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Antioxidant Activity in Rice Cultivar, Wild Rice, and Barley

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ABSTRACT: The antioxidant activities of methanol extracts of sixteen samples were tested using 1.1-diphenyl-2-picryl hydrazyl(DPPH) reactivity and TBARS substances assay in vitro. The methanol extracts of the rice brans from three wild rice -*O. minuta, O. rufipogon,* and *O. barthii*- were found to be the most effective in DPPH radical scavenging activity. The next effective ones were the rice brans of Heugjinjubyeo and leaves of Tapgolbori. When tested on lipid peroxidation using a lipid peroxidation generation system mediated by H_2O_2/Fe^{2+} in rat liver homogenates, the brans and hull of wild rice (*O. minuta, O. rufipogon,* and *O. barthii*) and rice bran of Heugjinjubyeo exhibited protective activities against lipid peroxidation in the order of effectiveness.

Keywords **:** rice, barley, by-products(rice bran, rice hull, leaf of barley), DPPH radical scavenging activity, inhibition of lipid peroxidation.

Antioxidant substances are prevalent in plants and animals. Antioxidizer such as ascorbic acid, tocopherol and carotenoids, which in general, vegetables and fruits contain, delays or prevents fat oxidation. Furthermore, it is a primary factor that fights aging by preventing and delaying cancer, cardiac system disorder, and adult disease. Natural antioxidant materials existing in plants are extensively used for food, medical supplies and cosmetics. Oil and fat or food containing them are mostly acidified by the association with oxygen in air, and the plenty of synthetic or natural antioxidant substances have been developed to prevent it. However, the synthetic antioxidizer such as BHA(butylated hydroxyanisol) and BHT(butylated hydroxytoluene) are more frequently used due to their effects and economical reasons. The synthetic antioxidizer is in general used for commercial food because of the excellent anti-oxidation it has; however, its use has been legally regulated for safety reasons expecially when it is used for food. Natural materials such as tocopherol is safe, but it cannot prevent oxidant chain reactions well and costs a lot. Therefore, the natural antioxidizer, which can be substituted for synthetic anti-oxidizer has been continuously developed from an extract of natural flavor or cultivated edible plants, which are typically eaten by human and whose safety is recognized.

Recent reports revealed that compared with the commonly cultivated rice, the seeds of wild rice can survive even the long-term storage. It is also said that such viability resulted from antioxidant materials which are obtained by the wild rice so as to keep their species in nature. Ramarathnam(1989) said that the reason for strong viability of rice seeds is due to antioxidant materials called isovitexin, a kind of flavonoid which exists in the caryopsis of the seeds. Another research showed that natural pigment of the colored rice contains the high level of antioxidant and that new antioxidant substances named saponarin exist in the leaves of young barley (Choi, 1994; Ohkawa, 1998). However, no study has been conducted on whether or not by-products of the cultivated plants such as barley leaves. Rice bran, rice hull of wild rice, and colored rice have antioxidant effects.

The purpose of this research is to examine the degree of the antioxidant effects of by-products' MeOH Ex. of 16 cereal crops using DPPH and TBARS.

MATERIALS AND METHODS

Test samples

16 samples of by products in cereal crops were used for this test. These samples were plants that we usually cultivate. Their names, used parts, the quantity of methanol extracts and extract rate were presented in Table 1. Reagent DPPH (1.1-dipheny1-2-picry1 hydrazyl) and TBA(Thiobarbituric acid) were purchased from Sigma Chemical Co., and TCA (Trichloroacetic acid) is from Junsei Chemical Co. For the rest of the reagent, special grade or first class reagent was used.

Preparation of methanol extracts

Rice was pounded by pounding machine and 7% of rice bran and hull were used in the test. MeOH Ex is produced

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by the following procedures: when the 3 main leaves of barley came out and reached 7 cm, they were dried in the shade, cut, and preserved in good condition. Then, they are filtered, and their filtrate was collected, decompressed, and condensed by rotary vacuum evaporator (EYELA, Japan).

