

## Variations in Physicochemical Properties of Brown Rice (*Oryza sativa* L.) During Storage

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**Abstract** Present study deals with variations of physicochemicals including  $\gamma$ -aminobutyric acid (GABA),  $\gamma$ -oryzanol, free sugar, lipoxygenase activity, fat acidity, and germination rate from Korean brown rice cultivars. With increase of storage time and temperature, GABA,  $\gamma$ -oryzanol, lipoxygenase activity, and fat acidity increased, whereas free sugar and germination rate was reduced. Among cultivars, 'Gopumbyeo' exhibited the highest contents in GABA and  $\gamma$ -oryzanol during 12 weeks storage at 25°C (GABA: 28.6±5.6→170.4±4.6 mg/100 g,  $\gamma$ -oryzanol: 6.1±0.7→6.7±0.4 mg/g) and 'Ilpumbyeo' significantly decreased in free sugar during 12 weeks storage at 10°C (1,423.7→1,058.4 mg/100 g). Moreover, 'Taebongbyeo' exhibited the highest quality owing to low lipoxygenase activity, low fat acidity, and high germination rate. In free sugar compositions, sucrose exhibited the highest content (>70%), followed by fructose (>7%), raffinose (>5%), glucose (>3%), and maltose (>2%) during storage. Based on our results, changes of physicochemicals in stored brown rice may be important information in processing food and functional properties.

**Key words:** brown rice,  $\gamma$ -aminobutyric acid,  $\gamma$ -oryzanol, physicochemical, storage

### Introduction

Rice (*Oryza sativa* L.) has been one of the most leading crops (1-3). This crop possesses many valuable physicochemicals and its quality depends on storage condition (4,5). Especially, many studies have reported health benefits concerning human diseases from brown rice. In many physicochemicals of this species,  $\gamma$ -aminobutyric acid (GABA) is involved in the regulation of cardiovascular functions (6). Moreover, this component has potential beneficial effects on several physiological functions including neurotransmission and induction of hypotensive effects, diuretic effects, and tranquilizes effects (7-9).  $\gamma$ -Oryzanol has some bioavailabilities such as inhibition of tumor promotion, reduction of serum cholesterol levels and antioxidant properties (10-12). It is well established that 3 physicochemicals including lipoxygenase activity, fat acidity, and germination rate are responsible for the rice quality (5,13,14). Among them, lipoxygenase activity has suggested that degradation of lipid could play a key role in the deteriorative changes during storage (15,16). Fat acidity was mainly used as an index of quality deterioration during rice storage because lipid dissolution progressed more rapidly than those of other components (17).

There are several reports on the changes of physicochemicals including physical, chemical, biological, and

nutritional properties (13,18,19). Furthermore, numerous literatures showed changes in GABA and  $\gamma$ -oryzanol during germination (20,21). However, changes in GABA,  $\gamma$ -oryzanol, and free sugar have been few studied in different storage conditions. For this reason, we investigated changes of physicochemical contents from 5 brown rice cultivars during 4, 8, and 12 weeks storage at low (10°C) and room (25°C) temperatures.

### Materials and Methods

**Plant material** Five Korean brown rice cultivars, 'Taebongbyeo', 'Gopumbyeo', 'Hwasungbyeo', 'Samkwangbyeo', and 'Ilpumbyeo' were harvested in September 2007, in the experimental field of National Institute of Crop Science (NICS), Rural Development Administration (RDA), Suwon, Korea. All cultivars were grown in the same conditions to avoid variations of characters concerning environmental factors.

**Storage conditions** After harvesting, 5 cultivars were dried and hulled with a milling machine (MR 701; J World Tech, Seoul, Korea). The hulled grains (each 500 g) were packed in polyethylene bottles with screw caps. The bottles were placed in an incubator for 4, 8, and 12 weeks at 10 and 25°C.

**Reagents**  $\gamma$ -Aminobutyric acid (GABA) was prepared from Tokyo Kasei (Kogyo Co., Tokyo, Japan) and  $\gamma$ -oryzanol as well as 5 free sugar compositions were obtained from Sigma-Aldrich (St. Louis, MO, USA).

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Potassium phosphate, linoleic acid (purity>99%), and lipoxygenase (E.C. 1.13.11.12) were purchased from Sigma-Aldrich. Analytical grade diethyl ether, benzene, and phenolphthalein were purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

**Instruments** Brown rice was stored using an incubator (DS-53CP; Dasol Scientific, Ltd., Seoul, Korea). Chromatographic separation was achieved using an Agilent 1100 series (Hewlett-Packard, Boeblingen, Germany) equipped with a binary gradient high performance liquid chromatography (HPLC) pump (G1312A; Agilent Technologies, Palo Alto, CA, Germany) and an Agilent 1100 series diode array detector. In spectrophotometric experiment, GABA and lipoxygenase activity were monitored by an Infinite M200 spectrophotometer (Tecan Austria GmbH, Salzburg, Austria) and determined using an oxygen monitoring system (Qubit Systems Inc., Kingston, ON, Canada) to measure oxygen uptake. The extracts of brown rice were centrifuged with Vision apparatus (VS-30000MT; Vision, Seoul, Korea). The filtrate was evaporated under the round-bottom flask of a rotary evaporator (RE. IKA RV05basic 1B; Ika, Staufen, Germany) for fat acidity analysis.

