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# Characteristic Changes in Brown Rice (*Oryza sativa* L.) Cultivars of 3 Ecotypes During Different Storage Conditions

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**Abstract** The aim of this study investigated the fluctuations of 3 characters from 3 ecotypes [early ripening (ER), middle ripening (MR), and late ripening (LR)] of 20 Korean brown rice cultivars in different storage systems [time: 12 and 24 weeks, temperature: low (10°C) and room (25°C)]. With increase of storage time and temperature, lipoxygenase activity, and fat acidity increased, whereas germination rate was reduced. ER cultivars exhibited the highest lipoxygenase activity of 35.49±2.46 unit/mg protein during 24 weeks storage at 25°C, followed by LR (32.73±1.33) and MR (32.66±1.62) cultivars. The amounts of fat acidity also were observed by the same order (ER: 20.40±2.12>LR: 19.68±1.86>MR: 19.64±1.35 mg KOH/100 g). Germination rate slightly decreased with increase of time and temperature (MR>LR>ER), but MR and LR cultivars showed the most significant changes (ER: 60.90±23.47%, MR: 32.66±13.95%, and LR: 32.53±5.87%). On the basis of above results, MR cultivars were evaluated the highest quality, because high lipoxygenase activity, high fat acidity, and low germination rate have deteriorated in quality and generated off-odor. Thus, MR cultivars might be very important sources in food processing and stored dietary supplement aspects.

**Keywords:** brown rice, ecotype, lipoxygenase activity, fat acidity, germination rate, storage time, storage temperature

## Introduction

It is well-known that edible sources during storage generate spontaneous phenomena including changes in the biological activities, chemical components, and nutritional values (1,2). Especially, rice (*Oryza sativa* L.) is the most widely consumed crop in many parts of the world owing to the purpose as material for food processing (3,4). Among many rice processing, storage is the one of the most important processes, because unsuitable storage causes quantitative and qualitative grains losses (5-7). Attempts to explain the changes in functionally associated with rice have focused on the properties of components including lipid, starch, and protein during storage (8). In rice components, many researchers have suggested that degradation of lipid could play a key role in the deteriorative changes during storage (9,10). Lipid degradation also related to the off-odor of stored rice owing to carbonyl compounds such as hexanal, propanal, and pentanal (11). In addition, rice shows the highest germination rate after harvest, however, as time goes on, germination rate diminution, fat acidity growth, and rice quality begins to deteriorate (12,13).

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There are numerous reports on the changes of the physicochemical properties of rice such as fat acidity, germination rate, starch, protein, and lipid during storage (2,6). Recently, lipoxygenase activity, fat acidity, and germination rate were investigated from brown rice after storage of 6 and 12 weeks at high temperature (35°C) (12). However, few studies have examined the character properties in the low (10°C) and room (25°C) temperatures. Moreover, their comparative study concerning different maturity types of brown rice cultivars have not been reported.

In our continuing research, we investigated changes in lipoxygenase activity, fat acidity, and germination rate from 20 brown rice including early ripening (ER), middle ripening (MR), and late ripening (LR) cultivars during 12 and 24 weeks storage at 10 and 25°C.

## Materials and Methods

**Plant material** Twenty cultivars of Korean brown rice were used for this study. These cultivars were selected according to the maturity types including early ripening (ER), middle ripening (MR), and late ripening (LR), respectively. The maturity types of cultivars were shown in Table 1. All cultivars were harvested in the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Suwon, Korea in 2007 and grown in the same conditions to avoid variations of character due to environmental factors.

**Table 1. List of brown rice cultivars used in this study**

| Ecotype[1)] | Cultivars |
|---|---|
| ER | Gounbyeo, Jeogjinju, Jinbubyeo, Heugjinju, Odae 1, Taebongbyeo, Taesungbyeo |
| MR | Chung-abyeo, Chungdambyeo, Dasan 1, Gopumbyeo, Hwasungbyeo, Hongjinju, Seoan 1, Seokjeongbyeo |
| LR | Chucheongbyeo, Ilpumbyeo, Juan 1, Saechucheongbyeo, Samkwangbyeo |

[1)]ER, early ripening; MR, middle ripening; LR, late ripening.

**Reagents** Lipoxygenase (E.C. 1.13.11.12), linoleic acid (purity>99%), potassium phosphate, and phenolphthalein were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade diethyl ether, benzene, and sodium hypochlorite throughout the experiments were purchased from Merck (Darmstadt, Germany).

