Improvement of Palatability and Prevention of Ablrupt Increases in Postprandial Blood Glucose Levels by Hokurikukona243 after High Pressure Treatment

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Abstract: Diabetes is a lifestyle disease, and its prevention and treatment are extremely important. Since Hoku243 presents fewer short-branched glucans and more long chains in the amyllopectin starch, its starch is resistant to gelatinization and the boiled rice grains are non-sticky and brittle. The prevention of abrupt increases in postprandial blood glucose level (BGL) after consumption of Hoku243 (produced by high pressure treatment (HPT) after soaking in unsalted rice koji miso) was investigated in Sprague-Dawley (SD) rats. The BGL at 90 min and area under the curve (AUC) for 120 min after eating were significantly lower in SD rats fed Hoku243 than those in rats fed the control diet, consisting of Koshihikari rice (1% or 5% level). The addition of glucose, glutamic acid, and dietary fibers by soaking in an unsalted rice koji miso (unsalted miso) suspension made the boiled rice tasty and harder. After HPT, the texture of the boiled rice grains became harder, but sticky, which made the rice acceptable in terms of palatability and bio-functional in terms of digestion delay. It is now possible to produce palatable and bio-functional boiled rice grains by HPT and soaking in unsalted miso.

Key words: amylose-extender mutant rice, high-pressure-treatment, unsalted rice koji miso

INTRODUCTION

Diabetes is a lifestyle disease and, hence, its prevention and management are extremely important. Low glycemic index (GI) foods inhibit the rapid increase in blood glucose or insulin secretion after a meal. Several studies have highlighted high-resistant starch (RS)1,2 or high-amylose and high-dietary fiber rice3 that was developed by physical and high-dietary fiber rice that indigestible dextrin (ID)4 has physiological roles such as regulating the functions of the intestines, modulating postprandial BGL, lowering serum lipids, and reducing body fat.5

Amylose-extender (ae) mutant rice cultivar, Hokurikukona243 (Hoku243), has been developed by the National Agricultural and Food Research Organization (NARO). Although Hoku243 lacks starch-branching enzyme (BE) IIb, which leads to a decrease in short-chain glucans (a degree of polymerization (DP) < 17), the proportion of longer chains (DP ≥ 37) was lower than the other ae mutant rice.6 The glycemic effect of foods depends on numerous factors such

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Abbreviations: Ae mutants, amylose extender mutants of rice; AUC, the blood glucose response curve (area under curve); BGL, blood glucose level; GI, glycemic index; HPT, high pressure treatment; Hoku243, Hokurikukona243; RS, resistant starch; SD rats, Sprague-Dawley rats; unsalted miso, unsalted rice koji miso.

as the amylose and amyllopectin structure. The amyllopectin of ae mutants presents more long-chain glucans, thereby making the texture of the rice grains very hard and non-sticky after cooking and rendering it unpalatable as cooked rice.7,8) The gelatinization temperatures of ae mutant rice starches are very high, ae mutant rice contains a substantial amount of RS even after boiling,10,11) making it a material for low-GI foods such as bread and noodles, so as to prevent diabetes. Furthermore, the batter made from ae mutant rice flour absorbed less oil during frying compared with that made from wheat flour and other types of rice flour.12)

Miso is produced by fermenting soybeans with salt and koji. Koji is produced from cultured grains such as rice, barley, or soybeans inoculated with aspergillus oryzae. For many centuries, miso contributed to the health of Japanese people, providing important nutritional components such as proteins, vitamins, minerals, and dietary fibers. Momose et al. demonstrated the controlling effects of various types of miso on postprandial BGL and diabetes. Some types of miso have been shown to reduce GI.13) Miura et al. demonstrated that miso brown pigment (melanoidin) exerts a inhibitory effect on the α-amylase activity in human saliva.14) Soybeans, which have long been utilized as a nutritive food, have also been reported to contain several polyphenolic compounds that exert antioxidative effects.15) Esaki et al. isolated and identified potent antioxidants (8-hydroxysydaidzein, 8-hydroxy-ygenistein, and 6-hydroxydaidzein) in fermented soybeans.16) We reported that rice grains boiled after soaking in a 5%
barley-koji miso suspension maintained high amounts of RS and dietary fibers and were fortified with polyphenols and isoflavones.27)