**Measurement of GABA** GABA content in stored brown rice was extracted according to the method of Oh and Oh (22) with a slight modification. Shortly, the mixture organic solution (CH<sub>3</sub>OH 5 mL:CHCl<sub>3</sub> 10 mL:H<sub>2</sub>O 5 mL) was added to pulverized brown rice (1.0 g). The aqueous solution layer containing GABA was obtained through centrifugation (2,800×g, 4°C, 10 min) and the supernatant was then freeze dried. GABA content was measured by a 1.0 mL/assay system in spectrophotometric experiment at 340 nm (23).

**Measurement of  $\gamma$ -oryzanol** Change in  $\gamma$ -oryzanol from brown rice oil during storage was measured according to methods of Rogers *et al.* (24) and Chotimarkorn *et al.* (25). Oil was weighted exactly 100 mg and dissolved in 1.0 mL of propanol before filtering through 0.45- $\mu$ m syringe filter (Millipore MSI, Westboro, MA, USA). The HPLC analysis was performed by Agilent 1100 series equipped with Hypersil octadecyl saline (ODS) column (4.0×250 mm, 5  $\mu$ m, Agilent Technologies) with the wavelength set at 330 nm. The mobile phase was used MeOH:CH<sub>3</sub>CN:CH<sub>2</sub>Cl<sub>2</sub>:CH<sub>3</sub>COOH (50:44:3:3) at 1.0 mL/min.  $\gamma$ -Oryzanol was identified by the retention time and calculated by comparing the peak areas of samples with the standard calibration curve.

**Measurement of free sugar** For free sugar analysis, the homogenized brown rice (0.5 g) was extracted with 20 mL of 80% ethanol for 6 hr at 25°C, and then centrifuged at 5,000×g for 20 min (26). The supernatant was filtered through a 0.45- $\mu$ m membrane filter (Millipore) and the filtrate (20  $\mu$ L) was injected into a HPLC system equipped with a RID-10A refractive index detector. The operating conditions were as follows: column temperature, 30°C; mobile phase, CH<sub>3</sub>CN:H<sub>2</sub>O (75%:25%); flow rate, 1 mL/min. Free sugar contents including glucose, sucrose, fructose, raffinose, and maltose were calculated by comparing the HPLC peak areas with the standard calibration curves.

**Measurement of lipoxygenase activity** The lipoxygenase activity was performed as previously reported method (27) with slightly modification. Briefly, the pulverized brown rice (1.0 g) was homogenized with 6 mL of 0.1 M phosphate buffer (pH 7.0) for 1 hr at 4°C. After centrifugation at 12,000×g for 25 min, the supernatant was stored at -75°C. The substrate consisted of 10  $\mu$ L linoleic acid, 4 mL H<sub>2</sub>O, 1 mL 0.1 N NaOH, and 5  $\mu$ L Tween 20. The mixture substrate solution was mixed by vortex and diluted to 25 mL with 0.2 M phosphate buffer. The reaction mixture contained 1.8 mL 0.1 M phosphate buffer of pH 6.8, 50  $\mu$ L linoleic acid, and 150  $\mu$ L enzyme extracts, respectively. After mixing, the absorbance value was measured at 234 nm. The activity (1 unit) was defined as 1  $\mu$ mol of oxygen consumed/min at 25°C (28).

**Measurement of fat acidity** The analytical method of fat acidity was determined by Association of Official Analytical Chemicals (AOAC) (26). Determination of fat acidity was measured as mg of KOH needed to neutralize free fatty acid involved in 100 g (mg KOH/100 g) of pulverized brown rice. The powder (10 g) was stirred in benzene (25 mL) at 25°C for 10 min. The mixture solution was filtered through the filter paper (Whatman No. 42), and then this residue (15 mL) was mixed with solution of phenolphthalein in ethanol (15 mL). The mixture solution was titrated with 0.0178 KOH. Ending point of the titration was determined when the solution turned to pink color.

**Measurement of germination rate** The grains of brown rice cultivars (100 g) were decontaminated with sodium hypochlorite solution. The sterilized grains were placed on a petri dish covered with a wet filter paper (Advantec No. 2, 90-mm). The petri dish was placed in an incubator set at 25°C and the ratio of germinated grains was counted after 7 days (9).

**Statistical analysis** All measurements were repeated 3 times and the results were as the mean±standard deviation (SD). Results were analyzed by using Sigma plot 2001 (SPSS Inc., Chicago, IL, USA).

