Texture of Cooked Rice Prepared from Aged Rice and Its Improvement by Reducing Agents

Toshihisa OHNO,1,1 Makoto TOMATSU,1 Kazuki TOEDA,1 and Naganori OHISA2

1Institute for Food and Brewing, Akita Prefectural Agriculture, Forestry and Fisheries Research Center, 4-26 Aza-sanuki, Araya-machi, Akita 010-1623, Japan
2School of Food, Agricultural and Environmental Sciences, Miyagi University, 2-2-1 Hatatate, Taihaku, Sendai, Miyagi 982-0215, Japan

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The textures of cooked rice prepared from aged rice grains and their improvement by reducing agents were investigated. For aged rice that was stored for 5 months without air by the operation of a vacuum packing machine, the stickiness/hardness ratio of cooked rice was as low as that of aged rice stored in air. The results of electrophoresis showed that oxidation of proteins in the former was advanced to the same degree as in the latter. The stickiness/hardness ratios of the aged rice were increased by the addition of sodium sulfite, cysteine, and dithiothreitol to the cooking water. Sodium sulfite, cysteine, and dithiothreitol cleave disulfide bonds to sulfhydryl groups. Therefore, cleaving disulfide bonds to sulfhydryl groups improved the texture. The addition of them to the cooking water also increased the extractable solids at the time of heating. Consequently, the gelatinized paste layer thickened and the thick paste layer softened the cooked rice.

Key words: aged rice; texture; oxidation of proteins; reducing agent; extractable solid

The taste of cooked rice is known to be related to textural factors such as hardness and stickiness.1) Because the texture of cooked rice that has been stored is generally hard and non-sticky as compared to that of fresh rice, Japanese people dislike cooked rice prepared from aged rice. Many factors have been proposed as causing these changes in cooking properties: an increase in free fatty acids during storage and their inhibitory effect on the gelatinization of rice starch;2) changes in the physicochemical properties of the rice starch itself;3) and changes in the structure-maintaining components containing the cell wall.4) In contrast, some investigators have specifically studied proteins associated with these changes, and have concluded that denatured proteins were associated with them.5) In short, the mechanisms responsible for these changes in aged rice have not yet been clarified.

To mitigate the changes in cooked rice prepared from aged rice, various procedures have been attempted: the addition of rice vinegar or fruit juice to the cooking water;6) high-pressure treatment of the rice;7) enzymatic degradation of the cell wall;8) and removal of the external layer from the rice grains.9,10) A reducing agent, dithiothreitol (DTT), influences the texture of cooked rice and the gelatinization properties of rice flour.11,12) In addition, acetic acid produces a textural change through the dissolution of proteins.13) We have reported that the texture of cooked rice prepared from aged rice was improved by the addition of sodium sulfite to the cooking water.10) We assumed that the textural changes in aged rice were in part attributable to the oxidation of proteins, but this assumption must be tested. In addition, the mechanism of the improvement due to reducing agents remains unknown.

Hence we investigated the texture of cooked rice prepared from aged rice, stored with and without air. We also carefully investigated the textural changes in cooked rice prepared from aged rice due to the addition of sodium sulfite or reducing agents to the cooking water. Based on these facts, not only the changes in rice grains which were soaked and heated, but also the behaviors of proteins were examined. We inferred the factors involved the textural changes in aged rice.

Materials and Methods

Experimental materials. Japonica type rice (Oryza sativa L. japonica, cv. Akitakomachi) harvested in Akita Prefecture, Japan, was used in these experiments. After

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1 To whom correspondence should be addressed. Tel: +81-18-888-2000; Fax: +81-18-888-2008; E-mail: ohno@arif.pref.akita.jp

Abbreviations: CBB, Coomassie Brilliant Blue; DTT, dithiothreitol; SDS–PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; S/H, stickiness/hardness
brown rice grains harvested in 2002 were polished to wash-free grade (about 1.5% more polished than normal polished rice grains), they were tested as New Rice A. New Rice A grains, stored for 5 months at 30°C in a closed aluminum pouch with air, were tested as Aged Rice B. New Rice A grains, prepared with a vacuum packing machine (FVC II-G; Furukawa Mfg. Co., Tokyo) and then stored at 30°C for 5 months in a closed aluminum pouch without air were tested as Aged Rice C. Aged Rices B and C were stored as polished rice. Thereafter, samples were stored below −5°C until use in experiments. In the samples, no significant changes in texture were detected for storage below −5°C.

