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Antioxidant and Tyrosinase Inhibitory Activities from Seed Coat of Brown Soybean

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Abstract Soybeans with brown, black, and yellow seed coats were compared to total phenolic contents and antioxidant activities including 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radicals. Also, 3 seed coats were examined for inhibitory activities on tyrosinase and lipoxygenase-1 on the basis of spectrophotometric and polarographic methods. Among seed coat extracts, 80% methanol extract of brown soybean seed coat showed the highest total phenolic contents (68.9±3.29 mg GAE/g) as well as exhibited potent scavenging effects on the DPPH (IC_{50}=4.3 μg/mL) and ABTS (IC_{50}=3.7 μg/mL) radicals. In a polarographic experiment, this extract was potentially inhibited the oxidation of L-tyrosine and L-3,4-dihydroxy-phenylalanin (L-DOPA) catalyzed by mushroom tyrosinase with IC_{50} values of 12.4 and 63.7 μg/mL, respectively. It was also detected inhibition of the tyrosinase catalyzed oxidation of L-DOPA with an IC_{50} value of 120.3 mg/mL in UV spectrophotometric experiment. In addition, this extract inhibited the linoleic acid peroxidation catalyzed by lipoxygenase-1 with an IC_{50} value of 4.0 μg/mL. These results suggest that brown soybean may possess more beneficial effect on human health than black and yellow soybeans.

Keywords: brown soybean seed coat, antioxidant, tyrosinase, lipoxygenase, oxygen consumption, total phenolic content

Introduction

Soybeans [*Glycine max* (L.) Merr.] and soy products, which are very rich in isoflavones, anthocyanins, and protein, are very popular foods in Korea (1-5). Recently, many studies have shown that daily intakes of soy foods were associated with the positive aspects such as antioxidative (6), antibacterial (7), antifungal agents (8), tyrosine protein kinase inhibitors (9), and cancer preventive property (10). It is well established that isoflavone and anthocyanin of soybean components are responsible for the physiological action, prevention of osteoporosis and circulatory disorders, and inflammatory diseases (11-13). Moreover, in our previous studies demonstrated that pterocarpans of soybean roots showed potent low-density lipoprotein (LDL) oxidative and human Acyl-CoA: cholesterol acyltransferase (hACAT) inhibitory activities (14,15).

In generally, studies of the antioxidants contained in soybeans have been conducted with the normal soybean and isoflavones are reported to be the most abundant antioxidants in soybean cotyledons and hypocotyls (16, 17). However, the biological activities in soybean seed coats have received a limited amount of attention because they have been assumed to be unimportant. Soybeans have various seed coat colors such as brown, black, yellow, green, and yellowish-whiter due to anthocyanins, chlorophyll (18). Among them, brown and black soybeans have been widely utilized as food and material for Oriental medicine, contain anthocyanins in the seed coat (19). Especially, it has been reported that anthocyanins from seed coat of black soybean inhibited the expression of tumor necrosis factor α (TNF-α)-induced genes and protected myocardiac injury from ischemia-reperfusion (I/R) (20). Even though black and yellow soybeans are known to have pharmaceutical effects (2,21), biological activities of brown soybeans are few reported except radical scavenging activity (22). In our continuing search for biological activity sources from natural plants, we found that methanol extract of brown soybean seed coat showed potent tyrosinase and lipoxygenase inhibitory activities and radical scavenging activities.

Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme widely distributed in natural that catalyzes 2 distinct reactions of melanin biosynthesis, the hydroxyla-tion of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones (23,24). This enzyme is also known as a polyphenol oxidase, and the browning of some fruits, beverages, and vegetables due to tyrosinase cause a significant decrease in their nutritional and aesthetic value (25). Therefore, the control of tyrosinase is important in relation to browning control of fresh materials. Additionally, tyrosinase inhibitors have become increasingly important in medicinal and constituents of cosmetic products in relation to hyperpigmentation (26).

Lipoxygenase (EC 1.13.11.12) is an enzyme that is found in many plants and animals, which catalyses the oxygenation of polyunsaturated fatty acids to form fatty acid hydroperoxides (27). This enzyme is suggested to be

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involved in the early event of atherosclerosis by inducing plasma LDL oxidation (28). On the other hand, in food protection, it is well known that lipid peroxidation is one of the major factors in deterioration during the storage and processing of foods, because it can lead to the development of unpleasant rancid or off flavors as well as potentially toxic end products (29).

In this study, we investigated that the radical scavenging activities in 3 soybean seed coats including brown, black, and yellow from different solvent systems. Furthermore, 3 soybean seed coats were for the first time evaluated for their inhibitory activities on tyrosinase and lipoxygenase through oxygen consumption and spectrophotomeric experiment. We were also evaluated for total phenolic contents using methanol extracts with several water percentages.

