Regular Paper

Improvement of Palatability and Inhibition of Abrupt Increase in Postprandial Blood Glucose Level by the Boiled Rice after Soaking with Functional Food Ingredients

(Received December 15, 2014; Accepted January 27, 2015)
(J-STAGE Advance Published Date: February 5, 2015)

Sumiko Nakamura1 and Ken’ichi Ohtsubo1,†

1Faculty of Agriculture, Niigata University
(8050 Ikarashi-ninocho, Nishi-ku, Niigata 950–2181, Japan)

Abstract: Diabetes is a lifestyle disease, and its prevention and treatment are extremely important. It is reported that inhibition of abrupt postprandial increase of glucose is effective to prevent the initiation of type 2 diabetes. We evaluated the efficacy of boiled rice soaked in MSTR (0.1% unsalted rice koji miso, 0.6% water-soluble extracts from egw seaweed, 5.6% water-soluble extracts from tomato, 5.6% water-soluble extracts from red onion, and 0.1% rice bran oil). Rice cultivars, including Japonica (Koshihikari) and ae mutants, (Hokurikukonaka243) were evaluated to assess the physical properties and chemical composition of the boiled rice grains. We investigated the inhibition of abrupt increases in postprandial blood glucose after consuming boiled rice using Sprague-Dawley rats. Blood glucose levels increase after consuming boiled rice soaked in MSTR were significantly lower than those of Koshihikari soaked in distilled water. The soaked boiled rice contained high levels of dietary fibers, glutamic acid, glucose content and a high 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity. This treatment would improve the bio-functional properties of boiled rice. Furthermore, we developed formulae for estimating the postprandial blood glucose level based on the contents of resistant starch and glucose, and physical property (toughness) of boiled rice grains. It would lead to an easy method for selecting the palatable and bio-functional boiled rice.

Key words: soaking in a MSTR, the blood glucose levels, dietary fiber, polyphenols, bio-functional, palatable boiled rice

INTRODUCTION

Diabetes is a lifestyle disease, and its prevention and treatment are extremely important. Low glycemic index (GI) foods inhibit rapid increases in blood glucose or insulin secretion after meals. The glycemic effect of foods depends on numerous factors such as the structure of amylose and amylopectin.1 The amylopectin found in ae mutants contains more long-chain glucans, thereby making the texture of the rice grains very hard and non-sticky after boiling, which renders it unpalatable as boiled rice.2 Thus, the gelatinization temperatures of ae mutant rice starches are very high. However, they are promising for the production of low-GI foods, such as bread or noodles, to prevent diabetes because they contain a substantial amount of resistant starch even after boiling.3,4 Several studies have reported the development of high ratio of resistance starch rice;5,6 as well as high-amylose and high-dietary fiber rice7 via physical or chemical mutation.

The consumption of a Western diet, which is characterized by a high intake of red meat and high-fat dairy products, would contribute to obesity and metabolic syndrome, as well as increasing the risk of type 2 diabetes and cardiovascular disease. By contrast, the traditional Asian diet, which is rich in soy and fish but low in animal protein and fat, helps to reduce the frequency of several chronic diseases.8 In addition, fiber-rich food choices would have significant health benefits. Various types of brown algae, such as “hijiki” (Sargassum fusiforme), “kombu” (Laminaria japonica), and “wakame” (Undaria pinnatifida), which have been popular foods in the traditional Japanese diet for many years, are high in dietary fiber. The consumption of seaweed has been linked to a lower incidence of chronic diseases, such as cancer, hyperlipidemia, and coronary heart disease, mainly on the basis of epidemiological studies that compared Japanese and Western diets.9 Flammang et al. showed that the incorporation of viscous fiber into a crispy bar helped to improve blood glucose levels by reducing the postprandial glucose, insulin, and C-peptide responses in subjects with type 2 diabetes.9

Vegetables and fruits are rich sources of plant polyphenols
or flavonoids, which are suggested to be the main agents that reduce the risk of cardiovascular disease.\textsuperscript{19} Kobori \textit{et al.} showed that chronic dietary intake of quercetin reduced body weight gain, and visceral and liver fat accumulation, as well as improving hyperglycemia, hyperinsulinemia, and dyslipidemia in mice fed a Western style diet.\textsuperscript{11} Lycopene also prevents oxidative damage, cancer, and aging. These activities are considered to be attributable to its high capacity for scavenging active oxygen species. Mascio \textit{et al.} demonstrated the effects of various types of \textit{miso} in controlling the postprandial blood glucose level, and in preventing diabetes, while some types of \textit{miso} may have the ability to reduce the GI\textsuperscript{13}. Previously, we reported that rice boiled after soaking in a 5% barley-\textit{koji}. \textit{Koji} is formed from cultured grains such as rice, barley, and soy beans after inoculation with a fungus, \textit{Aspergillus oryzae}. For many centuries, \textit{miso} has contributed to the health of Japanese people, because of its important components, such as protein, vitamins, minerals, and dietary fibers. Momose \textit{et al.} showed that lycopene, a biologically occurring carotenoid, has the highest physical quenching rate constant with singlet oxygen, and its plasma level is slightly higher than that of \(\beta\)-carotene.\textsuperscript{12} Furthermore, \textit{miso} is made by fermenting soy beans with salt and “\textit{koji}”. \textit{Koji} is formed from cultured grains such as rice, barley, and soy beans after inoculation with a fungus, \textit{Aspergillus oryzae}. For many centuries, \textit{miso} has contributed to the health of Japanese people, because of its important components, such as protein, vitamins, minerals, and dietary fibers. Momose \textit{et al.} demonstrated the effects of various types of \textit{miso} in controlling the postprandial blood glucose level, and in preventing diabetes, while some types of \textit{miso} may have the ability to reduce the GI\textsuperscript{13}. Previously, we reported that rice boiled after soaking in a 5% barley-\textit{koji} \textit{miso} suspension maintained high levels of dietary fiber and it was fortified with polyphenols and isoflavones.\textsuperscript{14}

In this study, we investigated on the correlation between the postprandial blood glucose levels and the physicochemical properties of boiled rice. We challenged to develop a novel method to improve the palatability of boiled rice, and, at the same time, to inhibit the abrupt increase in the postprandial blood glucose level after eating by soaking the rice in functional food ingredients.

**MATERIALS AND METHODS**

**Materials.** The \textit{ae} mutant rice cultivar, Hokurikukwana243 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 of the rice samples were stored at 4°C before the experiments. Carlose milled rice was purchased in California, USA.

Commercial \textit{miso} samples (unsalted rice \textit{koji miso}) were produced by Ishiyama Miso Co., Ltd. (Nigata, Japan). Commercial samples of “fu-nori” and “ego-nori” (ego) seaweed were produced in Sado Island and kombu and Wakame were produced in Hokkaido. Rice bran oil was produced by Tsuno Food Industrial Co., Ltd. (Wakayama, Japan). The tomato and onion-samples were produced in Niigata City, Japan.

