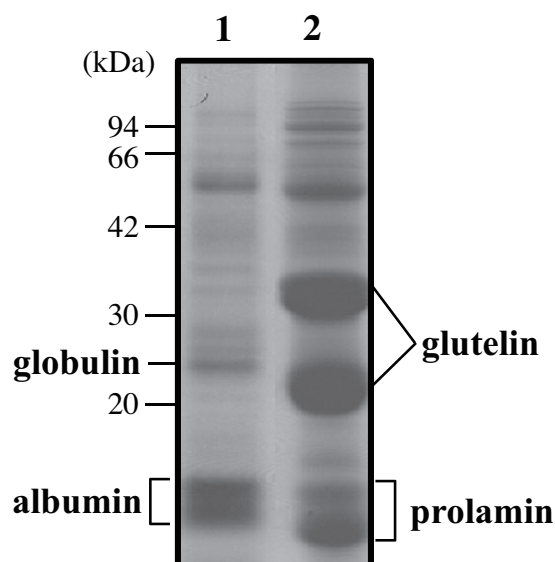
some non-gluten food products. Consequently, the total amount of protein intake as a result of rice consumption is not considered negligible.

Low-protein rice products have been developed for those whose intake of dietary protein at daily meal is limited such as patients with chronic kidney disease. One example is to produce a product from polished rice in which the surface is scraped off. Scraping off removes proteins considerably since they are mainly localized on the seed surface. Another low-protein product is the "starch rice", an imitation rice made from starch particles.

Certain rice cultivars such as *Shunyo*, *LGC-I* (*Low Glutelin Content-I*) and *LGC-soft* have been established by mutagenesis and crossbreeding<sup>8)</sup> methods to select the variety in which glutelin, the major protein in rice, is relatively low. *Shunyo* has the same total protein content as other rice cultivars; however, the synthesis of digestible glutelin is reduced while the indigestible prolamin composition has increased. For *LGC-I*, the expression of glutelin genes has proved to be suppressed by the RNA silencing mechanism<sup>9)</sup>. *Shunyo* and *LGC-I* have a low protein content but the taste of these rice cultivars is inferior with normal varieties. By contrast, cooked *LGC-soft* grains, which has less glutelin (by about 50%) and more prolamin (twice the normal variety), have a better texture than *Shunyo* and *LGC-I* by virtue of breeding and selection methods to reduce its amylase content<sup>10)</sup>.

Various kinds of cereals have been used as food materials and sources of carbohydrate. Among them rice,

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**Fig. 1. SDS-PAGE profile of rice seed proteins.** Albumins and globulin are contained in phosphate buffered saline (PBS)-soluble fraction (lane 1) and glutelin (acidic and basic subunits) and prolamin are contained in the SDS-soluble fraction (lane 2).

wheat, and corn are the most important cereals all around the world. In the case of wheat, it contains functional proteins such as gluten-related glutenin and gliadin. It is not advisable to remove gluten-related proteins in wheat because gluten formation is necessary to make dough for processed foods like bread and noodles. In contrast, since rice does not have gluten-related proteins, so it is expected that there is no characteristic change in its functions even if the amount of rice seed proteins decrease.

In this study, we tried to reduce the protein content of rice flour on the basis of the solubility of rice major proteins. We used different kinds of organic acid such as ascorbic acid, citric acid, malic acid, and gluconic acid for the extraction of rice proteins, especially the major and acid-soluble glutelin. Quantitative analyses indicated that the protein amount proved to have decreased in the acid-treated rice. Rice flour obtained through our methodologies may provide some benefits to those who should limit their intake of dietary protein. Furthermore, trial processed-products were made from rice flour with lesser protein and evaluation was carried out.