High pressure treatment (HPT) is very useful in the food industry.18) HPT is the technological process that has the least effect on tested hydrox soluble vitamins, thus contributing to the preservation of the nutritional quality in foodstuffs.19, 20) Yun et al. showed that the HPT process could be a suitable alternative to traditional pretreatment for improving boiled rice flavor.21) The combination of high pressure and protease treatments is effective for the removal of allergenic proteins from rice grains.22) Gamma aminobutyric acid (GABA), a bio-functional component in brown rice, is increased by HPT.23) Yamakura et al. showed that HPT before cooking results in an increase in some free amino acids and stickiness of cooked rice.24)

We attempted to develop a new method to improve the texture of Hoku243 boiled rice grains, which maintaining the high RS and dietary fiber content of boiled rice by the combination of HPT and soaking in unsalted rice koji miso (unsalted miso). Although the ac mutant, Hoku243, is not suitable as boiled rice and as a staple food because of its low palatability, it would be promising material for palatable and bio-functional food such as a low GI food after HPT and soaking in miso.

MATERIALS AND METHODS

Materials. The ac mutant cultivar, Hoku243, and high-quality premium rice Koshihikari were cultivated in an experimental field at Hokuriku Research Center in the Central Agricultural Research Center, NARO, Japan in 2013. All rice samples were stored at 4°C before the experiments.

Preparation of miso samples. Commercial miso samples (unsalted rice koji miso) were produced by Ishiyama Miso Co., Ltd. in Niigata City, Japan.

Measurement of polyphenol content. The polyphenol content of boiled milled rice was determined using the Folin-Ciocalteu method.25) Boiled rice flour samples were prepared by pulverization after lyophilization. The polyphenol content was extracted from the rice flour sample (0.1 g) by shaking with 4 mL of 80% ethanol at room temperature for 30 min, and then centrifuged for 10 min at 3,000 × G. The supernatant (1 mL) was mixed with the same volume of Folin-Ciocalteu solution (1 mL) and incubated at room temperature for 3 min, followed by the addition of 5 mL of sodium carbonate and incubation at 50°C for 5 min. Finally, the sample solution was cooled and allowed to stand for 1 h at 10°C. The absorbance was measured at 765 nm, and gallic acid (0.1 mg/mL) was used for calibration.

Measurement of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. The antioxidative activity of boiled milled rice was determined as described by Suda et al.26) Boiled rice flour samples were prepared by pulverization after lyophilization. The antioxidative activity was measured after extraction from the rice flour samples (0.1 g) by shaking with 2 mL of 60% ethanol at room temperature for 30 min, and then centrifugation for 10 min at 3,000 × G. The supernatant (0.5 mL) and 0.5 mL of buffer A (0.4 mM DPPH: 0.2 M 2-(N-morpholino) ethane sulfonic acid (MES, at pH 6.0); 20% ethanol = 1:1:1 ratio) were mixed at room temperature for 20 min in a dark room, followed by adding another extraction solution (0.5 mL) and 0.5 mL of buffer B (99.5% ethanol: 0.2 M MES (at pH 6.0); 20% ethanol = 1:1:1 ratio) and then mixing at room temperature for 20 min in a dark room. The absorbance was measured at 520 nm and trolox (0.042 mM) was used for calibration. The absorbance corresponding to the DPPH radical scavenging capacity was calculated as the difference between A (the absorbance of buffer A) and B (the absorbance of buffer B).

Preparation of rice samples. Brown rice was polished using an experimental friction-type rice milling machine (Yamamotoseisakusyo Co., Yamagata, Japan) to a milling yield of 90–91%. The rice flour used in chemical analyses and to evaluate the pasting properties was prepared by an SFC-S1 cyclone mill (Udy Corporation, Fort Collins, USA) with a screen with 1-mm diameter pores.

Control sample (Hoku243, sample B) polished rice grains (100 g) were added to 140 g (1.4 times, w/w) of distilled water (DW) at 15°C, and the control sample (Koshihikari, sample A) polished rice grains (100 g) were added to 130 g (1.3 times, w/w) of DW (15°C) before soaking for 1 h at 15°C. The samples were then boiled in a KSHA5 electric rice cooker (Sharp Corporation, Osaka, Japan). The boiled rice samples were kept in the vessel for 2 h at 25°C and then used for the measurements. Similarly, Hoku243 (100 g) were soaked in 0.5% unsalted miso suspension 140 g (1.4 times, w/w) for 0.5 h at 55°C, followed by treatment at 200 MPa for 2 min in an HP machine (Ishikawajima-Harima Heavy Industries Co., Ltd., Tokyo, Japan) and boiling in a rice cooker (sample F). After blending, milled rice grains of Hoku243 and Koshihikari (2:1 ratio, w/w; HK 100 g) were soaked in 0.5, 1.0, and 2.0% unsalted miso suspension (140 g, 1.4 times, w/w) for 0.5 h at 55°C, respectively, and subjected to HPT and boiling in a rice cooker (samples C, D, and E, respectively).

