Effects of high-temperature cooking on the gamma-aminobutyric acid content and antioxidant capacity of germinated brown rice (Oryza sativa L.)

Tomoyasu Toyoizumi, Toru Kosugi, Yusuke Toyama and Teruko Nakajima

Shizuoka Prefectural Research Institute of Agriculture and Forestry, Iwata, Japan

ABSTRACT
Germinated brown rice (GBR) has a high gamma-aminobutyric acid (GABA) content and antioxidant capacity; however, the effects of high-temperature cooking on these characteristics are unclear. We investigated alterations in GABA content, antioxidant capacity, 15 proteinogenic amino acids, TP and reducing sugar content, and color of cooked GBR at 105°C to 135°C and at different times (40 to 90 min) at 105°C. The contents of GABA and 9 proteinogenic amino acids decreased via thermal decomposition with increasing cooking temperatures. The hydrophilic and lipophilic antioxidant capacities (DPPH and L-ORAC) were enhanced at temperatures >125°C. L-ORAC values were strongly associated with the glucose content and $L^*$, $a^*$, and $b^*$ values. Additionally, prolonged treatment time decreased the content of 12 amino acids but increased DPPH and L-ORAC. Our results on functional ingredients and activity are useful for both home cooking and manufacturing processes and may lead to the development of better-processed products.

1. Introduction

Rice (Oryza sativa L.) is one of the most commonly consumed grain products worldwide, and more than 500 million tons of rice was produced in 2019 (milled basis) (Food and Agriculture Organization of the United Nations, 2020). Rice is also the staple food for nearly half of the world’s population and is the primary grain consumed in Asia (Callaway, 2014). Rice is consumed mainly as white rice, which is prepared by removing the bran layer from the surface of brown rice (BR) through a food milling process (Xia et al., 2019). However, this removed material contains fiber, vitamins, minerals, and several bioactive compounds, including gamma-aminobutyric acid (GABA), which exhibits hypotensive effects. Intake of 10 mg GABA was found to lower blood pressure in patients with mild hypertension (Inoue et al., 2003). Further, this removed material contains many antioxidants, such as phenolic acids, tocotrienols, and γ-oryzanol (Goufo & Trindade, 2014; Patil & Khan, 2011); these compounds exhibit antioxidant capacity and antihypertensive effects, among other bioactivities (Saleh et al., 2019). Reactive oxygen species (ROS), excessively produced by inflammatory reactions in living organisms, are deeply involved in the development of lifestyle-related diseases, such as arteriosclerosis and cancer, by damaging DNA and proteins. As such, ingesting antioxidants from food is important to reduce stress caused by ROS (Seifried et al., 2007). Thus, BR, which contains bran, is thought to have beneficial health effects in humans.

The functional compounds in BR are altered by food processing, including soaking, plasma treatment, and ultrasound (Xia et al., 2019). For example, soaking treatment leading to germination is an economical processing technology. BR absorbs water during soaking, resulting in activation of glutamic acid decarboxylase (GAD) (Komatsuzaki et al., 2007) and conversion of glutamic acid (Glu) to GABA. Notably, GABA content in germinated BR (GBR) is several times higher than that in BR (Moongngarm & Saetung, 2010). Furthermore, GBR exhibits an...
increased total phenolic (TP) compound content compared to BR following germination at approximately 30°C (Owolabi et al., 2018). Thus, GBR may be a useful food material and source of GABA and antioxidants and is expected to have greater health-promoting effects than BR.

Germination treatment can also mitigate the hard texture effects of BR. During treatment, endogenous hydrolytic enzymes are activated to break down starch, fibers, and proteins, resulting in the modification of textural characteristics. Additionally, under germination, the sweetness of BR increased by the effects of α-amylase (Jiamyangyuen & Ooraikul, 2008). Accordingly, both the compositional and cooking characteristics of GBR differ substantially owing to changes in the structural characteristics of different plants. Moreover, although many reports have described the functionality of GBR (Patil & Khan, 2011), GBR is consumed after cooking, including drying, boiling, and steaming; thus, the effects of cooking on GABA content and antioxidant capacity should be assessed to determine the health-promoting effects of these factors in practice.

Boiling using a rice cooker is one of the popular methods of processing rice, including GBR, in Asia (Kim, 2013). Although the cooking temperature is generally around 100°C when a rice cooker is used, pressure cookers or earthenware pans can reach around 130°C. Furthermore, recently developed rice cookers with superheated steam systems have been reported to heat to temperatures of 130°C or more (Soponronnarit et al., 2006) and have been used to improve texture during rice cooking. Alternatively, GBR can also be used to make other food products and dishes, including bread (generally 160°C or more), doughnuts (generally 160°C or more), cookies (generally 170°C or more), Italian risotto, Spanish paella, and Brazilian feijoada (Patil & Khan, 2011), for which other cooking processes apart from boiling may be used. During these processes, functional ingredients in GBR can be affected due to high temperatures. Accordingly, elucidation of the effects of cooking temperature on GABA content and antioxidant properties is essential for determining the characteristics of cooked foods prepared at high temperatures.

Unlike BR, few studies have assessed cooking processes for GBR (Surh & Koh, 2014). Additionally, few reports have evaluated the effects of cooking on GABA content and that of other related amino acids (Mabashi et al., 2007). In particular, no reports have investigated the effects of heating at temperatures above 100°C on GABA loss and the loss of related amino acids. Notably, during the high-temperature cooking of GBR, reducing sugars (glucose and fructose) and amino acids react in a nonenzymatic browning reaction (the Maillard reaction), thereby contributing to the strong antioxidant property and brown color (Ujihara et al., 2013). However, studies on changes in antioxidant capacity and polyphenol contents have only been conducted within a limited temperature range (Gujral et al., 2012), and the behaviors of these factors at higher temperatures are unknown. The antioxidant capacity of GBR cooked at 100°C or higher is the sum of the antioxidant capacity derived from polyphenols and that derived from the Maillard reaction. Therefore, clarification of the effects of cooking GBR at temperatures above 100°C may help determine the actual GABA content and antioxidant capacity in cooked foods and processed products.

In the present study, we investigated the change in GABA content and antioxidant capacity and other related indices, including proteinogenic amino acids, TP and reducing sugars content, and color of the cooked GBR at 105°C, 115°C, 125°C, and 135°C. In addition, we evaluated the effect of cooking time (40, 60 and 90 min) at 105°C on the above indices using GBR. To our knowledge, this is the first time changes in the GABA content and antioxidant capacity of cooked GBRs have been evaluated under high cooking temperatures above 105°C.

2. Materials and methods

2.1. Chemicals

Sodium acetate and ethanol were purchased from Kanto Chemical Co. (Tokyo, Japan), and 2-Morphioloethanesulfonic acid was from Chemical Dojin Co. (Kumamoto, Japan). The fluorescein sodium salt and (-)-6-hydroxy-2,5,7,8-tetramethyl-2-chromen-2-carboxylic acid (Trollox) were from Sigma-Aldrich Co. (Milwaukee, WI, USA). Folin-Ciocalteu’s phenol reagent was from Merck Co. (Darmstadt, Germany). Other chemicals were from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Sample preparation

BR (O. sativa L., ‘Koshihikari’), harvested in Shizuoka in 2018, was purchased from a rice store (Fujieda, Shizuoka, Japan). ‘Koshihikari’ is one of the most popular cultivars of Japonica rice cultivated in Japan. To prepare GBR, BR was soaked in distilled water (rice and water ratio, 1:2 w/v) in a 100-mL conical beaker at 30°C for 24 h for germination (Cho & Lim, 2016). BR and the obtained GBR samples were used in subsequent experiments.

