Studies on Textural and Chemical Changes in Aged Rice Grains

Toshihisa OHNO* and Naganori OHISA

Akita Research Institute of Food and Brewing, 4–26 Aza-sanuki, Araya-machi, Akita, 010–1623, Japan

Received May 13, 2005; Accepted November 23, 2005

Chemical properties and textures of cooked rice prepared with aged rice grains were investigated and compared with those of cooked rice prepared with new rice grains. Differences in stickiness/hardness (S/H) ratios between new rice and aged rice were eliminated by the removal of the external layer of rice grains. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that the proteins of the external layer in aged rice grains were oxidized to a greater extent than those of new rice grains. Addition of a reducing agent to cooking water increased the S/H ratio of aged rice to approximately that of new rice. The reducing agent cleaves the disulfide linkages of the proteins. Therefore, textural changes in aged rice were inferred to be due to oxidation of proteins in the external layers of grains.

Keywords: aged rice, texture in cooked rice, oxidation of proteins, intermolecular disulfide linkages, external layer of grains

Introduction

The texture of cooked rice that has been prepared from stored rice grains is generally hard and non-sticky. Japanese people prefer cooked rice that is prepared from newly harvested grains because of its highly sticky and soft texture. Many factors have been proposed as being responsible for these changes in cooked rice properties, including the increase in free fatty acids during storage and their inhibitory effect on the gelatinization of rice starch (Yasumatsu et al., 1964), changes in the physicochemical properties of rice starch itself (Villereal et al., 1976), and changes in the structure-maintaining components (Shibuya and Iwasaki, 1982).

Some investigators have specifically studied proteins associated with these changes. Moritaka and Yasumatsu (1972) suggested that disulfide bond formation is responsible for textural changes in cooked rice prepared from rice that has been stored. Arai et al., (1993) reported that denatured proteins were associated with these changes. Furthermore, Komeyasu (1994) reported that the water uptake ratio in aged rice was improved by electrolytic treatment, and Hamaker and Griffin (1990) reported that reducing agents influence the texture of cooked rice.

The mechanisms responsible for changes in aged rice that produce differently textured cooked rice have not yet been clarified. Therefore, this study is aimed at detailed clarification of the textural and chemical changes in aged rice. We investigated the texture of cooked rice using aged grains. Using reducing and oxidizing agents, textural and chemical properties of aged rice were investigated.

Materials and Methods

Experimental materials A Japonica type rice (Oryza sativa L. japonica, cultivar Akitakomachi) harvested in Akita Prefecture was used in the experiments. After brown rice grains harvested in 2001 were polished to wash-free grade (about 1.5% further polished than normal polished rice grains), they were tested as New Rice A. New Rice A grains, stored for 2 months at 30°C in a closed aluminum pouch, were tested as Aged Rice A (milled rice storage). Thereafter, samples were stored below −5°C until use in experiments. In samples, no significant changes in texture were detected for storage below −5°C. Chemical components of Aged Rice A were expected to resemble those of New Rice A because they had the same origin. The chemical composition of polished rice is shown in Table 1.

Cooking and texture measurement Unwashed 10-g samples of polished rice were soaked in 16 mL of distilled water for 1 hr in an aluminum cup covered with aluminum foil. Next, samples were cooked in an electric rice cooker (SR-W100, Matsushita Electric Industrial Co., Ltd., Osaka, Japan) for about 12 min with 75 mL of water added to the inner pan. After steaming for 30 min, the cooked rice was transferred to a closed dish and kept at 25°C for 90 min.

The hardness (H) and stickiness (S) of the individual cooked rice grains was then measured using 90% deformation (Okadome et al., 1996) with a compression tester (Tensipresser TTP-50BX2, Taketomo Electric Corp., Tokyo). The most important parameter, the stickiness/hardness (S/H) ratio, was calculated (Okabe, 1977). More than 40 cooked rice grains of every type were measured. Measurement conditions were: plunger and stage, aluminum; bite speed, 2 mm/s; and sample temperature at measurement, 25°C.