The boiled rice samples were stored in a freezer at −80°C. Subsequently, each sample was lyophilized using a freeze dryer (FD-1, Eyela, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) before pulverization using an SFC-S1 cyclone mill with a screen with 1-mm diameter pores.

Measurement of physical properties of cooked rice grains. The physical properties of boiled rice grains were measured based on bulk measurement (10 g), using a My Boy System Tensipresser (Taketomo Electric Co., Tokyo, Japan) according to the method described by Odahara et al.27) The bulk measurements were repeated five times, and the mean value was calculated.

Measurement of dietary fiber content. The dietary fiber content of the samples was measured according to the AOAC (Association of Official Analytical Chemists) method, using a total dietary fiber assay kit (Megazyme International Ireland, Wicklow, Ireland). Each sample (1 g) was digested by α-amylase, protease, and amyloglucosidase, mixed with 95% ethanol, and then filtered to collect the deposit before determining the protein and ash components. The total dietary fiber content was calculated by subtracting the protein and ash from the total weight of the deposit.

Measurement of glucose content. The boiled rice flour sample was prepared by pulverization after lyophilization. The solution was centrifuged (1,500 × G, 15 min) and the supernatant was used as the sample solution for the glucose
Measurements. The glucose content in the sample solution was measured using the NADPH enzyme assay method with a glucose assay kit (Roche Darmstadt Deutschland GmbH, Mannheim, Germany).

**Measurement of RS.** The RS in the samples was measured according to the slightly modified AOAC method by a resistant starch assay kit (Megazyme). Each sample (100 mg) was digested by pancreatin and amyloglucosidase at 37°C for 6 h (reduced from 12 h to 6 h) and the glucose content was measured using a spectrophotometer at 510 nm. The boiled rice flour samples were prepared by pulverization after lyophilization.

**Measurement of L-glutamic acid.** The L-glutamic acid content was measured using an F-kit (Roche Diagnostics). Each sample (1 g) was extracted by shaking with DW (1 mL) for 30 min at room temperature. The L-glutamic acid content of the sample was measured based on the formation of formazan, according to the manufacturer’s instructions. The absorbance was measured at 510 nm.

**GABA measurement.** Free amino acids were extracted from 0.6 g of boiled rice with 5 mL of 2% sulfosalicylic acid and then centrifuged. The supernatant was filtered through a 0.45 μm filter (GL chromate-disk 25A, GL Sciences Co., Ltd., Tokyo, Japan). The prepared sample solution was analyzed using an amino acid analyzer (Amino Tac JLC-500/v, JEOL, Ltd., Tokyo, Japan).

**Animal feeding test and diets.** Five-week-old Sprague-Dawley (SD) male rats were obtained from Japan SLC, Inc. Rats were housed individually in an air-conditioned room at 23–24°C under a 12-h light cycle. After acclimatization with a commercial rodent diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) for 7d, the rats were divided into 5 groups of 6 rats each. Potato starch (200 mg/mL/DW) was autoclaved (200 mg/mL/DW) (1:1). This mixture was given orally in a single dose through a gastric tube (20 mL/kg). The BGL was measured at 30, 60, 90, and 120 min after feeding using an Accu-Chek Aviva (Roche Diagnostics). The blood glucose response curve and area under the curve (AUC) were also calculated. The animal feeding test was conducted with the formal approval of the Ethics Committee on Animal Care according to the “Guide for the Care and Use of Laboratory Animals” of the Japan Food Research Laboratories.

**Statistical analyses.** All results, including the significance of regression coefficients, were subjected to t-test and one-way ANOVA in Excel Statistics (ver. 2006, Microsoft Corporation, Tokyo, Japan).

## RESULTS

### Effects of the different treatments on Hoku243 and Koshihikari boiled rice.

The component analysis of boiled rice is shown in Table 1. The RS of Hoku243 (4.26%) was much higher than that of the control ordinary rice, Koshihikari (0.30%). The RS of Hoku243 boiled rice increased 1.4 times after soaking in 0.5% unsalted miso for 0.5 h at 55°C (sample F) followed by HPT compared with the control soaked in DW (sample B), while the glucose content (0.8 times), dietary fiber content (2.9 times), L-glutamic acid content (1.4 times), and polyphenol content (1.1 times) also changed.