## Results and Discussion

**Changes in GABA during storage** It is well established that GABA is enhanced by germinated brown rice in comparison with nongerminated brown rice (22). Moreover, many researchers studied the effects of pretreatment conditions on GABA changes (29). However, changes in GABA content from brown rice of different storage systems have been few studied. In order to find out the effects of the various storage conditions, we proceeded to examine GABA from 5 cultivars during 12 weeks at 2 temperatures and their results showed in Table 1. Among the 5 cultivars, 'Gopumbyeo' exhibited the highest content and variation (128.6±5.6→170.4±4.6 mg/100 g). Interestingly, 'Hwasungbyeo' and 'Samkwangbyeo' were not observed GABA in postharvest state and 4 weeks storage. However, these cultivars slightly increased during 8 and 12 weeks storage at 25°C. As can be seen in Table 1, 'Hwasungbyeo' ranged between 3.3±0.2 (8 weeks, 25°C) and 4.2±0.4 mg/100 g (12 weeks, 25°C) and 'Samkwangbyeo' showed in

**Table 1. Changes of GABA and  $\gamma$ -oryzanol in 5 Korean brown rice cultivars under different storage conditions**

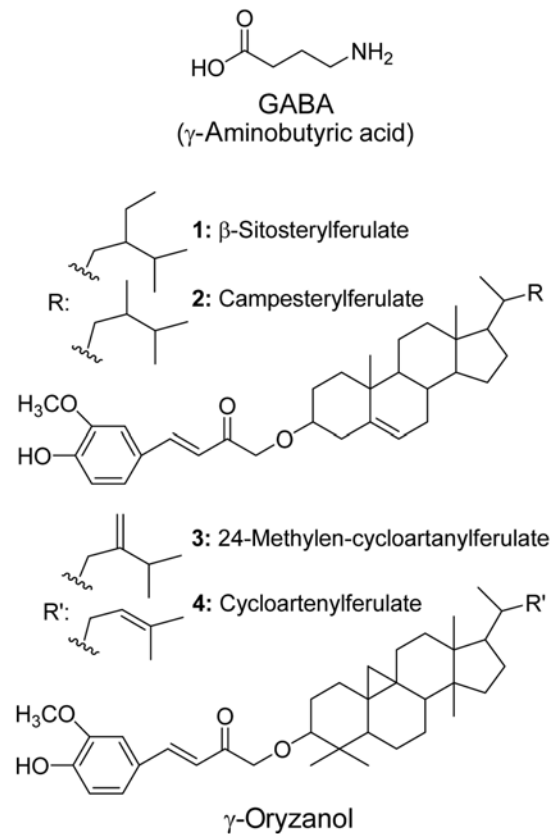
| Cultivar  | Postharvest | 4 weeks Storage |           | 8 weeks Storage |           | 12 weeks Storage |           |
|---|-------------|-----------------|-----------|-----------------|-----------|------------------|-----------|
|   |             | 10°C            | 25°C      | 10°C            | 25°C      | 10°C             | 25°C      |
| GABA content (mg/100 g) <sup>1)</sup>           |             |                 |           |                 |           |                  |           |
| Taebongbyeo                                     | 80.1±3.4    | 82.5±2.9        | 92.4±5.4  | 88.5±2.2        | 99.3±4.1  | 101.2±4.7        | 103.5±2.9 |
| Gopumbyeo                                       | 128.6±5.6   | 134.2±4.5       | 149.7±4.9 | 143.2±3.6       | 164.2±3.2 | 146.9±2.9        | 170.4±4.6 |
| Hwasungbyeo                                     | ND          | ND              | ND        | ND              | 3.3±0.2   | ND               | 4.2±0.4   |
| Samkwangbyeo                                    | ND          | ND              | ND        | ND              | 2.4±0.2   | ND               | 2.6±0.1   |
| Ilpumbyeo                                       | 95.2±6.8    | 101.30±4.90     | 129.0±4.5 | 109.5±3.7       | 143.6±3.1 | 113.4±4.4        | 149.2±4.1 |
| $\gamma$ -Oryzanol content (mg/g) <sup>1)</sup> |             |                 |           |                 |           |                  |           |
| Taebongbyeo                                     | 4.2±0.56    | 4.2±0.4         | 4.3±0.2   | 4.4±0.7         | 4.6±0.7   | 4.4±0.4          | 4.7±0.3   |
| Gopumbyeo                                       | 6.1±0.7     | 6.3±0.4         | 6.3±0.4   | 6.3±0.4         | 6.4±0.7   | 6.3±0.3          | 6.7±0.4   |
| Hwasungbyeo                                     | 4.1±0.6     | 4.0±0.6         | 4.2±0.4   | 4.3±0.4         | 4.3±0.2   | 4.3±0.2          | 4.9±0.2   |
| Samkwangbyeo                                    | 4.0±0.9     | 4.3±0.7         | 4.2±0.6   | 4.3±0.4         | 4.4±0.5   | 4.4±0.3          | 4.7±0.4   |
| Ilpumbyeo                                       | 4.7±0.8     | 4.7±0.3         | 4.8±1.0   | 4.9±0.4         | 5.1±0.3   | 4.9±0.4          | 5.3±0.4   |

<sup>1)</sup>The values indicate the mean±SD ( $n=3$ ) for GABA and  $\gamma$ -oryzanol contents of each sample; ND, not detected.

the range of 2.4±0.2 and 2.6±0.1 mg/100 g. These above results suggested that GABA was significantly influenced by 25°C in comparison with 10°C storage. In other words, this component has a great impact on high temperature as soaking and steaming effects through heat treatment (20). Moreover, GABA increased substantially with increase of storage time (4<8<12 weeks). As a result, this study was first documented that GABA in stored brown rice showed considerable fluctuations according to storage time and temperature.