## MATERIALS AND METHODS

**Rice flour.** Rice flour was commercially available from Namisato Co. and used for our analyses.

**Treatment of rice flour with dilute organic acid solution.** Rice flour (50 g) was suspended in 500 ml of various concentrations of solutions of ascorbic acid, citric acid, malic acid and gluconic acid (Wako Junyaku Co.) for 2 hours at 25°C. Suspension was centrifuged at 1,600×g for 10 minutes and supernatant was collected. Distilled H<sub>2</sub>O was added to the precipitation of rice flour and suspended. The suspension was then centrifuged at 1,600×g for 10 minutes and supernatant was disposed. These procedures

were repeated two more times. The resulting precipitate of rice flour was spread on filter paper and dried at 50°C in a convection oven. The treated rice flour was used for the following analyses.

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE).** Organic acid-treated rice flour was suspended in SDS-PAGE sample buffer (20 mM Tris-HCl, pH6.8, 1% SDS, 6% glycerol, 0.012% bromophenol blue) and sonicated thrice for three minutes with one minute interval. Centrifugation was carried out at 16,000×g for 10 minutes and resulting supernatant was transferred into new tubes. For dilute organic acid supernatant, SDS-PAGE sample buffer and β-mercaptoethanol were added to each sample followed by heating at 90°C for 3 minutes. SDS-PAGE was done according to the method by Laemmli et al<sup>11)</sup>. using 4.5% stacking gel and 15% separating gel with a constant current of 10 amperes (A) and 20A, respectively. The gel was later stained with Coomassie brilliant blue (CBB)-staining solution (2-propanol: acetic acid: distilled H<sub>2</sub>O=5:1:5, 0.25% CBB-R250) for 20 minutes followed by destaining in a buffer solution (7% methanol and 5% acetic acid) and gently shaken overnight using an electric shaker.

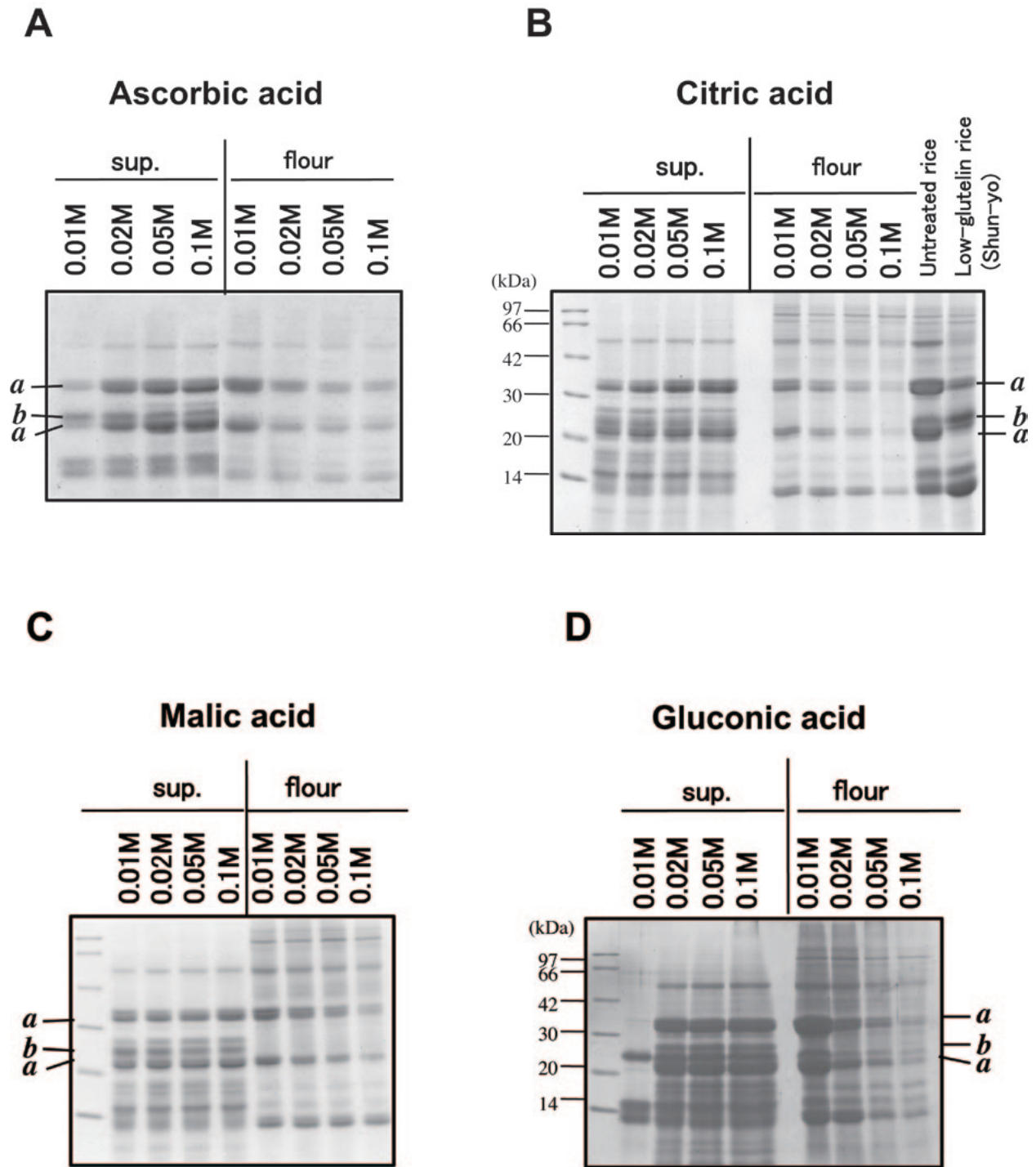
**Protein quantification.** Protein amount of acid-treated rice flour was quantified by bicinchoninic acid (BCA) method. Protein was extracted from rice flour with an extraction buffer (20 mM Tris-HCl, pH6.8, 1% SDS) and quantified using BCA Protein Assay Kit (PIERCE) according to manufacturer's protocol. BSA (bovine serum albumin) solution was used as a standard protein. The absorbance values at 562 nm were measured using a spectrophotometer (Shimadzu UV-1600). Protein amount of each sample was calculated from the standard curve.