**Instruments** Lipoxygenase activity was performed on UV-VIS spectrophotometer Evolution 500 (Thermo Electron Co., Woburn, MA, USA) and determined using an oxygen monitoring system (YSI Model 5300; YSI Inc., Yellow Springs, OH, USA) to measure oxygen uptake. The harvested brown rice was hulled with a milling machine (Jworld Tech, Ansan, Korea). Brown rice grains were ground using a coffee grinder (HR2860; Philips, Netherlands). The extracts of grounded grains were centrifuged with Vision apparatus (VS-1500CFNII; Vision, Seoul, Korea) and germination rate was determined using an incubator (DS-53CP; Dasol Scientific, Ltd., Seoul, Korea).

**Storage condiditons** The 300 g of hulled grains were put into a polyethylene bottle with screw cap. These bottles were kept in an incubator for 12 and 24 weeks at 10 and 25°C, and then 3 characters including lipoxygenase activity, fat acidity, and germination rate were measured in stored grains.

**Determination of lipoxygenase activity** The lipoxygenase activity was performed as previously reported method (14) with slightly modification. Briefly, powder (1.0 g) of brown rice 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 until activity analysis. The substrate consisted of 10 μL linoleic acid, 4 mL $H_2O$, 1 mL 0.1 N NaOH, and 5 μL 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 μL linoleic acid, and 150 μL enzyme extracts, respectively. After mixing, the absorbance value was measured at 234 nm. The activity of 1 unit was defined as the change of 0.001 unit/min.

**Determination of fat acidity** The analytical method of fat acidity was determined by Association of Official Analytical Chemicals (AOAC) (15). It was measured by the amount of KOH which was needed to reduce free fatty acid involved in 100 g (mg KOH/100 g) of grounded brown rice. The powder (10 g) was stirred in benzene (25 mL) at room temperature 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.

**Determination of germination rate** Each brown rice (100 g) was decontaminated 1% sodium hypochlorite solution for 2 hr. The sterilized grains were put on a petri dish covered with filter paper (Advantec No. 2, 90-mm), and then 10 mL distilled $H_2O$ was added. The petri dish was placed in an incubator (Dew Chamber) set at 25°C and the ratio of germinated grains was examined after 7 days (7).

**Statistical analysis** All measurements were repeated 3 times and the results were as the mean±standard deviation (SD) for the 3 experiments. Significance was determined by analysis of variance (ANOVA) and Duncan's multiple-range tests.

## Results and Discussion

**Fluctuations in lipoxygenase activity during storage** Changes in lipoxygenase activity of brown rice after 6 and 12 weeks at high temperature have been subject to extensive investigation (12). In order to find out the maturity types concern to the highest quality, we proceeded to examine the lipoxygenase activity from different maturity of brown rice during 12 and 24 weeks at 2 temperatures (10 and 25°C). As shown in Table 2, 20 Korean cultivars on 3 ecotypes showed significant differences. In accordance with the increased of storage time, lipoxygenase activity was a consistent increased in all sample. This result was in agreement with a report by Kim *et al.* (12). The temperature effect also showed significant difference and the stored brown rice at 25°C exhibited higher activities than 10°C storage (Table 2). Among the 3 ecotypes, the average activity of ER cultivars was significantly higher than their of MR and LR cultivars.

In the case of ER cultivars, the average lipoxygenase activity showed rapidly increased during 24 weeks storage at 25°C (35.49±2.46 unit/mg protein) in comparison with other storage conditions and this value was measured about 3 times more activity than postharvest state (13.12±3.87 unit/mg protein). MR and LR cultivars (postharvest; MR: 7.68±2.61 and 7.81±2.61 unit/mg protein) also strongly increased by increasing storage time and exhibited the highest lipoxygenase activities (MR: 32.66±1.62 and LR: 32.73±1.33 unit/mg protein) under the same storage condition as ER cultivars. Figure 1A showed average activity change in 20 cultivars on 3 ecotypes for 12 and 24 weeks storage at 2 temperatures and their values ranged from 9.41±4.10 to 33.56±2.28 unit/mg protein.

From these above results, it was suggest that lipoxygenase activity exhibited higher level by increasing storage time and temperature. However, it was well established that this

**Table 2. Comparison of average lipoxygenase activity in brown rice cultivars on 3 ecotypes from different storage conditions**

| Cultivar | Lipoxygenase activity (unit/mg protein) | | | | |
|---|---|---|---|---|---|
| | Postharvest | 12 weeks storage | | 24 weeks storage | |
| | | 10°C | 25°C | 10°C | 25°C |
| ER | 13.12±3.87[a1)] | 16.36±2.86[a] | 21.45±1.72[a] | 21.63±2.39[a] | 35.49±2.46[a] |
| MR | 7.68±2.61[b] | 11.36±0.58[b] | 16.96±1.76[c] | 17.55±0.82[b] | 32.66±1.62[b] |
| LR | 7.81±3.06[b] | 12.46±1.78[b] | 19.01±1.23[b] | 18.22±1.21[b] | 32.73±1.33[b] |

[1)]Data were mean±SD ($n$=3) and analyzed statistically by ANOVA and the significance of the differences between means were estimated by Duncan's multiple range test (DMRT).