As shown in Table 1, the RS content of the boiled-rice blend (sample C, D, and E) showed lower values (0.8–0.9 times) than that of Hoku243 as control (sample B), while the glucose content (4.5–7.9 times), DPPH radical scavenging capacity (0.9–2.3 times), dietary fiber content (2.7–4.4 times), L-glutamic acid content (1.2–2.8 times) and polyphenol content (1.0–1.2 times) also increased.

### Physical properties of Hoku243 boiled rice.

Table 2 shows the physical properties of the boiled rice grains by the bulk measurement (10 g) with the Tensipresser. Hoku243 boiled rice grains after HPT and soaking in the miso suspension (sample F) were softer and stickier than

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### Table 1. Component analyses of boiled rice prepared using various soaking conditions.

<table>
<thead>
<tr>
<th></th>
<th>Resistant starch (%)</th>
<th>Glucose content (g/100 g)</th>
<th>DPPH radical scavenging activities Trolox equivalent (mg/100 g)</th>
<th>Dietary fiber (mg/100 g)</th>
<th>Glutamic acid (mg/100 g)</th>
<th>Polyphenol content (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kos by soaking in DW for 1 h at 15°C</td>
<td>0.30</td>
<td>0.02</td>
<td>0.16</td>
<td>0.00</td>
<td>8.35</td>
<td>0.18</td>
</tr>
<tr>
<td>Hoku243 by soaking in DW for 1 h at 15°C</td>
<td>4.26</td>
<td>0.01</td>
<td>0.19</td>
<td>0.00</td>
<td>8.09</td>
<td>0.70</td>
</tr>
<tr>
<td>HK by HPT after soaking in 0.5% unsalted miso for 0.5 h at 55°C</td>
<td>3.52</td>
<td>0.07</td>
<td>0.93</td>
<td>0.02</td>
<td>7.41</td>
<td>0.17</td>
</tr>
<tr>
<td>HK by HPT after soaking in 1.0% unsalted miso for 0.5 h at 55°C</td>
<td>3.85</td>
<td>0.04</td>
<td>1.11</td>
<td>0.02</td>
<td>13.80</td>
<td>0.23</td>
</tr>
<tr>
<td>HK by HPT after soaking in 2.0% unsalted miso for 0.5 h at 55°C</td>
<td>3.60</td>
<td>0.02</td>
<td>1.51</td>
<td>0.04</td>
<td>18.71</td>
<td>0.44</td>
</tr>
<tr>
<td>H243 by HPT after soaking in 0.5% unsalted miso for 0.5 h at 55°C</td>
<td>5.80</td>
<td>0.03</td>
<td>0.15</td>
<td>0.01</td>
<td>8.40</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Correlation is considered significant at 5% by the method of Tukey. Same letter means not significantly different (p > 0.05). Kos, Koshihikari; Hoku243, HokurikuKona243; DW, distilled water; unsalted miso, unsalted rice koji miso; HPT, high pressure treatment; HK, HokurikuKona243 and Koshihikari rice blend (2:1 ratio, w/w).
that of Hoku243 as control (sample B), while their hardness and toughness were lower than those of Hoku243 as control (sample B). Moreover, the ratio of hardness to stickiness of the Hoku243 boiled rice was decreased 0.6 times by HPT after soaking in 0.5% unsalted miso for 0.5 h at 55°C (sample F), compared with the control Hoku243 soaked in DW. The ratio of hardness to adhesion of the Hoku243 boiled rice showed a similar value.

The hardness and toughness of the boiled rice blend (sample C, D, and E) decreased to 0.5–0.7 times compared with Hoku243 as control (sample B), and adhesion and stickiness of the boiled rice blend (sample C, D, and E) increased to 1.2–2.7 times when compared to that of Hoku243 as control (sample B).

Changes in postprandial BGL in rats.

Changes in postprandial BGL in rats are shown in Fig. 1. As shown in Figs. 1(a) and (b), BGL at 30 min and at 60 min showed no significant difference. As shown in Fig. 1(c), BGL at 90 min of animals fed sample F was significantly lower than that of animals fed the control diet Koshihikari, sample A at the 5% level. Thus, as shown in Figs. 1(d), the AUC for 120 min after eating the diets in rats fed sample F was significantly lower than that of animals fed the control diet A and the blended rice E at the 5% level. Thus, the sample F (Hoku243 boiled rice after HPT and soaking in a 0.5% unsalted miso for 0.5 h at 55°C) was the most promising to inhibit the abrupt increase in BGL. In contrast, rice blend samples show no significant inhibition of the abrupt increase of BGL.

Correlation between the chemical components, and physical properties of the different diets, and the BGL.