*To whom correspondence should be addressed.
E-mail: ohno@arif.pref.akita.jp
The influence of the use of either an oxidizing agent (5 mM potassium iodate) or a reducing agent (8 mM sodium sulfite) in place of distilled cooking water on the S/H ratio was also investigated. Cooking methods and measurement of the S/H ratio were carried out as described above.

**Removal of external layer of polished rice** Samples were cooked after the rice was further polished in a mill: a Grain Testing Mill (Satake Co. Ltd., Higashihiroshima, Japan) or an HS-4 Mill (Chiyoda Engineering Co. Ltd., Hiroshima, Japan). Rate of removal of the external layer of the polished rice was 7%. The methods of cooking and measuring the S/H ratio were as described above.

**Electrophoresis of proteins extracted from the flour** From rice flour derived from 5% of the external layer of the New Rice A grains by a mill as mentioned above, 20 mg were mixed with distilled water, an aqueous solution of 8 mM sodium sulfite, or an aqueous solution of 5 mM potassium iodate and held for 30 min. After the mixture had been centrifuged, the pellet was rinsed in distilled water and centrifuged again. The precipitate was mixed with 0.4 mL of solution A (10 mM sodium hydroxide, 1% SDS) and shaken for 2 hr. The mixtures were then centrifuged and the resulting supernatants were mixed with solution B (0.1 M tris-HCl, pH 6.8, 0.02% bromophenol blue, 40% glycerol) and subjected to electrophoresis. Then, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 5–20% gradient, or 12.5% isocratic polyacrylamide gels with Laemmli’s buffer containing 0.1% SDS (Laemmli, 1970) for 1.5 hr at 20 mA. Coomassie brilliant blue was used as the staining agent.

Using flour derived from 5% of the external layer of rice samples by a mill method mentioned above, 20 mg was mixed with distilled water and held for 30 min. After the mixture had been centrifuged, the pellet was rinsed in distilled water and centrifuged again. The precipitate was mixed with 0.4 mL of solution A and shaken for 2 hr. The mixtures were then centrifuged and the resulting supernatants were subjected to electrophoresis using the method described above.

**Electrophoresis of the proteins extracted from rice grains** After 20 grains of New Rice A were mixed with distilled water, an aqueous solution of 8 mM sodium sulfite, or an aqueous solution of 5 mM potassium iodate and held for 30 min, the grains were rinsed in distilled water. Next, the grains were mixed with 1 mL of solution A and held for 1 hr. The solutions were then centrifuged and the resulting supernatants were subjected to electrophoresis using the method described above.

After 20 grains of polished rice samples were mixed with distilled water and held for 30 min, the grains were rinsed in distilled water. Next, the grains were mixed with 1 mL of solution A and held for 1 hr. The solutions were then centrifuged and the resulting supernatants were subjected to electrophoresis using the method described above.

**Electrophoresis of proteins cut from gels** After the protein bands of gels were excised, the proteins were extracted with solution C (55 mM tris-HCl, pH 7.0, 1% SDS, 5% mercaptoethanol) (Miyazaki, 1998). Then, the mixtures were centrifuged, and the resulting supernatants were subjected to electrophoresis by the method described above.

**Statistical analysis** Significant differences between data of new rice and of respective aged rice samples were analyzed using Student's t-test at statistical significance levels of 5%, 1%, and 0.1%.

Significant differences among S/H ratios cooked in various waters were analyzed using Tukey’s multiple-range test with a probability level of 0.05.

### Results and Discussion

S/H ratios of various types of cooked rice The textures of cooked rice were evaluated. Table 2 shows the textural parameters of cooked rice prepared from different types of rice grains. Aged Rice A became hard and the S/H ratio of Aged Rice A became lower than that of New Rice A. Japanese people generally prefer cooked rice to have a high S/H ratio (Okabe, 1977). Therefore, Aged Rice A was inferior to New Rice A. This change in texture has been reported previously (Watanabe et al., 1991; Toyoshima et al., 1998).

