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Varietal Variation in Antioxidative Activity of Rice Grain by DPPH and TBA Methods

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ABSTRACT: This study was to investigate antioxidative activity of rice grain using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) method and germination ability for screening rice varieties with high antioxidative activities on korean native and foreign rice varieties harvested in 1998 and 1999. The average antioxidative activity of foreign rice varieties (DPPH 63.5% and TBA 55.2%) was significantly higher than that of native rice varieties (DPPH 47.2% and TBA 45.6%) on varieties harvested in 1999. The promptness index (PI) of native rice varieties was higher in stored rices for three months (mean PI=160.7) than that of stored rices for a year (mean PI=141.6). On the other hand, the PI of foreign rice varieties was higher in stored rices stored for a year (mean PI=176.7) than that of stored rices for three months (mean PI=157.5). Varieties with high redness of hulled rice (a-value) showed significant lipid peroxidation inhibitory activity to DPPH in a stored rices for a year (r=0.5744) and stored rices for three months (r=0.5630**). These results indicate that the pigments of hulled rice varieties may play important antioxidative roles and colored rice varieties with higher antioxidative potentials can be developed and also may provide information with rice breeder to breed rice variety with a high antioxidative activity for a rapid screening of a small amounts of a large number of samples using color value.**

Keywords : rice, antioxidative activity, DPPH, TBA.

Rice (*Oryza sativa* L.) is the most important food crop in Korea. The well nourishment of world's population depends on the development of better rice varieties and improved technology in rice production and utilization. The high quality rice is of great agricultural importance, especially in the aspect of a decrease in nutritional quality and the oxidation of harvested grain during storage. The conditions for post-harvest storage of rice vary from place to place depending on the technological or biochemical properties of rice grain, and the palatability of cooked rice. Rice seeds contain phenolic compounds such as flavonoids, isovitexin, cyanidin, oryzanol, α-tocopherol, and phytic acid, which exhibit strong natural antioxidant properties (Ramarathnam *et al.*, 1986, 1989; Wu *et al.*, 1994; Cho *et al.*, 1996; Choi *et al.*, 1994, 1996; Choi and Oh, 1996; Osawa *et al.*, 1985). Amongst these compounds, isovitexin and phytic acid isolated from rice hulls were found to be very strong antioxidants inhibiting lipid peroxidation (Ramarathnam *et al.*, 1989). For this reason, rice hulls received more attention as an economically attractive source of natural antioxidants. It is common practice to store rice after harvest before being consumed. During the storage the rate of aging depends partly on the moisture content, but mainly on the temperature (Barber, 1972). Storage conditions influence the loss of dormancy after short-term storage in freshly harvested rice seeds (Navasero *et al.*, 1975). It is therefore thought that the higher antioxidative ability, the longer storing in rice. In order to breed rice varieties containing high antioxidative substances, efficient screening methods must be established.

Among the methods of several antioxidative activity test, antioxidants react with the DPPH radical directly and restore it with the transfer of electrons or hydrogen. Also, because the thiobarbituric acid (TBA) method is simple and sensitive, and it has a higher correlation with sensory evaluation results as compared with other methods, it is the most widely used method for measuring the development of lipid oxidation (Park, 1995). It measures the amount of a secondary lipid oxidation product, malonaldehyde (MA), which forms a complex with TBA in an acidic heating process. Malonaldehyde, a primary reactant with TBA, produces a red chromogen with an maximum absorbance at 532 nm. In spite of being widely used for measuring lipid oxidation, the TBA method is not specific because a diverse compounds other than MA from both lipid and non-lipid sources could react with TBA. This method works well when applied to a defined membrane system or to liver microsomes, but its application to tissue extracts has caused many problems.

Little information is available on breeding rice varieties with higher antioxidative potential in Korea. Thus, the objective of this study was to evaluate antioxidative activity using DPPH and TBA method for screening rice varieties with high antioxidative activities on native and foreign rice varieties harvested in 1998 and 1999.