**Measurement of the polyphenol content.** The polyphenol content of boiled milled rice was determined using the Folin-Ciocalteu method.\textsuperscript{15} Boiled rice flour samples were prepared by pulverizing after lyophilization. The polyphenol content was measured by extracting from the rice flour sample (0.1 g) by shaking with 4 mL of 80% ethanol at room temperature for 30 min, and then centrifuging for 10 min at 3,000 \(\times\) 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 adding 5 mL of sodium carbonate and incubating 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 \textit{et al.}\textsuperscript{10} Boiled rice flour samples were prepared by pulverizing after lyophilization. The antioxidative activity was measured after extracting from the rice flour samples (0.1 g) by shaking with 2 mL of 60% ethanol at room temperature for 30 min, and then centrifuging for 10 min at 3,000 \(\times\) 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 corresponded 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 the rice samples.** Brown rice was polished using an experimental friction-type rice milling machine (Yamamotoseisakusyoo 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 Co., Fort Collins, USA) with a screen with 1-mm diameter pores. 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 pulverizing by an SFC-S1 cyclone mill with a screen with 1-mm diameter pores.

**Measurement of the physical properties of the boiled rice grains.** Control sample (Koshikihari) rice grains (100 g) were added to 130 g (1.3 times, w/w) of distilled water at 15°C, and other control sample of (Hokurikukwana243) rice grains (100 g) were added to 180 g (1.8 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 Co., Ohsaka, Japan). The boiled rice samples were kept in the vessel for 2 h at 25°C and then used for the measurements.

Samples of fu-nori, ego-nori, kombu, and Wakame (5 g for each sea weed) were added to 450 mL of DW (15°C), and soaked for 1 h at 15°C. The samples were then heated at 80°C for 1 h in a steamer (T-fal VC100571, France) and strained through a gauze cloth. The liquids obtained were diluted 2.0 times with DW (i.e., 0.6% fu-nori, 0.6% ego-nori, 0.6% kombu, and 0.6% Wakame). Koshikihari (100 g) was then added to 130 g (1.3 times, w/w) of each of the 0.6% seaweed extracts and soaked for 1 h at 15°C. The samples were then boiled in the same manner as the control samples.

The tomato and red onion samples were mixed with an equal-weight of DW before adding 0.1% rice bran oil (w/w). This mixture was then heated for 1 h at 80°C for 1 h in a steamer and strained through a gauze cloth (this treated sample was designated as TOR). This mixture liquid was diluted 9.0 times with DW (TOR-a). Koshikihari was then...
soaked in TOR-a for 1 h at 15°C and then boiled in the same manner as the control sample, while Hokurikukona243 was also treated in the same manner.

The TOR-a liquid and 0.5% unsalted rice koji miso were also mixed. Koshihikari was soaked in this liquid mixture for 1 h at 15°C and then boiled in the same manner as the control sample (0.5% miso-TOR-a at 15°C-60), while Hokurikukona243 was also treated in the same manner.

Furthermore, 0.6% ego-nori and 0.1% unsalted rice koji miso were mixed, and kept for 1 h at 15°C. Koshihikari was soaked in this mixture liquid for 1 h at 15°C and then boiled in the same manner as the control sample (0.1% miso-ego-nori at 15°C-60), while Hokurikukona243 was treated in the same manner.

In addition, 0.6% ego-nori and the TOR liquid were mixed (8:1 ratio, w/w) and 0.1% unsalted rice koji miso was added, before keeping for 1 h at 15°C. Koshihikari was soaked in this liquid mixture for 1 h at 15°C and then boiled in the same manner as the control sample containing MSTR (0.1% unsalted rice koji miso. 0.6% water-soluble extracts from ego-nori, 5.6% water-soluble extracts from tomato, 5.6% water-soluble extracts from red onion, and 0.1% rice bran oil) for 1 h at 15°C, while, Hokurikukona243 was treated in the same manner.

A blend of Hokurikukona243 and Koshihikari (2:1 ratio, w/w; HK 100 g) was heated at 100°C for 15 min in a steamer. The sample was then mixed with 180 g (1.8 times, w/w) of MSTR liquid and soaked for 1 h at 15°C. The sample was then boiled in the same manner as the control sample (MSTR at 15°C-60 after HT).

The physical properties of the boiled rice grains were measured based on single-grain measurements and bulk measurements (10 g), which included a low compression test (compression ratio = 90%) using a My Boy System Tensipresser (Taketomo Electric Co., Tokyo, Japan) and a high compression test (compression ratio = 25%) using a high compression test (compression ratio = 25%) using a high compression test (compression ratio = 25%) using a high compression test (compression ratio = 25%). The boiled rice flour sample was prepared by pulvcrizing after lyophilization.

**Measurement of dietary fiber.** The dietary fiber content of the samples was measured according to the AOAC method, using a total dietary fiber assay kit (Megazyme International Ireland Limited, 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 the glucose content.** The rice flour sample (0.1 g) was mixed with 1 mL of 60% ethanol and glucose was extracted from the mixture by rotation for 1 h at 20°C. The boiled rice flour sample was prepared by pulverizing 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, Germany).

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

**Measurement of L-glutamic acid.** The L-glutamic acid content was measured using an F-kit (Roche Diagnostics, GmbH, Mannheim, Germany). Each sample (1 g) was subjected to the extraction 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 generation of formazan, according to the manufacturer's instructions. The absorbance was measured at 510 nm.

**Sensory evaluation.** Eating quality of cooked rice was evaluated according to the method developed by the Food Agency with a slight modification. It was evaluated by eight expert panelists according to above-mentioned "five-point-method" marking 5 for very good, 4 for good, 3 for mean, 2 for bad and 1 for very bad, in which attributes are appearance, aroma, taste, stickiness, hardness, and overall evaluation.

**Animal feeding test and diets.** Five-week-old Sprague-Dawley (SD) male rats were obtained from Japan SLC, Inc. The 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) for 7d, the rats were divided into 5 groups of 6 rats each. Potato starch (200 mg/mL/DW) was autoclaved for 5 min at 95°C, and then mixed with sample rice flour (200 mg/mL/DW) (1:1). This mixture was given orally in a single dose through a gastric tube (20 mL/kg). The blood glucose levels were measured at 30, 60, 90, and 120 min after the meal using an Accu-Chek Aviva (Roche Diagnostics K.K., Japan). 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 of the 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**

**Physicochemical properties of Koshihikari rice boiled after soaking with various seaweeds extracts.** As shown in Table 1, the resistant starch content of Koshihikari boiled rice was increased 5.7–7.7 times by soaking in 0.6% seaweed extract for 1 h at 15°C, compared with the control soaked in DW, while the glucose content (5.7–6.1 times), dietary fiber content (3.4–4.8 times) increased. In terms of physical properties of the boiled rice, hardness (1.5–1.6 times) and toughness (1.2–1.3 times) also increased, whereas the adhesion (0.40–0.83 times) and stickiness (0.62–0.99 times) decreased. In particular, the dietary fiber content of Koshihikari boiled rice soaked in 0.6% ego-nori increased, but the stickiness and adhesion
rarely decreased.