GBR was cooked at a reference temperature of 105°C for 40 min, as previously described (Kinoshita & Yamamoto, 2012), with minor modifications. To evaluate the effects of cooking temperature, GBR was cooked at 5°C (noncooking group, kept in the refrigerator), 105°C, 115°C, 125°C, or 135°C for 40 min using an autoclave (HV-50; Hirayama Manufacturing, Saitama, Japan). Due to the performance of the processing equipment, it was not possible to set the temperature higher than 135°C. The respective texture value parameters were for the 105°C, 115°C, 125°C, and 135°C temperatures were as follows: hardness: 9.3 ± 1.1, 8.9 ± 1.0, 7.3 ± 0.7, and 4.8 ± 0.3 × 10⁻¹ N/m²; cohesiveness: 0.32 ± 0.01, 0.38 ± 0.02, 0.45 ± 0.05, and 0.68 ± 0.07; and adhesiveness: 187 ± 31, 178 ± 167, 22 ± 4, and 36 ± 15 J/m². Food physical properties of the samples used in this study were classified as Universal Design Foods (UDF) category 2 ("can be broken up using the gums") per the Universal Design Food Guidelines in Japan (http://www.udf.jp).

Next, to evaluate the effects of cooking time, GBR samples were cooked at 105°C for 0 (noncooking group), 40, 60, or 90 min. The respective texture parameters for the 40, 60, and 90 min durations were as follows: hardness: 10.9 ± 2.2, 13.2 ± 1.7, and 11.8 ± 1.1 × 10⁻¹ N/m²; cohesiveness: 0.33 ± 0.02, 0.35 ± 0.10, and 0.34 ± 0.054, and adhesiveness: 176 ± 113, 32 ± 16, and 18 ± 7 J/m², respectively. These values also met the UDF category 2 classification criteria.

Samples were then crushed using a mixer (IFM-530G; Iwatani, Tokyo, Japan) after freeze-drying. BR and GBR prepared by soaking treatment only were not freeze-dried. The resulting powder samples were stored at −80°C until analysis of the antioxidant capacity, amino acid content, reducing sugars and TP content, and color value. In addition, the amount of moisture in the powder was measured using a moisture analyzer (ML-50; A&D, Tokyo, Japan) to correct the antioxidant capacity values and contents of these indicators, except for...
color. The experiment was independently repeated five times to evaluate the effects of cooking.

2.3. **Determination of amino acid contents**

Powder samples were diluted 10-fold with 0.1 M hydrochloric acid and extracted by ultrasound sonification (US-13KS; SND, Nagano, Japan) for 10 min at 37°C. The mixture was centrifuged at 13,040 × g for 5 min, and the collected supernatants were deproteinized with 10 w/v% 5-sulfosalicylic acid. Samples were then centrifuged at 13,040 × g for 5 min, and the collected supernatants were then passed through a 0.2 µm PTFE filter for subsequent analysis.

The following 15 proteinogenic amino acids, as well as GABA, were separated and quantified using high-performance liquid chromatography (HPLC; Waters, Milford, MA, USA), as previously described (Toyoizumi et al., 2020): GABA, aspartic acid (Asp), glutamate (Glu), serine (Ser), asparagines (Asn), glycine (Gly), glutamine (Gln), arginine (Arg), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), lysine (Lys), and phenylalanine (Phe). The amino acid contents were determined using HPLC with a fluorescence detection (FLD) system using pre-column derivatization and gradient methods. The column temperature was 39°C. The FLD system was set for exposure at 250 nm and emission at 395 nm.

2.4. **Evaluation of antioxidant capacity**

Antioxidants deactivate radicals through two major mechanisms: single-electron transfer (SET) and hydrogen atom transfer (HAT) (Huang et al., 2005). The antioxidant capacity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, which can detect hydrophilic antioxidants and oxygen radical absorbance capacity for lipophilic and hydrophilic compounds (L- and H-ORAC) assays based on SET and HAT methods, respectively.

The extraction and DPPH assay procedures were performed as previously described (Ito et al., 2011; Toyoizumi et al., 2020), with minor modifications. Briefly, the samples were diluted fourfold with 80 v/v% acetone solution and extracted by stirring for 1 min at room temperature. The mixture was centrifuged at 13,040 × g for 5 min, and the collected supernatant was diluted with an equal amount of 20 v/v% acetone solution. The diluted sample, trolox calibration solution, or blank (100 µL volume) was reacted with 200 mM 2-morpholineethanesulfonic acid buffer (pH 6.0, 50 µL) and 800 µM DPPH solution (50 µL) for 20 min at room temperature. Then, the absorbance at 520 nm was determined using a microplate reader (Spark, Tecan, Männedorf, Switzerland). The DPPH values were expressed as micromoles of Trolox equivalent (TE) per gram (µmol TE/g).

The extraction and L- and H-ORAC assays were performed as described by Watanabe et al. (2014). To determine L-ORAC values, samples were diluted 10-fold with 7 w/v% methyl-β-cyclodextrin in 50 v/v% aqueous acetone and then further diluted with 6.3 w/v% methyl-β-cyclodextrin in 45 v/v% aqueous acetone and 10 v/v% dimethyl sulfoxide. To determine H-ORAC values, samples were diluted 10-fold with assay buffer solution (75 mM phosphate buffer solution, pH 7.4) and then further diluted with 10 v/v% methanol:water:acetic acid (90:9:5:0.5) in assay buffer solution.

For the diluted sample mentioned above, Trolox calibration solution or blank (35 µL) was added to the wells of a 96-well plate. Then, for L- and H-ORAC assays, 115 µL of 77.5 and 110.7 nM fluorescein solution and 50 µL of 82.4 and 31.7 mM 2,2’-azobis(2-aminopropane) dihydrochloride solution were respectively added to each well, and the plates were incubated at 37°C. The fluorescence intensity was monitored every 2 min for 120 (L-ORAC) and 90 (H-ORAC) min using a microplate reader with excitation at 485 nm and emission at 528 nm. The H- and L-ORAC values were expressed as micromoles of TE per gram (µmol TE/g).

2.5. **Determination of TP content**

The extraction procedures and determination of TP were performed as described previously (Ito et al., 2011; Toyoizumi et al., 2020).

The TP content of the diluted sample was determined according to the Folin–Ciocalteu method using gallic acid (GA) as a standard substance. Briefly, the sample, Trolox calibration solution, or blank (150 µL) was reacted with 150 µL of 50 v/v% Folin–Ciocalteu’s phenol reagent solution for 3 min at room temperature. The mixture was further mixed with 150 µL of 10 w/v% sodium carbonate solution and incubated for 30 min at room temperature. The absorbance was measured at 760 nm, and the TP value was expressed as micrograms of GA equivalent (GAE) per gram (µg GAE/g).