Materials and Methods

Plant materials Three soybeans with black, brown, and yellow seed coats, *G max* cv. Heugcheongkong, Galmikong, and Saealkong were selected in this study. These cultivars were grown at the experimental field of Yeongnam Agricultural Research Institute, National Institute of Crop Science, Rural Development Administra-tion, Miryang, Gyeongnam, Korea, in 2005. After harvest, 3 soybeans were cleaned in distilled water to remove extraneous matters and subsequently dried at room temperature for overnight. The dried seeds were stored at 4°C until they were used.

Reagents Gallic acid, Folin-Ciocalteau's phenol reagent, mushroom tyrosinase (EC 1.14.18.1), L-tyrosine, ethanol, dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, linoleic acid (purity> 99%), Tween-20, and soybean lipoxygenase (type 1, EC 1.13.11.12) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tris buffer and boric acid were obtained from Fisher-Scientific Co. (Fair Lawn, NJ, USA). L-DOPA, $NaH_2PO_4 \cdot H_2O$, and Na_2HPO_4 were purchased from Aldrich Chemical Co. (St. Louis, MO, USA). 4-*tert*-Butylcatechol was purchased from Fluka Chemical Co. (Milwaukee, WI, USA). 13(*S*)-Hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13-HPOD: λ_{max}=234 nm, ε=25/mM/cm) was prepared enzymatically by described procedure (30) and stored in ethanol at −18°C.

Instruments In spectrophotometric experiment, radical scavenging and enzyme inhibitory activities were monitored by an Infinite M200 spectrophotometer (Tecan Austria GmbH, Salzburg, Austria). Also, tyrosinase-dependent O_2 uptake and lipoxygenase-dependent O_2 uptake were performed using an OX_1LP Dissolved O_2 package (Qubit Systems Inc., Kingston, ONT, Canada).

Soybean seed coat extraction Three seed coats of *G max* cv. Hegcheongkong, Galmikong, and Saealkong, a breeding lines of black, brown, and yellow soybeans were peeled manually. The pulverized seed coats (each 0.1 g) were extracted with add a certain amount of water to the methanol in order to improve the extraction of secondary metabolites from seed coats. The crude extracts were filtrated and stored at 4°C until they were used.

Determination of total phenolic content Total phenolic content was measured according to the modified Folin-Ciocalteau colorimetric method (30). Briefly, each sample (1.0 mL) was mixed with Folin and Ciocalteu's phenol reagent (1.0 mL). After 5 min, 2 mL of 2% Na_2CO_3 solution was added to the mixture and the volume brought up to 10 mL by adding distilled water. After the reaction mixture was kept in the dark for 2 hr, absorbance was measured at 724 nm. The concentration of total phenolic content in the extracts was calculated using the following linear equation based on the calibration curve: y=0.0026x −0.00045, R^2=0.999, where, y was the absorbance and x was the total phenolic contents in mg of gallic acid equivalents (mg GAE/g extract).

DPPH radical scavenging activity Antioxidant activity of extract was measured on the basis of the scavenging activity of the stable DPPH free radical following the method described by Braca *et al.* (32). Various concentrations of extract were added to a concentration of 0.15 mM in EtOH, and the mixture was shaken vigorously. Absorbance at 517 nm was determined after 30 min, and the radical scavenging effect was calculated as $[A_c-A_t/A_c]\times 100$, where A_t and A_c were the absorbance of samples with and without sample extracts, respectively.

Trolox equivalent antioxidant capacity (TEAC) scavenging activity The TEAC assay was based on the relative ability of antioxidants to scavenge the radical cation $ABTS^{\cdot+}$ in comparison to a standard (Trolox) (33). The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate. The reaction mixture was maintained for 4-8 hr until the mixture was complete and the absorbance is stable. $ABTS^{\cdot+}$ solution was diluted with ethanol and the absorbance was read at 734 nm. For the photometric assay 0.9 mL $ABTS^{\cdot+}$ solution and 0.1 mL compounds were mixed for 45 sec and the absorbance measured immediately after 1 min at 734 nm. Antioxidant activity of each extract was calculated by determining the decrease in absorbance at different concentrations using the following equation: $E=[(A_c-A_t)/A_c]\times 100$, where A_t and A_c were absorbance of samples with and without sample extracts, respectively. Antioxidant activity was expressed as TEAC values.

Tyrosinase inhibitory activity The mushroom tyrosinase was purified by anion-exchange chromatography using DEAE-Sepharose Fast Flow (Pharmacia, Uppsala, Sweden) as previous described (34). Throughout the experiment, L-DOPA or L-tyrosine was used as a substrate. In a spectrophotometric experiment, the enzyme activity was monitored by dopachrome formation at 475 nm with an Infinite M200 spectrophotometer at 30°C. All samples were dissolved in ethanol and used for the experiment with dilution. The final concentration of ethanol in the test solution was always 3.3%. First, 0.06 mL of a 5.0 mM L-DOPA or L-tyrosine aqueous solution was mixed with 0.6 mL of 0.25 M phosphate buffer (pH 6.8) and 2.14 mL water, incubated at 30°C for 10 min. Then, 0.1 mL of the sample solution

and 0.1 mL of the same phosphate buffer solution of the mushroom tyrosinase (138 units) were added in this order to the mixture. Tyrosinase-dependent O_2 uptake was performed using an OX_1LP Dissolved O_2 package (Qubit Systems Inc.) at 30°C, under constant stirring with a rotating magnetic bar. The reaction mixture was essentially the same procedures in the spectrophoto-metric experiment. Calibration of an oxygen electrode was performed by using 4-*tert*-butylcatechol and excess tyrosinase according to the previous report (34).