**Changes in  $\gamma$ -oryzanol during storage** As shown in Fig. 1,  $\gamma$ -oryzanol in brown rice was a mixture of phytosterol and campesteryl ferulate such as cycloartenyl ferulate, 24-methylenecycloartenyl ferulate, and campesteryl ferulate (10). Also, this component displayed antioxidant properties, improvement of plasma lipid, and high density lipoprotein-cholesterol (30). On the basis of the above health benefits, many researchers have been focused on  $\gamma$ -oryzanol (21,31). Although germination effects have been carried out previous report (21), to the best of our knowledge, there has been no report on the variations of  $\gamma$ -oryzanol from brown rice under the various storage conditions. As can be seen in Table 1, with increase of storage time and temperature,  $\gamma$ -oryzanol was slightly increased and temperature showed more effect than time. Thus, temperature may be discriminating factor concern to  $\gamma$ -oryzanol in brown rice during storage. Among cultivars, ‘Gopumbyeo’ showed the highest content in postharvest state (6.1±0.1 mg/g) and 12 weeks storage (6.7±0.4 mg/g) at 25°C. In contrast, ‘Samkwangbyeo’ was observed the lowest content (postharvest state: 4.0±0.9 mg/g and 12 weeks storage at 25°C: 4.7±0.4 mg/g). From these results, ‘Gopumbyeo’ showed the highest contents in GABA and  $\gamma$ -oryzanol during storage and might be very important source in processing food and functional supplement.

**Changes in free sugar during storage** It is well-known that storage conditions of temperature, time, and air are important factors for hydrolysis and oxidation (32). Therefore, hydrolysis, degradation, and decomposition in

**Fig. 1. Chemical structures of GABA and  $\gamma$ -oryzanol.**

stored rice occurred, which caused an increase in reducing free sugar content (33). However, few studies have examined the variations of free sugar in different storage conditions (6). Changes in 5 free sugar compositions of brown rice cultivars under the various storage conditions are summarized in Table 2. As can be seen, with increase of storage time, free sugar content decreased. Moreover, free sugar at 10°C storage showed higher content than that of 25°C. Among cultivars, ‘Ilpumbyeo’ [1,423.7→1,058.4

mg/100 g (12 weeks, 10°C) and 1,423.7→1,126.5 mg/100 g (12 weeks, 25°C)] showed the highest variations, while 'Samkwangbyeo' [877.9→639.3 mg/100 g (12 weeks, 10°C) and 877.9→779.0 mg/100 g (12 weeks, 25°C)] was observed the lowest during different storage conditions.

The contents of free sugar compositions also showed significant differences during storage. Sucrose (>70%) exhibited the predominant content, followed by fructose (>7%), raffinose (>5%), glucose (>3%), and maltose (>2%). These results suggested that free sugar content diversified

