Highly Polymerized Procyanidins in Brown Soybean Seed Coat with a High Radical-Scavenging Activity

Yasuhiro Takahata,[§] Mayumi Ohnishi-Kameyama,[‡] Shu Furuta,[†] Masakazu Takahashi,[†] and Ikuo Suda*[,†]

National Agricultural Research Center for Kyushu Okinawa Region, National Agricultural Research Organization (KONARC/NARO), Nishigoshi, Kumamoto 861-1192, Japan and National Food Research Institute, Kannondai, Tsukuba 305-8642, Japan

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity of the 70% aqueous acetone extract from the seed coat of the brown soybean variety, Akita-Zairai, was investigated. The activity of the seed coat of Akita-Zairai was much higher than that of three other reddish-brown varieties, but lower than that of two black varieties, and was closely dependent on the content of phenolic compounds. In the LH20 column chromatography of Akita-Zairai, high DPPH radical-scavenging activities were detected in the fractions eluted with MeOH and 70% aqueous acetone. Proanthocyanidins were also detected in fractions showing high radical-scavenging activities. Matrix-assisted laser desorption/ionization−time-of-flight mass spectrometry analysis showed that the degree of polymerization (DP) of the procyanidins contained in the brown or black soybean seed coat was as high as DP30.

Keywords: *Soybean; Glycine max; procyanidin polymer; radical-scavenging activity; brown seed coat; MALDI−TOFMS*

INTRODUCTION

Soybeans have been regarded as a nutritionally rich food and are often used in the daily Japanese diet. Recently, attention has been given to the significant potential of the soybean in the prevention of chronic diseases such as atherosclerosis, coronary heart disease, and cancer. Oxidative damage is thought to represent one of the mechanisms leading to chronic diseases. Therefore, research has focused on the antioxidants in soybeans or their radical-scavenging activity. Studies of the antioxidants contained in soybeans have generally been conducted with the normal soybean, which has a yellowish-white seed coat. Isoflavones are reported to be the most abundant antioxidants in soybean cotyledons and hypocotyls (*1*, *2*). Antioxidants in the yellowish-white seed coat have received a limited amount of attention because they have been assumed to be unimportant. However, the antioxidants in the darker seed coats are worth studying because they are thought to contain anthocyanins and/or other secondary metabolites. Soybeans with brown or black seed coats have traditionally been a part of the Japanese diet even though they are sometimes consumed only occasionally. There are a few reports describing the anthocyanin content (*3*) or radical scavenging activity (*4*) in the black soybean; however, the radical-scavenging activities of extracts of the seed coats of the brown soybean have not been reported.

The objective of this work was to investigate and compare the radical-scavenging activity in the brown soybean seed coat with that of the black soybean seed coat, which has been reported to have a high degree of activity. Furthermore, we have characterized the proanthocyanidins, which are assumed to be the principal radical-scavenging components in the seed coat.

MATERIALS AND METHODS

Plant Materials and Extraction of Antioxidants from the Seed Coat. All soybean varieties listed in Table 1 were grown and harvested in an experimental field at the National Agricultural Research Center for Kyushu Okinawa region (KONARC), Nishigoshi, Kumamoto, Japan. Seed coats (ca. 150 mg) were removed and cut into small pieces for extraction of antioxidants. Because Ariga et al. (*5*) have reported that a high content of polymeric proanthocyanidins were extracted with 70% aqueous acetone from the black soybean, and our preliminary extraction experiments also indicated that this solvent was superior to 80% EtOH for the extraction of antioxidants, we used 70% aqueous acetone as an extraction solvent. Cut seed coats were extracted with 5 mL of 70% aqueous acetone overnight at 25 °C in the dark. The supernatant was concentrated by evaporation of acetone and adjusted to a final volume of 5 mL using an EtOH for dilution. The concentration of EtOH was set at ca. 80%. This 80% EtOH-based crude solution was used for all experiments in the present study.

Determination of Total Polyphenol Content. The total polyphenol content was determined by the Folin−Ciocalteu method using (+)-catechin as a standard. Thus, 2 mL of a 10% Folin reagent (Wako Pure Chemical Industries, Ltd., Japan) was added to 400 μL of a diluted sample solution, and the contents of the test tube were mixed. After an interval of 3 min, 2 mL of a 10% aqueous sodium carbonate solution was added, and the mixture was kept for 60 min. The absorbance was measured at 750 nm, and the total polyphenol content was expressed as a (+)-catechin equivalent.

* Corresponding author. Tel: +81-96-242-7742. Fax: +81-96-249-1002. E-mail: ikuosu@affrc.go.jp.

[†] National Agricultural Research Organization.

[‡] National Food Research Institute.

[§] Present address: Laboratory of Upland Crop Genetic Resources, Yokoichi 6651-2, Miyakonojo, Miyazaki 885-0091, Japan.