2.6. **Color measurement**

A color reader (CR-20; Konica Minolta, Tokyo, Japan) was used to measure the color of the GBR powder sample, and the results are presented as L*, a*, and b* values according to international standards for color measurement developed by the Commission Internationale de l’Eclairage in 1976. The measurement consists of a lightness component (L* value = 0 yields black and L* value = 100 denotes white), along with two chromatic components: the a* value (from green [-] to red [+]) and the b* value (from blue [-] to yellow [+]).

2.7. **Determination of reducing sugar content**

The glucose and fructose contents in the samples were determined according to our previous method (Toyoizumi et al., 2015, 2020). Briefly, the samples were diluted 10-fold with 80 v/v% ethanol and extracted under 80°C for 15 min. The mixture was centrifuged at 310 × g for 10 min at room temperature, and the supernatant was collected. The residue was extracted three times with 80 v/v% ethanol, and the collected supernatants were then passed through a 0.2-µm PTFE filter for subsequent analysis.

Glucose and fructose were separated on a Shodex packed column for sugars (SC101; Shodex, Tokyo, Japan) and analyzed by HPLC (Waters) with a refractive index detector (RID) according to the following conditions. The mobile phase was composed of ultra-purified water. The column and RID temperatures were 90°C and 50°C, respectively.

2.8. **Statistical analysis**

Mean and standard deviation (SD) values were calculated from five independent experiments. Differences in amino acid content, TP and sugar content, and antioxidant capacity
between the noncooked BR and GBR were examined using Welch’s t-test (two-tailed) after examining the homogeneity of variances using the F test. For all analyses, differences between the averages of the GBR noncooked and cooked groups were compared using Dunnnett’s tests or Steel tests (two-tailed) after evaluating dispersibility based on Bartlett’s tests. The correlation of cooking temperature with each measurement index (amino acids, antioxidant capacity, TP, La*αb*, and sugars), as well as that of cooking time with each measurement index (amino acids, antioxidant capacity, TP, La*αb*, and sugars) was determined using Pearson’s or Spearman’s rank-order correlations after evaluating the normality using Shapiro–Wilks tests. Further, correlation analysis was performed for antioxidant capacity and related parameters (TP, La*αb*, and sugars) in the same manner. All statistical analyses were conducted using Excel Statistics for Windows, versions 7.08 and 8.00 (Esumi, Tokyo, Japan). Differences with p-values of less than 0.05 were considered significant.

3. Results

3.1. Amino acid contents after germination and at different cooking temperatures

Table 1 shows the contents of 15 proteinogenic amino acids and GABA in BR and GBR samples. Compared to the BR group, the noncooked GBR group showed significantly increased GABA, Asn, Gly, Arg, Ala, Pro, Val, Ile, Leu, Lys, and Phe content and significantly decreased Asp and Glu content.

Compared to the noncooked GBR group, all cooked groups had significantly decreased levels of GABA, Asp, Ser, Asn, Pro, Ile, Leu, and Lys and significantly increased levels of Ala. Gly content was increased only in the 135°C group, Val content was decreased in all groups except the 125°C group, and Phe content was decreased in all groups except the 125°C and 135°C groups compared to the noncooked GBR group.

Glu content was increased in all groups except the 135°C group compared to the noncooked GBR group.

3.2. Antioxidant capacity and TP content following germination and at different cooking temperatures

Table 2 shows DPPH free radical scavenging, L-ORAC values, and TP content in BR and GBR samples. Compared to the BR group, DPPH-free radical scavenging and H-ORAC values in the noncooked GBR group were significantly decreased. In contrast, DPPH-free radical scavenging activity was significantly lower in GBR samples cooked at temperatures of 105°C and 115°C than in the noncooked group. Moreover, L-ORAC values were significantly higher in the 125°C and 135°C groups than in the noncooked group, and H-ORAC values were significantly higher in the 115°C group than in the noncooked group. Compared to the noncooked GBR group, all cooked groups showed significantly decreased TP levels.

3.3. Reducing sugar content following germination and at different cooking temperatures

Table 3 shows the glucose and fructose content of BR and GBR samples. Compared to the BR group, both values were significantly decreased in the noncooked GBR group. Moreover, the glucose content of all treated groups and fructose content of the 105°C and 115°C groups were significantly decreased compared to the noncooked group.

3.4. Color changes at different cooking temperatures

Table 4 shows the color parameters of La*, a*, and b* for GBR samples cooked at different temperatures (105–135°C). Compared to the noncooked group, the La* values in the 115°C, 125°C, and 135°C groups were significantly

Table 1. Changes in amino acid contents following germination of brown rice (BR) and in germinated brown rice (GBR) at different cooking temperatures.

| Amino acid | BR | GBR |
|------------|----|-----|
|            | Noncooked | Noncooked | 105°C | 115°C | 125°C | 135°C |
| GABA       | 38 ± 6 | 215 ± 12 ** | 144 ± 8 †† | 131 ± 8 †† | 129 ± 7 †† | 126 ± 13 †† |
| Asp        | 152 ± 44 | 46 ± 5 ** | 22 ± 5 †† | 24 ± 9 †† | 25 ± 4 †† | 33 ± 3 †† |
| Glu        | 120 ± 32 | 8 ± 4 ** | 17 ± 3 †† | 15 ± 3 †† | 14 ± 2 †† | 14 ± 3 |
| Ser        | 27 ± 1 | 3 ± 6 | 13 ± 5 †† | 16 ± 3 †† | 18 ± 4 †† | 19 ± 5 †† |
| Asn        | 79 ± 37 | 242 ± 25 ** | 190 ± 25 † | 181 ± 21 † | 173 ± 40 † | 184 ± 21 † |
| Gly        | 17 ± 7 | 60 ± 9 ** | 57 ± 5 | 52 ± 9 | 62 ± 11 | 79 ± 1 †† |
| Arg        | 51 ± 4 | 133 ± 34 ** | 99 ± 10 | 142 ± 96 | 123 ± 41 | 95 ± 19 |
| Ala        | 42 ± 8 | 109 ± 2 ** | 175 ± 40 †† | 180 ± 4 †† | 173 ± 30 † | 177 ± 33 †† |
| Pro        | 52 ± 10 | 145 ± 12 ** | 46 ± 25 †† | 52 ± 30 †† | 70 ± 27 † | 76 ± 2 †† |
| Tyr        | 13 ± 9 | 26 ± 11 | 18 ± 8 | 29 ± 13 | 26 ± 7 | 23 ± 13 |
| Val        | 9 ± 7 | 39 ± 6 ** | 28 ± 5 †† | 25 ± 5 †† | 36 ± 6 | 27 ± 5 †† |
| Met        | 12 ± 15 | 17 ± 6 | 1 ± 3 | 10 ± 5 | 16 ± 9 | 8 ± 6 |
| Ile        | 4 ± 5 | 7 ± 2 ** | 8 ± 2 †† | 8 ± 1 †† | 11 ± 1 †† | 9 ± 1 †† |
| Leu        | 0.4 ± 0.4 | 34 ± 6 ** | 15 ± 6 †† | 14 ± 4 †† | 17 ± 3 †† | 16 ± 2 †† |
| Lys        | 1.3 ± 0.7 | 67 ± 12 ** | 40 ± 15 †† | 33 ± 9 †† | 33 ± 10 †† | 36 ± 4 †† |
| Phe        | 0.4 ± 0.2 | 26 ± 9 ** | 15 ± 3 †† | 8 ± 6 †† | 16 ± 7 | 16 ± 8 |

The mean values were obtained from five independent experiments.