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MATERIALS AND METHODS

Plant materials and color measurement

Fifty-four korean native varieties including colored rice, 'Jangsamdo' and common rice, 'Arongbyeo', and 28 foreign rice including colored rice, 'HweiJu', and common rice, 'GPNO 12856', were cultivated at the Konkuk University farm by the standard methods of rice cultivation in middle area of Korea. The harvested seeds in October 1998 were dried and stored for one year at room temperature (24°C). Also, the harvested seeds in 1999 as the same above mentioned method were stored for three months at room temperature (24°C) until need. After all the stored seeds were hulled by rice huller machine (HMF 185 type, Japan), the color value of hulled rice surface was measured. To measure the grain color of the hulled rice, grain (1.7 g) were placed in a Granular Material Attachment (22 mm I.D.) and their color were measured with a color difference meter (Chroma meter CR-310, Minolta, Tokyo, Japan). The white standard plate (Calibration Plate CR-310, Minolta, Tokyo, Japan) was used for calibration of the equipment. (L) value indicates the whiteness of a sample with 100 as white and 0 as black. (a) and (b) value expresses redness and yellowness, respectively.

Tests of antioxidative activity

Sample preparation for DPPH and TBA activity tests

The sample preparation for antioxidative activity test in rice varieties by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) is applied with a slight modification of the method developed by Chung *et al.* (1998). After color measurement, the sample were ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass through a 40-mesh screen. The ground powder samples (10 g) of each were extracted with 200 mL of 80% MeOH for 24 hours in a vibrating water bath at 20°C after the stored brown rice were ground in a Wiley mill through a 40 mesh screen. The extracts were then filtered (Whatman No. 4) and evaporated to dryness in vacuum at 40. The separated fractions were weighed to determine the respective yields of the soluble component. The crude samples were then redissolved with 80% MeOH to make 1% (w/v) extract solution. The 1% extract solution was used in DPPH (1.0 mL) and TBA (0.8 mL) methods for the antioxidative assay.

Radical scavenging activity test by DPPH method

Antioxidative activity by DPPH was measured by the Yoshida *et al.* (1989) method which use 0.25 mL of each 1% substrate solution after mixing 2.5 mL of DPPH (0.35 mM) with 50% ethanol. The DPPH value was measured by an absorbance at 517 nm after shaking for exactly 10 minutes at room temperature. The activity was calculated on the control, which used 80% MeOH. Inhibition percentage was calculated from the following equation:

$$\text{Inhibition percentage (\%)} = (1 - A / B)\ 100$$

where A is the absorbance of samples and B is the absorbance of the control.

Radical scavenging activity test by TBA method

Antioxidative activity was measured with TBA by the Wong *et al.* (1981) method which use 0.8 mL of each 1% substrate solution for the assay. Each sample was added to a solution mixture of linoleic acid, 99% ethanol and 100 mM potassium phosphate buffer (pH 7.0). The mixed solution was shaken in a water bath for 24 hours at 40. Two mL of the solution was then mixed with 2 mL 0.75% TBA and 1 mL 35% trichloroacetic acid (TCA), and boiled in the water bath for 40 minutes at 95. After mixing with 2 mL chloroform and 1 mL acetic acid, the solution was centrifuged at 3,000 rpm for 5 minutes. The TBA value was determined by reading the absorbance at 532 nm. The inhibition activity was calculated against the TBA value of the control (Mitsuda *et al.*, 1966). Inhibition percentage was calculated from the following equation:

$$\text{Inhibition percentage (\%)} = (1 - A / B)\ 100$$

where A is the absorbance of samples and B is the absorbance of the control.