**Table 2. Changes of free sugar in 5 Korean brown rice cultivars under different storage conditions**

| Cultivar     | Storage period (week) | Free sugar composition content (mg/100 g) |              |           |            |          |         |
|--------------|-----------------------|---|--------------|-----------|------------|----------|---------|
|              |                       | Glucose                                   | Sucrose      | Fructose  | Raffinose  | Maltose  | Total   |
| 10°C Storage |                       |   |              |           |            |          |         |
| Taebongbyeo  | Postharvest           | 35.3±2.4 <sup>1)</sup>                    | 723.4±14.3   | 80.5±1.5  | 54.3±1.6   | 24.8±0.9 | 918.3   |
|              | 4                     | 24.7±1.9                                  | 610.9±11.1   | 56.2±1.3  | 46.5±1.0   | 11.7±0.7 | 750.0   |
|              | 8                     | 18.6±1.1                                  | 574.2±16.1   | 49.3±1.1  | 38.9±1.1   | 9.2±0.5  | 690.2   |
|              | 12                    | 16.2±1.0                                  | 530.9±15.4   | 43.7±1.0  | 37.1±1.2   | 9.0±0.8  | 636.9   |
| Gopumbyeo    | Postharvest           | 49.4±3.4                                  | 884.5±16.2   | 98.5±2.0  | 91.4±4.0   | 16.9±1.5 | 1,140.7 |
|              | 4                     | 31.3±2.1                                  | 710.2±15.5   | 71.0±2.2  | 74.7±2.5   | 14.0±0.5 | 901.2   |
|              | 8                     | 28.5±1.8                                  | 652.8±8.5    | 61.5±2.3  | 68.3±2.9   | 12.8±1.3 | 823.9   |
|              | 12                    | 26.5±1.2                                  | 640.8±4.6    | 57.9±1.7  | 61.5±2.0   | 11.1±0.9 | 797.8   |
| Hwasungbyeo  | Postharvest           | 74.6±2.9                                  | 820.8±14.1   | 90.9±3.7  | 80.3±3.1   | 14.9±0.9 | 1,081.5 |
|              | 4                     | 50.9±1.7                                  | 730.5±9.9    | 72.4±3.5  | 68.5±4.3   | 13.0±1.4 | 935.3   |
|              | 8                     | 41.7±2.0                                  | 680.9±11.2   | 61.7±2.8  | 57.9±2.5   | 10.4±1.8 | 852.6   |
|              | 12                    | 32.9±1.7                                  | 630.6±8.9    | 54.1±2.2  | 50.8±1.7   | 9.9±0.9  | 778.3   |
| Samkwangbyeo | Postharvest           | 50.1±2.0                                  | 610.3±18.5   | 110.7±4.1 | 106.8±84.2 | ND       | 877.9   |
|              | 4                     | 28.1±1.7                                  | 532.8±10.9   | 88.2±3.5  | 79.4±4.3   | ND       | 728.5   |
|              | 8                     | 21.5±2.1                                  | 493.8±4.4    | 74.9±2.7  | 70.1±3.8   | 7.0±1.0  | 667.3   |
|              | 12                    | 18.8±1.5                                  | 476.9±4.0    | 68.1±1.9  | 64.9±4.0   | 10.6±0.6 | 639.3   |
| Ilpumbyeo    | Postharvest           | 54.8±2.4                                  | 1,194.2±18.0 | 90.8±9.4  | 75.9±2.9   | 8.0±1.4  | 1,423.7 |
|              | 4                     | 30.2±1.9                                  | 970.4±14.7   | 58.77±6.7 | 50.8±3.2   | ND       | 1,110.2 |
|              | 8                     | 26.4±1.4                                  | 964.0±15.0   | 48.87±3.0 | 46.9±1.9   | ND       | 1,086.1 |
|              | 12                    | 20.9±1.5                                  | 955.7±11.9   | 41.63±2.4 | 40.1±1.9   | ND       | 1,058.4 |
| 25°C Storage |                       |   |              |           |            |          |         |
| Taebongbyeo  | Postharvest           | 35.3±2.4                                  | 723.4±14.3   | 80.5±1.5  | 54.3±1.6   | 24.8±0.9 | 918.3   |
|              | 4                     | 30.0±2.1                                  | 680.7±8.9    | 95.0±2.8  | 43.8±2.1   | 16.1±1.4 | 865.6   |
|              | 8                     | 28.7±1.9                                  | 640.2±8.1    | 110.6±3.2 | 39.4±1.8   | 13.9±1.5 | 832.8   |
|              | 12                    | 27.8±2.1                                  | 564.8±7.0    | 130.9±3.8 | 34.0±2.3   | 13.8±1.4 | 771.3   |
| Gopumbyeo    | Postharvest           | 49.4±3.4                                  | 884.5±16.2   | 98.5±2.0  | 91.4±4.0   | 16.9±1.5 | 1,140.7 |
|              | 4                     | 35.7±1.2                                  | 736.5±8.8    | 108.7±2.0 | 80.6±2.3   | 13.5±1.1 | 974.7   |
|              | 8                     | 32.5±1.6                                  | 693.7±8.1    | 126.8±2.9 | 75.6±2.0   | 12.9±0.7 | 941.5   |
|              | 12                    | 30.0±0.9                                  | 688.1±7.6    | 133.1±2.5 | 70.9±2.4   | 12.4±1.0 | 934.5   |
| Hwasungbyeo  | Postharvest           | 74.6±2.9                                  | 820.8±14.1   | 90.9±3.7  | 80.3±3.1   | 14.9±0.9 | 1,081.5 |
|              | 4                     | 60.5±1.5                                  | 765.5±11.6   | 89.5±2.5  | 72.7±.5    | 14.0±1.0 | 1,002.2 |
|              | 8                     | 53.7±1.6                                  | 716.8±9.4    | 104.8±3.1 | 65.4±2.1   | 13.2±1.2 | 953.9   |
|              | 12                    | 44.8±2.0                                  | 695.8±15.9   | 103.8±2.8 | 58.9±1.9   | 10.4±0.9 | 913.7   |
| Samkwangbyeo | Postharvest           | 50.1±2.0                                  | 610.3±18.5   | 110.7±4.1 | 106.8±84.2 | ND       | 877.9   |
|              | 4                     | 40.7±1.4                                  | 550.5±5.8    | 194.6±3.9 | 89.8±3.2   | ND       | 875.6   |
|              | 8                     | 33.5±1.5                                  | 518.9±4.9    | 183.5±3.0 | 78.2±2.6   | ND       | 813.9   |
|              | 12                    | 26.7±1.2                                  | 498.8±3.7    | 184.7±2.5 | 68.7±2.1   | ND       | 779.0   |
| Ilpumbyeo    | Postharvest           | 54.8±2.4                                  | 1,194.2±18.0 | 90.8±9.4  | 75.9±2.9   | 8.0±1.4  | 1,423.7 |
|              | 4                     | 39.9±2.1                                  | 1,090.7±17.5 | 98.4±3.6  | 68.9±3.0   | 6.9±0.7  | 1,304.8 |
|              | 8                     | 31.5±1.7                                  | 992.2±18.3   | 112.0±3.5 | 62.7±2.9   | 6.5±1.0  | 1,204.9 |
|              | 12                    | 28.7±1.0                                  | 925.8±16.7   | 116.7±2.8 | 49.5±2.0   | 5.8±0.7  | 1,126.5 |

<sup>1)</sup>The total values indicate the mean±SD (n=3) for free sugar content of each sample; ND, not detected.