Lipoxygenase inhibitory activity Lipoxygenase-dependent O_2 uptake (35) was performed using an OX_1LP Dissolved O_2 package at 25°C as essentially the same procedures in the tyrosinase assay. The reaction mixture (3.0 mL) contained 60 μM linoleic acid, inhibitor and 0.1 M Tris-HCl (pH 8.0). After 2 min pre-incubation, the reaction was started by addition of lipoxygenase-1 (4.35 nM).

Statistical analysis and curve fitting All measurements were repeated 3 times and the results were as the mean ±SD for the 3 experiments. Significance was determined by ANOVA analysis and a Duncan's multiple range tests ($p \leq 0.05$). Each assay was conducted in triplicate of separate experiments. The data analysis performed by using Sigma Plot 2001 (SPSS Inc., Chicago, IL, USA). The IC_{50}s were obtained by fitting experimental data to the logistic curve by Langmuir isotherm as follows (36).

$$\text{Activity (\%)} = 100[1/\{1+([I]/IC_{50})\}]$$

where [I] is the inhibitor concentration.

Results and Discussion

Determination of total phenolic content The total phenolic content was investigated at different soybean seed coats and various conditions of extracting solvent system. Also, different extraction solvents of seed coat on black, brown, and yellow soybeans were expressed as mg/g of gallic acid (GAE/g). There were significant differences among soybean cultivars and different extracting solvent systems as well as these interactions were detected for total phenolic content (Table 1). Total phenolic content in seed coat of black soybean such as *G. max* cv. Heugcheongkong was observed the highest amount in 80% MeOH extract. The highest total phenolic content in brown soybean seed coat such as *G. max* cv. Galmikong was also observed in 80% MeOH extract and the lowest content was found in 100% MeOH extract. In yellow soybean seed coat such as *G. max* cv. Saealkong, total phenolic content was exhibited 6-10 folds lower than those in black and brown seed coats. As shown in Table 1, total phenolic content of black soybean seed coat in different extraction solvents was in the decreasing order of the 80% MeOH extract (52.1±1.84 mg GAE/g) >50% MeOH extract (46.6±1.95 mg GAE/g) >30% MeOH extract (40.5±2.84 mg GAE/g) >100% MeOH extract (7.5±0.99 mg GAE/g). In brown soybean seed coat, total phenolic content showed in the decreasing order of the 80% MeOH extract (68.9±3.29 mg GAE/g) >50% MeOH extract (49.0±2.42 mg GAE/g) >30% MeOH extract (43.0±1.89 mg GAE/g) >100% MeOH extract (15.4 ±0.69 mg GAE/g) (Table 1). Also, total phenolic content in

Table 1. Total phenolic contents of three soybean seed coats in different extraction solvents

Seed coat of *G. max*	Extraction solvent (% MeOH)	Total phenolic content (mg of GAE/g)[1]
Black soybean cv. Heugcheongkong	30	40.5±2.8e[2]
	50	46.6±2.0cd
	80	52.1±1.8b
	100	7.5±1.0g
Brown soybean cv. Galmikong	30	43.0±1.9de
	50	49.0±2.4bc
	80	68.9±3.3a
	100	15.4±0.7f
Yellow soybean cv. Saealkong	30	8.3±0.9g
	50	6.1±0.7g
	80	5.3±0.7g
	100	3.6±0.3g
Seed coat (S) LSD**		
Extraction solvent (E) LSD**		
S×E LSD**		

[1]The values indicate the mean's of 3 replications of the experiment for the total phenolic content of each extraction solvent (mean±SD, n=3).
[2]Values in the same column followed by different letters differ significantly at $p \leq 0.05$. **Significant at the $p<0.001$.

yellow seed coat using different extraction solvents were found to be 3.6-8.3 mg GAE/g and 100% MeOH extract of yellow seed coat showed the lowest total phenolic content in comparison to black and brown seed coats (Table 1). Even though previous study has shown that total phenolic content in black soybean seed coat extract was 2 folds more than that of 70% acetone extract from brown seed coat (22), in this study, brown seed coat had a higher total phenolic content than black seed coat ($p<0.05$). These might indicate that procyanidin content in seed coat was present in *G. max* cv. Galmikong (brown soybean) more than *G. max* cv. Heugcheongkong (black soybean).