The correlation between the chemical components and physical properties of the diets and BGL is shown in Table 3. The postprandial BGL at 30 min was significantly and negatively correlated with hardness (p < 0.05) and toughness (p < 0.05). Postprandial BGL at 60 min was positively correlated with postprandial BGL at 90 min (p < 0.05). The postprandial BGL at 90 min was positively correlated with the AUC (p < 0.01) and negatively correlated with RS (p < 0.05). The postprandial BGL at 120 min was positively correlated with toughness (p < 0.05) and negatively correlated with the dietary fiber content (p < 0.05). RS content showed a significant negative correlation with adhesion (p < 0.05) and stickiness (p < 0.05). The polyphenol content was positively correlated with the dietary fiber content (p < 0.05) and DPPH radical scavenging activity (p < 0.05).

Formulas to estimate the BGL and AUC based on physical or chemical parameters of boiled rice.

As shown in Fig. 2, the formulae to estimate the postprandial BGL at 30, 60, and 90 min, and AUC were developed. The BGL at 30, 60, and 90 min or AUC was used as the response variable and the physical or chemical parameters such as RS content, hardness, and glucose content were used.

Table 2. Physical properties of boiled rice prepared using various soaking conditions.

<table>
<thead>
<tr>
<th></th>
<th>Hardness (gw/cm²)</th>
<th>Toughness (gw/cm²)</th>
<th>Adhesion (gw/cm²)</th>
<th>Stickiness (gw/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kos</td>
<td>11.34 ± 1.72</td>
<td>30.66 ± 3.73</td>
<td>40.30 ± 4.11</td>
<td>46.25 ± 3.02</td>
</tr>
<tr>
<td>H243</td>
<td>17.24 ± 1.59</td>
<td>39.99 ± 1.69</td>
<td>12.57 ± 3.50</td>
<td>14.43 ± 1.88</td>
</tr>
<tr>
<td>HK</td>
<td>9.13 ± 3.38</td>
<td>21.44 ± 3.28</td>
<td>34.40 ± 7.02</td>
<td>24.18 ± 5.55</td>
</tr>
<tr>
<td>HK</td>
<td>11.64 ± 5.91</td>
<td>23.63 ± 5.04</td>
<td>23.80 ± 10.72</td>
<td>16.17 ± 3.43</td>
</tr>
<tr>
<td>HK</td>
<td>8.68 ± 2.41</td>
<td>20.45 ± 1.28</td>
<td>22.69 ± 8.60</td>
<td>18.13 ± 3.99</td>
</tr>
<tr>
<td>HK</td>
<td>16.50 ± 1.62</td>
<td>26.24 ± 3.50</td>
<td>12.90 ± 0.71</td>
<td>21.60 ± 1.80</td>
</tr>
</tbody>
</table>

Correlation is considered significant at 5% by the method of Tukey. Same letter means not significantly different (p > 0.05). Kos, Koshihikari; Hoku243, Hokurikukona243; DW, distilled water; unsalted miso, unsalted rice koji miso; HPT, high pressure treatment; HK, Hokurikukona243 and Koshihikari rice blend (2:1 ration, w/w).

Table 3. Correlation between chemical components, and physical properties of the diets, and rat blood glucose levels.