As shown in Table 2 (A), the overall evaluation score in the sensory test for Koshihikari boiled rice soaked in 0.6% ego-nori was significantly better than that for Koshihikari soaked in DW, as well as those soaked in the other 0.6% seaweeds extract \((p < 0.05)\). Thus, palatable boiled rice fortified with resistant starch and dietary fiber can be produced by soaking in 0.6% ego-nori extract.

**Table 1.** Components and physical properties of boiled rice by various seaweeds soaking.

|                        | Resistant starch (%) | Glucose content (g/100 g) | Dietary fiber (g/100 g) | Hardness (g/cm²) | Toughness (g/cm²) | Adhesion (g/cm²) | Stickiness (g/cm²) |
|------------------------|----------------------|---------------------------|-------------------------|------------------|------------------|-----------------|------------------|
| Boiled rice of Kos by soaking in a DW | 0.30\(^a\) | 0.02 | 0.16\(^a\) | 0.00 | 0.44\(^a\) | 0.03 | 8.58\(^a\) | 2.10 | 25.57\(^a\) | 1.70 | 23.90\(^a\) | 9.50 | 21.30\(^a\) | 3.20 |
| Boiled rice of Kos by soaking in 0.6% Fu-nori | 1.79\(^a\) | 0.01 | 0.97\(^a\) | 0.01 | 1.48\(^a\) | 0.08 | 12.42\(^a\) | 2.61 | 29.51\(^b\) | 2.92 | 13.31\(^b\) | 4.16 | 13.29\(^b\) | 7.86 |
| Boiled rice of Kos by soaking in 0.6% Ego-nori | 1.71\(^b\) | 0.06 | 0.95\(^b\) | 0.02 | 2.10\(^b\) | 0.10 | 13.09\(^b\) | 2.29 | 32.12\(^c\) | 2.37 | 19.93\(^b\) | 3.54 | 20.07\(^b\) | 1.84 |
| Boiled rice of Kos by soaking in 0.6% Kombu | 2.32\(^a\) | 0.11 | 0.93\(^b\) | 0.00 | 1.57\(^b\) | 0.00 | 12.88\(^b\) | 1.73 | 34.21\(^a\) | 1.62 | 9.49\(^b\) | 2.25 | 15.42\(^b\) | 2.80 |
| Boiled rice of Kos by soaking in 0.6% Wakame | 1.92\(^b\) | 0.04 | 0.91\(^b\) | 0.04 | 1.92\(^c\) | 0.00 | 13.33\(^b\) | 1.06 | 32.67\(^a\) | 1.72 | 18.04\(^a\) | 4.67 | 21.26\(^a\) | 1.83 |

Correlation is significant at 5% by the method of Tukey. Same letter means not significantly different \((p < 0.05)\). The physical properties of boiled rice grains were measured by bulk measurement with a Tensipresser. Kos, Koshihikari; DW, distilled water; 0.6% Funori, 0.6% water-soluble extracts from fu-nori; 0.6% Ego-nori, 0.6% water-soluble extract from ego-nori; 0.6% Kombu, 0.6% water-soluble extracts from kombu; 0.6% Wakame, 0.6% water-soluble extracts from wakame.

|                        | Appearance | SD | Taste | SD | Aroma | SD | Hardness | SD | Stickiness | SD | Overall evaluation | SD |
|------------------------|------------|----|-------|----|--------|----|-----------|----|-------------|----|-------------------|----|
| Boiled rice of Koshihikari by soaking in a DW | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 |
| Boiled rice of Koshihikari by soaking in 0.6% Funori | 3.8\(^b\) | 0.1 | 2.4\(^b\) | 0.1 | 2.1\(^b\) | 0.3 | 2.3\(^b\) | 0.2 | 2.7\(^b\) | 0.1 | 2.4\(^b\) | 0.1 |
| Boiled rice of Koshihikari by soaking in 0.6% Ego | 4.2\(^b\) | 0.2 | 3.1\(^b\) | 0.1 | 3.0\(^b\) | 0.1 | 3.2\(^b\) | 0.1 | 3.3\(^b\) | 0.2 | 4.2\(^b\) | 0.1 |
| Boiled rice of Koshihikari by soaking in 0.6% Kombu | 4.1\(^b\) | 0.1 | 4.3\(^b\) | 0.2 | 1.8\(^b\) | 0.3 | 2.9\(^b\) | 0.1 | 2.8\(^b\) | 0.1 | 2.3\(^b\) | 0.2 |
| Boiled rice of Koshihikari by soaking in 0.6% Wakame | 4.1\(^b\) | 0.1 | 2.8\(^b\) | 0.1 | 2.2\(^b\) | 0.1 | 3.1\(^b\) | 0.2 | 3.1\(^b\) | 0.2 | 2.4\(^b\) | 0.2 |

Correlation is significant at 5% by the method of Tukey. Same letter means not significantly different \((p < 0.05)\). 1, inferior; 2, rather inferior; 3, ordinary; 4, rather good; 5, good. DW, distilled water; 0.6% Funori, 0.6% water-soluble components extracted from fu-nori; 0.6% Ego-nori, 0.6% water-soluble extract from ego-nori; 0.6% Kombu, 0.6% water-soluble extracts from kombu; 0.6% Wakame, 0.6% water-soluble extracts from wakame.

|                        | Appearance | SD | Taste | SD | Aroma | SD | Hardness | SD | Stickiness | SD | Overall Evaluation | SD |
|------------------------|------------|----|-------|----|--------|----|-----------|----|-------------|----|-------------------|----|
| Boiled rice of Koshihikari by soaking in a DW at 15°C-60 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 | 3.0\(^a\) | 0.0 |
| Boiled rice of Koshihikari by soaking in MSTR at 15°C-60 | 3.8\(^b\) | 0.1 | 3.2\(^b\) | 0.1 | 2.8\(^b\) | 0.3 | 3.6\(^b\) | 0.2 | 3.2\(^b\) | 0.1 | 3.8\(^b\) | 0.1 |

**Physical properties and chemical composition of boiled rice treated with various soaking conditions.**

As shown in Table 3, the physical properties of the boiled rice grains were measured by the low-compression (25%) and high-compression (90%) methods\(^b\) with a My Boy System Tensipresser. The stickiness of the surface layer (-H1) and overall layer (-H2) of the boiled rice of Koshihikari soaked in 0.1% miso-ego-nori for 1 h at 15°C were higher than that of boiled rice of Koshihikari soaked in 0.6% ego-nori for 1 h at 15°C. The addition of 0.1% miso to
### Table 3. Physical properties of boiled rice by various soaking conditions.

