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Antioxidant Activity and Total Phenolic Compounds in Grain Extracts of Wheat, Barley, and Oat

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ABSTRACT: Cereal grains are rich in phenolic compounds that give beneficial effect in human health. Although several research works have been reported on the effects of phytochemicals of plant origin, such as fruits, vegetables, few studies have examined the antioxidative effects of whole cereal grains. The objective of this study was to determine total antioxidant capacity of 80% ethanolic extracts of cereal grains by testing the ability of the extracts to inhibit UV-induced lipid peroxidation *in vitro* using linoleic acid in comparison to well-known antioxidant such as ascorbic acid and tannic acid. The total phenolic content of the cereal grain (80% ethanolic extracts) investigated in this study varied from 2.1 mg/g (wheat cv. Olgeurumil) to 10.4 mg/g (barely cv. Seodunchalbori). Highly positive relationship between total phenol compounds and antioxidant activity was found. When the antioxidant activities of all investigated extracts were measured with application of same quantity of phenol compounds, oat grain extracts showed similar antioxidant activity of barely cultivars. However, barely extract appeared as the most potent antioxidant activity of inhibition of UV-induced lipid peroxidation. This indicated that factors such as phenolic compound composition and their individual antioxidant activity could play a crucial role in the total antioxidant activity of cereal grains.

Keywords : cereal grain, wheat, barely, oat, phenolic compounds, antioxidative capacity

Many experimental and epidemiological studies show that grains, vegetables and fruits contain a large variety of substances called "plant chemicals" or "phytochemicals" (Johnson, 1994; Sung *et al.*, 1995; Wei *et al.*, 1999). The term "phytochemical" refers to every naturally occurring chemical substance present in plants, especially those that are biologically active (Caragay, 1992). Major phytochemicals include phenolic acids, flavonoids, and coumarin derivatives as well as many other polyphenols.

Phenolic compounds are derived from cinnamic acid, which is formed from phenylalanine by the action of phenylalanine ammonia-lyase (PAL), the branch point enzyme between shikimate pathway and phenylpropanoid metabolism. They are usually conjugated to sugars, cell wall carbohydrates, or organic acids and it provides rigidity as well as rendering the walls hydrophobic and water permeable. Despite the importance of lignin to land plant growth, phenol compounds are synthesized when the cells recognize unfavorable environment, and this means that when the environment during seed maturation is not suitable for the development of the seeds, the seeds make phenolic compounds and avoid the danger (Whetten, 1995; Dixon, 1995).

It is suspected that phenolic compounds may be at least partially responsible for the beneficial effects derived from the consumption of whole grains, fruits, vegetables, and beverages. Phenolic compounds have strong *in vitro* antioxidant properties associated to their ability to scavenge free radicals and chelate metals: the stabilization of such radicals by other functional groups in the structure enhances the antioxidant activity. An increased consumption of phenolic compounds has been correlated with a reduced risk of cardiovascular diseases and certain types of chronic disease (Ferguson and Harris, 1999; Osada *et al.*, 2001; Osawa, 1999). Consequently, dietary phytochemicals may actively contribute to the control of oxidative reactions and provide protection *in vivo*. Antioxidants also play an important role in preventing undesirable changes in flavor and nutritional quality of foods (Handelman *et al.*, 1999). The chemical composition and bioavailability of phytochemical varies between species and varieties of grains and may be affected by forms of processing as feed and food (Bravo, 1998).

Cereal grains are thought to be particularly rich source of phenolic compounds, which are located in aleulroan layer; the total amounts may up to 500 mg/kg of edible cereals (Senter *et al.*, 1983). Cereal grains also provide significant quantity of energy, protein and selected micronutrients to the animals and human diet, and have various types of protective components. Whole grains are rich source of phytoestrogens and fermentable carbohydrates including dietary fiber, resistant starch, and Oligosaccharides. Oat and barely contain about one-third soluble fiber which is associated with cholesterol-lowering effect and improved glucose re-

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sponse (Welch, 1995). Therefore considerable epidemiological evidence implicated that consumption of whole grains are related to reduced risk for diabetes, obesity and cancer, especially those of the gastrointestinal tract and breast (Slavin *et al.*, 1999; Sung *et al.*, 1998). In cereal crops, flavonoids are present in small quantities. Barley contains measurable amounts of catechins and some di- and trimer procyanidins that has shown antioxidant properties (Ferguson and Harris, 1999; Maillard *et al.*, 1996; Thompson, 1994, Salunkhe *et al.*, 1982). Although numerous studies have been reported on the effects of phenolic compounds of other plant origin, such as fruits, vegetables, few studies have examined the antioxidative effects of whole grains.