<table>
<thead>
<tr>
<th></th>
<th>BGL 30 min</th>
<th>BGL 60 min</th>
<th>BGL 90 min</th>
<th>BGL 120 min</th>
<th>AUC</th>
<th>Hardness</th>
<th>Toughness</th>
<th>Adhesion</th>
<th>Stickiness</th>
<th>Resistant starch (%)</th>
<th>Glucose content (g/100 g)</th>
<th>Glutamic acid (mg/L)</th>
<th>Polyphenol (mg/100 g)</th>
<th>Dietary fiber (g/100 g)</th>
<th>DPPH radical scavenging activity (%)</th>
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</thead>
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<tr>
<td>BGL30 min</td>
<td>1.00</td>
<td>0.90</td>
<td>0.87</td>
<td>1.00</td>
<td></td>
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<tr>
<td>BGL60 min</td>
<td>-0.29</td>
<td>1.90</td>
<td>0.90</td>
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<tr>
<td>BGL90 min</td>
<td>0.06</td>
<td>0.97</td>
<td>1.00</td>
<td>1.00</td>
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<td>BGL120 min</td>
<td>-0.48</td>
<td>0.53</td>
<td>0.60</td>
<td>1.00</td>
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<tr>
<td>AUC</td>
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<td>0.76</td>
<td>0.96</td>
<td></td>
<td></td>
<td>0.44</td>
<td>1.00</td>
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<tr>
<td>Hardness</td>
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<td>-0.41</td>
<td></td>
<td></td>
<td>0.41</td>
<td>-0.61</td>
<td>1.00</td>
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<tr>
<td>Toughness</td>
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<td>0.41</td>
<td>0.26</td>
<td></td>
<td></td>
<td>0.36</td>
<td>0.04</td>
<td>0.76</td>
<td>1.00</td>
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<tr>
<td>Adhesion</td>
<td>0.54</td>
<td>0.23</td>
<td>0.59</td>
<td>-0.03</td>
<td></td>
<td>0.72</td>
<td>-0.31</td>
<td>-0.26</td>
<td>1.00</td>
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<tr>
<td>Stickiness</td>
<td>0.03</td>
<td>0.24</td>
<td>0.50</td>
<td>0.31</td>
<td></td>
<td>0.25</td>
<td>-0.05</td>
<td>0.07</td>
<td>0.78</td>
<td>1.00</td>
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<td></td>
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<tr>
<td>RS</td>
<td>-0.12</td>
<td>0.67</td>
<td>-0.38</td>
<td>-0.80</td>
<td></td>
<td>0.50</td>
<td>-0.08</td>
<td>-0.89</td>
<td>-0.98</td>
<td>1.00</td>
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<tr>
<td>Glucose</td>
<td>0.68</td>
<td>-0.04</td>
<td>0.07</td>
<td>-0.72</td>
<td></td>
<td>0.16</td>
<td>-0.72</td>
<td>-0.81</td>
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<tr>
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<td>0.37</td>
<td>0.22</td>
<td>-0.51</td>
<td></td>
<td>0.31</td>
<td>-0.63</td>
<td>-0.60</td>
<td>0.19</td>
<td>0.09</td>
<td>-0.21</td>
<td>0.75</td>
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<td>0.39</td>
<td>-0.62</td>
<td>-0.65</td>
<td></td>
<td>0.49</td>
<td>0.01</td>
<td>-0.48</td>
<td>-0.67</td>
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<td>0.66</td>
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<td>Dietary fiber</td>
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<td>-0.43</td>
<td>-0.86</td>
<td></td>
<td>-0.23</td>
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<td>-0.53</td>
<td>0.47</td>
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<td>DPPH radical</td>
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<td>-0.29</td>
<td>-0.37</td>
<td></td>
<td>0.27</td>
<td>0.18</td>
<td>-0.20</td>
<td>-0.64</td>
<td>-0.63</td>
<td>0.52</td>
<td>0.49</td>
<td>0.62</td>
<td>0.83</td>
<td>0.66</td>
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*Correlation is considered significant *at *or **at 5% or 1%, respectively by t-test.*
as predictor variables in multiple regression analyses.

The equation presented a determinant coefficient of 0.997, 1.000, 0.715, or 0.605 for each calibration, respectively. The following formulae used to estimate the BGL were obtained by using 6 types of boiled rice for calibration.

Estimation formulae:

1. \( \text{BGL at 30 min} = \) hardness \(-8.33\) + glutamic acid \(+17.23\) + glucose content \(+203.42\)
2. \( \text{BGL at 60 min} = \) RS \(-5.33\) + toughness \(+2.29\) + DPPH \(-0.45\)
3. \( \text{BGL at 90 min} = \) hardness \(+94.39\)
4. \( \text{AUC} = \) RS \(+7.68\) + glucose content \(+210.52\)

**DISCUSSION**

**Component analysis of Hoku243 boiled rice.**

Hoku243 contains more RS than Koshihikari because its amylopectin has less short-chain glucans and more long-chain glucans similar to the \(ae\) mutant rice, EM 10.\(^7\) Starch is composed of essentially two kinds of \(\alpha\)-glucans that have distinctive structures. Amylose is a small, linear, and slightly branched molecules, whereas amylopectin is a large, highly branched molecule. Particularly, amylose is one of the components of rice starch that greatly affects the quality and gelatinization properties of cooked rice.\(^8,9\) In addition to amylose, chain-length distribution of amylopectin markedly affects the rice physical properties.\(^9\) High-RS rice is digested slowly.\(^9\) Thus, Hoku243, especially Hoku243 boiled rice after HPT and soaking in unsalted miso, would be a promising material for bio-functional food in terms of diabetes prevention.