Radical scavenging activity on DPPH and ABTS The DPPH and ABTS systems have both been commonly used to measure total antioxidative status of various biological specimens because of their good reproducibility and easy quality control (33). Especially, DPPH radical scavenging assay could be used to evaluated antioxidant activity in a relatively short time compared to other methods. Table 2 showed DPPH radical scavenging activity in 3 soybean seed coats using different extraction solvents. Although it was well established that antioxidant activity in the 70% acetone extract black soybean seed coat was higher than seed coat of brown soybean from Takahata *et al.* (22), in this work, the 80% methanol extract of brown soybean seed coat had the highest DPPH radical scavenging activity in comparison with those in seed coats of black and yellow soybeans. These results also suggested that black soybean seed coat had lower procyanidin concentration than brown seed coat. As shown in Table 2, 80% MeOH extract showed the highest antioxidant activity (brown seed coat: 75.9±2.21%, black seed coat: 68.1±1.87%, and yellow seed coat: 10.0± 0.05%) and 100% MeOH extract had

Table 2. Antioxidant activities of three soybean seed coats on DPPH radical using different extraction solvents

Seed coat of *G. max*	Extraction solvent (% MeOH)	DPPH radical scavenging activity (%)[1)]
Black soybean cv. Heugcheongkong	30	47.8±0.9e[2)]
	50	58.1±1.7d
	80	68.1±1.9b
	100	12.4±0.6g
Brown soybean cv. Galmikong	30	40.7±1.5f
	50	62.1±1.8c
	80	75.9±2.2a
	100	38.0±1.7f
Yellow soybean cv. Saealkong	30	1.0±0.1h
	50	4.0±0.1h
	80	10.0±0.1g
	100	2.0±0.1h
Seed coat (S) LSD**		
Extraction solvent (E) LSD**		
S×E LSD**		

1)The values indicate the mean's of 3 replications of the experiment for DPPH radical scavenging activity of each extraction solvent (mean± SD, n=3).
2)Values in the same column followed by different letters differ significantly at $p \leq 0.05$. ** Significant at the $p<0.001$.

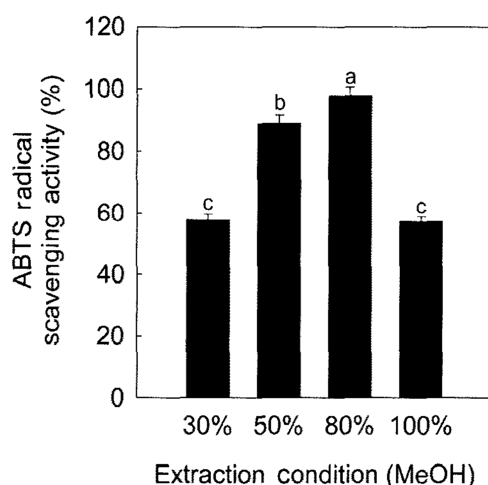

Fig. 1. ABTS radical scavenging activity of different methanol extracts from seed coat of brown soybean.

poor antioxidant activity in compared with other extraction solvents (brown seed coat: 38.0±1.67%, black seed coat: 12.0±0.56%, and yellow seed coat: 2.0±0.09%). The seed coat of brown soybean was observed the highest DPPH radical scaveng-ing activity and total phenolic content ($p \leq 0.05$). Therefore, this species clearly had a significant amount of DPPH radical scavenging activity and 80% MeOH solvent exhibited the best choice to extract procyanidin content from brown soybean seed coat. These results showed that procyanidins were quite likely to be the predominant compounds responsible for DPPH radical scavenging activity in brown soybean seed coat. To understand another parameter that was affected by radical

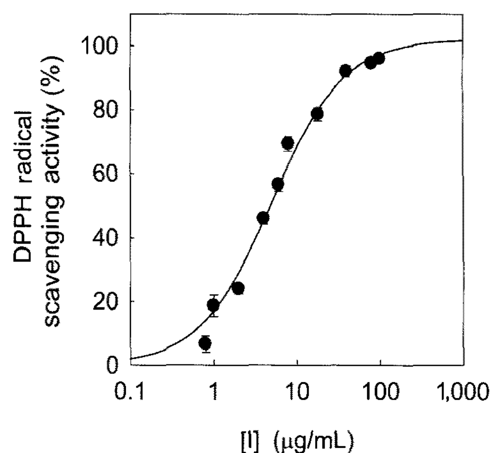

Fig. 2. Scavenging activity of 80% methanol extract against DPPH radical from seed coat of brown soybean.