**Processing products of rice flour.** Rice dumplings were made as follows: Fifteen ml of water was added to 18 g of rice flour, kneaded to form a dough, divided into small ball portions and tossed in boiling water for a few minutes. After floating on the surface of the boiled water, the cooked dumplings were cooled in ice-cold water.

**Sensory evaluation.** A seven-scale evaluation sheet was prepared for eight panelists who joined the sensory evaluation (-3; "extremely bad" to +3; "extremely good") of the processed products. The results were evaluated using a two-factor analysis of variance (ANOVA). A 5% test of significance was applied.

## RESULTS AND DISCUSSION

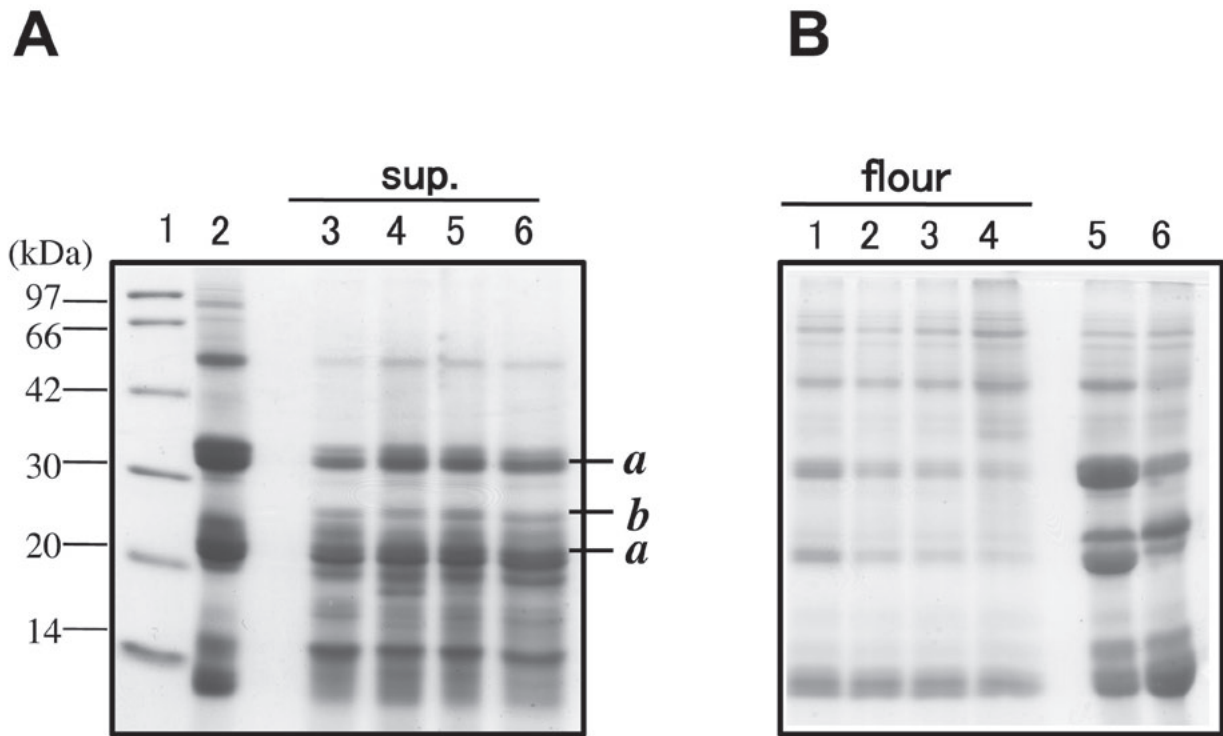
**Treatment of rice flour with organic acid.** The protein content of polished rice is about 6% according to the Standard Tables of Food Composition in Japan. In order to reduce the protein content of rice, we used ascorbic acid, citric acid, malic acid and gluconic acid, all of which are applicable to food. Ascorbic acid is contained in many food sources and is one of the vital nutrients in the group of water-soluble vitamins. Ascorbic acid is often used as an anti-oxidative agent. Citric acid is contained in citrus fruits such as lemon and grapefruit. Malic acid is a dicarboxylic acid originally found in apples. Because of their fine sour taste, citric acid and malic acid are sometimes used as acidifiers in processed foods and beverages. Gluconic acid is an oxidized product of D-glucose and is



**Fig. 2. Extraction and reduction of rice proteins by organic acid solution.** Dilute organic acid solution with the indicated concentration was added to rice flour. Proteins in acid solution and treated rice flour were detected by CBB staining in SDS-PAGE. Glutelin and  $\alpha$ -globulin were denoted by “a” and “b”, respectively. (A) ascorbic acid; (B) citric acid; (C) malic acid; (D) gluconic acid.

contained in honey, vinegar, miso, soy source, and so on. It is also used as a food additive. Dilute acid-soluble glutelin was mainly dissolved in organic acid solution. Two subunits, namely, the acidic and basic subunits of glutelin (denoted by “a”) and the salt-soluble  $\alpha$ -globulin (denoted by “b”) were extracted in the acid solution (Fig. 2). A higher concentration (0.1 M) of each acid resulted in rice

grains having reduced proteins relative to ordinary rice (Figs.2-A, -B, -C, and -D). The protein content of these rice grains after treatment was lower than *Shunyo*, a commercially available low-glutelin rice cultivars (Fig. 2-B). By treating with the solution of citric acid, malic acid and gluconic acid, it was shown that most of the glutelin and the  $\alpha$ -globulin in rice were reduced according to the