Germination tests

Germination tests were performed on the Korean native and foreign rice seeds harvested in 1998 and 1999. The seeds were surface sterilized in a 1 : 10 (v/v) dilution of commercial hypochlorite bleach for 10 min and rinsed several times with distilled water. The seeds were then allowed to imbibe on moistened paper towels for 2 h. Filter paper (Whatmam No. 42) containing 50 seeds was placed in each sterilized 9 cm petri dish. Five mL of distilled water were added to a petri dish and distilled water was used as a control. All petri dishes were placed in a lighted growth chamber at 24°C. Germination was determined by counting the number of germinated seeds at 24 h intervals over a 5d period. To prevent confusion, if the radicle protruded totally from the seed coat as observed with the naked eye, the seed was counted as a germinated seed. Promptness index (PI) of germination was calculated as follows:

$$PI = (D_1 \times 5) + (D_2 \times 4) + (D_3 \times 3) + (D_4 \times 2) + (D_5 \times 1)$$

where D is the number of germinated seeds on the day of observation.

Statistical analysis

Analysis of variance for all data was accomplished using the general linear model procedure of the statistical analysis system program (SAS Institute, 1986). The experiments were repeated in a completely randomized design with 6 replications except color measurement and the pooled mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level. Since there was no significant difference (similar inclination) between the types of each variety and antioxidative activity, these results were combined and the average used for a correlation analysis.

RESULTS AND DISCUSSION

Antioxidative activity tests on seed extracts

Eight among 54 korean native rice varieties and 13 among 28 foreign rice varieties were colored rice (Table 1). Color values of 8 korean native and 13 foreign rice varieties stored for one year ranged 30.18~44.77 in (L), 1.38~4.96 in (a), 5.38~12.54 in (b) and 30.29~44.43 in (L), (1.14~5.96) in (a) and (5.12~11.61) in (b), respectively. Also, color values of these stored for three months ranged 30.95~45.00 in (L), 1.27~5.33 in (a), 6.39~12.37 in (b) and 30.49~43.24 in (L), (1.75~5.72) in (a) and (5.99~10.29) in (b), respectively. On the other hand, the color values of the 46 common rice varieties among 54 korean native rice varieties stored three months ranged 40.0~045.68 in (L), 0.60~1.92 in (a) and 6.95~12.00 in (b). The color values of the 15 common rice varieties among 28 foreign rice varieties stored for three months ranged 41.07~46.11 in (L), 0.86~1.83 in (a) and 9.06~12.08 in (b). The antioxidative activity of tested rice materials by DPPH and TBA method are given in Table 2. Radical scavenging activity in both native and foreign colored rices was higher than that in common rice varieties. The antioxidative activity of foreign rice varieties (DPPH 63.5% and TBA 55.2%) were higher than those of korean native rice varieties (DPPH 47.2% and TBA 45.6%) in a three month storage. Antioxidative activity of foreign colored rices was higher than that of native rice varieties, suggesting that foreign rices may contain more antioxidative compounds. This result is supported by the color values measured with a chroma meter of hulled rice, which is rep-

Table 1. Comparison of chroma meter estimates of hulled rice grains between native and foreign rice varieties.

Type		No. of var.	Stored one year			Stored three months		
			L†	a	b	L	a	b
Korean native rice varieties	**Colored rice**	8						
	Maximum		44.77	4.96	12.54	45.00	5.33	12.37
	Minimum		30.18	1.38	5.38	30.95	1.27	6.39
	Mean		41.14	2.40	11.03	41.01	2.26	10.38
	Common rice	46						
	Maximum		46.97	1.95	13.52	45.68	1.92	12.00
	Minimum		40.01	1.51	11.26	40.00	0.60	6.95
	Mean		42.42	1.51	11.26	41.75	1.55	9.96
CV (%)			7.7	15.5	2.2	1.3	5.8	7.8
LSD (0.05)			5.24	0.42	0.38	0.89	0.16	1.28
Foreign rice varieties	**Colored rice**	13						
	Maximum		44.43	5.96	11.61	43.24	5.72	10.29
	Minimum		30.29	1.14	5.12	30.49	1.75	5.99
	Mean		36.58	3.40	8.22	37.32	3.32	8.50
	Common rice	15						
	Maximum		43.91	1.92	12.09	46.11	1.83	12.08
	Minimum		40.20	1.26	9.97	41.07	0.86	9.06
	Mean		42.30	1.38	10.87	42.77	1.62	10.29
Total		82						
CV (%)			1.4	7.1	2.7	1.3	5.6	2.8
LSD (0.05)			0.88	0.26	0.43	0.87	0.22	0.40
CV (%)			6.4	13.1	2.4	1.3	5.9	6.5
LSD (0.05)			4.26	0.40	0.41	0.89	0.18	1.03

†L; Lightness, a; Redness, b; Yellowness.