**: Significantly higher or lower than in the BR group at 1% level (Welch’s t test, two-tailed).
††: Significantly higher or lower than in the noncooked GBR group at 1% level (Dunnett’s test, two-tailed).

Los valores medidos se obtuvieron a partir de cinco experimentos independientes.

**: Significativamente mayor o menor que en el grupo BR a nivel de 1% (prueba t de Welch, de dos colas).
††: Significativamente mayor o menor que en el grupo GBR sin cocción a nivel de 1% (prueba de Dunnett, de dos colas).
Table 2. Changes in antioxidant capacity and total phenolic (TP) contents following germination of brown rice (BR) and in germinated brown rice (GBR) prepared at different cooking temperatures.

| Group | DPPH (µmol/g D.W.) | L-ORAC (µmol/g D.W.) | H-ORAC (µmol/g D.W.) | TP (µg/g D.W.) |
|-------|---------------------|-----------------------|----------------------|---------------|
|       | Mean ± SD           | Mean ± SD             | Mean ± SD            | Mean ± SD     |
| BR    | Noncooked           | 1.95 ± 0.19           | 6.4 ± 1.0            | 13.5 ± 0.8    | 217 ± 16 |
| GBR   | Noncooked           | 0.96 ± 0.06 **        | 6.0 ± 0.8            | 8.2 ± 2.1 **  | 193 ± 19 |
|       | 105°C               | 0.60 ± 0.05 ††        | 5.2 ± 1.1            | 9.9 ± 1.2     | 96 ± 4 †† |
|       | 115°C               | 0.60 ± 0.08 ††        | 6.8 ± 1.4            | 12.7 ± 1.2 †† | 113 ± 7 †† |
|       | 125°C               | 0.71 ± 0.20           | 8.3 ± 0.8 ††         | 9.2 ± 3.3     | 113 ± 4 †† |
|       | 135°C               | 1.10 ± 0.17           | 8.5 ± 0.8 ††         | 7.2 ± 0.2     | 143 ± 3 †† |

The mean values were obtained from five independent experiments. **: Significantly lower than in the BR group at 1% level (Welch’s t test, two-tailed). ††: Significantly higher or lower than in the noncooked GBR group at 1% level (Dunnett’s or Steel test, two-tailed). Los valores medios se obtuvieron a partir de cinco experimentos independientes. **: Significativamente menor que en el grupo BR a nivel de 1% (prueba t de Welch, de dos colas). ††: Significativamente mayor o menor que en el grupo GBR sin cocción a nivel de 1% (prueba de Dunnett o de Steel, de dos colas).

3.5. Correlations between cooking temperature and various parameters

Table 5 shows the correlations between cooking temperature and various parameters, including amino acid content, antioxidant capacity, TP content, L*/a*/b*/ values, and reducing sugars.

Among amino acids, GABA, Asp, Ser, Asn, Pro, Val, Ile, Leu, Lys, and Phe content decreased significantly depending on the cooking temperature, and Glu and Ala content increased significantly. Moreover, antioxidant capacity, related indices, and L-ORAC, a*, and b* values increased in a cooking temperature-dependent manner, whereas L* values and glucose content decreased as cooking temperature increased.

3.6. Correlations of antioxidant capacity with TP, L*/a*/b*/values, and reducing sugars

Table 6 shows the correlations between antioxidant capacity and related parameters following cooking at different temperatures (105–135°C). A significant negative correlation was observed between DPPH values and glucose contents, and there was a significant positive correlation between DPPH values and fructose contents. L-ORAC values were significantly negatively correlated with L* values and glucose content but significantly positively correlated with a* values, b* values, and fructose content. H-ORAC values were also significantly negatively correlated with TP and fructose content.

3.7. Amino acids content at different cooking times

Table 7 shows changes in amino acid content following cooking at 105°C for 0–90 min and the correlations between cooking temperature (0, 40, 60, and 90 min) and amino acid content.

GABA, Ser, Asn, Arg, Pro, Val, Met, Ile, Leu, Lys, and Phe content were significantly lower in all cooked GBR groups than in the noncooked group. Additionally, Asp content was significantly lower in the 90 min GBR group, whereas Tyr content was significantly lower in the 60 and 90 min groups. Furthermore, Glu and Ala contents were significantly higher in all cooked GBR groups than in the noncooked group.
Table 5. Correlation matrix between cooking temperatures and measurement parameters in germinated brown rice.

| Measurement parameter | Cooking temperature | Measurement parameter | Cooking temperature |
|-----------------------|---------------------|-----------------------|---------------------|
| GABA                  | −0.914 ††           | Leu                   | −0.844 ††           |
| Asp                   | −0.693 ††           | Lys                   | −0.785 ††           |
| Glu                   | 0.664 ††            | Phe                   | −0.544 ††           |
| Ser                   | −0.752 ††           | DPPH                  | −0.204              |
| Asn                   | −0.704 ††           | L-ORAC                | 0.453 †             |
| Gly                   | 0.189               | H-ORAC                | −0.137              |
| Arg                   | −0.083              | TP                    | −0.222              |
| Ala                   | 0.628 ††            | L*                    | −0.785 ††           |
| Pro                   | −0.781 ††           | α*                    | 0.641 ††            |
| Tyr                   | −0.030              | β*                    | 0.777 ††            |
| Val                   | −0.520 ††           | Glucose               | −0.938 ††           |
| Met                   | −0.368              | Fructose              | −0.052              |
| Ile                   | −0.837 ††           | -                     | -                   |

The mean values were obtained from 25 independent experiments.

† or ††: Significant differences in the correlation matrix between cooking temperature and each measurement parameter at 5% or 1% level (Pearson’s or Spearman’s rank-order correlation).

Los valores medios se obtuvieron a partir de 25 experimentos independientes.

† o ††: Diferencias significativas en la matriz de correlación entre la temperatura de cocción y cada parámetro de medición a nivel de 5% o de 1% (correlación de rango de Pearson o de Spearman).

The Glu and Ala content increased significantly depending on the cooking time, while other amino acids except Gly decreased significantly.

3.8. Antioxidant capacity and TP content at different cooking times

Table 8 presents the correlation between antioxidant capacity value and TP content. GBR cooked for 40 to 90 min at 105°C had significantly lower levels of DPPH and TP than did noncooked GBR. In addition, H-ORAC values were significantly lower in GBR cooked for 60 and 90 min. Furthermore, the DPPH, H-ORAC, and TP values decreased significantly in a time-dependent manner.

Table 6. Correlation matrix between antioxidant capacity and related parameters in germinated brown rice.

| Index     | DPPH  | L-ORAC | H-ORAC |
|-----------|-------|--------|--------|
| TP        | 0.407 | 0.165  | −0.582 †† |
| L*        | 0.014 | −0.622 †† | −0.174  |
| α*        | 0.347 | 0.64 †† | 0.037  |
| β*        | −0.139 | 0.544 † | 0.259  |
| Glucose   | −0.454 † | −0.469 † | 0.174  |
| Fructose  | 0.520 †† | 0.515 † | −0.430 † |

The mean values were obtained from 20 independent experiments.