Measurement of radical scavenging effect on DPPH

After MeOH Ex. of each sample was dried in a drying oven (Cheil Co., Korea), it was dissolved in 99.5% methanol, and manufactured after diluting it up to 1, 10, 100 times. Then, DPPH(1.5×-4 M) 300 μl was added to 900 μl of each sample, mixed by a vortex mixer (Hwa Shin Med Lab., Korea) for 10 seconds, and then cultivated in 37°C water bath(Buchi) for 30 minutes. Optical density was measured by spectrophotometer (8452A Hewlett Packard, USA) at 520 nm. and free radical scavenging effect of each sample's extract was indicated as electron donating ability (EDA%).

Manufacture of rat liver homogenate

Test animals were Sprague-dawley male rats which weigh 200 ± 20 g, and were bred as solid sample (Samyang Oil & Feed Co.) in an animal's room at 20 ± 2° C (temperature) and 50 ± 10% (humidity) for one week. After the test, the animals were narcotized by ether and dissected. Ice cold KCI (0.15 M) was run through liver vein to remove and extract blood in the liver. The liver was cleansed using 0.15 M ice cold KCl, and ice cold KCI, which was ten times heavier than the liver, was promptly added, and the liver was cut and homogenized in an ice bath for five minutes.

TBARS (Thiobarbituric Acid Reactive Substances) Assay

Each of the following solution is produced up to 1,700 μl with Liver homogenate 500 μl (equivalent to 15 mg protein): 50 mM phosphate buffer (pH 7.4) 1,100 μl, 0.01 M L-ascorbic acid 100 μl, or 50 mM phosphate buffer (pH 7.4) 800 μl, 30 mM H_2O_2 300 μl and 0.01 M L-ascorbic acid 100 μl, or 50 mM phosphate buffer (pH 7.4) 800 μl, 3.3 mM FeSO4 300 μl, and 0.01 M L-ascorbic acid 100 μl, or 50 mM phosphate buffer (pH 7.4) 800 μl, 30 mM H_2O_2 150 μl, 3.3 mM $FeSO_4$ 150 μl, and reaction mixture of 0.01 M L-ascorbic acid 100 μl.

Sample by concentration was added to each round bottom flask test tube and cultivated in a water bath (37°C) for 20 minutes in order to create liquid peroxidation. Stop solution (0.38% TBA, 15% TCA, 0.25 N HCl) 4.0 mL was added to

Table 1. Methanol extract of 16 samples and their radical scavenging effects of the methanol extract on DPPH.

Scientific name (Varietiy name or common name)	Part used	Dry weight (g)	Total ex. weight (g)	Extract rate (%)	Sample concentration (mg)	EDA (%)	IC_{50}† (mg/mL)
1. *O. sativa* L.(Dongjinbyeo)	Rice bran	20.75	3.51	16.9	9.40 0.94 0.09	95.33 71.96 45.10	2.12
2. *O. sativa* L.(Ilpumbyeo)	Rice bran	20.90	3.52	16.8	9.40 0.94 0.09	93.23 70.68 46.25	2.08
3. *O. sativa* L.(Heugjinjubyeo)	Rice bran	21.14	3.53	16.7	9.40 0.94 0.09	74.45 49.94 40.26	0.96
4. *O. sativa* L.(Dongjinbyeo)	Rice hull	55.62	5.0	9.0	12.8 1.28 0.13	69.87 47.90 40.12	2.29
5. *O. sativa* L.(Ilpumbyeo)	Rice hull	55.47	4.9	8.9	12.8 1.28 0.13	69.80 46.71 41.35	2.46
6. *O. sativa* L.(Heugjinjubyeo)	Rice hull	55.14	5.2	9.4	12.8 1.28 0.13	69.81 47.85 38.10	2.39

Table 1. Continued.