**Table 3. Changes of lipoxygenase activity, fat acidity, and germination rate in 5 Korean brown rice cultivars under different storage conditions**

| Cultivar                                | Postharvest           | 4 weeks Storage |          | 8 weeks Storage |          | 12 weeks Storage |          |
|---|-----------------------|-----------------|----------|-----------------|----------|------------------|----------|
|   |                       | 10°C            | 25°C     | 10°C            | 25°C     | 10°C             | 25°C     |
| Lipoxygenase activity (unit/mg protein) |                       |                 |          |                 |          |                  |          |
| Taebongbyeo                             | 7.0±0.1 <sup>1)</sup> | 7.7±0.2         | 12.5±0.3 | 8.8±0.2         | 14.6±0.5 | 10.5±0.2         | 16.5±0.3 |
| Gopumbyeo                               | 11.5±0.1              | 11.9±0.3        | 17.0±0.6 | 13.6±0.3        | 18.6±0.4 | 14.4±0.3         | 22.0±0.3 |
| Hwasungbyeo                             | 7.1±0.1               | 8.7±0.4         | 14.8±0.5 | 9.8±0.3         | 16.1±0.8 | 11.4±0.2         | 17.9±0.3 |
| Samkwangbyeo                            | 5.7±0.1               | 8.1±0.3         | 14.4±0.4 | 10.1±0.1        | 15.9±0.3 | 11.4±0.2         | 18.1±0.3 |
| Ilpumbyeo                               | 8.4±0.2               | 9.7±0.3         | 14.3±1.1 | 10.7±0.3        | 15.0±0.6 | 11.6±0.2         | 18.0±0.4 |
| Fat acidity (mg KOH/100 g)              |                       |                 |          |                 |          |                  |          |
| Taebongbyeo                             | 3.3±0.2               | 3.5±0.5         | 6.1±0.3  | 4.7±0.9         | 7.4±0.7  | 6.9±0.1          | 10.7±0.2 |
| Gopumbyeo                               | 2.8±0.1               | 4.4±0.5         | 7.3±0.3  | 7.3±0.6         | 11.8±0.8 | 9.5±0.2          | 14.7±0.2 |
| Hwasungbyeo                             | 6.0±0.2               | 6.6±0.6         | 7.4±0.3  | 7.4±0.4         | 8.8±0.6  | 9.6±0.6          | 14.1±0.2 |
| Samkwangbyeo                            | 6.5±0.1               | 8.8±0.8         | 13.5±1.4 | 12.4±1.5        | 15.8±1.3 | 14.2±0.6         | 18.3±0.2 |
| Ilpumbyeo                               | 5.4±0.5               | 5.5±0.5         | 8.8±0.7  | 6.2±0.5         | 11.5±0.3 | 8.2±0.4          | 14.7±0.1 |
| Germination rate (%)                    |                       |                 |          |                 |          |                  |          |
| Taebongbyeo                             | 100±0.0               | 100±0.0         | 100±0.0  | 99±1.0          | 96±1.0   | 99±1.0           | 95±1.0   |
| Gopumbyeo                               | 100±0.0               | 99±1.0          | 96±1.0   | 99±1.0          | 96±4.0   | 98±2.0           | 94±2.0   |
| Hwasungbyeo                             | 98±1.0                | 98±3.0          | 97±2.0   | 98±1.0          | 96±2.0   | 97±1.0           | 94±2.0   |
| Samkwangbyeo                            | 98±1.0                | 98±1.0          | 94±0.0   | 97±1.0          | 93±3.0   | 96±0.0           | 94±0.0   |
| Ilpumbyeo                               | 100±0.0               | 100±0.0         | 97±1.0   | 99±1.0          | 96±2.0   | 98±2.0           | 92±2.0   |

<sup>1)</sup>The values indicate the mean±SD ( $n=3$ ) of each sample.

according to the cultivars and storage conditions.

**Changes in lipoxygenase activity during storage** Stored rice for a long time decreased  $\alpha$ -amylase and  $\beta$ -amylase as well as increased lipoxygenase, protease, and lipase activities (34). Among these enzymes, lipoxygenase could cause undesirable flavor and odor (35), but few scientific researches have been conducted on the effects under the various storage time and temperature. As shown in Table 3, lipoxygenase activity showed significant changes during storage and this value increased with increase of storage time and temperature. Especially, the effect of storage temperature showed significant difference in comparison with time (Table 3). Briefly, lipoxygenase activity during 12 weeks storage at 25°C increased approximately 10 unit/mg protein more level than that of postharvest state. Moreover, this character exhibited average 5 unit/mg protein less activity at 10°C than 25°C storage. In this study, to brown rice quality, the available storage conditions concerning lipoxygenase activity were observed up to 4 weeks storage at 10°C. In 5 cultivars, 'Gopumbyeo' exhibited the highest activity (11.9±0.3 unit/mg protein), while 'Taebongbyeo' showed the lowest (7.7±0.2 unit/mg protein) for 4 weeks storage at 10°C. Thus, 'Taebongbyeo' might be very important source in processing aspect because increasing of lipoxygenase activity resulted in numerous changes in physical as well as chemical aspects and deteriorated quality (15,16).