Therefore, the objective of this study was to determine total antioxidant capacity of 80% ethanolic extracts of cereal grains by testing the ability of these extracts to inhibit UV-induced lipid peroxidation *in vitro* using linoleic acid in comparison to well-known antioxidants. Relationship between quantity of phenolic compounds content in ethanolic extracts of cereal grains and total antioxidant capacity was examined.

MATERIALS AND METHODS

Grain samples

Grain samples of wheat, barely, oat were provided by the National Crop Experimental Station at Suwon. The samples included four wheat cultivars, ten barely cultivars and one oat cultivar (Table 1). We used hulless grain for wheat cultivars and whole meal for oat and barely cultivars. Grains were grounded in a laboratory mill type KM75 (KRUPS, Germany) and were stored at 4 until used.

Preparation of extracts

Ground samples (5 g) were extracted with 80% aqueous ethanol (150 mL) by agitating (150 rpm) at 20°C for 24 h. Extracts were filterscreened and ethanol soluble extractant was evaporated under vacuum. The residues were refrigerated and freeze-dried. The lyophilizates were dissolved in methanol (2.5 mg/mL) for measuring electron donating ability, capability of inhibition of UV-induced lipid peroxidation in linoleic acid and determining total phenolic compounds.

Determination of total phenolic compounds (TPCs)

Total phenolic compounds were quantified according to the method of Shahidi and Naczk (1995). A 0.25 mL aliquot of the extract solution (2.5 mg/mL methanol) was mixed with 0.25 mL of Folin-Ciocalteu reagent (previously diluted with water 1 : 1 v/v) and 0.5 mL of saturated sodium carbonate (Na_2CO_3) solution and 4 mL of water. Mixture was set at room temperature for 25 min followed by centrifugation at 5000 rpm for 10 min. Supernatant absorbance was measured at 725 nm and expressed as (±) tannin equivalents.

Table 1. Total phenol contents and antioxidant activity of cereal grain extracts.

Cereal grain	Total phenol contents (mg/g seed)†	Electron donating ability (%)‡	Relative EDA to phenol content¶	TBARS (Inhibition %)§
Oat	2.69	27.34 ± 3.13*	10.15	26.74 ± 20.62*
Wheat (Urimil)	2.84	14.43 ± 4.31*	5.07	-11.26 ± 26.55
Wheat (Tapdongmil)	2.89	20.63 ± 10.55*	7.15	-38.47 ± 11.66
Wheat (Alchanmil)	3.08	16.82 ± 2.26*	5.45	-2.59 ± 10.27
Wheat (Olgeurumil)	2.48	22.75 ± 15.95*	9.17	-7.92 ± 6.16
Barely (Namhyangbori)	5.25	76.45 ± 10.39*	14.57	6.93 ± 8.71*
Barely (Jinyangbori)	6.24	76.79 ± 0.48*	12.29	16.40 ± 9.58*
Barely (Doosan #8)	4.27	69.72 ± 0.19*	9.80	16.86 ± 8.56*
Barely (Doosan #29)	6.89	80.04 ± 2.74*	18.76	45.58 ± 22.23*
Barely (Saeolbori)	7.11	78.93 ± 0.53*	11.44	16.40 ± 13.14*
Barely (Saessalbori)	4.57	77.21 ± 1.07*	16.91	9.86 ± 3.45*
Barely (Saegangbori)	6.42	75.82 ± 0.95*	11.81	23.16 ± 16.02*
Barely (Olbori)	8.73	78.69 ± 0.79*	9.01	34.48 ± 16.02*
Barely (Seodunchal)	10.16	80.67 ± 2.15*	7.94	22.18 ± 1.15*
Barely (Chalbori)	6.65	81.38 ± 1.67*	12.24	33.88 ± 15.24*
Tannic acid	-	91.24 ± 0.19*	-	82.22 ± 1.99*
Ascorbic acid	-	92.77 ± 0.27*	-	74.01 ± 0.46*

†Total phenol contents by Folin-Ciocalteu Methods performed duplicate, ‡EDA (%) = (1 - sample Abs./control Abs.) × 100, §Inhibition (%) = (1 - sample Abs./control Abs.) × 100, ¶EDA (%)/total phenolic amount.
*Means are significantly different from control at $p<0.05$ as determined by Fisher's least significantly different test.