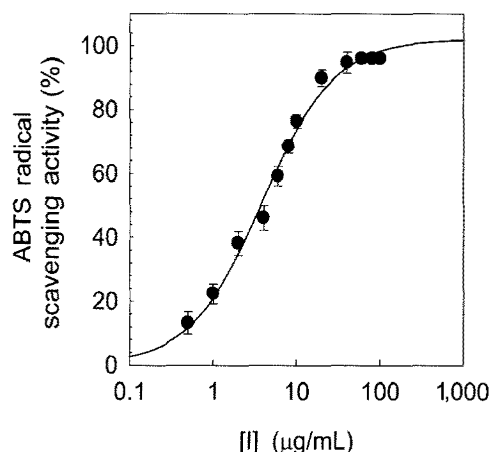

Fig. 3. Scavenging activity of 80% methanol extract against ABTS radical from seed coat of brown soybean.

scavenging activity, the seed coat of brown soybean was applied to ABTS radical scavenging activity. As shown in Fig. 1, the highest ABTS radical scavenging activity in brown seed coat extract was observed in 80% MeOH extract (97.8±2.72%) and different extraction solvents were in the decreasing order of 50% MeOH extract (89.1±2.55%) >30% MeOH extract (57.6±2.14%) >100% MeOH extract (57.1±1.66%). It was observed that seed coat of brown soybean showed a higher ability to scavenger ABTS radical than DPPH radical ($p<0.05$). Also, in most of the assays to determine their antioxidative properties, ABTS radical scavenging activity was strongly correlated with that of DPPH. Due to 80% MeOH extract of brown seed coat showed potent antioxidant activity, this extract was investigated at dose-dependent activities against DPPH and ABTS radicals (Fig. 2 and 3). As shown in Fig. 2 and 3, at a concentration of 1 mg/mL, this extract showed radical scavenging activity more than 20% and exhibited DPPH and ABTS radical scavenging activities with IC_{50} values of 4.3 and 3.7 μg/mL, respectively. To understand another parameter determining DPPH and ABTS radical scavenging activities, 80% MeOH extract of the highest antioxidant activity from brown seed coat was measured time-

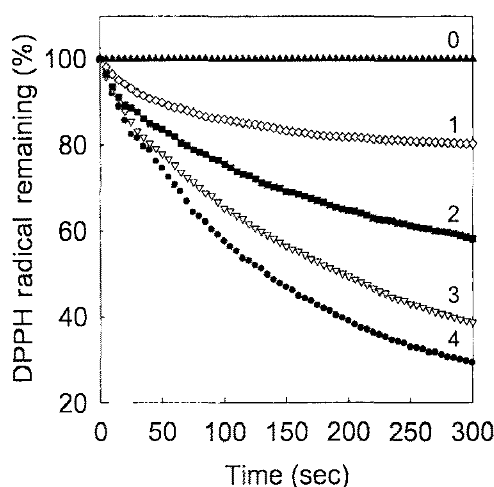

Fig. 4. Time-dependent scavenging of the DPPH radical in the presence of 80% methanol extract from seed coat of brown soybean. Concentrations of brown seed coat extract for curves 0-4 were 0, 2, 6, 40, and 100 mg/mL, respectively.

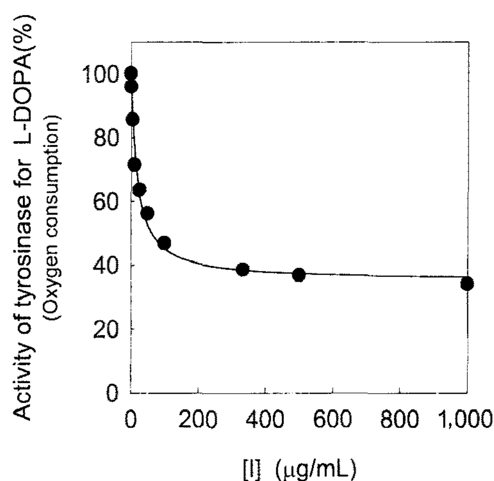

Fig. 5. Effects of 80% methanol extract from seed coat of brown soybean on the activity of the tyrosinase for catalysis L-DOPA at 30°C by measuring oxygen consumption.

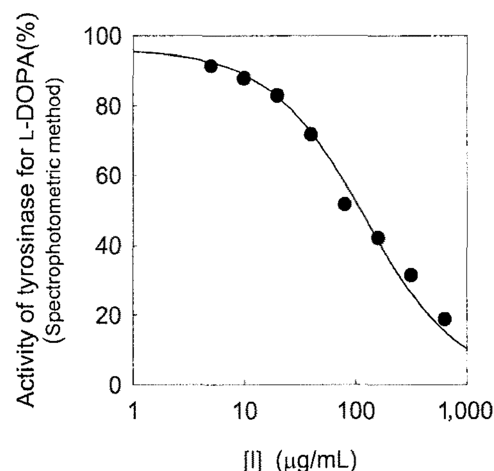

Fig. 6. Effects of 80% methanol extract from seed coat of brown soybean on the activity of the tyrosinase for catalysis L-DOPA at 30°C by spectrophotometric method.

dependent radical remaining effects. Although ABTS radical remaining effect was not measured due to fast radical scavenging activity, DPPH was carried out dose-dependent concentrations (100, 40, 6, 2, and 0 μg/mL) for 300 sec. The time course of DPPH radical remaining in the presence of different concentrations from 80% MeOH extract was shown in Fig. 4. After 300 sec, 83 and 58% DPPH radicals were remained at the presence of 2 and 6 μg/mL, while 39 and 29% DPPH radicals were remained at the presence of 40 and 100 μg/mL. As a result, we have shown that 80% MeOH extract of brown soybean seed coat exhibited a strong antioxidant activity in DPPH assay. These results suggested that antioxidant in soybean seed coat might play an important role in protection against oxidative damage and contributed to enhance the value of soybean as dietary supplement.