Scientific name (Varietiy name or common name)	Part used	Dry weight (g)	Total ex. weight (g)	Extract rate (%)	Sample concentration (mg)	EDA (%)	IC_{50}† (mg/mL)
					8.3	95.71	
7. *O. minuta* (wild) rice	Rice bran	39.12	4.96	12.7	0.83	63.63	0.16
					0.08	43.08	
					8.3	95.70	
8. *O. rufipogon* (wild)	Rice bran	39.16	4.98	12.7	0.83	63.60	0.17
					0.08	43.09	
					8.3	95.70	
9. *O. barthii* (wild)	Rice bran	39.15	4.96	12.6	0.83	63.63	0.17
					0.08	43.09	
					6.3	73.89	
10. *O. minuta* (wild)	Rice hull	55.62	4.75	8.5	0.63	47.48	0.80
					0.06	43.92	
					6.3	69.72	
11. *O. rufipogon* (wild)	Rice hull	55.62	4.60	8.2	0.63	46.53	0.87
					0.06	45.16	
					6.3	69.72	
12. *O. barthii* (wild)	Rice hull	55.62	4.60	8.2	0.63	46.53	0.87
					0.06	45.16	
					10.6	70.05	
13. *H. vulgare* L. (Kangbori)	Leaf	23.77	6.93	29.1	1.06	48.61	1.20
					0.11	44.21	
					11.0	76.69	
14. *H. vulgare* L. (Tapgolbori)	Leaf	23.70	6.90	29.1	1.0	46.57	1.39
					0.11	43.08	
					11.0	76.25	
15. *H. vulgare* L. (Namhyangbori)	Leaf	23.70	6.91	29.1	1.0	46.57	1.38
					0.11	43.08	
					11.0	76.65	
16. *H. vulgare* L. (Sacheon 6)	Leaf	23.70	6.89	29.1	1.0	45.50	1.39
					0.11	43.08	
					5.00	98.51	
17. L-ascorbic acid					0.50	99.19	<0.07
					0.05	99.29	

†IC_{50} values were calculated by extrapolation.

the created liquid peroxidation respectively and boiled at 100°C for 15 minutes to allow color reaction. After cooling it in ice water for ten minutes, absorptivity of supernatant, which was centrifuged (3,000 rpm) for ten minutes, was measured by spectrophotometer (535 nm).

RESULTS AND DISCUSSION

The antioxidant capability of the samples was obtained by comparing the following: measurement by EDA(%) on DPPH and calculation of inhibition intensity of each sample on free radical inhibition by 50%. The results are presented in Table 1.

When comparing the brans and hull of standard cultivated rice, the brans and hull of wild rice, and the intensity of free radical scavenging effects of MeOH Ex. from barely leaf at IC_{50}, the IC_{50} value of bran and hull of wild rice, *O. minuta,* was 0.16 and 0.80 mg/mL respectively. This indicated that it

Table 2. Effect of sample extracts on the lipid peroxidation induced buffer in the normal rat liver homogenate *in vitro*.

Common name	Part used	Sample concentration (mg)	Lipid peroxidation inhibition (%)	IC_{50}† (mg/ml)
1. Standard	L-ascorbic acid	1.0	64.15	0.66
		0.5	39.58	
		0.25	33.90	
2. *O. sativa* L. (Dongjinbyeo)	Rice bran	1.0	65.56	-
		0.5	< -	
		0.25	65.38	
3. *O. sativa* L. (Ilpumbyeo)	Rice bran	1.0	66.99	-
		0.5	65.16	
		0.25	70.89	
4. *O. sativa* L. (Heugjinjubyeo)	Rice bran	1.0	43.19	1.5
		0.5	30.54	
		0.25	< -	
5. *O. sativa* L.(Dongjinbyeo)	Rice hull	1.0	21.40	-
		0.5	24.97	
		0.25	31.89	
6. *O. sativa* L. (Ilpumbyeo)	Rice hull	1.0	22.21	-
		0.5	25.86	
		0.25	32.18	
7. *O. sativa* L. (Heugjinjubyeo)	Rice hull	1.0	22.19	-
		0.5	26.35	
		0.25	33.41	
8. *O. minuta* (wild)	Rice bran	1.0	37.45	0.07
		0.5	71.46	
		0.25	67.43	
9. *O. rufipogon* (wild)	Rice bran	1.0	37.46	0.09
		0.5	71.46	
		0.25	66.15	
10. *O. barthii* (wild)	Rice bran	1.0	37.39	0.09
		0.5	71.46	
		0.25	66.15	
11. *O. minuta* (wild)	Rice hull	1.0	64.20	0.2
		0.5	70.89	
		0.25	59.98	
12. *O. rufipogon* (wild)	Rice hull	1.0	63.21	0.2
		0.5	69.96	
		0.25	60.87	
13. *O. barthii* (wild)	Rice hull	1.0	64.20	0.2
		0.5	70.72	
		0.25	60.45	
14. *H. vulgare* L. (Kangbori)	leaf	1.0	51.17	0.94
		0.5	40.68	
		0.25	51.74	
15. *H. vulgare* L. (Tapgolbori)	leaf	1.0	51.24	-
		0.5	41.72	
		0.25	49.68	
16. *H. vulgare* L. (Namhyangbori)	leaf	1.0	52.31	-
		0.5	43.16	
		0.25	47.25	
17. *H. vulgare* L. (Sacheon 6)	leaf	1.0	52.49	-
		0.5	44.18	
		0.25	46.25	

†IC_{50} values were calculated by extrapolation.

had stronger free radical scavenging effects than cultivated rice.