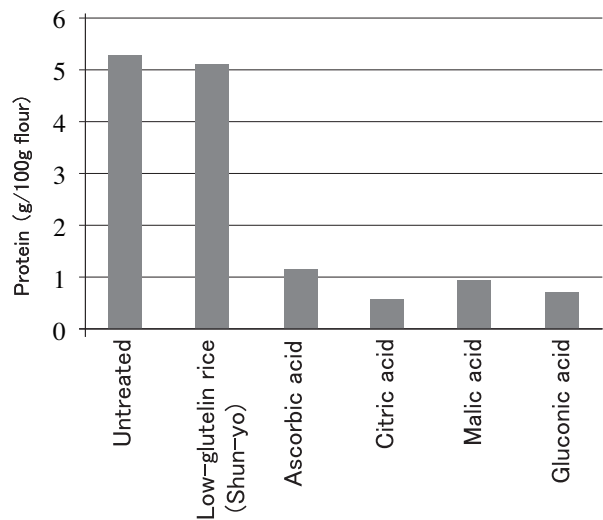


**Fig. 3. Comparison of organic acid solution for efficiency of protein reduction in rice flour.** (A) After addition of dilute organic acid solution to the rice flour followed by mixing, the suspensions were centrifuged; then the supernatant obtained was subjected to SDS-PAGE. Lane 1: Molecular weight marker; lane 2: SDS-soluble proteins of ordinary rice (control); lane 3: 0.1 M ascorbic acid; lane 4: 0.1 M citric acid; lane 5: 0.1 M malic acid; lane 6: 0.1 M gluconic acid. Glutelin and  $\alpha$ -globulin were denoted by “a” and “b”, respectively. (B) SDS-soluble proteins were extracted after organic acid treatment of rice flour and these samples were subjected to SDS-PAGE. Lane 1: 0.1 M ascorbic acid; lane 2: 0.1 M citric acid; lane 3: 0.1 M malic acid; lane 4: 0.1 M gluconic acid; lane 5: SDS-soluble proteins of ordinary rice (control); lane 6: SDS-soluble proteins of *Shun-ryo*.

result of the gel electrophoresis (Figs. 3-A and -B). These results showed that in addition to glutelin, the most major protein,  $\alpha$ -globulin, which is one of the allergenic proteins in rice, decreased by treatment with organic acid solution.

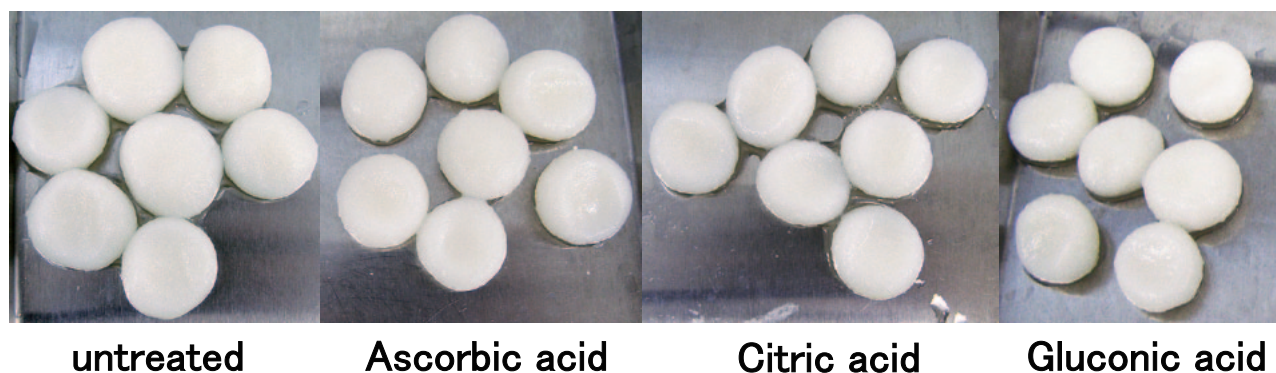
**Quantitative analyses of the acid-treated rice flour.** In order to clarify the reductive effect of organic acid on the protein content of rice, protein quantification by BCA method was carried out. Acid-treated rice flour was used for analysis. Quantification analyses clarified that the four kinds of organic acid solutions markedly reduced the amount of rice protein to 12-20% less than the ordinary rice (Fig. 4). This means that appropriate concentrations of organic acid solutions could lead to a reduction in the main protein glutelin and in the  $\alpha$ -globulin of rice flour. Treatment with citric acid and gluconic acid reduced protein amount up to 12% and 14%, respectively. These results showed that dilute acid treatment of rice flour can reduce protein content to low levels. This new method of treating rice flour may be useful in the preparation of low-protein rice-based products. Hence, it is necessary to confirm whether the low-protein rice flour is almost identical to ordinary rice and if it is applicable for processing.