|                  | Surface layer | Overall | Surface layer | Overall | Surface layer |
|------------------|---------------|---------|---------------|---------|---------------|
|                  | Hardness (H1) | SD      | Hardness (H2) | SD      | Stickiness (H1) | SD      |
| Kos by soaking in a DW at 15°C-60 | 69.39a | 23.23 | 18480.00 | 163.90 | -15.87 | 9.15 |
| Kos by soaking in 0.6% Ego-nori at 15°C-60 | 53.13a | 15.66 | 18630.00 | 221.00 | -14.98 | 5.85 |
| Kos by soaking in 0.1% Miso-Ego at 15°C-60 | 75.16a | 15.81 | 19850.00 | 188.90 | -22.65 | 6.21 |
| Kos by soaking in a TOR at 15°C-60 | 67.93a | 29.30 | 17860.00 | 164.30 | -8.07 | 1.85 |
| Kos by soaking in 0.5% Miso-TOR at 15°C-60 | 69.90a | 22.94 | 19120.00 | 321.60 | -10.50 | 4.00 |
| H243 by soaking in a DW at 15°C-60 | 60.97a | 17.37 | 17720.00 | 254.50 | -5.73 | 1.83 |
| H243 by soaking in 0.6% Ego-nori at 15°C-60 | 63.09a | 22.83 | 16760.00 | 181.90 | -6.38 | 2.21 |
| H243 by soaking in 0.1% Miso-Ego at 15°C-60 | 64.49a | 26.51 | 17400.00 | 184.40 | -6.12 | 1.68 |
| H243 by soaking in a TOR at 15°C-60 | 59.69a | 21.21 | 1712.55 | 132.30 | -2.91 | 1.52 |
| H243 by soaking in 0.5% Miso-TOR at 15°C-60 | 61.42a | 22.50 | 1833.75 | 136.20 | -3.79 | 1.58 |

|                  | Balance degree -H1/H2 | SD | Balance degree -H2/H2 | SD | Balance degree A3/A1 | SD | Overall Balance degree A6/A4 | SD |
|------------------|------------------------|----|------------------------|----|----------------------|----|-----------------------------|----|
| Kos by soaking in a DW at 15°C-60 | 0.22a | 0.08 | 0.21a | 0.03 | 0.47a | 0.20 | 0.14a | 0.05 |
| Kos by soaking in 0.6% Ego at 15°C-60 | 0.29a | 0.10 | 0.17a | 0.04 | 0.58a | 0.27 | 0.12a | 0.03 |
| Kos by soaking in 0.1% Miso-Ego at 15°C-60 | 0.31a | 0.08 | 0.19a | 0.02 | 0.55a | 0.18 | 0.15a | 0.04 |
| Kos by soaking in a TOR at 15°C-60 | 0.05a | 0.66 | 0.21a | 0.04 | 0.25a | 0.16 | 0.07a | 0.01 |
| Kos by soaking in 0.5% Miso-TOR at 15°C-60 | 0.17a | 0.08 | 0.20a | 0.06 | 0.31a | 0.22 | 0.10a | 0.06 |
| H243 by soaking in a DW at 15°C-60 | 0.10a | 0.03 | 0.17a | 0.04 | 0.18a | 0.08 | 0.06a | 0.02 |
| H243 by soaking in 0.6% Ego-nori at 15°C-60 | 0.10a | 0.02 | 0.16a | 0.05 | 0.16a | 0.06 | 0.06a | 0.02 |
| H243 by soaking in 0.1% Miso-Ego at 15°C-60 | 0.10a | 0.03 | 0.18a | 0.06 | 0.17a | 0.07 | 0.06a | 0.02 |
| H243 by soaking in a TOR at 15°C-60 | 0.02a | 0.01 | 0.17a | 0.03 | 0.09a | 0.05 | 0.03a | 0.01 |
| H243 by soaking in 0.5% Miso-TOR at 15°C-60 | 0.07a | 0.02 | 0.17a | 0.03 | 0.12a | 0.06 | 0.04a | 0.02 |

### Table 4. Physical properties and chemical composition of boiled rice treated with various soaking conditions.

|                  | Resistant starch | SD | Glucose content | SD | Glutamic acid | SD | Polyphenol/gallic acid | SD | Dietary fiber | SD | BPPM radial scarring Trinos (mg/100 g) | SD |
|------------------|------------------|----|-----------------|----|---------------|----|----------------------|----|---------------|----|-----------------------------|----|
| Kos by soaking in a DW at 15°C-60 | 0.30a | 0.02 | 0.16a | 0.00 | 0.25a | 0.03 | 1.96a | 0.03 | 3.53a | 0.03 | 35.6a | 0.18 |
| H243 by soaking in a DW at 15°C-60 | 4.20a | 0.05 | 0.19a | 0.00 | 0.68a | 0.00 | 6.4a | 0.03 | 8.0a | 0.04 | 8.0a | 0.70 |
| Kos by soaking in a MSTR at 15°C-60 | 1.17a | 0.04 | 0.27a | 0.00 | 0.80a | 0.02 | 58.7a | 0.51 | 0.73a | 0.03 | 12.6a | 1.96 |
| H243 by soaking in a MSTR at 15°C-60 | 3.60a | 0.02 | 0.46a | 0.00 | 1.06a | 0.02 | 66.68a | 1.53 | 2.40a | 0.06 | 10.64 | 1.47 |
| HK by soaking in a MSTR at 15°C-60 after HT | 1.96a | 0.01 | 0.17a | 0.00 | 0.49a | 0.00 | 70.29a | 1.53 | 1.90a | 0.05 | 19.26a | 1.11 |

Correlation is significant at 5% by the method of Tukey. Same letter means not significantly different (p < 0.05). The physical properties of boiled rice grains were measured by a low compression test (25%) and a high compression test (90%) with Tempresser. Kos, Koshihikari; DW, distilled water; 0.6% Ego, 0.6% water-soluble extracts from ego-nori; 0.1% Miso-Ego, 0.1% unsalted rice koji miso, 0.6% ego-nori; 0.5% Miso-TOR-A, 0.5% unsalted rice koji miso; TOR-a, TOR mixture liquid was diluted with DW 90 time; TOR, 5.6% water-soluble extracts from tomato, 5.6% water-soluble extracts from red onion, and 0.1% rice bran oil.
soaking solutions increased stickiness of cooked rice grains soaked with ego-nori. The stickiness of the surface layer (-H1) and the hardness of the surface layer (H1) of the boiled rice soaked in TOR-a for 1 h at 15°C were lower than that soaked in DW for 1 h at 15°C. The hardness of the surface layer (H1) and the overall layer (H2) of the boiled rice soaked in 0.5% miso-TOR-a for 1 h at 15°C were higher than that of boiled rice soaked in TOR-a for 1 h at 15°C, while the stickiness of the surface layer (-H1) increased.\textsuperscript{14,20}

As shown in Table 4, the resistant starch contents of boiled rice of Koshihikari and Hokurikukona243 rice soaked in MSTR increased 3.9 and 0.83 times respectively, after soaking for 1 h at 15°C compared with the control soaked in DW for 1 h at 15°C, while there were also increases in: glucose content of 1.7 and 2.4 times, respectively; glutamic acid of 3.1 and 1.8 times, polyphenols content of 1.1 and 1.0 times; dietary fiber of 1.6 and 3.3 times; DPPH radical scavenging capacity of 1.5 and 1.3 times; hardness of 2.2 and 0.7 times, toughness of 1.3 and 0.6 times; adhesion of 0.9 and 0.7 times; and stickiness of 1.0 and 0.7 times.