Tyrosinase inhibitory activity Three soybean seed coats were evaluated for their inhibitory activities on tyrosinase. Among them, black and yellow seed coats were not inhibitory activities, whereas brown seed coat showed potent inhibitory activity. More specifically, 80% MeOH extract showed the highest tyrosinase inhibitory activity. The effect of brown seed coat on the oxidation of L-DOPA (100 μM) catalyzed by the purified mushroom tyrosinase was studied first. The enzyme activity was monitored by measuring oxygen consumption. The 80% MeOH extract of brown seed coat showed a dose-dependent inhibitory effect on this oxidation (Fig. 5). As concentrations of this extract increased, the enzyme activity was rapidly decreased, and then slowed until a nearly straight line was approached. The remaining enzyme activity was about 34% when this extract concentration reached 1 mg/mL. The inhibitory concentration (IC_{50}) leading to 50% activity lost was estimated to be 63.7 μg/mL. It should be noted the enzyme activity was also usually monitored by measuring dopachrome formation at 475 nm accompanying the oxidation of L-DOPA (100 μM). Although dopachrome was a relatively stable intermediate, it was gradually oxidized further; and hence, the spectrophotometric method measures only the very initial rate of dopachrome formation (37). The spectrophotometric method was convenient and sensitive but may not be long enough to evaluate brown seed coat from a practical point of view. As a result, 80% MeOH extract of brown seed coat showed a dose-dependent inhibitory effect on this oxidation as shown in Fig. 6. As concentrations of brown seed coat extract increased, the enzyme activity was rapidly decreased with completely suppressed. The inhibitor concentration leading to 50% activity lost (IC_{50}) was estimated to be 120.3 μg/mL. Therefore, tyrosinase inhibitory activity of this extract was closely related with both radical scavenging activity and total phenolic content. To monophenolase inhibitory activity, 80% MeOH extract of brown soybean seed coat was tested for inhibit the hydroxylation of L-tyrosine. Hence, the effect of extract on the tyrosinase-catalyzed oxidation of L-tyrosine (100 μM) was studied. The enzyme activity was monitored by measuring oxygen consumption at 475 nm and showed a dose-dependent inhibitory effects on this

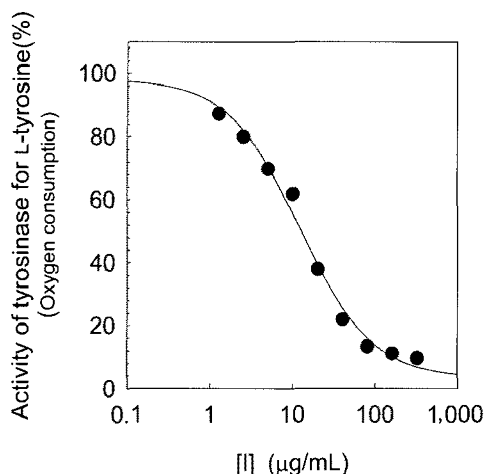

Fig. 7. Effects of 80% methanol extract from seed coat of brown soybean on the activity of the tyrosinase for catalysis L-tyrosine at 30°C by measuring oxygen consumption.

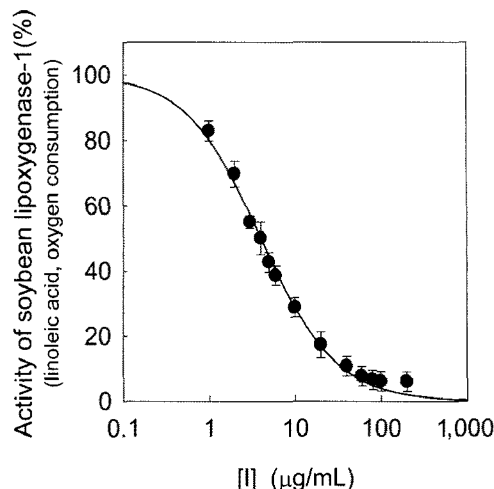

Fig. 8. Effects of 80% methanol extract from seed coat of brown soybean on the activity of soybean lipoxygenase-1 for the catalysis of linoleic acid at 25°C by measuring oxygen consumption.

oxidation as illustrated in Fig. 7. Brown soybean seed coat, never reported as tyrosinase inhibitory, showed potent activity with IC_{50} value of 12.4 μg/mL. These might explain the current observed effects of the seed coat extract in brown soybean on the tyrosinase-catalyzed oxidation of L-DOPA and L-tyrosine.