**Table 3. Comparison of average fat acidity in brown rice cultivars on 3 ecotypes from different storage conditions**

| Cultivar | Fat acidity (mg KOH/100 g) | | | | |
|---|---|---|---|---|---|
| | Postharvest | 12 weeks storage | | 24 weeks storage | |
| | | 10°C | 25°C | 10°C | 25°C |
| ER | 6.82±1.74[a1)] | 12.83±2.34[a] | 16.25±2.38[a] | 17.07±3.33[a] | 20.40±2.12[a] |
| MR | 3.72±1.35[b] | 8.40±1.70[b] | 13.03±2.32[c] | 13.22±2.21[b] | 19.64±1.35[b] |
| LR | 5.46±2.46[b] | 10.33±1.59[b] | 14.63±0.85[b] | 14.58±1.92[b] | 19.68±1.86[b] |

[1)]Data were mean±SD ($n$=3) and analyzed statistically by ANOVA and the significance of the differences between means were estimated by Duncan's multiple range test (DMRT).

**Table 4. Comparison of average germination rate in brown rice cultivars on 3 ecotypes from different storage conditions**

| Cultivar | Germination rate (%) | | | | |
|---|---|---|---|---|---|
| | Postharvest | 12 weeks storage | | 24 weeks storage | |
| | | 10°C | 25°C | 10°C | 25°C |
| ER | 98.10±1.21[a1)] | 93.30±4.11[b] | 85.00±2.66[c] | 80.50±4.43[c] | 60.90±3.47[b] |
| MR | 99.20±0.91[a] | 97.20±1.82[a] | 94.20±2.71[a] | 86.70±3.57[a] | 32.66±3.95[a] |
| LR | 99.00±0.63[a] | 96.40±2.79[a] | 91.70±5.79[b] | 84.30±4.25[b] | 32.53±5.87[b] |

[1)]Data were mean±SD ($n$=3) and analyzed statistically by ANOVA and the significance of the differences between means were estimated by Duncan multiple range test (DMRT).

enzyme degraded to carbonyl compounds such as pentanol and hexanol as well as fat-soluble vitamins and essential fatty acids in rice (16). It also generated off-flavors and odors due to unsaturated fatty acids (16,17). Therefore, increasing lipoxygenase activity during storage resulted in numerous changes in physical as well as chemical aspects and deteriorated quality and cooking of rice. Among the 3 ecotypes, it was considered that MR cultivars were better in the quality and taste properties of brown rice than other ecotype cultivars during storage.

**Fluctuations in fat acidity during storage** Changes of fat acidity in brown rice cultivars according to rice storage conditions are well documented (12). On the basis of this result, we focused on diversity in ecotypes of various brown rice cultivars at low and room temperatures. The amounts of fat acidity were observed significantly increase according to storage time. Furthermore, the fat acidity ratio of room temperature exhibited strongly higher than that of low temperature (Table 3). ER cultivars showed fat acidity of the highest level (6.82±1.74 mg KOH/100 g), while LR cultivars were the lowest (5.46±2.46 mg KOH/100 g) in postharvest. After 24 weeks storage at 25°C, MR cultivars exhibited 6 times more fat acidity than postharvest state and ER and LR cultivars also increased nearly 3 times. Moreover, 3 ecotypes cultivars for 12 weeks storage at 10°C were observed 2 times more fat acidity than postharvest state and their levels showed much higher when temperature was higher.

The average fat acidity of 3 ecotypes cultivars exhibited 4.00 mg KOH/100 g values more level in room than low temperature storage. Figure 1B exhibited average fat acidity changes in 3 ecotype cultivars and their levels ranged from 5.34±2.22 to 19.89±1.74 mg KOH/100 g. As shown in Table 3, fat acidity showed significant differences on ecotypes during storage and this amount exhibited ER cultivars the highest level, followed by LR and MR cultivars. This character was commonly known as index of quality deterioration because lipid dissolution progressed more rapidly than that of protein and starch (18). Thus, increasing fat acidity caused of the numerous changes in physical and chemical as well as quality aspects (7,12,19). Although our results were in consistency with the previously reported (7), the elucidations of fat acidity from different ecotypes have not been studied extensively. On the basis of above results, MR cultivars were better in the quality than other ecotypes during storage concern to food processing aspect.