As shown in Table 4, boiled rice of Koshihikari and Hokurikukona243 soaked in MSTR showed significant increases in resistant starch of 3.9 times (Koshihikari), in glucose of 1.7 and 2.4 times, in glutamic acid of 3.1 and 1.8 times, and in dietary fiber of 1.6 and 3.3 times, compared with those soaked in DW, respectively.

The hardness and toughness of boiled rice of Koshihikari soaked in MSTR were significantly increased 2.2 times and 1.3 times than that soaked in DW, on the contrary, Hokurikukona243 showed significantly decreased 0.7 times and 0.6 times than that soaked in DW.

Moreover, for the blend of rice (HK, 2:1 ratio) with HT, the polyphenol content and DPPH radical scavenging capacity were at the highest levels, while there were also decreases in the dietary fiber of 0.79 times, resistant starch of 0.54 times, while the adhesion of 1.35 times, and stickiness of 1.56 times, compared with Hokurikukona 243 soaked in MSTR.

As shown in Table 2 (B), the overall evaluation score in the sensory test was significantly better for boiled Koshihikari rice soaked in MSTR compared with Koshihikari soaked in DW (p < 0.05). Thus, palatable boiled rice fortified with resistant starch and dietary fiber could be produced by soaking in MSTR.

Changes in postprandial blood glucose level in rat.

Changes in postprandial blood glucose levels in rats are shown in Fig. 1. A, Boiled rice of Koshihikari produced by soaking in DW for 1 h at 15°C; B, Boiled rice of Hokurikukona243 produced by soaking in DW for 1 h at 15°C; C, Boiled rice of Koshihikari produced by soaking in MSTR for 1 h at 15°C; D, Boiled rice of Hokurikukona243 produced by soaking in MSTR for 1 h at 15°C; E, Boiled rice of HK rice produced by soaking in MSTR for 1 h at 15°C after HT. As shown in Fig. 1 (A), the blood glucose level at 30 min after eating the control diet of Koshihikari was significantly higher (all, p < 0.05) than those of B, C, D, and E. And as shown in Fig1(B), the changes in the postprandial blood glucose levels in rats at 60 min after eating control diet of Koshihikari was significantly higher (p < 0.05) than those of B and D. Moreover, as shown in Fig 1(C), the blood glucose response curve (AUC) for 120 min after eating control diet of Koshihikari was significantly higher (p < 0.05) than that of B.

Correlation among the chemical components, physical properties, and blood glucose levels with different diets.

Table 5 shows that there were significant correlations among the chemical components, physical properties, and blood glucose levels with different diets. The blood glucose level at 30 min after eating showed a positive correlation with adhesion (p < 0.05) and stickiness (p < 0.05) of boiled rice grains, whereas it was negatively correlated with the polyphenols content (p < 0.05). The blood glucose level at 60 min after eating showed a positive correlation with

![Fig. 1. Changes in the postprandial blood glucose level in rat.](image-url)
### Table 5. Correlation between chemical components, physical properties and blood glucose levels with different diets.

|                          | Resistant starch (%) | Glucose content (g/100 g) | Polyphenol gallic acid (mg/100 g) | Dietary fiber (g/100 g) | DPPH scavenging (mg/g) | Adhesion (gw/cm²) | Stickiness (gw/cm²) |
|--------------------------|----------------------|---------------------------|-----------------------------------|-------------------------|------------------------|-------------------|-------------------|
| RK                        | 0.33                 | 0.50                      | 0.65                              | 0.47                    | 0.37                   | 0.80              | 0.88              |
| HK                        | 0.30                 | 0.50                      | 0.68                              | 0.20                    | 0.30                   | 0.80              | 0.88              |
| Carlose                   | 0.47                 | 0.50                      | 0.47                              | 0.20                    | 0.30                   | 0.80              | 0.88              |
| Koshihikari              | 0.33                 | 0.50                      | 0.65                              | 0.47                    | 0.37                   | 0.80              | 0.88              |
| Boiled rice of HK soaked in DW for 1 h at 15°C after HT | 0.30 | 0.50 | 0.68 | 0.20 | 0.30 | 0.80 | 0.88 |
| Boiled rice of HK soaked in MSTR for 1 h at 15°C after HT | 0.30 | 0.50 | 0.68 | 0.20 | 0.30 | 0.80 | 0.88 |
| Boiled rice of Carlose soaked in DW for 1 h at 15°C after HT | 0.30 | 0.50 | 0.68 | 0.20 | 0.30 | 0.80 | 0.88 |
| Boiled rice of Carlose soaked in MSTR for 1 h at 15°C after HT | 0.30 | 0.50 | 0.68 | 0.20 | 0.30 | 0.80 | 0.88 |

Adhesion (\(p < 0.05\)) of boiled rice gains and the blood glucose level at 30 min (\(p < 0.05\)), but correlated negatively with the polyphenol contents (\(p < 0.05\)). The blood glucose response curve (AUC) showed a negative correlation with the resistant starch content (\(p < 0.05\)). The resistant starch content also correlated negatively with adhesion (\(p < 0.05\)) and stickiness (\(p < 0.05\)). The polyphenol content showed a negative correlation with adhesion (\(p < 0.05\)).

### Formula for estimating the blood glucose level (AUC) based on the resistant starch, hardness and glucose content of boiled rice.

As shown in Fig. 2, the formula for estimating the blood glucose level (AUC) was developed as follows. The blood glucose level (AUC) was used as the response variable and the resistant starch content, hardness, and glucose content were used as predictor variables in multiple regression analyses. The equation had a multiple regression coefficient of 0.85 for calibration. The following formula for estimating the blood glucose level (AUC) was obtained by using eight types of boiled rice for calibration.

\[
\text{Estimation formula: blood glucose level (AUC)} = -7.647 \times \text{resistant starch} - 0.980 \times \text{hardness} + 38.285 \times \text{glucose content} + 198.757.
\]

The Table 6 (A) shows resistant starch content, hardness, glucose content and blood glucose level (AUC) of samples for estimating formula. And Table 6 (B) shows that the application of the above-mentioned formula to thirteen types of different boiled rice samples with those used for calibration. (1, Boiled rice of Carlose soaked in DW; 2, Boiled rice of HK soaked in DW; 3, Boiled rice of HK soaked in MSTR; 4, Boiled rice of Koshihikari soaked in DW for 1 h at 15°C after HT; 5, Boiled rice of Carlose rice soaked in MSTR for 1 h at 15°C; 6, Boiled rice of Hokusukikona243 soaked in MSTR for 1 h at 15°C after HT; 7, Boiled rice of HK soaked in MSTR for 1 h at 15°C after HT; 8, Boiled rice of HK soaked in 0.6% water-soluble extracted from eco seaweed. The equation had a multiple regression coefficient of 0.85 based on the calibration. Estimation formula: Blood glucose level (AUC) = -7.647 × Resistant starch - 0.980 × Hardness + 38.285 × Glucose content + 198.757.
Table 6. Chemical components, physical properties and blood glucose levels (AUC) of samples for estimation formula and examination estimate formula.