### Table 1. Chemical Composition of Polished Rice.

<table>
<thead>
<tr>
<th></th>
<th>Water* (%)</th>
<th>Crude protein* (%)</th>
<th>Crude lipid (%)</th>
<th>Ash* (%)</th>
<th>Carbohydrate* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Rice A</td>
<td>15.8</td>
<td>6.1</td>
<td>0.4</td>
<td>0.2</td>
<td>77.5</td>
</tr>
</tbody>
</table>

* Drying at 135°C for 3 h

### Table 2. Textural Parameters of Cooked Rice.

<table>
<thead>
<tr>
<th></th>
<th>Hardness (N)</th>
<th>Stickiness (N)</th>
<th>S/H ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Rice A</td>
<td>26.7 ± 4.5</td>
<td>9.4 ± 0.7</td>
<td>0.335 ± 0.047</td>
</tr>
<tr>
<td>Aged Rice A</td>
<td>34.2 ± 4.7***</td>
<td>9.7 ± 0.6*</td>
<td>0.289 ± 0.044***</td>
</tr>
</tbody>
</table>

* and *** denote significance at 5% and 1% levels, respectively.

### Table 3. The S/H Ratios of further Polished Rice Grains.

<table>
<thead>
<tr>
<th></th>
<th>S/H Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Rice A</td>
<td>0.319 ± 0.036</td>
</tr>
<tr>
<td>Aged Rice A</td>
<td>0.333 ± 0.040</td>
</tr>
</tbody>
</table>

New Rice A was used as a reference for statistical analysis in Aged Rice A. There was no significant difference between New Rice A and Aged Rice A.
After the polished rice had been further polished in a mill, it was cooked using ordinary methods. Table shows the S/H ratios of samples. After removing 1/8 of the external layer from the polished rice, the S/H ratio of Aged Rice A was increased to a value slightly higher than that of New Rice A, primarily because hardness was decreased. Inoue and Suzuki (+) also reported improvement in the S/H ratio in aged rice following removal of 1/8 of the external layer. These improvements of S/H ratios in aged rice by removal of the external layer mean that the external layer of the aged rice, in particular, deteriorates and that the external layer of aged rice is responsible for the decreased S/H ratio.

Electrophoresis of the proteins extracted from the flour treated with reducing agent or oxidizing agent

The SDS-PAGE result for rice flour treated with distilled water is shown in Fig. 1. Flour treated with distilled water separated into five main protein bands of different molecular weights on SDS-PAGE: 14 kDa, band A; 20 kDa, band B; 21 kDa, band C; 32 kDa, band D; and 48 kDa, band E. After protein bands were excised from the gel and dissolved with a reducing agent, they were subjected to electrophoresis again. The SDS-PAGE results shown in Fig. 2 clarify that bands A, B, C and D were 14-, 24-, 22- and 33-kDa proteins, respectively, and that band E was comprised of 22- and 33-kDa proteins. According to Tanaka (1982) and Sugimoto et al., (1991), assignment of bands were made as follows: band A was prolamin, band B was globulin, band C was a basic subunit of glutelin, band D was an acidic subunit of glutelin, band E was formed from a basic subunit of glutelin and an acidic subunit of glutelin by disulfide linkage.

SDS-PAGE results for rice flour treated with distilled water, reducing agent, or oxidizing agent are shown in Fig. 3. The SDS-PAGE results of flour treated with reducing agent (lane 1) showed a decrease in the amount of protein. Because proteins were dissolved in water using a reducing agent in advance, the amount of proteins extracted by solution A was decreased. SDS-PAGE of flour treated with oxidizing agent (lane 3) revealed a decrease in low molecular proteins (21 kDa and 32 kDa) and an increase in high molecular proteins (48 kDa, 92 kDa and larger than 92 kDa) in comparison with flours treated with reducing agent or distilled water. Thus, intermolecular disulfide linkages of rice proteins were formed easily in the presence of an oxidizing agent. The amount of glutelin was much greater than for other proteins (Kurasawa, 1982). For this reason, these phenomena clearly appeared in glutelin. Further, not only glutelin, but also other proteins had an increase in disulfide link-
SDS-PAGE results for flour derived from 5% external layer of rice samples are shown in Fig. 4. The SDS-PAGE of flours derived from Aged Rice A revealed an increase in 21-kDa protein to a certain extent compared with that of flours derived from New Rice A. Nevertheless, the SDS-PAGE pattern of Aged Rice A was very similar with that of New Rice A. Therefore, we estimated that the surface of rice grains might be oxidized more clearly in aged rice. For this reason, we tried to extract proteins from rice grains, as explained below.