After brown rice grains harvested in 2002 were polished to normal grade, they were stored for 5 months at 30°C in a closed aluminum pouch with air. They were tested as Aged Rice D.

Cooking and texture measurement. New Rice A, Aged Rice B, and Aged Rice C were used as samples. Unwashed 10 g samples of polished rice were soaked in 16 ml of distilled water for 1 h in an aluminum cup covered with aluminum foil, and then cooked as described in our previous paper. The hardness and stickiness of the individual cooked rice grains were then measured using 90% deformation with a compression tester (Tensipresser TTP-50BX2; Taketomo Electric, Tokyo). The most important parameter, the stickiness/hardness (S/H) ratio, was calculated. More than 30 cooked rice grains of each type were measured. The measurement conditions were as follows: load cell, max. 10 kgf; plunger, 25 mm diameter; plunger and stage, aluminum; bite speed, 2 mm/s; sample temperature at measurement, 25°C. The textural parameters as cooked with 8 mM sodium sulfite were also measured. In addition, after rice samples were soaked in 8 mM sodium sulfite for 1 h, they were washed with distilled water. Subsequently, they were cooked and their textural parameters were measured.

The respective influences of various solutions used in place of distilled water on S/H ratios were also measured by the method described above. Aged Rice D was used as a sample. Distilled water, disodium hydrogen phosphate, ascorbic acid, sodium sulfite, cysteine, or DTT was used as a sample. Distilled water, disodium hydrogen phosphate and 1% SDS, were washed 2 times with 10 ml of distilled water. Then the solutions and solids taken from the rice grains were collected. Next the rice grains were washed 2 times with 10 ml of distilled water. Solution, solids, and washing water were collected and the protein contents were measured by the Kjeldahl method.

Electrophoresis of proteins extracted from the external layer of rice grains and electrophoresis of proteins excised from the gels. Rice samples, New Rice A, Aged Rice B and Aged Rice C were further polished in a mill; the Grain Testing Mill (Satake, Higashi-hiroshima, Japan). The rate of removal of the external layer of the polished rice was 4%. Then 20 mg of rice flour of the external layer was mixed with 0.4 ml of a solution composed of 10 mM sodium hydroxide and 1% SDS, and the mixture was shaken for 2 h. The mixtures were then centrifuged and the resulting supernatants were mixed with equal volumes of a solution composed of 0.1 M Tris–HCl, pH 6.8, 0.02% bromophenol blue, and 40% glycerol. They were subjected to electrophoresis immediately without a reducing agent. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) was performed on 5–20% gradient polyacrylamide gel (Atto, Tokyo) with Laemmli’s buffer containing 0.1% SDS. Coomassie Brilliant Blue (CBB) R250 (Bio-Rad Laboratories, Hercules, CA) was used as the staining reagent. Standard protein as molecular weight markers for SDS–PAGE were α-lactalbumin (14.4 kDa), trypsin inhibitor (20.1 kDa), carbonic anhydrase (30.0 kDa), ovalbumin (45.0 kDa), albumin (66.0 kDa), and phosphorylase B (97.0 kDa). After the extraction described above, the flour was washed once with a solution composed of 10 mM sodium hydroxide and 1% SDS, with subsequent washing with distilled water two times. Then the residual ratios of the proteins against total proteins were measured by the combustion method (Sumigraph NCH-21; Sumika Chemical Analysis Service, Osaka, Japan).