**Evaluation of the processed products made from low-protein rice flour.** When the treated rice flour was dried, it was not at all different from ordinary rice flour in outward appearance as seen with the naked eye. In order to confirm whether or not the properties of organic acid-



**Fig. 4. Protein quantification of rice treated by organic acid solution.** Proteins were extracted from rice flour followed by BCA protein assay. Standard curve was obtained using standard solutions of bovine serum albumin. Protein content per 100 g of rice flour was calculated.





**Fig. 5. Dumplings made from rice flour.** Rice flour treated with dilute organic solution was processed into trial products. These dumplings were subjected to sensory evaluation.

treated rice flour are the same with the ordinary rice flour, rice flour particles were observed under the microscope. The shape and size of the particles have been found to be almost identical, indicating that the acid treatment didn't change the outward properties of rice flour (data not shown).

In order to check whether the flour from treated rice is applicable for processing, a trial processed product was produced with rice as the main ingredient. At first, dumplings were prepared from both ordinary and acid-treated rice flour. These dumplings were then evaluated (Fig. 5). Sensory evaluation based on outward appearance, aroma, taste, texture and overall evaluation was done by eight panelists to compare the quality of the processed products. A seven-scale score sheet (-3, extremely bad to +3, extremely good) was used for the evaluation. Results of the evaluation for outward appearance and texture revealed no significant differences with untreated samples, whereas average evaluation points for aroma and taste decreased slightly after treatment with organic acid solutions. We infer that these changes may have been caused by the acid treatment causing a decrease in or removal of peculiar compounds in rice that may be related to aroma and flavor. Results of overall evaluation were determined statistically by two-factor ANOVA. The average overall evaluation points for the dumplings made from ordinary rice, ascorbic acid, citric acid, and gluconic acid-treated rice flour were 1.14, 0.14, 0.14, 0.29, respectively. Based on ANOVA, there was no significant differences among samples based on the panelists' scores for overall estimation. These results show that the rice flour treated with organic acid solutions could be used as a food material in the manufacture of low-protein processed foods.

In some cases, intake of protein is restricted in patients with kidney failure. Good sources of proteins are those that contain essential amino acids which are normally found in meat, eggs, fish, milk and soybeans. Rice and wheat also contain some proteins of less than 10%. Since rice is a staple food for nearly one-half of the world's population and it provides fully 60% of the food intake in Southeast Asia and about 35% in East Asia and South Asia<sup>12)</sup>, it is important to consider protein intake from rice particularly for patients with kidney disease. In this regard, rice products have been developed for those patients, for example, rice grains in which the outer portion of the endosperm was removed. Because most of the rice

proteins are localized in the outer portion of the endosperm in the grain, protein content of the surface-removed rice grains could be reduced. In this study, we have succeeded in reducing the protein content of rice flour based on the solubility of rice seed proteins by the use of dilute organic acid solutions.

Recently rice flour has gained widespread popularity in Japan as one of the main materials in various kinds of foods. Rice flour treated by the method shown in this study may be applicable to processed foods intended for those whose intake of protein is restricted. Rice does not contain gluten and related proteins for viscoelasticity of foods; however, it may be a good alternative to such people preferring a gluten-free diet such as those with Celiac disease or dermatitis. On the other hand, it would be interesting to come up with processed products from rice that are formulated to have viscoelasticity or gluten-like properties. We have been doing pilot research to develop processed products such as cookies and steamed breads using rice flour instead of wheat as a main material. We envision the production of substitute gluten-free foods for those who cannot eat products from wheat, barley, rye and oats, among others because of gluten intolerance and allergies. Furthermore, wheat allergy patients are sometimes also allergic to other cereals such as rice and barley. One of the main rice allergenic protein, the  $\alpha$ -globulin, has been reduced in rice flour treated by organic acid solution in this study, hence, this rice flour may found its versatility in many food preparations and provide benefits to allergy patients caused by multiple foods as well.

#### ACKNOWLEDGMENT

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