**Changes in fat acidity during storage** Fat acidity was one of the best indicators, which was usually used to determine rice quality (19). As shown in Table 3, fat acidity increased with increase of storage time and temperature.

Especially, the storage temperature showed significant difference in comparison with time and the average fat acidity content showed approximately 3.00 mg KOH/100 g less level in 10°C than 25°C storage. In general, increasing fat acidity caused of the numerous changes in physical and chemical aspects (9). Therefore, 'Taebongbyeo' might be very important source in processing and storage aspects owing to the lowest contents (3.3±0.2→10.7±0.2 mg KOH/100 g) during 12 weeks storage. Although this study was similar to that of previous study (14), to our knowledge, variations in fat acidity under the various storage systems were first investigated.

**Changes in germination rate during storage** It is well documented that stored rice for a long time decreases germination rate and deteriorates in rice quality (7,36). Even though researchers mainly have focused on changes in germination rate from brown rice during storage (7), there was little reported under the various storage conditions. Although germination rate did not significantly changes in stored brown rice up to 4 weeks at 10°C, this character after 4 weeks was observed slightly decrease in all cultivars (Table 3). As shown in Table 3, this rate was kept more than 90% for 12 weeks storage at 2 temperatures and 'Taebongbyeo' exhibited the lowest variations (100±0.0→95±1.0%). Thus, this cultivar may have more effect on the quality than other cultivars.

In the current study, we have shown that changes in 6 physicochemicals including GABA,  $\gamma$ -oryzanol, free sugar, lipoxygenase activity, fat acidity, and germination rate during 4, 8, and 12 weeks storage at 10 and 25°C from 5 brown rice cultivars. Among physicochemicals, GABA,  $\gamma$ -oryzanol, lipoxygenase activity, and fat acidity increased,

while free sugar and germination rate decreased according to increase of storage time and temperature. Especially, free sugar, lipoxygenase activity, and fat acidity showed the predominant variations. Among cultivars, 'Gopumbyeo' and 'Taebongbyeo' may possess high potential in functional and processing foods. Also, the obtained results may provide a basic understanding of functional properties in stored brown rice.