Several protein band peaks per total area of the SDS–PAGE result were analyzed using densitometric software (Image-J; NIH, Bethesda, MD). The relative intensity was calculated against each band of New Rice A.

After the gels’ protein bands were excised, the three higher-molecular-weight protein bands, those at 48 kDa, 99 kDa, and 170 kDa, were extracted with a solution composed of 55 mM Tris–HCl, pH 7.0, 1% SDS, and 5% mercaptoethanol. Then the mixtures were centrifuged, and the resulting supernatants were subjected to electrophoresis by the method described above. Coomassie G250 stain (Bio-Rad Laboratories) was used as the staining reagent.

Measurement of extractable proteins after soaking. New Rice A, Aged Rice B, and Aged Rice C were used as samples. Unwashed rice grains (10 g) were soaked in 16 ml of distilled water or 8 mM of sodium sulfite for 1 h in an aluminum cup. Then the solution and solids taken from the rice grains were collected. Next the rice grains were washed 2 times with 10 ml of distilled water. Solution, solids, and washing water were collected and the protein contents were measured by the Kjeldahl method.

Measurement of extractable solids after heating. New Rice A, Aged Rice B, and Aged Rice C were used as samples. Unwashed rice samples (5 g) were soaked in 8 ml of distilled water or 8 mM of sodium sulfite for 1 h in an aluminum cup, and then placed in a water bath at 80°C for 5 min, with occasional shaking. After 10 ml of distilled water was added, the solution and the solids taken from the rice grains were collected and dried at 105°C for 3 h. Then the amount of solids was measured as extractable solids.
The influences of various solutions used in place of distilled water on the extractable solids were also measured. Aged Rice D was used as a sample. Distilled water, disodium hydrogen phosphate, ascorbic acid, sodium sulfite, cysteine, or DTT was used as the cooking solution. The concentration of the solution was 10 mM, except for DTT, the concentration of which was 5 mM.

Electrophoresis of proteins dissolved in soaking solutions. Unwashed rice samples (10 g) of Aged Rice D were soaked in 16 ml of various solutions for 1 h in an aluminum cup, and the solution was collected. The concentration of solution was 10 mM. After the solution was centrifuged and the resulting supernatants were mixed with a solution composed of 0.1M Tris–HCl, pH 6.8, 0.02% bromophenol blue, 40% glycerol, and 5% 2-mercaptoethanol, the mixture was subjected to electrophoresis on 14% polyacrylamide gel containing 0.1% SDS by the method described above. Coomassie G250 stain was used as the staining reagent.

Electrophoresis of proteins mixed with various solutions. The protein solution (10 mM sodium hydroxide, 1% SDS) extracted from New Rice A was mixed with equal volumes of various solutions and reacted for 3 h. Then the mixture was mixed with a solution composed of 0.1 M Tris–HCl, pH 6.8, 0.02% bromophenol blue, and 40% glycerol, and the mixture was subjected to electrophoresis on 5–20% gradient polyacrylamide gel by the method described above. Then CBB R250 stain was used as the staining reagent.

Statistical analysis. Significant differences among textural parameters and the amount of extractable solids were analyzed by Tukey’s multiple-range test. Significance was inferred for a probability level of < 0.05.

Results and Discussion

Textural parameters of various types of cooked rice and changes in aged rice

The textures of cooked rice were evaluated. The white bars in Fig. 1 show the textural parameters of cooked rice for New Rice A, Aged Rice B, and Aged Rice C cooked with distilled water. Aged Rices B and C were significantly hard and the S/H ratios of Aged Rices B and C became significantly low compared to the respective values of New Rice A. The cooked rice prepared from aged rice generally became hard and non-sticky,7,17) but there were no significant differences in stickiness as between New Rice A and Aged Rice C. Because we measured the texture by the individual method, the stickiness in new rice was measured as not as stickiness, but as softness. We estimated that Aged Rice C stored without air showed decreased textural change. But Aged Rice C showed similar changes to those of the aged rice stored with air.