(A) Estimation formula

| Sample Description | Resistant starch | SD | Glucose content | SD | Hardness | SD | AUC (mg/dL) | SD |
|--------------------|------------------|----|-----------------|----|----------|----|-------------|----|
| 1. Boiled rice of Koshihikari soaking in DW | 0.30a | 0.02 | 0.16a | 0.00 | 11.34a | 1.92 | 186.82a | 42.53 |
| 2. Boiled rice of Koshihikari soaking in MSTR | 0.21a | 0.04 | 0.27b | 0.00 | 24.03b | 14.35 | 182.08a | 22.98 |
| 3. Boiled rice of Hokurikukona243 soaking in DW | 4.26b | 0.01 | 0.19a | 0.00 | 17.24b | 1.59 | 156.88a | 22.98 |
| 4. Boiled rice of Hokurikukona243 soaking in MSTR | 3.60b | 0.02 | 0.46c | 0.00 | 11.67b | 2.30 | 172.04b | 18.63 |
| 5. Boiled rice of Carlsoe soaking in MSTR | 0.55a | 0.04 | 0.30b | 0.02 | 22.04b | 3.47 | 189.90b | 25.78 |
| 6. Boiled rice of Hokurikukona243 by soaking in MSTR after HT | 2.72b | 0.02 | 0.20b | 0.01 | 6.41c | 3.32 | 187.70b | 14.20 |
| 7. Boiled rice of HK soaking in 0.5% unsalted rice | 1.96b | 0.01 | 0.17a | 0.00 | 11.37a | 1.63 | 176.42b | 10.71 |
| 8. Boiled rice of HK soaking in 0.6% water-soluble extract | 2.24b | 0.01 | 0.34b | 0.00 | 9.24b | 2.59 | 187.70b | 14.20 |

(B) Examination estimate formula with unknown samples

| Sample Description | Resistant starch | SD | Glucose content | SD | Hardness | SD | Estimate AUC |
|--------------------|------------------|----|-----------------|----|----------|----|--------------|
| 1. Boiled rice of Carlose soaking in DW | 0.59a | 0.01 | 0.20a | 0.00 | 15.40a | 2.83 | 186.78a |
| 2. Boiled rice of HK soaking in DW | 3.42b | 0.01 | 0.21a | 0.01 | 9.67b | 3.30 | 171.11b |
| 3. Boiled rice of HK soaking in MSTR | 1.55a | 0.05 | 0.24a | 0.00 | 10.39a | 1.54 | 185.71a |
| 4. Boiled rice of HK soaking in TOR-a | 2.96b | 0.01 | 1.02b | 0.00 | 9.50a | 1.17 | 205.95b |
| 5. Boiled rice of HK soaking in 0.5% unsalted rice koji miso, TOR-a and 1.2% wakame seaweed | 1.92c | 0.04 | 0.91a | 0.04 | 13.33a | 1.06 | 205.93a |
| 6. Boiled rice of HK soaking in 0.5% unsalted rice koji miso, TOR-a and 1.2% kombu seaweed | 2.32a | 0.11 | 0.93a | 0.00 | 12.88a | 1.73 | 203.79a |
| 7. Boiled rice of HK soaking in 0.5% unsalted rice koji miso, TOR-a and 1.2% fu-nori seaweed | 1.79a | 0.01 | 0.97a | 0.01 | 12.42a | 2.61 | 210.15a |
| 8. Boiled rice of HK soaking in 0.5% unsalted rice koji miso, TOR-a and 1.2% ego seaweed | 1.71a | 0.06 | 0.95a | 0.02 | 13.09a | 2.29 | 209.04a |
| 9. Boiled rice of HK soaking in 2.0% unsalted rice koji miso (adding water 1.5 times of weight) | 4.58d | 0.16 | 0.96c | 0.03 | 15.63a | 3.17 | 185.25a |
| 10. Boiled rice of HK soaking in 2.0% unsalted rice koji miso and Tor-a (adding water 1.5 times of weight) | 4.21d | 0.04 | 1.31a | 0.03 | 16.63a | 2.25 | 200.34a |
| 11. Boiled rice of HK soaking in 0.5% unsalted rice koji miso (adding water 1.5 times of weight) | 4.18d | 0.03 | 0.68c | 0.02 | 12.46a | 2.38 | 180.76a |
| 12. Boiled rice of HK soaking in 1.0% unsalted rice koji miso (adding water 1.5 times of weight) | 4.52d | 0.07 | 0.84a | 0.02 | 11.95a | 2.80 | 184.53a |
| 13. Boiled rice of HK soaking in 1.5% unsalted rice koji miso (adding water 1.5 times of weight) | 4.36d | 0.02 | 1.03b | 0.03 | 14.28a | 1.77 | 190.77a |

4. Boiled rice of HK soaked in TOR-a; 5. Boiled rice of HK soaking in 0.5% unsalted rice koji miso, TOR-a and 1.2% wakame extract; 6. Boiled rice of HK soaked in 0.5% unsalted rice koji miso, TOR-a and 1.2% kombu extract; 7. Boiled rice of HK soaked in 0.5% unsalted rice koji miso, TOR-a and 1.2% fu-nori extract; 8. Boiled rice of HK soaked in 0.5% unsalted rice koji miso, TOR-a and 1.2% ego extract; 9. Boiled rice of HK soaked in 2.0% unsalted rice koji miso (adding water 1.5 times of rice weight); 10. Boiled rice of HK soaked in 2.0% unsalted rice koji miso and Tor-a (adding water 1.5 times of rice weight); 11. Boiled rice of HK soaked in 0.5% unsalted rice koji miso (adding water 1.5 times of rice weight); 12. Boiled rice of HK soaked in 1.0% unsalted rice koji miso (adding water 1.5 times of rice weight); 13. Boiled rice of HK soaked in 1.5% unsalted rice koji miso (adding water 1.5 times of rice weight).

DISCUSSION

Physicochemical properties of Koshihikari boiled rice after soaking with various seaweed extracts.

Dietary fiber comprises the ingested dietary plant material that is resistant to enzymatic digestion. Alginates are dietary fibers, which are refined from brown seaweed. Miranda et al. showed that substantial changes in the fiber content of the diabetic diet may lead to marked changes in diabetic control, and thus increasing the dietary fiber content may be a useful way to lower plasma glucose in some diabetic patients. Sugiuura et al showed that the intake of at least 5 g of depolymerized sodium alginate can increase the defecation frequency and effectively reduce abdominal discomfort in people who experience functional constipation. Maeda et al. showed that an agar diet led to marked weight loss due to the maintenance of a reduced calorie intake and an improvement in metabolic parameters.