† or ††: Significant differences in the correlation matrix between each antioxidant index and related parameters at 5% or 1% level (Pearson’s or Spearman’s rank correlation).

Los valores medios se obtuvieron a partir de 20 experimentos independientes.

† o ††: Diferencias significativas en la matriz de correlación entre cada índice antioxidante y los parámetros relacionados a un nivel de 5% o de 1% (correlación de rango de Pearson o de Spearman).

3.9. Correlations between antioxidant capacity and TP content

Correlation matrix between antioxidant capacity and TP content following cooking at 105°C for 0–90 min was calculated using Spearman’s rank correlation. A significant positive correlation was observed between DPPH values and the TP content at 1% level (0.740). Meanwhile, there were no significant correlations between TP content and L-ORAC (−0.150) or H-ORAC (0.273) values.

4. Discussion

In this study, we aimed to clarify the effects of high-temperature cooking at temperatures above 105°C on the GABA content and antioxidant capacity of GBR. We found that the GABA content and that of nine proteinogenic amino acids decreased as the heating temperature increased due to thermal decomposition. Moreover, the hydrophilic and lipophilic antioxidant capacities derived from DPPH and L-ORAC.

Table 7. Amino acid contents in germinated brown rice at different cooking times.

| Amino acid | 0 (Noncooked) | 40 | 60 | 90 |
|------------|---------------|----|----|----|
| GABA       | 272 ± 16      | 143 ± 17 ** | 135 ± 10 ** | 140 ± 14 ** | −0.822 †† |
| Asp        | 64 ± 14       | 42 ± 14    | 44 ± 15    | 39 ± 11    | −0.574  |
| Glu        | 12 ± 7        | 28 ± 6    | 27 ± 5    | 29 ± 8    | 0.637 † |
| Ser        | 102 ± 18      | 41 ± 12    | 35 ± 10    | 34 ± 5    | −0.824 † |
| Asn        | 204 ± 18      | 163 ± 15   | 166 ± 15   | 156 ± 15  | −0.722 † |
| Gly        | 65 ± 8        | 64 ± 6    | 66 ± 5    | 69 ± 5    | 0.214  |
| Arg        | 119 ± 11      | 107 ± 10   | 104 ± 2   | 98 ± 8    | −0.709 † |
| Ala        | 77 ± 24       | 163 ± 30   | 153 ± 8   | 153 ± 12  | 0.692 † |
| Pro        | 216 ± 49      | 74 ± 14    | 84 ± 15   | 81 ± 10   | −0.758 † |
| Tyr        | 40 ± 12       | 30 ± 7    | 25 ± 6    | 24 ± 6    | −0.627 †† |
| Val        | 63 ± 7        | 41 ± 6    | 39 ± 11   | 43 ± 9    | −0.630 † |
| Met        | 19 ± 2        | 7 ± 3    | 9 ± 6    | 8 ± 2    | 0.665 † |
| Ile        | 18 ± 4        | 7 ± 1    | 7 ± 1    | 9 ± 2    | 0.708 † |
| Leu        | 121 ± 20      | 51 ± 6    | 48 ± 10   | 56 ± 3    | −0.758 † |
| Lys        | 39 ± 5        | 25 ± 3    | 26 ± 3   | 23 ± 4    | −0.786 † |
| Phe        | 41 ± 3        | 20 ± 2    | 21 ± 6    | 24 ± 11   | −0.594 † |

The mean values were obtained from five independent experiments.

* or **: Significantly higher or lower than in noncooked germinated brown rice (0 min) at the 5% or 1% level (Dunnett’s test, two-tailed).

† or ††: Significant differences in the correlation matrix between cooking time and amino acid contents at 5% or 1% level (Pearson’s correlation).

Los valores medios se obtuvieron a partir de 5 experimentos independientes.

* o ††: Significativamente mayor o menor que en el arroz integral germinado no cocido (0 min) a nivel de 5% o de 1% (prueba de Dunnett, de dos colas).

† o ††: Diferencias significativas en la matriz de correlación entre el tiempo de cocción y el contenido de aminoácidos a nivel de 5% o de 1% (correlación de Pearson).
were enhanced at temperatures above 125°C. Notably, L-ORAC values were highly associated with glucose contents and L*α*TRb values derived from the Maillard reaction. Additionally, prolonged treatment time decreased the content of most amino acids, including GABA, and increased DPPH and L-ORAC values. These results demonstrated that cooking GBR at temperatures above 105°C decreased GABA content owing to thermal decomposition and that cooking at temperatures above 125°C enhanced antioxidant capacity via the Maillard reaction.

In this study, we found that GABA, Asn, Gly, Arg, Ala, Pro, Val, lle Leu, Lys, and Phe content were significantly increased in GBR compared to BR; in contrast, Glu and Asp content were significantly decreased. As evaluated in this study, germination of BR by soaking has been reported to modulate the proteinogenic amino acids content as well as GABA in ‘Haiminori’ (Komatsuzaki et al., 2007). Similar to this report, proteinogenic amino acids content in our study would also increase via protein degradation due to activation of proteases during germination (Cho & Lim, 2016). Meanwhile, increased GABA content and decreased Glu and Asp content, as observed in the current study, may be related to activation of the GABA production pathway via GAD (Cho & Lim, 2016; Fait et al., 2008). Only the compositions of Ser and Asn were inconsistent with those reported in a previous report (Komatsuzaki et al., 2007), and this discrepancy may be related to differences in the metabolic capacities of the various cultivars.

In contrast to a previous report (Owolabi et al., 2018), we found that the hydrophilic antioxidant capacity of BR was reduced by germination of BR. However, in some foods other than BR, the hydrophilic antioxidant capacity has also been shown to decrease following soaking (Tiansawang et al., 2016). The increase in TP content may be related to the activation of phenylalanine ammonia-lyase, which is responsible for the synthesis of phenolics during germination (Pramai et al., 2018), and the specific cultivar used in this study may exhibit a low activity of this enzyme. Additionally, immersion can promote the generation of reactive oxygen species by anaerobic metabolism (Das & Roychoudhury, 2014), thereby accelerating the oxidation of phenolic compounds and leading to decreased antioxidant capacity. Furthermore, the increases observed in glucose and fructose content in this study were consistent with the results of prior reports demonstrating the saccharification of starch by amylase and decomposition of disaccharides into monosaccharides by germination with anoxia (Cho & Lim, 2016; Magneschi & Perata, 2009). Taken together with the results of amino acid analysis, our findings indicate that BR was affected by endogenous enzymes and showed sufficient quality after germination.

Our results showed that GABA contents in GBR decreased after cooking at temperatures of 105°C or above, with obvious temperature-dependent changes. Although no prior reports have described changes in BR after cooking, some germinated legumes and sesame have been reported to show decreased GABA contents after boiling, steaming, microwave cooking, or open pan roasting via elution and thermal decomposition (Tiansawang et al., 2016). In our study, the GBR sample was cooked in a beaker, and GABA elution was not affected. Accordingly, we assumed that our results strongly reflected the effects of thermal decomposition.