Hence we investigated the proteins extracted from the 4% external layer of the rice samples. The respective residual ratios of proteins in New Rice A, Aged Rice B, and Aged Rice C against the total proteins were 11.2%, 10.7%, and 11.6%, so the residual rates were almost identical. The SDS–PAGE result and the densitograph for each lane are shown in Fig. 2A and B respectively. The densities of proteins in the flour derived from Aged Rices B and C revealed decreased 21 kDa and 32 kDa proteins, along with increased 48 kDa, 99 kDa, and 170 kDa proteins as compared to those of the flour...
derived from New Rice A. Hence the relative intensities of the five protein bands mentioned above to those of New Rice A were calculated (Fig. 2C). The 21 kDa and 32 kDa proteins of Aged Rice B and C decreased to about 60% as compared to those of New Rice A; the 48 kDa proteins increased to about 115%; the 99 kDa proteins increased to about 140%; and the 170 kDa proteins increased to about 250%. Then the protein bands at 48 kDa, 99 kDa, and 170 kDa were excised from the gels and dissolved using a reducing agent; they were subjected to electrophoresis again. The SDS–PAGE result confirmed that the 48 kDa proteins included 22 kDa proteins and 32 kDa proteins. Hence we assigned the 22 kDa protein as the basic subunit of glutelin. The 32 kDa protein was the acidic subunit of glutelin, and the 48 kDa protein was formed from a basic subunit of glutelin and an acidic subunit of glutelin by the disulfide linkage. Our extraction method used no urea or heating process. Consequently, the acidic subunit of glutelin was detected not as a 37–39 kDa protein, but rather as a 32 kDa protein. The SDS–PAGE result also confirmed that the 99 kDa and 170 kDa proteins comprised a 22 kDa protein and 32 kDa protein (Fig. 2D). The 32 kDa protein included several bands, because the acidic subunit of glutelin included several molecular weights.\textsuperscript{19,20} As inferred from these facts, the 99 kDa and 170 kDa proteins were also formed from glutelin subunits by disulfide linkages.

Fig. 2. SDS–PAGE Analyses of Proteins Extracted from the Rice Samples.
A, SDS–PAGE analysis of proteins extracted from the external layer of rice samples under non-reducing conditions. Sodium hydroxide/SDS-extracts from New Rice A (lane 1), Aged Rice B (lane 2), and Aged Rice C (lane 3) were subjected to 5–20% gradient gel electrophoresis. The volume of the extracted solution applied was 2.5 μl. B, densitograph of each lane in Fig. 1A. C, relative intensity changes of five bands from Fig. 1B. The five peak-area/total-area was analyzed on the basis of Fig. 1B. The relative intensity against each band of New Rice A was calculated. Five peaks were apparent, for 21 kDa, 32 kDa, 48 kDa, 99 kDa, and 170 kDa proteins. D, SDS–PAGE analysis of the protein bands excised from gels under reducing conditions. After the 48 kDa, 99 kDa, and 170 kDa protein bands of Fig. 1A were excised from the gel, proteins were extracted with 5% mercaptoethanol-containing buffer. Then they were subjected to 5–20% gradient gel electrophoresis.
In the proteins of the external layers of Aged Rices B and C, the 48 kDa, 99 kDa, and 170 kDa proteins were higher than in those of New Rice A, and their 21 kDa and 32 kDa proteins were lower than those of New Rice A (Fig. 2C). The 21 kDa and 32 kDa proteins must have included the basic subunit of glutelin and acidic subunit of glutelin respectively. This electrophoresis was performed under non-reducing conditions. Consequently, the basic subunit of glutelin was detected not as 22 kDa, but as 21 kDa. These data revealed that the glutelin of Aged Rices B and C was polymerized by oxidation from the glutelin subunit and the low-molecular glutelin respectively, to glutelin and high-molecular glutelin. Aged Rice C revealed textural changes similar to those of Aged Rice B because the oxidation in Aged Rice C was advanced to the degree of that in Aged Rice B.