We believe that the increase in dietary fiber, L-glutamic acid, and polyphenol content of boiled rice soaked in unsalted miso was derived from the added unsalted miso (Table 1). Hoku243 was prepared by soaking in 0.5% unsalted miso suspension for 0.5 h–at 55°C. This boiled rice contained more RS and dietary fibers, and was fortified with glutamic acid and polyphenols (Table 1).

As \(\alpha\)-amylase is localized only in the outer layer of the brown rice grain, it does not markedly affect the molecular structure of starch and physical properties of the cooked white rice grain.\(^10\) As the \(\alpha\)-amylase activity of unsalted miso was very high,\(^17\) it was presumed to have increased the glucose content of the boiled rice (sample C, D, and E), while RS content of sample F was the highest, suggesting the inactivation of \(\alpha\)-amylose and \(\alpha\)-glucosidase leading to a decrease in the glucose content of sample F.

Takeuchi et al. reported that miso contained antioxidants, including saponin, isoflavone, melanoidin, and tocopherol (vitamin E).\(^15\) Esaki reported that miso contains various kinds of bio-functional components, such as melanoidin, angiotensin converting enzyme inhibitory peptides, and isoflavones.\(^31\)

Asaka et al. showed that the activity of polyphenol oxidase in pear fruits is increased by HPT.\(^32\) Dietary polyphenols are the most abundant antioxidants in the human diet.\(^33\) Dietary polyphenol consumption is of interest because it is associated with lower rates of diabetes and cardiovascular diseases.\(^34,35\)
Physical properties of Hoku243 boiled rice.

As shown in Table 2, Hoku243 boiled rice grains soaked in DW without HTP (sample B) showed a harder and less sticky texture compared with Koshihikari rice (sample A).

The group of high-amylose starches includes two types of rice starches with similar apparent amylose contents (AAC), but different super-long chain (SLC) amylopectin contents.\(^{28}\)

The cooked grains of \(^{ae}\) mutant rice cultivars are too hard and non-sticky, because \(^{ae}\) mutant rice cultivars lack the starch branching enzyme – IIb and contain amylopectin that consists of long-chain glucans.\(^{6,8,9}\)

Hoku243 boiled rice grains after HPT and soaking in 0.5% unsalted miso for 0.5 h at 55 °C (sample F) showed a higher stickiness (1.5 times) and lower toughness (0.66 times) than those of Hoku243 used as control (sample B). Additionally, sample F showed a lower ratio of hardness to stickiness (0.64 times) and ratio of toughness to stickiness (0.44 times) than sample B. Thus, sample F was markedly improved in terms of texture compared with sample B.

These changes between sample B and F might be due to the activation of \(\alpha\)-amylase by HPT and soaking in unsalted miso. Another reason for these changes would be the improvement of water absorption of the rice grains through the change in microstructure of starch granules by HTP as previously reported.\(^{24}\)

Rice sample treated with high pressure (sample C, D, E, and F) showed a higher RS content (Table 1), while Koshihikari milled rice without soaking in 0.5% unsalted miso and HPT (sample A) showed a lower RS content.

It is very interesting and meaningful that Hoku234 rice grains presented higher RS and dietary fiber content than sample B (soaked in DW without HPT). Nevertheless, sample F presented a softer and stickier texture than sample B.

The application of HPT to indica rice grains changed the cooking properties as well as enzymatic treatments.

![Fig. 2. Formula to estimate the BGL and AUC based on the chemical components and Physical Properties of Boiled Rice.](image)

(a) Estimation formula

\[
\text{BGL at 30 min} = -8.33 \times \text{Hardness} + 6.42 \times \text{RS} - 17.23 \times \text{Glutamic acid} - 10.38 \times \text{Glucose content} + 203.42
\]

(b) Estimation formula

\[
\text{BGL at 60 min} = -5.33 \times \text{RS} + 1.13 \times \text{Toughness} + 2.29 \times \text{DPPH radical scavenging} - 0.45 \times \text{Hardness} + 94.39
\]

(c) Estimation formula

\[
\text{BGL at 90 min} = -7.41 \times \text{RS} + 129.48
\]

(d) Estimation formula

\[
\text{AUC} = -7.68 \times \text{RS} + 210.52
\]