The levels of nine amino acids were decreased in the cooked groups compared to the noncooked group, and these decreases were dependent on temperature. This finding explained the effects of consumption on the Maillard reaction and thermal decomposition, similar to the trends observed for GABA. Additionally, it may be possible to increase amino acids content other than GABA in cooked rice through the decomposition of protein in rice by induction of proteases during cooking. For example, previous studies have reported increases in the content or activities of endogenous aspartic proteases and cysteine proteases with an optimum temperature of around 50°C in rice (Asakura et al., 1997; Mikola & Suolinni, 1969). In contrast, increases in Glu and Ala levels may be related to the inactivation of metabolic enzymes involved in degradation by heating. Based on these findings, we assumed that the observed reductions were related to the effects of consumption on the Maillard reaction and thermal decomposition.

The DPPH values of samples cooked at 105°C and 115°C were significantly lower than those in noncooked samples but increased at higher temperatures. DPPH activity in GBR has been reported to be reduced by extrusion cooking at 100°C and 120°C (Gujral et al., 2012). Moreover, autoclaving at 115°C has been shown to decrease the DPPH value of soaked beans compared to the noncooked group (Siah et al., 2014), similar to the results observed in our study. Furthermore, DPPH activities in samples cooked at 125°C or higher were similar to those in noncooked samples. Cooking at 100°C or higher is known to produce melanoidin from the condensation reaction with reducing sugars and amino acids in many foods, resulting in high antioxidant capacity, such as
that mediated by the SET mechanism (Shen et al., 2018). Our results confirmed that amino acids acting as substrates for the Maillard reaction were decreased, and the DPPH activity of cooked samples prepared at temperatures of 125°C or higher was greater than that in samples prepared at 115°C or lower, which may be related to the promotion of the Maillard reaction. Therefore, we believe that DPPH activity decreased at low temperatures owing to the decomposition of antioxidant components, such as polyphenols, whereas increases in activity at high temperatures may involve the formation of Maillard reaction products.

L-ORAC values were significantly increased in the 125°C and 135°C groups compared to the noncooked group, and temperature-dependent effects were observed. Polymerization of Maillard reaction products has been reported to proceed depending on the heating temperature (Es-Safi et al., 2000). Additionally, the chain length of the melanoidin molecule, as a Maillard reaction product, increases with polymerization progression (Motai, 1976), potentially because of increased hydrophobicity. Because the L-ORAC method can detect lipophilic antioxidants based on the HAT mechanism, increases in L-ORAC values at temperatures above 125°C suggest that antioxidant ability is enhanced by the formation of Maillard reaction products based on the HAT mechanism.

TP has been reported to be responsible for the antioxidant capacity of grains, fruits, and vegetables (El Gharras, 2009). In this study, we found that TP contents were significantly decreased by cooking. Consistent with this, in a previous study, TP content was found to be decreased in GBR upon extrusion at 100°C, and the TP content decreased further when the extrusion temperature was increased from 100°C to 120°C (Gujral et al., 2012). Another study also demonstrated that cooking at 120°C reduced the contents of TP and polyphenols, such as p-hydroxybenzoic acid, chlorogenic acid, vanillic acid, trans-ferulic acid, and cis-ferulic acid, in BR because of decomposition (Zeng et al., 2016). Therefore, we concluded that this result was also related to thermal decomposition. In contrast, when the cooking temperature is high, the TP content is obviously high; increased TP contents were observed after cooking at 135°C compared to at 105°C, potentially because of the decomposition of polyphenols by heating. This may be why no temperature-dependent changes were observed. Additionally, there were no positive correlations between TP contents and DPPH or H-ORAC values. Overall, these findings suggest that the observed changes in TP contents owing to different heating temperatures likely had little effect on antioxidant capacity.

The H-ORAC value was maximal at 115°C and decreased at higher temperatures. As described above, the Maillard reaction occurs more at 125°C or higher. However, browning substances are produced even at low temperatures and can be detected using the H-ORAC method (Unno, 2015). Thus, products of the Maillard reaction may have contributed to the increased H-ORAC values, although the thermal stability may not be high. We need to identify the structure of products from the Maillard reaction and evaluate their relationship with thermal decomposition in the future.

Notably, our results showed that the Maillard precursors, glucose and fructose, were lost during cooking at temperatures above 105°C. Glucose content showed a temperature-dependent decrease. This change may be attributed to the loss of both glucose and fructose during the Maillard reaction and degradation reaction by heating. Although a previous study showed that glucose and fructose content was increased by heating (Lamberts et al., 2008), our experimental conditions were different, including a higher heating temperature and a longer treatment time. Therefore, the Maillard reaction progressed more, and consumption of both sugars was increased, which may have led to this discrepancy compared with the study by Lamberts et al. (2008).

Increases in redness (a* value) and yellowness (b* value) and decreases in lighting (L* value) were also observed after cooking at 115°C or higher. Similar results were reported for parboiled brown, red, and milled rice using five cultivars, indicating changes according to the Maillard reaction (Lamberts et al., 2006, 2008). Furthermore, we observed changes in L*/a*/b* values in a temperature-dependent manner, consistent with previous reports (Es-Safi et al., 2000). These changes could be explained by the Maillard reaction.

In this study, we observed correlations between L-ORAC values and glucose and L*/a*/b* values, suggesting that Maillard reactants contributed significantly to the increased lipophilic antioxidant capacity. However, analysis of fructose content did not support the promotion of the Maillard reaction. Fructose is also expected to be consumed as a substrate in the Maillard reaction; however, above 125°C, its consumption was equivalent to that in the noncooked group and showed no negative correlation. Starch and sucrose in GBR are expected to increase in absolute amounts of glucose and fructose due to enzymatic decomposition and saccharification during temperature rise (Cho & Lim, 2016). Furthermore, it is also believed that glucose-fructose isomerization reaction induces glucose to fructose conversion during the heating process (Lamberts et al., 2008). Therefore, this result implies that the high-temperature group was prone to glucose isomerization when the temperature rises. As a result, the amount of fructose produced exceeded the amount of fructose consumed in the Maillard reaction, showing no negative correlation. Although we observed a negative correlation between DPPH value and glucose content, it is believed that glucose consumption, as a substrate for the Maillard reaction, increased the production of substances showing DPPH activity. It was considered that there was no correlation with fructose is considered because of the same effect described above. Further, there are close relationships between DPPH activity and the Maillard reaction in other foods, indicating increased DPPH activity (Ujihara et al., 2013). Thus, our current findings indicated that the formation of antioxidants derived from the Maillard reaction strongly contributed to the increase in L-ORAC values and to increases in DPPH values at high temperatures.

Notably, our results showed that with cooking at 105°C for 40–90 min, the contents of all amino acids, except Glu, Gly, and Ala, decreased in a time-dependent manner. This effect was thought to be related to thermal decomposition and consumption of substances by the Maillard reaction. Therefore, we could infer that if the heating temperature is raised, the amino acid contents may decrease further.