## References

- Friedman M. The nutritional value of proteins from different food sources. *J. Agr. Food Chem.* 43: 3-29 (1996)
- Gregorio GB, Senadhira D, Htut T, Graham RD. Improving iron and zinc value of rice for human nutrients. *Agr. Dev.* 23: 68-87 (1999)
- Chung YM, Lee JC, Kim KS, Eun JB. Chemical compositions of 26 varieties of Korean rice straw. *Food Sci. Biotechnol.* 10: 267-271 (2001)
- Sowbhagya CM, Bhattacharya KR. Changes in pasting behavior of rice during aging. *J. Cereal Sci.* 34: 115-124 (2001)
- Zhou ZK, Robards K, Helliwell S, Blanchard C. Aging of stored rice: Changes in chemical and physical attributes. *J. Cereal Sci.* 35: 65-78 (2002)
- Tran TU, Suzuki K, Okadome H, Ikezaki H, Homma S, Ohtsubo K. Detection of changes in taste of *Japonica* and *Indica* brown and milled rice (*Oryza sativa* L.) during storage using physicochemical analyses and a taste sensing system. *J. Agr. Food Chem.* 53: 1108-1118 (2005)
- Noh GI, Kim KY, Shin SH, Park HS, Kim BK, Shin MS, Ko JK. Genetic analysis on germination rate and fat acidity of *Japonica* rice seeds after high temperature storage. *Korean J. Breed. Sci.* 40: 228-233 (2008)
- Ramezanzadeh FM, Rao RM, Windhauser M, Prinyawiwatkul W, Tulley R, Marshall WE. Prevention of hydrolytic rancidity in rice bran during storage. *J. Agr. Food Chem.* 47: 3050-3052 (1999)
- Genkawa T, Uchino T, Inoue A, Tanaka F, Hamanaka D. Development of a low-moisture-content storage system for brown rice: Storability at decreased moisture contents. *Biosys. Eng.* 99: 515-522 (2008)
- Xu Z, Hua N, Godber JS. Antioxidant activity of tocopherols, tocotrienols, and  $\gamma$ -oryzanol components from rice bran against cholesterol oxidation accelerated by 2,20-azobis(2-methylpropionamide) dihydrochloride. *J. Agr. Food Chem.* 49: 2077-2081 (2001)
- Jakobs C, Jaeken J, Gibson KM. Inherited disorders of GABA metabolism. *J. Inherit. Metab. Dis.* 16: 704-715 (1993)
- Okada T, Sugishita T, Murakami T, Murai H, Saikusa T, Horio T. Effect of the defatted rice germ enriched with GABA for sleepless, depression, autonomic disorder by oral administration. *Nippon Shoku. Kougyou. Kaishi* 47: 596-603 (2000)
- Omori M, Yano T, Okamoto T, Tsushida T, Murai T, Higuchi M. Effect of anaerobically treated tea (gabaron tea) on blood pressure of spontaneously hypertensive rats. *Nippon Nogeik. Kaishi* 61: 1449-1451 (1987)
- Tanaka A, Koto A. Mass spectra of several ferulates. *Yukagaku* 24: 570-574 (1975)
- Xu Z, Godber JS. Purification and identification of components of  $\gamma$ -oryzanol in rice bran oil. *J. Agr. Food Chem.* 47: 2724-2728 (1999)
- Yasukawa K, Akihisa T, Kimura Y, Tamura T, Takido M. Inhibitory effect of cycloartenyl ferulate, a component of rice bran, on tumor promotion in two stage carcinogenesis in mouse skin. *Biol. Pharm. Bull.* 21: 1072-1076 (1998)
- Shin MG, Yoon SH, Rhee JH, Kwon TW. Correlation between oxidative deterioration of unsaturated lipid and *n*-hexanal during storage of brown rice. *J. Food Sci.* 51: 460-463 (1986)
- Villareal RM, Resurreccion AP, Suzuki LB, Juliano BO. Changes in physicochemical properties of rice during storage. *Starch* 28: 88-94 (1976)
- Kim KY, Lee GM, Noh KI, Ha KY, Son JY, Kim BK, Ko JK, Kim CK. Varietal difference of germination, fat acidity, and lipoxygenase activity of rice grain stored at high temperature. *Korean J. Crop Sci.* 52: 29-35 (2007)
- Komatsuzaki N, Tsukahara K, Toyoshima H, Suzuki T, Shimizu N, Kimura T. Effect of soaking and gaseous treatment on GABA content in germinated brown rice. *J. Food Eng.* 78: 556-560 (2007)
- Lee YR, Kim JY, Woo KS, Hwang IG, Kim KH, Kim KJ, Kim JH, Jeong HS. Changes in the chemical and functional components of Korean rough rice before and after germination. *Food Sci. Biotechnol.* 16: 1006-1010 (2007)
- Oh SH, Oh CH. Brown rice extracts with enhanced levels of GABA stimulate immune cells. *Food Sci. Biotechnol.* 12: 248-252 (2003)
- Zhang G, Brown AW. The rapid determination of  $\gamma$ -aminobutyric acid. *Phytochemistry* 44: 1007-1009 (1997)
- Rogers EJ, Rice SM, Nicolosi RJ, Carpenter DR, McClelland CA, Romanczyk Jr. Identification and quantitation of  $\gamma$ -oryzanol components and simultaneous assessment of tocopherols in rice bran oil. *J. Am. Oil Chem. Soc.* 70: 301-307 (1993)
- Chotimarkorn C, Benjakul S, Silalai N. Antioxidant effects of rice bran extracts on refined tuna oil during storage. *Food Res. Int.* 41: 616-622 (2008)
- AOAC. Official Methods of Analysis of AOAC Int. 16<sup>th</sup> ed. Method 969.33. Association of Official Analytical Chemists, Arlington, VA, USA (1990)
- Suzuki Y, Matsukura U. Lipoxygenase activity in maturing and germinating rice seeds with and without lipoxygenase-3 in mature seeds. *Plant Sci.* 125: 119-126 (1997)
- Lee YS, Song SJ, Rhee CO. Lipoxygenase activity in black rices. *Agr. Chem. Biotechnol.* 42: 130-133 (1999)
- Choi HD, Park YK, Kim YS, Chung CH, Park YD. Effect of pretreatment conditions on  $\gamma$ -aminobutyric acid content of brown rice and germinated brown rice. *Korean J. Food Sci. Technol.* 36: 761-764 (2004)
- Wennermark B, Ahlmen H, Jagerstad M. Improved vitamin E retention by using freshly milled whole-meal wheat flour during drying. *J. Agr. Food Chem.* 43: 1348-1351 (1994)
- Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso EF, Mendonça CRB, Ramis-Ramos G. Composition, industrial processing, and applications of rice bran  $\gamma$ -oryzanol. *Food Chem.* 115: 389-404 (2009)
- Sowbhagya CM, Bhattacharya KR. Lipid autoxidation in rice. *J. Food Sci.* 41: 1018-1023 (1976)
- Barber S. Milled rice and changes during aging. pp. 215-263. In: *Rice Chemistry and Technology*. 1<sup>st</sup> ed. Houston DF (ed). American Association of Cereal Chemists, St. Paul, MN, USA (1972)
- Dhalival YS, Sekhon KS, Nagi HPS. Enzymatic activities and rheological properties of stored rice. *Cereal Chem.* 68: 18-21 (1991)
- Suzuki T, Honda Y, Mukasa Y, Kim SJ. Effects of lipase, lipoxygenase, peroxidase, and rutin on quality deteriorations in buckwheat flour. *J. Agr. Food Chem.* 53: 8400-8405 (2005)
- Zhang Y, Yu Z, Lu Y, Wang Y, She D, Song M, Wu Y. Effect of the absence of lipoxygenase isoenzymes on the storage characteristics of rice grains. *J. Stored Prod. Res.* 43: 87-91 (2007)