As shown in Table 1, the boiled rice in 0.6% seaweed extracts had high levels of dietary fiber, as well as greater
glucose and resistant starch contents, whereas the adhesion and stickiness of boiled rice grains were decreased, and the hardness and toughness were increased, compared with the control soaked in DW. Moreover, the overall evaluation score in the sensory test for Koshihikari boiled rice soaked in 0.6% ego-nori was significantly better than Koshihikari soaked in DW. Thus, it became possible to prepare bio-functional and palatable boiled rice by soaking in 0.6% ego-nori extract solution.

**Physical properties and chemical composition of boiled rice treated with various soaking conditions.**

In a previous study, Nomura et al. showed that among the flavonoids examined, the flavones, apigenin and luteolin; the flavonols, kaempferol, quercetin and fisetin; an isoflavone, genistein; a flavanonol, silibin; and the flavanols, (-)-epigallocatechin gallate and theaflavins, significantly inhibited insulin-stimulated glucose uptake. Dyslipidemia is a major risk factor for the development of several obesity-related diseases. Kim et al. showed that a potent PPARα activator derived from tomato juice, 13-oxo-ODA, decreased the plasma and hepatic triglyceride levels in obese diabetic mice. 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 may promote human health. Moreover, viscous types of dietary fiber may slow down the speed of ingested sugar absorbed in the intestine, thereby moderating the rapid elevation in the blood glucose level after meals. Resistant starch is starch that escapes digestion in the small intestine, which may be fermented in the large intestine, thereby reducing the postprandial blood glucose level of palatable rice, such as Koshihikari, by preparing boiled rice grains soaked in MSTR.

**Changes in postprandial blood glucose level in rat.**

Yamanaka et al. showed that lactic acid concentration in the cecal contents of rats fed a diet containing viscous exudates of mekabu was lower than that in rats fed a diet without the additive. Moritaka et al. also found that for 120 min after eating boiled rice, the blood glucose response curve exhibited a less rapid increase in the blood glucose level when agar was added to the diet, and the maximum blood glucose level was lower. Polyphenols exist ubiquitously in plants and their regular consumption leads to a reduced risk of various chronic diseases, including cancer, cardiovascular disease and neurodegenerative disorders. Bazuine et al. showed that the isoflavone-derivative, genistein, which is widely applied as an inhibitor of tyrosine kinases, also affects the function of other proteins, such as glucose transporter. In another study, the triglyceride levels in the livers of rats fed high-amylose starch were not significantly lower than those in the controls, whereas the triglyceride levels in the livers of rats fed heat-moisture-treated starch were significantly lower than those in the controls.

In this study, we tried to lower the postprandial glucose of the boiled rice by the addition of above-mentioned MSTR including polysaccharide, isoflavone on cooking of rice. As a result, the blood glucose level increase for 30 min after consuming boiled rice soaked in MSTR was significantly lower than that of Koshihikari soaked in DW (p < 0.05). Therefore, we developed a novel method for lowering the postprandial blood glucose levels of palatable rice, such as Koshihikari, by preparing boiled rice grains soaked in MSTR.

**Correlation of chemical components, physical properties, and blood glucose levels with different diets.**

The viscosities of the diets supplemented with high-amylose starch or steamed starch were significantly lower than that of the control diet. The low viscosity of high-amylose starch or steamed starch is known to enhance cecal fermentation. Jeong et al. showed that the plasma glucose levels were significantly lower in a group that absorbed low levels of quercetin compared with a control group, whereas those in a high quercetin group were reduced even further compared with the low quercetin group in mice, which would be useful for the prevention of type 2 diabetes mellitus.

In this study, the blood glucose levels at 30 or 60 min after eating had negative correlation with the polyphenols, and resistant starch contents, but positive correlations with adhesion and stickiness of boiled grains. Therefore, we propose the palatable and low-GI boiled rice by the increase of polyphenol and resistant starch and reducing the adhesion and stickiness of the boiled rice grains by the addition of water-soluble dietary fibers and polyphenols of seaweed and miso in soaking water. Hokurikukona243 is chalky rice and absorb water very rapidly, which accelerates the penetration of enzyme solution such as MSTR into the inner layer of rice grain during soaking and cooking. And enzymes, such as amylases, proteases, decompose the starch and proteins, which lead to the softness of cooked rice (Tables 3 and 4). In the case of Hokurikukona243 of MSTR soaking, BGL after 30 min and
AUC are higher than those of it soaking in DW. On the contrary, Koshihikari grain is crystalized and absorb less enzyme solution than Hokusukikoukona243. Therefore, cooked rice grains of Koshihikari by soaking MSTR show harder texture than it soaked in DW, which would lead to the lower BGL after 30 min and AUC due to the effect by coating by MSTR.

**Formula for estimating the blood glucose level (AUC) based on the resistant starch, hardness and glucose content of boiled rice.**

We developed formula for estimating the postprandial blood glucose level (AUC) based on the resistant starch content, hardness and glucose content, which may help to improve the bio-functional properties of boiled rice. As shown in supplemental Table 1 (B), the equation can be applied well to unknown samples. Our estimation formula would be useful to estimate the results of in vivo test for postprandial blood glucose level using mice or rats based upon only the physicochemical in vitro measurements.

Boiled rice soaked in MSTR contained more dietary fiber, and was fortified with polyphenols, and glutamic acid, while it also had a high DPPH radical scavenging capacity. The blood glucose level increases for 30 min after eating this treated boiled rice were lower than that of the control diet of Koshihikari soaked in DW \((p < 0.05)\). Thus, it is possible to produce palatable and bio-functional boiled rice grains by soaking in MSTR. These results would be due to complex effects by \(\alpha\)-amylase and polyphenol in miso, dietary fiber in seaweeds, protease and dietary fiber in tomato, and isoflavones in red onion.

By using the estimation formula based upon the results of in vitro measurements, it would be possible to select the promising samples as a primary test, which would lead to the saving of the lives of precious rats and saving of the time and costs necessary for in vivo experiments to estimate the postprandial blood glucose.

**ACKNOWLEDGEMENTS**

This work was supported partly by a research project on the development of agricultural products and foods with health-promoting benefits (NARO) and Strategic Innovation Program (SIP, Cabinet Office, Government of Japan).

**REFERENCES**

1 ) A. Nishi, Y. Nakamura, N. Tanaka, and H. Satoh: Biochemical and genetic analysis of the effects of amylase-extender mutation in rice endosperm. *Plant Physiol.*, **127**, 459–472 (2001).

2 ) S. Nakamura, H. Satoh, and K. Ohitsu: Palatable and bio-functional wheat/rice products developed from pre-germinated brown rice of super-hard cultivar EM10. *Biosci. Biotechnol. Biochem.*, **74**, 1164–1172 (2010).

3 ) A.C. Nilsson, E.M. Ostman, Y. Granfeldt, and I.M. Bjorck: Effect of cereal test breakfast differing in glycemic index and content of indigestible carbohydrates on longday glucose tolerance in healthy subjects, 1, 2, 3. *Am. J. Clin. Nutr.*, **87**, 645–654 (2008).