DPPH and H-ORAC values, as well as TP contents, decreased in a time-dependent manner. From previous reports, TP
contents are thought to decrease owing to thermal decomposition (Gujral et al., 2012). In addition, a positive correlation was observed between TP content and DPPH value. That is, decreased TP contents greatly contributed to decreased antioxidant capacity owing to the extension of heating time. However, L-ORAC values did not change with time. As described above, when processed at 125°C or higher, the production of Maillard reaction products showing antioxidant capacity was increased. Overall, if the cooking temperature is increased, changes in incubation time would be expected to contribute to increasing DPPH and L-ORAC values as long as the substrate was present.

Based on the results described above, we predicted that when GBR was cooked at 105 to 135°C for less than 40 min, the levels of 13 amino acids, including GABA and TP, as well as the DPPH and H-ORAC values, would only minimally decrease. Furthermore, the results suggest that the effect of increasing DPPH and L-ORAC values at temperatures above 125°C and H-ORAC values at temperatures above 115°C during the Maillard reaction would also be small. Some researchers have reported that GABA contents and antioxidant capacity differ among BR cultivars (Cho & Lim, 2016; Owolabi et al., 2018). In this study, changes in both indicators were observed based on processing conditions, reflecting chemical reactions, not the metabolic activities of endogenous enzymes; this suggests that differences in reactivity between cultivars would be expected to be small. Accordingly, we believe it is likely that other cultivars would yield results similar to those found here.

Our results revealed that heating reduced GABA content by approximately 33–59%, and a cooking temperature of 105°C yielded the highest residual GABA among the temperatures investigated. To maintain the GABA content equivalent of uncooked GBR, it is necessary to process it at less than 100°C. In the future, we need to further clarify functional processing conditions for GBR below 100°C. In contrast, temperatures of 125°C or higher (DPPH and L-ORAC) and 115°C (H-ORAC) enhanced antioxidant capacity. Thus, these findings may be useful for consumers and food manufacturers considering the intake of GABA and the antioxidant capacity of GBR after boiling and cooking using other approaches. On the other hand, there are foods, such as bread and cookies, that are processed in a temperature range higher than 135°C; hence, we need to perform evaluations considering such temperatures in the future. Furthermore, based on this knowledge, functional foods may be developed with a lot of GABA content or enhanced antioxidant activity after processing in the future, utilizing useful cultivars showing high increases in GABA and antioxidant activity following germination treatment. However, since the useful cultivars are yet unknown, we need to disclose the cultivars with high functionality even after processing.

5. Conclusions

Our findings in the current study indicated that increased heating temperature during GBR cooking enhanced the loss of GABA content by thermal decomposition, whereas cooking at a temperature of 125°C or higher enhanced antioxidant capacity based on DPPH and L-ORAC methods. Further, cooking at 115°C increased antioxidant capacity based on the H-ORAC method. In addition, Maillard reaction products were involved in the enhancement of antioxidant capacity, and the possibility of further increases by prolonging the cooking time was also indirectly demonstrated. Thus, the processing of GBR can provide useful information for determining the functional ingredients and activities of rice when cooking at home and developing processed products in the future.

Acknowledgments

We thank Ms. Michiko Kanazawa for her technical support.

Declaration of interest

No potential conflict of interest was reported by the authors.

Funding

This work was supported by JSPS KAKENHI (grant no. 18K13043).

ORCID

Tomoyasu Toyozumi http://orcid.org/0000-0003-0127-1382

References

Asakura, T., Watanabe, H., Abe, K., & Arai, S. (1997). Oryzasin as an aspartic proteinase occurring in rice seeds: Purification, characterization, and application to milk clotting. Journal of Agricultural and Food Chemistry, 45(4), 1070–1075. https://doi.org/10.1021/jf960582x

Callaway, E. (2014). The birth of rice. Nature, 514(7524), 558–559. https://doi.org/10.1038/514558a

Cho, D. H., & Lim, S. T. (2016). Germinated brown rice and its bio-functional compounds. Food Chemistry, 196, 259–271. https://doi.org/10.1016/j.foodchem.2015.09.025

Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers of Environmental Science and Engineering, 3(2), 1–13. https://doi.org/10.3389/fenvs.2014.00053

El Gharras, H. (2009). Polyphenols: Food sources, properties and applications—a review. International Journal of Food Science & Technology, 44(12), 2512–2518. https://doi.org/10.1111/j.1365-2621.2009.02077.x

Es-Safi, N. E., Cheynier, V., & Moutounet, M. (2000). Study of the reactions between (+)-catechin and furfural derivatives in the presence or absence of anthocyanins and their implication in food color change. Journal of Agricultural and Food Chemistry, 48(12), 5946–5954. https://doi.org/10.1021/jf000394d

Fait, A., Fromm, H., Walter, D., Gallili, G., & Fernie, A. R. (2008). Highway or byway: The metabolic role of the GABA shunt in plants. Trends in Plant Science, 13(1), 14–19. https://doi.org/10.1016/j.tplants.2007.10.005

Food and Agriculture Organization of the United Nations. (2020). Cereal supplies to remain ample in 2020/21 despite this month’s cut in global production forecast. http://www.fao.org/faostat/en/#home

Goufo, P., & Trindade, H. (2014). Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. Food Science & Nutrition, 2(2), 75–104. https://doi.org/10.1002/fsn3.86

Gujral, H. S., Sharma, P., Kumar, A., & Singh, B. (2012). Total phenolic content and antioxidant activity of extruded brown rice. International Journal of Food Properties, 15(2), 301–311. https://doi.org/10.1080/10994291.2010.483617

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 53(6), 1841–1856. https://doi.org/10.1021/jf030723c

Inoue, K., Shirai, T., Ochiai, H., Kasao, M., Hayakawa, K., Kimura, M., & Sansawa, H. (2003). Blood-pressure-lowering effect of a novel fermented rice containing γ-aminoxybutyric acid (GABA) in mild hypertensives. European Journal of Clinical Nutrition, 57(3), 490–495. https://doi.org/10.1038/sj.ejcn.1601555