Measurement of electron donating ability (EDA)

The measurement of electron-donating ability was measured based on the method of Blios (1958) who measured EDA of ethanol extracted cereal samples, ascorbic acid, tannic acid to DPPH (1,1-diphenyl-2-picryl-hydrazyl). Briefly, 20 mg of DPPH was dissolved in 150 ml of ethanol, and sample solutions were prepared to give concentration of 2.5 mg/ml. DPPH solution (0.5 ml) was added to vials containing an aliquot (50 μl) of each sample solution (final conc. 1 mM) as an initiator of radicals and mixed thoroughly for 5 sec. After 30 min of incubation at room temperature, absorbance was measured at 570 nm. Absorbance of the vial containing DPPH compound was compared to that of the vial containing DPPH only. Percent EDA was calculated using the following formula;

EDA (%)
= [1 - (sample absorbance/control absorbance)] × 100

Thiobarbituric acid-reactive substances (TBARS)

Inhibitory action of lipid peroxidtion of samples was measured by the decreased formation of thiobarbituric acid reactive substances using linoleic acid as the substrate. Linoleic acid (0.1% w/v) was dispersed in sodium lauryl sulfate solution (0.8% w/v). To an aliquot (0.8 ml) of above linoleic acid solution, 0.1 ml EDTA (0.1 mM) and 0.1 ml of sample solution were added. Ultraviolet lamp (40 W) was illuminated at a distance of 30 cm for 90 min. A half ml of trichloroacetic acid (0.44 M) and 0.5 ml of 0.8% 2-thiobarbituric acid were mixed and incubated for 15 min at 100°C. The absorbance was measured at 532 nm.

Statistical analysis

All experiments were performed two or three times with each assay in triplicates. The results were expressed as the mean ± standard deviation (SD). The differences between treatment groups were compared by analysis of variance. The p value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The total phenolic content

The total phenolic contents of 80% ethanolic extracts of the cereal grains were varied from 2.1 mg/g (wheat cv. Olgeurumil) to 10.4 mg/g (barely cv. Seodunchalbori) (Table 1). The highest amounts of phenolic components were found in barley followed by oat and wheat. The oat and wheat grain contained about 2~3 times smaller total phenolics as compared to barley. The lowered phenolic contents in wheat might be associated with using dehulled grains. Relatively low phenolic content was also found in naked barely. Generally, phenolic compounds were accumulated high in the outer layers of the grains than in the endosperm (Slavin *et al.*, 1999; Thompson, 1994). The formation of flavone and flavonol glycosides greatly depends on light: therefore, the highest concentrations of these compounds are found generally in leaves and outer parts of plants, with only trace amounts in the subterranean parts of plants. Amount of phenolic compounds in cereals was various among cultivars within the species. The presence of phenolic compounds in plant food is largely influenced by genetic factors and environmental conditions. Other factors, such as germinations, degree of ripeness, variety, processing, and storage, also influenced the content of plant phenolics (Bravo, 1998). Phenolic conetents of three malting barely cultivars 'Namhyang', 'Jinyang', 'Doosan8' were lower than other barely cultivars except 'Saessalbori' that was naked barely. These barely cultivars are selected for low protein and phenolic contents because beer precipitates resulted by conjugation between phenolic compounds and proteins upon cooling (Kasha *et al.*, 1993).

Electron donating ability of cereal grain extracts

The comparisons were done in respect to the ascorbic acid and tannic acid. Ascorbic acid and tannic acid are synthetic antioxidant with phenolic structure and have been used in various health food products. EDA of ascorbic acid and tannic acid revealed 91.2 and 92.7%. The contents of total phenolic compounds and EDA values of lyophilizates of 80% ethanol extracts obtained from the whole grain are shown in Fig. 1. The relationship between total phenol compounds and antioxidant activity of all 80% ethanol extracts is given by the equation $y = 138.75x - 2.17$, with $R^2 = 0.82$. This result indicated that there was a highly positive relationship between total phenol compounds and antioxidant activity. The estimated values of EDA based on the relative abilities of the barely extracts to scavenge the DPPH (1-diphenyl-2-picrylhydrazyl) in comparison with ascorbic acid were similar and showed a higher antioxidant activity than other cereals. About two fold and five fold decreased activities than that of barely were found in oat and wheat, respectively. However, when the antioxidant activities of all investigated extracts were measured with application of same quantity of phenol compounds, oat grain extracts exhibited similar activity as barely did. This result might be associated with different accumulation of phenolic acids composition in different plant parts, such as groat and hull. Xing and White (1997) reported in respect to oat, that caffeic acid was only found in