The IC_{50} value of the brans and hull of wild rice, *O. rufipogon and O. barthii,* were 0.17 and 0.87 mg/mL respectively, which showed that these had stronger free radical scavenging effects than Dongjinbyeo and Ilpumbyeo.

These results were similar to Ramarathnam's that addressed the character of wild rice possessing the antioxidant substances. The rice hull of Heugjinjubyeo showed a higher antioxidant capability than Dongjinbyeo and Ilpumbyeo. This was believed to be caused by antioxidant capability of bran layer's natural pigment.

Among colored rices, the bran layer of Heugjinjubye that contained the highest natural pigment of C3G (cyanidin 3-glucoside) exhibited a superior DPPH free radical scavenging capability compared with a standard cultivated rice's bran layer.

Choi (1994) and Wang (1996) supported the above result by reporting that C3G pigment had the most powerful antioxidant activities among the natural pigments.

On the other hand, when examining antioxidant capability of barley leaves by species, by comparing Tapgolbori containing high saponarin component and Kangbori, Namhyangbori, Sacheon 6 containing low saponarin component, Tapgolbori has a superior DPPH free radical scavenging capability (Ryu, 2001). Therefore, the research and reviews of relationship saponarin component contents and antioxidant capability should be conducted by developing more various species.

Effect of sample extracts on the lipid peroxidation

The lipid peroxidation inhibition effect of sample by density on the lipid peroxidation that comes from buffer/H_2O_2/Fe^{+2}/L-ascorbic acid system was compared and analyzed with L-ascorbic acid as standard substances using a liver homogenate of a normal rat at *in vitro.*

As showed in Table 2, as for the lipid peroxidation inhibition effect by buffer, the rice bran of a wild rice, *O. rufipogen,* was 70 μg/ml of IC_{50}, which was fairly high, and that the brans and hull of *O. minuta* and of a wild rice, *O. barthii,* showed somewhat high inhibition activities around 90 μl/ml of IC_{50}m. This represented stronger inhibition activities than L-ascorbic acid. Heugjinjubyeo, red-purpled rice exhibited high inhibition activities as well. The results implied that if antioxidant capability of by-products such as the bran, the rice hull, etc., was defined in great detail, it could also be used for industrial purposes.

As shown in Table 3, the inhibition ratio of lipid and oxidation induced by H_2O_2 could not obtain the IC_{50} value for the following reasons: inhibition activity of peroxidation is

Table 3. Effect of sample extracts on the lipid peroxidation induced by H_2O_2 in rat liver homogenate *in vitro.*

Common name	Part used	Sample concentration (mg)	Lipid peroxidation inhibition (%)	IC_{50}† (mg/mL)
1. Standard	L-ascorbic acid	1.0	-	-
		0.5	-	
		0.25	23.15	
2. *O. minuta*	Rice bran	1.0	64.50	-
		0.5	91.48	
		0.25	83.35	
3. *O. rufipogen*	Rice bran	1.0	59.83	-
		0.5	88.52	
		0.25	82.15	
4. *O. barthii*	Rice bran	1.0	61.35	-
		0.5	88.79	
		0.25	84.38	
5. *O. sativa* L. (Heugjinjubyeo)	Rice bran	1.0	62.66	0.47
		0.5	51.90	
		0.25	57.85	
6. *H. vulgare* L. (Tapgolbori)	leaf	1.0	68.09	2.25
		0.5	73.24	
		0.25	72.43	

†IC_{50} values were calculated by extrapolation.

too high or there was no reaction to capacity within measurement density except for 3 types of sample extracts. The above ratio was calculated using 2 of density which indicate the rice bran's capacity reaction.