Ito, M., Ohara, E., Kobayashi, A., Yamazaki, A., Kaji, R., Yamaguchi, M., Ishizaki, K., Nara, E., & Ohtsubo, K. (2011). Antioxidant capacities and polyphenol content of colored rice cultivars. Journal of Japan Society
of Nutrition and Food Science, 58(12), 576–582. (Japanese). https://doi.org/10.3136/nskkk.58.58.576
Jiamyangyuen, S., & Oorakul, B. (2008). The physico-chemical, eating and sensorial properties of germinated brown rice. The Journal of Food Agriculture and Environment, 6(2), 119–124.
Kim, H. (2013). Functional foods and the biomedicalisation of everyday life: A case of germinated brown rice. Sociology of Health & Illness, 35(6), 842–857. https://doi.org/10.1111/j.1467-9566.2012.01533.x
Kinoshita, A., & Yamamoto, K. (2012). Comparison of germinated and non-germinated brown rice with fatty acids. Journal for the Integrated Study of Dietary Habits, 23(2), 117–120. (Japanese) https://doi.org/10.2740/jsidh.23.117
Komatsuaki, N., Tsukahara, K., Toyoshima, H., Suzuki, T., Shimizu, N., & Kimura, T. (2007). Effect of soaking and gaseous treatment on GABA content in germinated brown rice. Journal of Food Engineering, 78(2), 556–560. https://doi.org/10.1016/j.foodeng.2005.10.036
Lamberts, L., De Bie, E., Derycke, V., Veraverbeke, W. S., De Man, W., & Delcour, J. A. (2006). Effect of processing conditions on color change of brown and milled parboiled rice. Cereal Chemistry Journal, 83(1), 80–85. https://doi.org/10.1093/ccc/83.0080
Lamberts, L., Rombouts, I., Bijis, K., Gebruers, K., & Delcour, J. A. (2008). Impact of parboiling conditions on Maillard precursors and indicators in long-grain rice cultivars. Food Chemistry, 110(4), 916–922. https://doi.org/10.1016/j.foodchem.2008.02.080
Maboshi, Y., Ookura, T., and Kasai, M. (2007). Effects of rate of temperature increase and holding temperature during cooking on the amounts of chemical components in rice grains. Journal of Cookery Science of Japan, 40(5), 323–328. (Japanese). https://doi.org/10.114102/cookeryscience1995.40.5.323
Magneschi, L., & Perata, P. (2009). Rice germination and seedling growth in the absence of oxygen. Annals of Botany, 103(2), 181–196. https://doi.org/10.1093/aob/mcn121
Mikola, J., & Suolimaa, E. M. (1969). Purification and properties of a trypsin inhibitor from barley. European Journal of Biochemistry, 9 (4), 555–560. https://doi.org/10.1111/j.1432-1033.1969.tb00645.x
Moonggarm, A., & Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. Food Chemistry, 122(3), 782–788. https://doi.org/10.1016/j.foodchem.2010.03.053
Motai, H. (1976). Viscosity of melanoidins formed by oxidative browning: Validity of the equation for a relationship between color intensity and molecular weight of melanoidin. Agricultural and Biological Chemistry, 40(1), 1–7. https://doi.org/10.1007/BF023169.1976.10862002
Owolabi, I. O., Saibandith, B., Wichienchat, S., & Yupanqui, C. T. (2018). Nutritional compositions, polyphenolic profiles and antioxidant properties of pigmented rice varieties and adlay seeds enhanced by soaking and germination conditions. The Functional Foods in Health and Disease, 8(2), 561–578. https://doi.org/10.31989/fhdv.812.564
Patil, S. B., & Khan, M. K. (2011). Germinated brown rice as a value added rice product: A review. Journal of Food Science and Technology, 48(6), 661–667. https://doi.org/10.1007/s13197-011-0232-4
Pramai, P., Hamid, N. A. A., Mediani, A., Maulidini, M., Abas, F., & Jiamyangyuen, S. (2018). Metabolite profiling, antioxidant, and α-glucosidase inhibitory activities of germinated rice: Nuclear-magnetic-resonance-based metabolomics study. Journal of Food and Drug Analysis, 26(1), 47–57. https://doi.org/10.1016/j.jfda.2016.11.023
Saleh, A. S., Wang, P., Wang, N., Yang, L., & Xiao, Z. (2019). Brown rice versus white rice: Nutritional quality, potential health benefits, development of food products, and preservation technologies. Comprehensive Reviews in Food Science and Food Safety, 19(4), 1070–1096. https://doi.org/10.1111/1541-4337.12449
Selfried, H. E., Anderson, D. E., Fisher, E. I., & Milner, J. A. (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. The Journal of Nutritional Biochemistry, 18(9), 567–579. https://doi.org/10.1016/j.jnutbio.2006.10.007
Shen, Y., Chen, G., & Li, Y. (2018). Bread characteristics and antioxidant activities of Maillard reaction products of white pan bread containing various sugars. LWT, 95, 308–315. https://doi.org/10.1016/j.lwt.2018.05.008
Siah, S., Wood, J. A., Agboola, S., Konczak, I., & Blanchard, C. L. (2014). Effects of soaking, boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (Vicia faba L.) differing in seed coat colours. Food Chemistry, 142, 461–468. https://doi.org/10.1016/j.foodchem.2013.07.068
Soponronnarit, S., Natharakarnkule, A., Jirajindalert, A., & Taechapairoj, C. (2006). Parboiling brown rice using super heated steam fluidization technique. Journal of Food Engineering, 75(3), 432–433. https://doi.org/10.1016/j.jfoodeng.2005.04.058
Surh, J., & Koh, E. (2014). Effects of four different cooking methods on anthocyanins, total phenolics and antioxidant activity of black rice. Journal of the Science of Food and Agriculture, 57(3), 3296–3304. https://doi.org/10.1002/jsfa.6690
Tiansawang, K., Luangpituka, P., Varanyanond, W., & Hansawasdi, C. (2016). GABA (γ-aminobutyric acid) production, antioxidant activity in some germinated dietary seeds and the effect of cooking on their content. Food Science and Technology, 36(2), 313–321. https://doi.org/10.1590/1678-457X.0080
Toyozumi, T., Ohba, S., Takano-Ishikawa, Y., Ikegaya, A., & Nakajima, T. (2020). Placental tissue of greenhouse muskmelon (Cucumis melo L.) contains more gamma-aminobutyric acid with antioxidant capacity than the fleshe pulp. Bioscience, Biotechnology, and Biochemistry, 83(1), 1211–1220. https://doi.org/10.1007/s13113-020-17290-89
Toyozumi, T., Yamamoto, H., & Sasaki, M. (2015). Exploring of steaming condition for elderly people on carrot using low temperature steam cooking. Journal of Japan Society of Nutrition and Food Science, 62(7), 341–348. (Japanese) https://doi.org/10.3136/nskk.62.341
Ujihara, K., Yoshimoto, M., Wada, K., Takahashi, M., & Suda, I. (2013). Enhancement of DPPH-radical scavenging activity in heat-processed sugarcane molasses. Nippon Shokuhin Kagaku Kaigi Kaishi, 60(4), 159–164. (Japanese). https://doi.org/10.3136/nskkk.60.159
Unno, T. (2015). Antioxidant activity of different grades of maple syrup as determined by the hydrophilic oxygen radical absorbance capacity method. Food Science and Technology, 21(3), 495–498. https://doi.org/10.3136/fstr.21.495
Watanabe, J., Oki, T., Takebayashi, J., & Takeano-Ishikawa, Y. (2014). Extraction efficiency of hydrophilic and lipophilic antioxidants from lyophilized foods using pressurized liquid extraction and manual extraction. Journal of Food Science, 79(9), C1665–C1671. https://doi.org/10.1111/1750-3841.12570
Xia, Q., Green, B. D., Zhu, Z., Li, Y., Gharibzahedi, S. M. T., Roochinejad, S., & Barba, F. J. (2019). Innovative processing techniques for altering the physicochemical properties of wholegrain brown rice (Oryza sativa L.)— Opportunities for enhancing food quality and health attributes. Critical Reviews in Food Science and Nutrition, 59(20), 3349–3370. https://doi.org/10.1080/104008398.2018.1491829
Zeng, Z., Liu, C., Luo, S., Chen, J., & Gong, E. (2016). The profile and bioassailability of phenolic compounds in cereals influenced by improved extraction cooking treatment. PLoS One, 11(8), 1–11. https://doi.org/10.1371/journal.pone.0161086