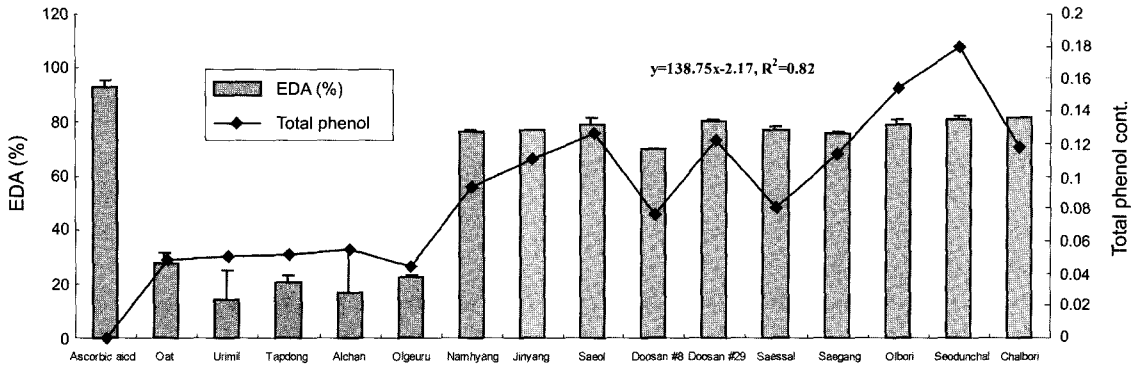

Fig. 1. Total phenol compounds and electron donating abilities (EDA) of cereal grain extracts to 1-diphenyl-2-picrylhydrazyl (DPPH) radicals.

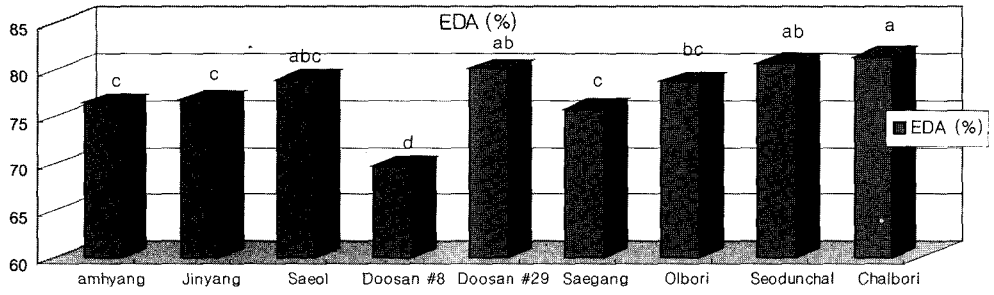

Fig. 2. Comparison of Electron donating abilities (EDA) within barely cultivars.

the groat extract, but not the hull extracts. Hull extract contained the other kind of phenolic compounds such as *o*-coumaric, sinapic, and salicylic acids. As another group of phenolic antioxidants in oat cinnamoyl-anthranilic acid derivatives called avenanthramides can enhance the antioxidant activities. Oat groat contains at least 25 avenanthramides whose antioxidant capacity are about 60% of that of α-tocopherol while hull extracts contain only 20 of these compounds.

We compared the antioxidative activities among barely cultivars except 'Saessalbori', naked barely in Fig. 2. Antioxidative activities were various among barely cultivars and high in 'Doosan9', 'Seodunchal', 'Chalbori', 'Saeolbori'.

In wheat cultivars, any significant difference between cultivars for antioxidative activities were not found. Mukoda (2001) and Onyeneho (1992) reported antioxidative effect of hull part of wheat. However it is important for dehulled fraction of wheat to have antioxidative activities because dehulled fraction was major cereal part being used in food processing and food industry.