Increases in disulfide bonds and the decrease in sulfhydryl groups in glutelin during rice storage have been reported. This measurement typically requires several steps to extract glutelin, and it is performed by the spectrophotometric method. These results reveal the same phenomena after electrophoresis with extraction in one step.

The aluminum pouch was deaerated sufficiently. Therefore, these results reveal that the storage of rice without air by a packing machine is not sufficient to prevent textural deterioration. Several possible reasons why the oxidation of proteins was advanced are these: the small amount of oxygen that remained in the aluminum pouch was sufficient for oxidation of rice; oxygen was included in the rice grain itself; the aluminum pouch transmitted oxygen very slowly; and enzymes, such as peroxidase and catalase, were related to the oxidation.

The black bars in Fig. 1 show the textural parameters of cooked rice of New Rice A, Aged Rice B, and Aged Rice C cooked with 8 mM sodium sulfite. In all the samples, hardness was significantly low and the S/H ratio was significantly high in comparison with the values for rice cooked with water. Consequently, there were no significant differences in hardness and stickiness as between new rice and aged rice, although there were significant differences in S/H ratios between them. Such improvements in S/H ratios due to the use of sodium sulfite have been reported.

Next, after the rice grains were soaked in 8 mM sodium sulfite, they were washed with distilled water. Then they were cooked with distilled water. The gray bars in Fig. 1 show their textural parameters. In this case, the hardness of Aged Rices B and C became low and their S/H ratios increased significantly in comparison with the respective values of rice cooked with water. Because the S/H ratios of cooked rice soaked in sodium sulfite and cooked with distilled water were improved, we assumed that textural improvement might occur at the time of soaking.

Measurement of extractable proteins and solids
The results for extractable proteins after soaking are shown in Fig. 3A. The proteins, dissolved in solution and taken from rice grains, increased with 8 mM of sodium sulfite in place of distilled water. This result also supports the assumption that the textural improvement that occurred due to the use of sodium sulfite might have taken place at the time of soaking.

The results for the extractable solids after heating are shown in Fig. 3B. The extractable solids of New Rice A were greater than those of Aged Rices B or C. In addition, the extractable solids increased with 8 mM sodium sulfite in place of distilled water. These solids finally formed a gelatinized paste layer of starch at the surface of the rice grains after cooking. The hardness of the cooked rice was measured by the crushing method. Therefore, as well as increasing the stickiness, the large gelatinized paste layer of cooked rice must soften the cooked rice. From the data obtained as above, we inferred that not only the softening by sodium sulfite but...
also the hardening of aged rice might be related to the amount of extractable solids.

**Electrophoresis of proteins dissolved in various solutions and the textural parameters of rice cooked with various solutions**

In view of the inference mentioned above, the proteins dissolved to soaking water were investigated. The results are shown in Fig. 4. The amount of proteins dissolved in sodium sulfite was large. This result coincides with the result shown in Fig. 3A. The amount of proteins dissolved in disodium hydrogen phosphate was large, as was that in sodium sulfite; consequently, we selected disodium hydrogen phosphate as the cooking water additive. Sodium sulfite is a reducing agent, so we investigated ascorbic acid, cysteine, and DTT in the cooking water in addition to disodium hydrogen phosphate.

The textural parameters are shown in Fig. 5A, B, and C. Compared with the values as cooked in distilled water, the hardness of the rice cooked with sodium sulfite, cysteine, and DTT decreased significantly, and consequently the S/H ratios increased significantly. On the other hand, the addition of disodium hydrogen phosphate to cooking water only slightly increased the S/H ratio. The addition of cysteine, which dissolved the proteins less than sodium sulfite and disodium hydrogen phosphate added to soaking water did (Fig. 4), improved the S/H ratio. Therefore, the dissolving ability of proteins at the time of soaking only slightly influenced the cooked-rice texture. The addition of sodium sulfite to the soaking water improved the S/H ratio, but not because the amount of proteins dissolved to the soaking water was large. The residual sodium sulfite in the rice grains might be attributable to the textural improvement in the rice that was soaked in sodium sulfite then cooked after rinsing. Increases in extracted proteins and textural improvement due to soaking in salt solution have been reported, but the density was greater than 50 mM. At low density, such as 10 mM, it was not the dissolving