A, Koshihikari boiled rice soaking in DW for 1 h at 15°C; B, Hoku243 boiled rice after soaking in DW for 1 h at 15°C; C, HK boiled rice after HPT and soaking in 0.5% unsalted miso for 0.5 h at 55°C; D, HK boiled rice after HPT and soaking in 1.0% unsalted miso for 0.5 h at 55°C; E, HK boiled rice after HPT and soaking in 2.0% unsalted miso for 0.5 h at 55°C; F, Hoku243 boiled rice after HPT and soaking in 0.5% unsalted miso for 0.5 h at 55°C. Correlations are considered significant at \(p < 0.05\) according to Tukey's test. The same letter indicates no significant difference \((p > 0.05)\). HK, Hokurikukona243 and Koshihikari rice blend (2:1 ratio, w/w); HPT, high pressure treatment.
Therefore, HPT can be applied to the production of boiled indica rice, which would be acceptable for Japanese customers. Yamakura et al. showed that treatment of boiled rice at 400 MPa for 10 min increased the ratio of stickiness to hardness. One of the characteristics of Ae mutant rice cultivars is that their grains are chalky and not transparent. Therefore, we consider that the decrease in hardness and increase in stickiness were accelerated from the structure of the chalky grain structure.

**Changes in postprandial blood glucose level in rats.**
As shown in Fig. 1 (a), sample C showed the highest BGL and sample B showed the lowest BGL, and only these two samples showed a significant difference. As shown in Table 2, sample B boiled rice grains were the hardest and the least sticky. In contrast, sample C boiled rice grains showed the lowest hardness and toughness. It seems that the hardness of the boiled rice grains markedly affects the BGL during the early stage of digestion.

As shown in Fig. 1(b), postprandial BGL at 60 min showed no significant difference among the rice samples. As shown in Figs. 1(c) and (d), postprandial BGL at 90 min and AUC for 120 min of animals fed sample F were significantly lower than those of animals fed the control diet. Thus, sample F (Hoku243 boiled rice after HPT and soaking in a 0.5% unsalted miso for 0.5 h at 55°C) was the most promising to inhibit the postprandial abrupt increase in BGL. In contrast, rice blend samples showed no significant inhibition of abrupt increase of BGL.

Moritaka et al. showed that the AUC for 120 min after eating boiled rice slowly increased by adding agar and the maximum AUC was decreased. Folium Mori extracts (150 mg/kg) significantly reduced the AUC of GK rat (Goto-Kakizaki rat). RS content of high-amylose corn starch (HAS) doubled after heat-moisture treatment. Short chain fatty acid (SCFA) concentration was higher in HAS groups than ordinary corn starch (OCS) group and the cecal pH was lower in HAS groups than in OCS group.

The physiological effects of dietary carbohydrates are highly dependent on the rate and extent of digestion and absorption in the small intestine as well as fermentation in the large intestine, which promote human health. Hoku243 boiled rice grains influence the first stage of digestion. We speculated that stimulation by mastication was conveyed to the central nervous system and the secretion of insulin significantly decreased. Additionally, in the next stage (90 min), RS contents were markedly affected and, in the third stage (120 min), dietary fibers became the dominant factor to inhibit the increase of blood glucose in rats.

**Correlation between the chemical components, and physical properties of the different diets, and postprandial BGL.**
As shown in Table 3, the hardness and toughness of boiled rice influenced the first stage of digestion. We speculated that stimulation by mastication was conveyed to the central nervous system and the secretion of insulin significantly decreased. Additionally, in the next stage (90 min), RS contents were markedly affected and, in the third stage (120 min), dietary fibers became the dominant factor to inhibit the increase of blood glucose in rats.

**Formulae to estimate the BGL and AUC based on physical or chemical parameters of boiled rice.**
As shown in Fig. 2, the formulae to estimate the postprandial BGL at 30, 60, and 90 min and AUC were developed. As described above, the physical properties of the boiled grains greatly affected the postprandial BGL at 30 min. Next, both RS and toughness of boiled rice grains strongly influenced postprandial BGL at 60 min, and RS was the most important factor affecting the postprandial BGL at 90 min.

In the early stage, the digestion of harder rice grains is delayed. In the late stage of digestion, RS and dietary fiber of the boiled rice grains are the most important factors affecting postprandial BGL.

**CONCLUSIONS**
We developed a new method to treat rice that resulted in a decrease of postprandial BGL. Figure 1 shows that after HPT and soaking in unsalted miso, ingestion of the treated boiled rice resulted in a decrease in postprandial BGL. Ae mutant rice, Hoku243, is a suitable cultivar because its starch contains amylopectin, which consists of long-chain glucans. The addition of glucose, glutamic acid, and dietary fibers by soaking in unsalted miso suspension increased the taste and hardness of boiled rice. The texture of Hoku243 boiled rice grains became harder, but sticky, which made Hoku243 acceptable in terms of palatability and bio-functional in terms of delaying the digestion. It is now possible to produce palatable and bio-functional boiled rice grains by HPT and soaking in unsalted miso.

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