Inhibitory effect of lipid peroxidation

Our study showed that 80% ethanol extracts of cereal grains exhibited antioxidant activity evaluated under UV-induced lipid peroxidation in linoleic acid (Fig. 3). The wheat extracts did not show any antioxidant properties using UV-

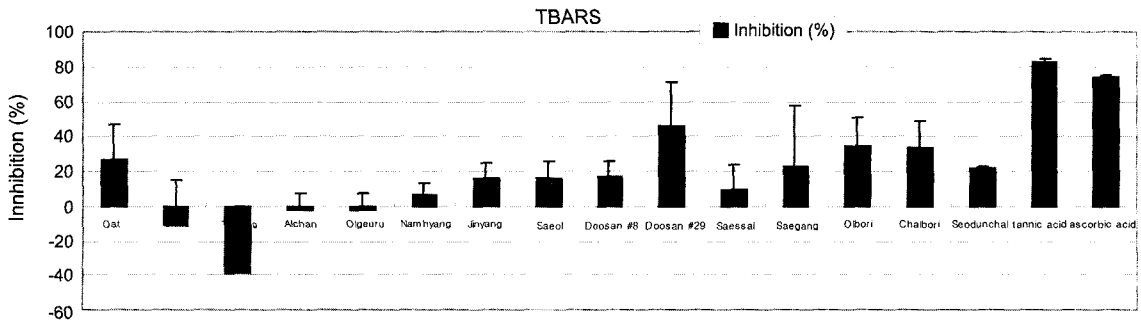

Fig. 3. Inhibitory effect of lipid peroxidation induced by U.V. radiation by cereal grain extracts and major antioxidants.

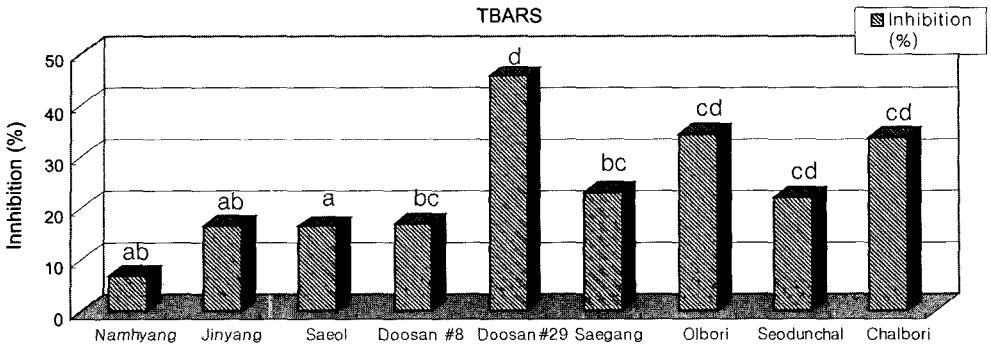

Fig. 4. Comparison of inhibitory effect of lipid peroxidation induced by U.V. radiation within barely cultivars.

induced lipid peroxidation. This result may due to using dehulled fraction of wheat, although other studies reported antioxidative activity of wheat bran (Onyeneho and Hettiarachchy, 1992). The highest antioxidant properties were observed in the extract from barely and significant differences of antioxidative activities among barely cultivars were also found. This result indicated that factors such as phenol compound composition, their individual antioxidant activity, and solubility together with other water and lipid-soluble antioxidant in the cereal grains could play a combined role in the inhibition of lipid peroxidation. It can be also explained by the fact that the Folin-Ciocalteu method measures phenolics with other constituents, which alleviate its specificity (Shahidi and Nack, 1995). Moreover, all the phenolics do not have the same antioxidant activity, some are powerful, others are weak, and they develop antagonistic or synergistic effects with themselves or with the other constituents of extracts (Wei *et al.*, 1999; Friedman and Jurgens, 2000). Cereal protein has been reported to exert strong antioxidant activities (Iwama *et al.*, 1987). The ability of antioxidants to inhibit UV-induced lipid peroxidation *in vitro* using the linoleic acid and the relative abilities of antioxidants to scavenge the DPPH radicals showed similar mode of reactivities (Table 1, Fig. 4). Doosan 29 showed highest antioxidative activity among barely cultivars and Namhyangbori showed the least antioxidative activity with equal amount of phenolic compound application. Although its phenolic contents were low, it had high antioxidative activities. Although most of malting barely have low phenolic contents, we can obtain beneficial health effect from beverages made with this type of barely which have low phenolic contents and high antioxidative activities, and this properties could be applied to breeding program for malting barley.

Since cereals are used as a main food source and consumed large amount, it may be important to evaluate their functional activities. In the present study, electron-donating ability and inhibition of lipid peroxidation of cereal extracts were measured, and the results showed significant antioxidative activities although they were significantly weaker antioxidant activities than ascorbic acid and tannic acid.

Evaluation of antioxidant properties of cereal grain extracts including some of the important properties of cereal constituents such as reducing agents, potential complexers of prooxidant metals, and quenchers of singlet oxygen is required to further elucidation of functional activities of cereal extracts.

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