Lipoxygenase inhibitory activity We examined lipoxygenase-1 inhibitory activity of extracts made with various solvents from 3 soybean seed coats in previous study (35). The inhibition activity of lipoxygenase-1 was measured by a polarographic method. In the current experiment, linoleic acid was used as a substrate and the buffer used for all experiments was 0.1 M Tris-HCl at pH 8.0. The bioassay with 80% MeOH extract of brown seed coat was monitored by measuring oxygen consumption (polarographic method). This study reported that 80% MeOH extract showed the highest effect in comparison with other extracts of brown seed coat, whereas black and yellow seed coats were not detected with lipoxygenase-1 inhibitory activities. As shown in Fig. 8, this extract showed a dose-dependent inhibitory effect on this oxidation. As concentrations of extract increased, the enzyme activity was rapidly decreased with completely suppressed. The inhibitor concentration leading to 50% activity lost (IC_{50}) was estimated to be 4.03 μg/mL. These results indicated that 80% MeOH extract of seed coat in brown soybean had also potent lipoxygenase-1 inhibitory activity. From in this study, we suggested that seed coat of brown soybean might be useful as natural tyrosinase inhibitor, not only to protect against the oxidation of foods, but also to protect the human health against oxidative damage by free radicals. Thus, in order to develop new food products, the seed coat of brown soybean had detrimental effects on food quality and food processing additives.

In summary, 3 soybean seed coats were compared for their total phenolic contents and antioxidant activities based on DPPH and ABTS radical. The brown seed coat showed the highest DPPH and ABTS radical scavenging activities (DPPH: IC_{50}=4.3 μg/mL, ABTS: IC_{50}=3.7 μg/mL) in comparison with those of black and yellow soybeans and 80% MeOH extract of brown seed coat showed the most total phenolic content (68.9 mg GAE/g). Moreover, we were for the first time evaluated for inhibitory activities on tyrosinase and lipoxygenase from soybean seed coat. Interestingly, among them, brown seed coat showed potent tyrosinase (substrate: L-DOPA, tyrosinase-dependent O_2 uptake: IC_{50}=63.7 μg/mL, spectrophoto-metric method: IC_{50}=120.3 μg/mL; substrate: L-tyrosine, tyrosinase-dependent O_2 uptake: IC_{50}=12.4 μg/mL) and lipoxygenase-1 (IC_{50}=4.0 μg/mL) inhibitory activities. In the future, follow-up studies should be done to purify and define the bioactive substances in seed coat from brown soybean that function as antioxidants as well as tyrosinase and lipoxygenase inhibitory activities agents.

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References

1. Yang SO, Chang PS, Lee JH. Isoflavone distribution and β-glucosidase activity in '*cheonggukjang*', a traditional Korean whole soybean-fermented food. Food Sci. Biotechnol. 15: 96-101 (2006)
2. Lee JH, Renita M, Fioritto RJ, Martin SKS, Schwartz SJ, Vodovotz Y. Isoflavone characterization and antioxidant activity of Ohio soybeans. J. Agr. Food Chem. 52: 2647-2651 (2004)
3. Satué-Gracia MT, Heinonen M, Frankel EN. Anthocyanins as antioxidants on human low-density lipoprotein and lechitin-liposome systems. J. Agr. Food Chem. 45: 3362-3367 (1997)
4. Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. J. Agr. Food Chem. 45: 304-309 (1997)
5. Lee CH, Yang L, Xu JZ, Yeung SYV, Huang Y, Chen ZY. Relative antioxidant activity of soybean isoflavones and their glycosides. Food Chem. 90: 735-741 (2005)
6. Hu CC, Hsiao CH, Huang SY, Fu SH, Lai CC, Hong TM, Chen HH, Lu FJ. Antioxidant activity of fermented soybean extract. J. Agr. Food Chem. 52: 5735-5739 (2004)
7. Kwon TW, Song YS, Kim JS, Moon GS, Kim JI, Honh JH. Current