Table 2. Comparison of DPPH and TBA inhibitory activities between foreign and native rice varieties.

Type	Varieties	DPPH	TBA
		-- Inhibition (%) --	
One year storage			
Korean native rice varieties	Colored rice	59.3	41.0
	Common rice	48.5	41.4
	Mean	53.9	41.2
Foreign rice varieties	Colored rice	69.5	47.4
	Common rice	47.1	45.1
	Mean	58.3	46.3
CV (%)		7.6	15.6
LSD (0.05)		6.40	11.19
Three months storage			
Korean native rice varieties	Colored rice	51.3	44.4
	Common rice	43.0	46.8
	Mean	47.2	45.6
Foreign rice varieties	Colored rice	71.8	56.4
	Common rice	55.1	54.0
	Mean	63.5	55.2
CV (%)		12.6	17.1
LSD (0.05)		10.41	13.85
CV (%)		19.1	27.5
LSD (0.05)		11.21	14.80

Table 3. Comparison of promptness index of germination between foreign and native rice varieties.

Type	Varieties	PI†
One year storage		
Korean native rice varieties	Colored rice	138.5
	Common rice	144.7
	Mean	141.6
Foreign rice varieties	Colored rice	175.0
	Common rice	182.4
	Mean	176.7
CV (%)		10.3
LSD (0.05)		26.17
Three months storage		
Korean native rice varieties	Colored rice	163.0
	Common rice	158.4
	Mean	160.7
Foreign rice varieties	Colored rice	151.2
	Common rice	163.8
	Mean	157.5
CV (%)		10.1
LSD (0.05)		25.67
CV (%)		15.7
LSD (0.05)		27.97

†PI; Promptness Index of germination.

resented in Table 1. The results of this study are supported by the data that there was significantly positive correlation between the value of DPPH and the color value of hulled rice (Tables 4 and 5). Thus, pigments in colored rices are thought to be largely involved in the high antioxidative activity. However, the greatest inhibition on DPPH and TBA value among common rice varieties was 67.10%, 33.61% for Sancheongdo in korean native varieties and 87.20%, 56.16% for IET 60 in foreign varieties during one year storage, and was 67.06%, 32.79% for IRI 301 for korean native varieties and 90.10%, 62.74% for IET 60 in foreign varieties during three months storage, respectively. This result points to the possibility that common rice seed may have potential antioxidative activity. The response is in agreement with the results from our isovitexin analysis study (did not shown data).

Also, the difference of antioxidative activity between DPPH and TBA methods cannot be explained readily. Chung *et al.* (1998), also concluded that antioxidative activity between the DPPH and TBA methods was different.

Measurement of promptness index by germination tests

As shown in Table 3, PI values of rice seeds were higher in three months storage (mean PI=160.7) than that in one year storage (mean PI=141.6) in korean native rices. On the other hand, PI values were higher in one year storage (mean PI=176.7) than that in three months storage (mean PI=157.5) in foreign rices. Hence, rice hulls might play an important role against damage caused by oxygen radicals.

Autooxidation of rice lipids is reported as one of several causes reducing the germination of rice seeds (Povarova *et al.*, 1975). One of the major factors responsible for loss of viability in stored rice seeds was ascribed to damage to the intracellular membrane structure (Ramarathnam *et al.*, 1986). This study suggests that an antioxidants of rice seeds, which have effective role of chemical protection to both the rice grain and the germ, is involved

Table 4. Correlation coefficients among antioxidative activity characters, promptness index and chroma values of hulled rice stored one year.