**Electrophoresis of the proteins extracted from rice grains** The SDS-PAGE results for polished rice grains treated with reducing agent, distilled water, or oxidizing agent are shown in Fig. 5. The SDS-PAGE of polished rice treated with the oxidizing agent revealed lower 21-kDa, 32-kDa and 48-kDa protein levels than those of polished rice treated with reducing agent or distilled water.

The 48-kDa protein in the polished rice treated with an oxidizing agent (Fig. 5, lane 3) differed from that of 48-kDa protein in flour treated with the oxidizing agent (Fig. 3, lane 3). We estimated that proteins were polymerized with the proteins of the inner layer and therefore became difficult to extract, resulting in a decrease of the 48-kDa protein in polished rice treated with oxidizing agent.

The SDS-PAGE results for polished rice samples are shown in Fig. 6. SDS-PAGE of Aged Rice A revealed a decrease in 21-kDa, 32-kDa and 48-kDa proteins as was shown for polished rice treated with oxidizing agent. Therefore, proteins of the external layer of Aged Rice A were inferred to have been oxidized during storage. Moritaka and Yasumatsu (1972) also demonstrated an increase in disulfide groups in aged rice. The results of this study fit with these findings because the external layer is more readily in contact with air than is the interior layer of grains.

The results obtained in this study clarified that the S/H ratio of aged rice was low when the external proteins of rice grains were oxidized and that the S/H ratio of aged rice was improved when the external layer was excluded. In this way, the textural changes in aged rice considerably coincide with the protein oxidation. The influence of
the addition of oxidizing or reducing agent on the S/H ratio was specifically investigated (Table 4). The addition of a reducing agent engendered a significant increase in S/H ratios because hardness was primarily decreased. However, the addition of an oxidizing agent to cooking water did not cause a significant change in the S/H ratios. The reducing agent, which cleaves the disulfide linkages of the proteins, resulted in a change in the S/H ratios. Therefore, textural changes in aged rice were inferred, in part, to be attributable to oxidation of proteins in the external grain layers. Collating the data obtained above, we attempted to infer the reason for textural changes in aged rice. Glutelins and prolamins are only slightly soluble in water (Kurasawa, 1982), so they remain inside of rice grains when the grains are soaked in water. Because the addition of oxidizing agent to cooking water did not cause a significant change in S/H ratios, we presume that the oxidation of glutelins and prolamins does not cause textural changes. In new rice grains, water-soluble and some salt-soluble proteins, such as albumins and globulins, are dissolved in water when rice grains are soaked in water (Arai et al., 1993). We presume that albumins and some globulins had been polymerized with intermolecular disulfide linkages by oxidation during storage and thereby became difficult to dissolve. We infer that those phenomena decreased the S/H ratio. Albumins and globulins were dissolved in water before oxidation; therefore, addition of oxidizing agent to cooking water did not cause a significant change in S/H ratios. Arai et al., (1993) also pointed out that albumins and globulins in aged grains had been denatured so as to be only slightly extractable with water. This supposition was not asserted previously because fatty acids also accumulate in the external layers of aged rice (Shibuya et al., 1974). Nevertheless, data obtained in this study show that the oxidation of proteins might be related to textural changes in aged rice.

Further investigations must be made to clarify our supposition. We will continue to investigate the mechanisms of rice aging.

Acknowledgments This work was financially supported in part by the Iijima Food Science Foundation.

References