research on the bioactive functions of soyfoods in Korea. J. Korean Soybean Digest 15: 1-12 (1998)
8. Weidenborner M, Hindorf H, Jha HC, Tsotsonos P, Egge H. Antifungal activity of isoflavonoids indifferent reduced stage on *Rhizoctonia solani* and *Sclerotium rolfsii*. Phytochemistry 29: 801-803 (1990)
9. Graber M, June CH, Samelson LE, Weiss A. The protein tyrosine kinase inhibitor Herbimycin-A, but not genistein, specifically inhibits signal transduction by the T-cell antigen receptor. Int. Immunol. 4: 1201-1210 (1992)
10. Hendrich S, Lee KW, Xu X, Wang HJ, Murphy PA. Defining food components as new nutrients. J. Nutr. 124: 1789S-1792S (1994)
11. Bettini V, Fiori A, Martino R, Mayellaro R, Ton P. Study of the mechanism whereby anthocyanosides potentiate the effect of catecholamines on coronary vessels. Fitoterapia 54: 67-72 (1985)
12. Anthony MS, Clarkson TB, Williams JK. Effects of soy isoflavones on atherosclerosis: Potential mechanisms. Am. J. Clin. Nutr. 68: 1390S-1393S (1998)
13. Setchell KDR, Cassidy A. Dietary isoflavone: Biological effects and relevance for human health. J. Nutr. 129: 758S-767S (1999)
14. Lee JH, Lee BW, Kim JH, Jeong TS, Kim MJ, Lee WS, Park KH. LDL-antioxidant pterocarpans from roots of *Glycine max* (L.) Merr. J. Agr. Food Chem. 54: 2057-2063 (2006)
15. Lee JH, Seo WD, Jeong SH, Jeong TS, Lee WS, Park KH. Human acyl-CoA: Cholesterol acyltransferase inhibitory effect of flavonoids from roots of *Glycine max* (L.) Merr. Agr. Chem. Biotechnol. 49: 57-61 (2006)
16. Miyazawa M, Sakano K, Nakamura S, Kosaka H. Antimutagenic activity of isoflavones from soybean seeds (*Glycine max* Merrill). J. Agr. Food Chem. 47: 1346-1349 (1999)
17. Kim JA, Hong SB, Jung WS, Yu CY, Ma KH, Gwag JG, Chung IM. Comparison of isoflavones composition in seed, embryo, cotyledon, and seed coat of cooked-with-rice and vegetable soybean (*Glycine max* L.) varieties. Food Chem. 102: 738-744 (2007)
18. Todd JJ, Vodkin LO. Pigmented soybean (*Glycine max*) seed coats accumulate proanthocyanidins during development. Plant Physiol. 102: 663-670 (1993)
19. Choung MG. Structural analysis of black common bean (*Phaseolus vulgaris* L.) anthocyanins. Food Sci. Biotechnol. 14: 672-675 (2005)
20. Kim HJ, Tosy I, Park JM, Chung JI, Shin SC, Chang KC. Anthocyanins from soybean seed coat inhibit the expression of TNF-α-induced genes associated with ischemia/reperfusion in endothelial cell by NF-κB-dependent pathway and reduce rat myocardial damages incurred by ischemia and reperfusion *in vivo*. FEBS Lett. 580: 1391-1397 (2006)
21. Takahashi R, Ohmori R, Kiyose C, Momiyama Y, Ohsuzu F, Kondo K. Antioxidant activities of black and yellow soybeans against low density lipoprotein oxidation. J. Agr. Food Chem. 53: 4578-4582 (2005)
22. Takahata Y, Ohnishi-Kameyama M, Furuta S, Takahashi M, Suda I. Highly polymerized procyanidins in brown soybean seed coat with a high radical-scavenging activity. J. Agr. Food Chem. 49: 5843-5847 (2001)
23. Mayer AM, Harel E. Phenoloxidases and their significance in fruit and vegetables. pp. 373-398. In: Food Enzymology. Fox PE (ed). Elsevier Press, Inc., London, UK (1998)
24. Robb DA. Tyrosinase. Vol. II, pp. 207-240. In: Copper Proteins and Copper Enzymes. Lontie R (ed). CRC Press, Inc., Boca Raton, EL, USA (1984)
25. Mayer AM. Polyphenol oxidases in plants-recent progress. Phytochemistry 26: 11-20 (1995)
26. Kubo I, Kinst-Hori I, Kubo Y, Yamagiwa Y, Kamikawa T, Haraguchi H. Molecular design of antibrowing agents. J. Agr. Food Chem. 48: 1393-1399 (2000)
27. Gardner HW. Biological roles and biochemistry of the lipoxygenase pathway. Hortscience 30: 197-205 (1995)
28. Kris-Etherton PM, Keen CL. Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. Curr. Opin. Lipidol. 13: 41-49 (2002)
29. Grechkin A. Recent developments in biochemistry of the plant lipoxygenase pathway. Prog. Lipid Res. 37: 317-352 (1998)
30. Gibian MJ, Galaway RA. Steady-state kinetics lipoxygenase oxygenation of unsaturated fatty acid. Biochemistry 15: 4209-4214 (1976)
31. Park YK, Lee WY, Park SY, Ahn JK, Han MS. Antioxidant activity and total phenolic content of *Callistemon citrinus* extracts. Food Sci. Biotechnol. 14: 212-215 (2005)
32. Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. J. Nat. Prod. 64: 892-895 (2001)
33. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 26: 1231-1237 (1999)
34. Ha TJ, Tamura S, Kubo I. Effects of mushroom tyrosinase on anisaldehyde. J. Agr. Food Chem. 53: 7024-7028 (2005)
35. Ha TJ, Kubo I. Lipoxygenase inhibitory activity of anacardic acids. J. Agr. Food Chem. 53: 4350-4354 (2005)
36. Coperland RA. Enzyme: A Practical Introduction to Structure, Mechanism, and Data Analysis. Wiley-VCH, New York, NY, USA. pp. 266-332 (2000)
37. Mayer AM, Harel E, Ben-Shaul R. Assay of catechol oxidase-A critical comparison of methods. Phytochemistry 5: 783-789 (1966)