**Fluctuations in germination rate during storage** Many studies have been conducted with regard to germination rate associated with rice storage (7,12,14). On the basis of previously described (12,14), the results of germination

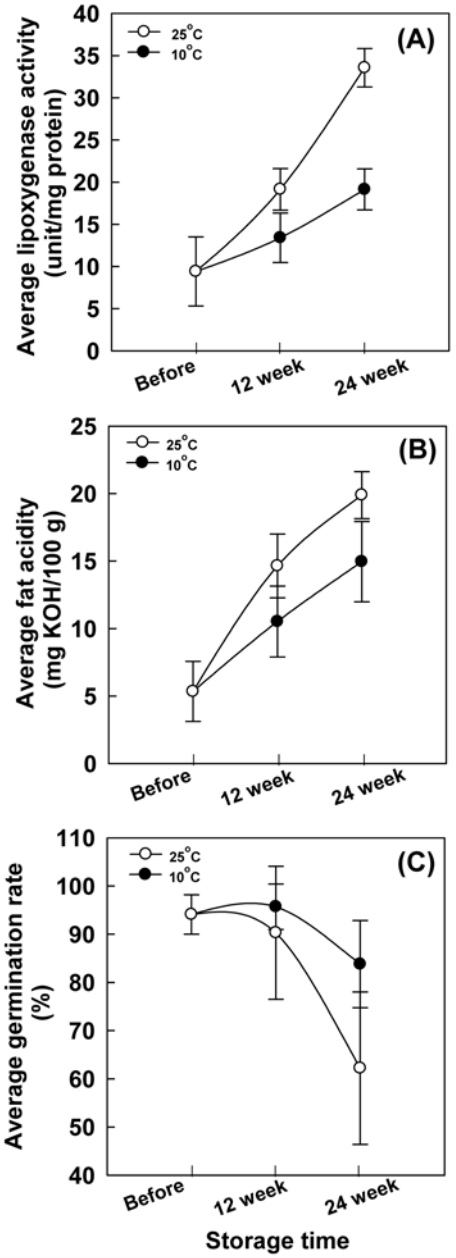


**Fig. 1. Average fluctuations of 3 characters from the grains of 20 brown rice cultivars according to the 3 ecotypes during storage time at 10 and 25°C.** (A) average lipoxygenase activity, (B) average fat acidity, and (C) average germination rate.

rate on 3 ecotypes of brown rice cultivars during 24 weeks storage at 10 and 25°C were given in Table 4. In postharvest state, germination rate of 3 ecotypes including ER, MR, and LR cultivars showed high rate with values of 98.10±1.21, 99.20±0.91, and 99.00±0.63%, respectively. During 12 weeks storage, MR cultivars were observed the highest rate (97.20±1.82%) at 10°C, while the lowest rate was measured by ER cultivars (85.00±2.66%) at 25°C.

During 24 weeks storage, MR cultivars exhibited the highest rate (86.70±3.57%) at 10°C, while LR cultivars were measured the lowest rate (32.53±5.87%) under 25°C. Interestingly, MR and LR cultivars (MR: 32.66±3.95% and LR: 32.53±5.87%) in 24 weeks storage at 25°C showed the most significant changes in comparison with other storage conditions. The measured average germination rate of 3 ecotypes in brown rice cultivars were shown in Fig. 1C and the germination rate decreased with augmentation of storage time and rate of 10°C storage were kept more than that of 25°C.

These results was similar to that of a previously (7,12), information for characterization of different maturity types in brown rice cultivars has been few studied. The average germination rate of MR cultivars showed the highest level, followed by LR and ER cultivars. Thus, MR cultivars may have more effect on the quality and dietary materials than other ecotypes cultivars in stored brown rice.

In summary, this work has shown the changes in the storage characters including lipoxygenase activity, fat acidity, and germination rate under different storage conditions from different maturity types of 20 Korean brown rice cultivars. Among the 3 ecotypes, MR cultivars were evaluated the highest quality, followed by LR and ER, because of low lipoxygenase activity, low fat acidity, and high germination rate, respectively. Thus, our results could provide potential sources for the development of the processed food and nutraceutical concern to rice. Moreover, the obtained results might provide a basic understanding of functional properties in different brown rice maturity types. In the future, to measure the chemical and functional compositions of MR cultivars of brown rice in comparison with other ecotype cultivars, we will investigate the changes of the free sugars, free amino acids, tocopherols, γ-oryzanol, γ-aminobutyric acid (GABA), and dietary fiber.

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