However, barley leaves and Heugjinjubyeo showed considerably higher antioxidant effects than L-ascorbic acid, compared with its peroxidation inhibition ratio under 0.25 mg/ml density.

Out of 20 species of sample extracts, IC_{50} values of liquid peroxidation inhibition could be obtained only for 7 species (refer to Table 4). In case of the rice bran of 3 varieties, wild rice shows similarity to DPPH radical scavenging capability in the following aspect: it exhibited high lipid peroxidation inhibition activities at 0.07 mg/ml of IC_{50} under 2 of density. Lastly, the hull of wild rice and the brans of Heugjinjubyeo, colored rice, showed high lipid peroxidation inhibition activities.

Conclusion

The research on 16 samples of edible plants were conducted regarding MeOH Ex.'s antioxidant effects by the parts using DPPH and TBA. The free radical scavenging effect using a DPPH was clearly observed in the brans and hull of wild rice, the brans of Heugjinjubyeo and leaf of Topgolbori. Furthermore, the lipid peroxidation inhibition effect induced by buffer of sample extracts showed that the inhibition effect rice bran of

Table 4. Effect of sample extracts on the lipid peroxidation induced by $FeSO_4$ in rat liver homogenate *in vitro*.

Common name	Part used	Sample concentration (mg)	Lipid Peroxidation inhibition (%)	IC_{50}† (mg/mL)
1. Standard	L-ascorbic acid	1.0	25.78	-
		0.5	19.76	
		0.25	25.43	
2. *O. sativa* L. (Dongjinbyeo)	Rice bran	1.0	83.93	-
		0.5	84.21	
		0.25	54.20	
3. *O. sativa* L.(Ilpumbyeo)	Rice bran	1.0	83.91	-
		0.5	85.20	
		0.25	60.72	
4. *O. sativa* L.(Heugjinjubyeo)	Rice bran	1.0	82.95	0.32
		0.5	90.28	
		0.25	23.80	
5. *O. sativa* L.(Dongjinbyeo)	Rice hull	1.0	83.45	-
		0.5	85.10	
		0.25	24.35	
6. *O. sativa* L.(Ilpumbyeo)	Rice hull	1.0	84.15	-
		0.5	86.10	
		0.25	30.16	
7. *O. sativa* L.(Heugjinjubyeo)	Rice hull	1.0	84.16	-
		0.5	86.89	
		0.25	25.16	
8. *O. minuta*	Rice bran	1.0	67.75	0.07
		0.5	60.03	
		0.25	55.96	
9. *O. rufipogen*	Rice bran	1.0	67.75	0.07
		0.5	60.03	
		0.25	55.96	
10. *O. barthii*	Rice bran	1.0	67.75	0.07
		0.5	60.03	
		0.25	55.96	

Table 4. Continued.

Common name	Part used	Sample concentration (mg)	Lipid Peroxidation inhibition (%)	IC_{50}† (mg/mL)
11. *O. minuta*	Rice hull	1.0	84.81	0.22
		0.5	91.92	
		0.25	55.23	
12. *O. rufipogen*	Rice hull	1.0	84.81	0.21
		0.5	90.92	
		0.25	56.23	
13. *O. barthii*	Rice hull	1.0	84.80	0.22
		0.5	91.92	
		0.25	55.24	
14. *H. vulgare* L.(Kangbori)	leaf	1.0	88.28	-
		0.5	85.10	
		0.25	79.81	
15. *H. vulgare* L.(Tapbori)	leaf	1.0	86.23	-
		0.5	84.10	
		0.25	75.10	
16. *H. vulgare* L.(Namhyanbori)	leaf	1.0	86.15	-
		0.5	80.15	
		0.25	74.16	
17.*H. vulgare* L.(Sacheon 6)	leaf	1.0	84.16	-
		0.5	79.15	
		0.25	73.84	

†IC_{50} values were calculated by extrapolation.

the wild rice, *O. minuta,* had the high IC_{50} value at 70 μg/ml.

The inhibition effect on the lipid peroxidation induced by H_2O_2 has the highest antioxidant inhibition effects in the hull of wild rice compared with a peroxidation inhibition ratio of L-ascorbic acid under 0.25 mg density. The lipid peroxidation inhibition effect induced by $FeSO_4$ was found particularly high in the 3 species of wild rice as IC_{50} of rice bran is 0.07 mg/ml.

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