**Fig. 4.** SDS–PAGE Analysis of Proteins Dissolved in Various Solutions.

Aged rice D was soaked in distilled water (lane 1), sodium chloride (lane 2), sodium hydrogen carbonate (lane 3), sodium sulfate (lane 4), disodium hydrogen phosphate (lane 5), potassium dihydrogen phosphate (lane 6), sodium sulfite (lane 7), or cysteine (lane 8). The concentration of solution was 10 mM. The volume of the extracted solution applied was 12.5 µl.

**Fig. 5.** Influence of Various Solutions on Textural Parameters and Extractable Solids.

Aged rice D was cooked or heated with various solutions. A, hardness; B, stickiness; C, S/H ratios; D, extractable solids after heating. DW, distilled water; DSHP, disodium hydrogen phosphate; AA, ascorbic acid; SS, sodium sulfite; CYS, cysteine; DTT, dithiothreitol. The data are shown as means ± SD (bars) of 30 replications in textural parameters and three replications in extractable solids. Different letters indicate a significant difference (p < 0.05).
ability of proteins but rather the other abilities of sodium sulfite, cysteine, and DTT that caused textural improvements.

**Electrophoresis of proteins mixed with various solutions**

Reducing agents besides ascorbic acid improved the S/H ratios. Hence we assumed that the reducing ability might be related to the cooked rice texture. We tried to mix the protein solution with equal volumes of distilled water, 20 mM disodium hydrogen phosphate, 20 mM ascorbic acid, 20 mM sodium sulfite, or 20 mM cysteine for 3 h. The results are shown in Fig. 6. The ascorbic acid solution did not cleave disulfide bonds to sulfhydryl groups even though ascorbic acid is a reducing agent. In contrast, the sodium sulfite and cysteine solutions cleaved disulfide bonds to sulfhydryl groups. The fact that DTT solution cleaves disulfide bonds to sulfhydryl groups is widely known. Ascorbic acid, a reducing agent, did not have that ability and did not improve the S/H ratio. From these facts, we assigned the textural improvement to the cleaving of disulfide bonds to sulfhydryl groups.

**Measurement of extractable solids with various solutions**

The results for extractable solids with various solutions after heating are shown in Fig. 5D. The extractable solids, using sodium sulfite, cysteine, and DTT in cooking water, showed significantly increased values. These results coincide with those for extractable solids described above (Fig. 3B).

The reducing agents, aside from ascorbic acid, increased the S/H ratios (Figs. 1C and 5C) and the extractable solids (Figs. 3B and 5D), and cleaved disulfide bonds to sulfhydryl groups (Fig. 6). Based on these facts, we assume that the latter ability increased the extractable solids; then these solids formed a gelatinized paste layer of starch at the surface of the cooked rice.
cooked rice. Consequently, the gelatinized paste layer thickened and the thick paste layer softened the cooked rice. The ability to cleave disulfide bonds to sulfhydryl groups was essential to textural improvement due to reducing agents.

We produced models of cooked rice prepared from new rice and aged rice with and without reducing agents, except for ascorbic acid (Fig. 7). Because the oxidative polymerization of proteins in new rice hardly advanced (Fig. 2C), the extractable solids which made the gelatinized paste layer were rather large (Fig. 3B). Therefore, the gelatinized paste layer thinned and the thick paste layer softened the cooked rice (Figs. 1 and 5A). Consequently, the gelatinized paste layer were rather large (Fig. 3B). The processing of a 57-kDa precursor peptide to subunits of rice glutelin. Cereal Chem., 70, 377–380 (1993).