	DPPH	TBA	PI	L	a
TBA	0.0262				
PI	-0.0747	-0.1908**			
L	-0.5163**	0.0559	-0.1085		
a	0.5744**	-0.0641	0.1312	-0.6604**	
b	-0.6486**	-0.0287	-0.1352*	0.7903**	-0.7757**

PI; Promptness Index of germination, L; Lightness, a; Redness, b; Yellowness.
*,**Significant at 5% and 1% level, respectively. df=491 for DPPH, TBA, PI and df=245 for L, a, b.

Table 5. Correlation coefficients among antioxidative activity characters, promptness index and chroma values of hulled rice stored three months.

	DPPH	TBA	PI	L	a
TBA	0.3657**				
PI	-0.0660	-0.1326*			
L	-0.5660**	-0.2011**	-0.0211		
a	0.5630**	0.2540**	-0.0250	-0.8724**	
b	-0.4561**	-0.2790**	-0.0505	0.8290**	-0.7065**

PI; Promptness Index of germination, L; Lightness, a; Redness, b; Yellowness.
*,**Significant at 5% and 1% level, respectively. df = 491 for DPPH, TBA, PI and df=245 for L, a, b.

in germination viability.

Correlation analysis between antioxidative activity tests and color value

The utility of DPPH and TBA methods for evaluating the antioxidative activity of rice grain was examined by the correlation analysis between the estimates of antioxidative activity and the color of hulled rice. In relationship between antioxidative activity and the color values of hulled rice, (L) and (b) value showed the highly negative correlations with DPPH estimates (r = -0.5163**, r = -0.6486**), while (a) revealed a highly positive correlation (r = 0.5744**) with the antioxidative activity (Table 4). However, (L) and (b) value showed highly negative correlations with DPPH (r = -0.5660**, r = -0.4561**) and TBA (r = -0.2011**, r = -0.2790**), while (a) value revealed the highly positive correlation with DPPH (r = 0.5630**) and TBA (r = 0.2540**) in three months stored rice (Table 5). The results of this study are supported by the report of Choi and Oh (1996) that pointed out the antioxidative activity of anthocyanin pigment in colored rices. Thus, color pigments such as the known rice antioxidant, cyanidin, are thought to be largely involved in the high antioxidative activity in colored rice extracts (Cho *et al.*, 1996; Choi *et al.*, 1994 and 1996). These results are similar to those of Ramarathnam *et al.* (1986 and 1989), Wu *et al.* (1994) and Cho *et al.* (1996), indicating that colored rices showed higher antioxidant activities and the pigments of hulled rice play an major role in antioxidative activity. Phenolic compounds such as flavonoids, isovitexin, cyanidin, oryzanol, α-tocopherol and phytic acid are known as antioxidants (Ramarathnam *et al.*, 1986 and 1989; Wu *et al.*, 1994; Choi and Oh, 1996). This study result implies that the pigments of colored rices is a potential natural antioxidant, the detailed antioxidant activity of diverse colored rices must be studied more extensively. Also, this study may be suitable rice breeder to breed rice variety with a high antioxidative activity for a rapid screening of a small amounts of a large number of samples using color value.

However, the data showed a negative correlation between PI and TBA (r = -0.1908**) in one year stored rices and (r = -0.1326*) in three month stored rices. This result is similar to that of Ramarathnam *et al.* (1986), who observed that there were no any positive correlation between the amount of natural antioxidants in rice hull and germination ability of rice seeds. Although antioxidative activity mechanism is distinct that he rice containing high antioxidant is thought to provide physical protection of grain from attack by insects and fungi as well as maintain high germination ability preventing rice lipid autoxidation during storage, the relationship between antioxidative activity and germination ability did not agree well with the germination data of the corresponding rice varieties.

By the result of this study, it can be supposed that many compounds of rice grain extracts might considerably contribute to the varietal or methodological difference of antioxidative activity. However, further investigations are required to identify the antioxidant substances, to examine the influence of environmental variations on varietal variation of antioxidative ability and the significance of these compounds on seed viability during storage.