4 ) C.J. Henry, H.J. Lightowler, E.A. Tydeman, and R. Skeath: Use of low-glycemic index bread to reduce 24-h blood glucose: implications for dietary advice to non-diabetic and diabetic subjects. *Int. J. Food Nutr.*, **57**, 273–278 (2006).

5 ) C.Z. Yang, X.L. Sha, L.L. Zhang, X.Y. Wang, H.J. Zhao, C.X. Ma, and D.X. Wu: Starch properties of mutant rice high in resistant starch. *J. Agric. Food Chem.*, **54**, 523–528 (2006).

6 ) M. Goddard, G. Yong, and R. Marcus: The effect of amylose content on insulin and glucose responses to ingested rice. *Am. J. Clin. Nutr.*, **39**, 388–392 (1984).

7 ) H.J. Kang, I.K.H. Wang, K.S. Kim, and H.C. Choi: Comparative structure and physicochemical properties of Ipumbyeo, a high-quality *Japonica* rice, and its mutant, Suwon 464. *J. Agric. Food Chem.*, **51**, 6598–6603 (2003).

8 ) H. Iso: Lifestyle and cardiovascular disease in Japan. *J. Atheroscler. Thromb.*, **18**, 2(2), 83–88 (2010).

9 ) A.M. Flammang, D.M. Kendall, C.J. Baumgartner, T.D. Slagle, and Y.S. Choe: Effect of a viscous fiber bar on postprandial glycemia in subjects with type 2 diabetes. *J. Am. Clin. Nutr.*, **25**, 409–414 (2006).

10 ) I.C. Arts and P.C. Hollman: Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr.*, **81**, 317S–325S (2005).

11 ) M. Kobori, S. Masumoto, Y. Akimoto, and H. Oike: Chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a Western-style diet in C57BL/6J mice. *Mol. Nutr. Food Res.*, **55**, 530–540 (2011).

12 ) P. Masicz, S. Kaiser, and S. Sies: Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.*, **274**, 532–538 (1989).

13 ) A. Momose, N. Goto, H. Hayase, T. Gomyo, and R. Miura. *Nippon Shokuhin Kagakukogaku Kaishi*, **57**, 617–621 (2010). (in Japanese)

14 ) S. Nakamura, Y. Nakano, H. Satoh, and K. Ohitsu: Improved palatability and bio-functionality of super-hard rice by soaking in a barley-koji miso suspension. *Biosci. Biotechnol. Biochem.*, **77**, 2419–2429 (2013).

15 ) O. Folin and W. Denis: A colorimetric method for the determination of phenols (and phenol derivatives) in purine. *J. Biol. Chem.*, **22**, 305–308 (1915).

16 ) I. Suda, T. Oki, Y. Nishibashi, M. Masuda, and M. Kobayashi: Polyphenol contents and radical-scavenging activity of extracts from fruits and vegetables in cultivated in Okinawa, Japan. *Nippon Shokuhin Kagakukogaku Kaishi*, **52**, 462–471 (2005). (in Japanese)

17 ) M. Odahara, H. Sokososhi, T. Takahashi, H. Okadome, and K. Ohitsu: The effect of sushi vinegar on texture of sushi rice before and after storage under low temperature. *Nippon Shokuhin Kagakukogaku Kaishi*, **51**, 620–625 (2004). (in Japanese)

18 ) H. Okadome, H. Toyoshima, and K. Ohitsu: Multiple measurements of physical properties of individual cooked rice grains with a single apparatus. *Cereal Chem.*, **76**, 855–860 (1999).

19 ) Syokuryotyo Institute: Spread of the Food Technologies Vol. 2, 50 (1964).

20 ) S. Nakamura, H. Satoh, and K. Ohitsu: Characteristics of pre-germinated Ae mutant rice flours prepared by boiling after preroasting. *J. Agric. Food Chem.*, **59**, 10665–10676 (2011).

21 ) P.M. Miranda and D.L. Horwitz: High-fiber diets in the treatment of diabetes mellitus. *Am. Intern. Med.*, **88**, 482–486 (1978).

22 ) Y. Sugiu, K. Kouzuma, T. Yasunuma, I. Tokimoto, M. Uematsu, and T. Hosoya: Analysis of minimal effective dose of mixture of depolymerized sodium alginate and partially hydrolyzed gum gum in treatment of defecation and abdominal discomfort in functional constipation. *J. Home Econ. Jpn.*, **59**, 143–153(2008).

23 ) H. Maeda, R. Yamamoto, K. Hirao, and O. Tochikubo: Effects of agar (kanten) diet on obese patients with impaired glucose tolerance and type 2 diabetes. *Diabetes Obes. Metabol.*, **7**, 40–46 (2005).

24 ) M. Nomura, T. Takahashi, N. Nagata, K. Tsutsumi, S. Kobayashi, T. Akiba, K. Yokogawa, S. Moritani, and K. Miyamoto: Inhibitory mechanisms of flavonoids on insulin-stimulated glucose uptake in MCT3-G2/Pa6 adipose cells. *Biol. Pharm. Bull.*, **31**, 1403–1409 (2008).

25 ) Y.I. Kim, S. Hirai, T. Goto, C. Ohyane, H. Takahashi, T. Tsuzane, C. Konishi, T. Fuji, S. Inai, Y. Iijima, K. Aoki, D. Shibata, N. Takahashi, and T. Kawada: Potent PPARα activator derived from tomato juice, 13-oxo-9,11-octadecadienoic acid, decreases plasma and hepatic triglyceride in obese diabetic mice. *PLoS ONE*, **7**(2), e31317, (2012).

26 ) N. Yamanaka, A. Inagaki, T. Sakata, and N. Ogawa: Effect of the viscous exudate of mekabu (sporophyll of Undaria pinnatifida) on the rate of cecal fermentation in rats. *J. Home Econ. Jpn.*, **53**, 991–999 (2002). (in Japanese)
27) H. Moritaka, Y. Nakanishi, M. Fuwa, and R. Tanii: Effects of agar on thermal property, sensory characteristic and glycemic index of cooked rice. *J. Cookery Sci. Jpn.*, **45**, 115–122 (2012). (in Japanese)

28) D. Vauzour, M.A. Rodriguez, G. Corona, and C.M.J. Oruna: Polyphenols and human health: Prevention of disease and mechanisms of action. *Nutrients.*, **2**, 1106–1131 (2010).

29) M. Bazuine, P.J. Van den Broek, and J.A. Maassen: Genistein directly inhibits GLUT4-mediated glucose uptake in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.*, **326**, 511–514 (2005).

30) Y. Itoh, N. Yamanaka, N. Ogawa, H. Tsuge, and T. Hayakawa: Effect of the heat-moisture-treated high-amylose starch on cecal fermentation and lipid metabolism. *Bull. Gifu Women’s Univ.*, **3**, 121–130 (2006).

31) S.M. Jeong, M.J. Kang, H.N. Choi, J.H. Kim, and J.I. Kim: Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. *Nutr Res Pract.*, **6**, 201–207 (2012).