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REFERENCES

Baber, S. 1972. *In* D. F. Houston (ed.) Rice chemistry and technology. Amer. Assoc. Cereal Chemists. p. 237.

Cho, M. H., H. H. Yoon, and T. R. Hahn. 1996. Thermal stability of the major color component, cyanidin-3-glucoside, from a korean pigmented rice variety in aqueous solution. *Agric.*

Chemistry and Biotechnology. 39(3) : 245-248.

Choi, H. C. and S. K. Oh. 1996. Diversity and function of pigments in colored rice. *Korean J. Crop Sci.* 41 : 1-9.

Choi, S. W., S. H. Nam, and H. C. Choi. 1996. Antioxidative activity of ethanolic extracts of rice brans. *Foods and Biotechnology.* 5(4) : 305-309.

Choi, S. W., W. W. Kang, T. Osawa, and S. Kawakishi. 1994. Antioxidative activity of crysanthemin in black rice hulls. *Foods and Biotechnology.* 3(4) : 233-237.

Chung, I. M., K. H. Kim, and J. K. Ahn. 1998. Screening Korean medicinal and food plants with antioxidant activity. *Korean J. Medicinal Crop Sci.* 6(4) : 311-322.

Mitsuda, H., K. Yasumoto, and R. Iwamik. 1966. Antioxidative action of indole components during the autotoxidations of linoleic acid. *J. Jpn. Soc. Food and Nutrition.* 19(3) : 60-65.

Navasero, E. P., L. C. Baun, and B. O. Juliano. 1975. Grain dormancy, peroxidase activity and oxygen uptake in *Oryza sativa. Phytochem.* 14 : 1899-1902.

Osawa, T., N. Ramarathnam, K. Shunro, N. Mitsuo, and T. Toru. 1985. Antioxidative defense system in rice hull against damage caused by oxygen radicals. *Agric. Biol. Chem.* 49(10) : 3085-3087.

Park, P. W. 1995. Toxic compounds derived from lipids. p. 363. *In* I. J Jeon, and W.G Ikins (eds) Analyzing food for nutrition labelling and hazardous contaminants. Marcel Dekker, Inc., New York.

Povarova, R. I., N. P. Krasnook, I. A. Vishnyakova, and E. K. Davidenko. 1975. Change in a lipid complex in relation to the loss of germinating power by rice grains. Izv. Vyssh. Uchbn. Zaved., *Pisch. Tekhnol.* 3 : 42-45.

Ramarathnam, N., T. Osawa, M. Namiki, and T. Tashiro. 1986. Studies on the relationship between antioxidative activity of rice hull and germination ability of rice seeds. *J. Sci. Food Agric.* 37 : 719-726.

Ramarathnam, N., T. Osawa, M. Namiki, and T. Tashiro. 1989. Chemical studies on novel rice hull antioxidants. 2. Identification of isovitexin, a *c*-glycosyl flavonoid. *J. Agric. Food Chem.* 37 : 316-319.

SAS Institute. 1986. SAS User's Guide; Basic. 5th ed. SAS Institute, Cary, NC.

Wong, S. F., B. Holliwell, R. Richimond, and W. R. Skowroneck. 1981. The role of superoxide and hydroxyl radicals in the degradation of hyaluronic acid induced by metal ions and ascorbic acid. *J. Inorganic Biochem.* 14 : 127-134.

Wu, K., W. Zhang, P. B. Addis, R. J. Epley A. M. Salih, and J. Lehrfeld. 1994. Antioxidant properties of wild rice. *J. Agric. Food Chem.* 42 : 34-37.

Yoshida, T., K. Mori, T. Hatano, T. Okumura, I. Ushara, K. Komagoe, Y. Fujita, and T. Okuda. 1989. Studies on inhibition mechanism of autooxidation by tannins and flavonoids. V. Radical scavenging effects of tannins and related polyphenols on 1,1-Diphenyl-2-picrylhydrazyl radical. *Chem. Pharm. Bull.* 37(7) : 1919-1923.