10.1021/jf010307x CCC: $20.00
Published on Web 11/03/2001

Table 1. DPPH Radical Scavenging Activity and Total Polyphenol Content of Different Soybean Varieties

variety	seed coat phenotype	radical scavenging activity[a]	total polyphenol content[b]
Akita-Zairai	brown	0.128 ± 0.023	41.6 ± 5.6
Shin-Tanbaguro	black	0.055 ± 0.006	81.3 ± 5.5
Shinanoguro	black	0.048 ± 0.004	94.8 ± 2.7
Akamame	reddish-brown	4.141 ± 0.856	6.55 ± 0.40
Akamame-Mirasaka	reddish-brown	3.414 ± 0.272	7.45 ± 0.05
Takada34	reddish-brown	2.661 ± 0.200	8.62 ± 0.24

[a] Radical scavenging activity is expressed as seed coat weight (g) required to cause a 50% decrease in the absorbance at 520 nm for the control (100%) (mean ± S. D., at least 3 replicates). A smaller seed coat weight indicates a higher radical scavenging activity. [b] mg/g seed coat, (+)-catechin equivalent (mean ± S. D., at least 3 replicates).

Assay of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical-Scavenging Activity. *Comparison for Varietal Differences.* The radical-scavenging activity was measured according to the method described by Suda et al. (*6*). DPPH was dissolved in 80% EtOH to give a 200 μM solution and formed a stable free radical. In the experiments to study varietal differences in DPPH radical-scavenging activity, the reaction mixture consisted of 0.5 mL of 200 μM of a DPPH solution and 0.5 mL of the test sample diluted in 80% EtOH (or 80% EtOH for the control). A series of diluted sample solutions was prepared before starting the reaction; for example, a crude solution was diluted as 10:490 (ratio of crude extract to 80%EtOH), 20:480, 30:470, 40:460, 50:450, and 100:400. The reaction was started by adding the diluted test sample. After the test tube had been kept for 20 min at 25 °C, the absorbance of the reaction mixture at 520 nm was measured, and the volume of crude extract required to cause a 50% decrease in the absorbance at 520 nm relative to the control (100%) was calculated. When the range of the dilution was unsuitable for determining a 50% decrease, the ratio of dilution was optimized, and the reaction was repeated until the proper value was obtained. The DPPH radical-scavenging activity was expressed as the seed coat weight, calculated from the added volume of the crude solution.

Evaluation of Eluates Fractionated on LH20. In the LH20 column fractionation described below, we simplified the method to evaluate the relative intensities of the DPPH radical-scavenging activity in the fractions derived from chromatography. After preliminary tests for an optimum condition to avoid complete fading of absorbance, we modified the method as follows. The reaction mixture consisted of 20 μL of each fraction and 1 mL of 100 μM DPPH in EtOH. For a blank mixture, 20 μL of EtOH, MeOH, or 70% acetone was used instead of a fraction. After the test tube had been kept for 60 min at 25 °C, the absorbance of the reaction mixture and the blank mixture at 520 nm was measured. Because a larger decrease in the absorbance at 520 nm means a higher radical-scavenging activity, differences between the reaction and the blank mixtures reflected the relative intensities of the DPPH radical-scavenging activity in the fractions. The data are expressed as differences in absorbance between the reaction and blank mixtures.

LH20 Column Fractionation. The crude solution was applied to an LH20 column (50 × 10 mm i.d.) by a modification of the method of Watanabe et al. (*7*). The LH20 resins (Pharmacia) were preequilibrated with EtOH. The brown soybean Akita-Zairai and the black soybean Shin-Tanbaguro were provided for analysis. In both varieties, crude solutions equivalent to 42 mg of seed coat were applied and sequentially eluted with 20 mL of EtOH, 20 mL of MeOH, and 20 mL of 70% acetone with collection of 1-mL fractions. The relative intensity of the DPPH radical-scavenging activity was evaluated for each fraction, as described above, and the proanthocyanidin content was estimated. The anthocyanin content was estimated only in the black soybean.

Evaluation of Proanthocyanidin and Anthocyanin Content. The proanthocyanidin content was evaluated using the HCl−butanol method (*8*). The reaction mixture consisted of 200 μL of each fraction and 800 μL of HCl−butanol (concentrated HCl/*n*-butanol, 1:5). Before the mixture was heated, the absorbance was measured at 548 nm. After the reaction mixture was heated at 95 °C for 30 min, the absorbance was again measured at 548 nm. The increase in absorbance at 548 nm was used for the measurement of proanthocyanidin content. In the case of the black soybean, because cyanidin-3-glucoside was reported to be the major anthocyanin (*3*), absorbance at 538 nm before heating, which is the maximum in the spectrum of cyanidin-3-glucoside, was also measured for evaluation of anthocyanin content.

Recovery of Radical-Scavenging Activities from LH20 Column Fractionation. To ascertain that all radical-scavenging activities eluted in the LH20 column fractionation, a recovery test of radical-scavenging activities was conducted on the brown soybean Akita-Zairai. After elution, all fractions eluted with MeOH and 70% acetone were combined and evaporated. The combined solution was finally adjusted to ca. 80% EtOH concentration and to 1.5 mL, which were the same conditions as those of the original extract used for the LH20 column chromatography. The DPPH radical-scavenging activity was determined by the same procedure for the comparison of varietal differences and compared to that in the original extract used for this test. The recovery test was replicated at least three times.

Matrix-Assisted Laser Desorption/Ionization−Time-of-Flight Mass Spectrometry (MALDI−TOFMS) Analysis. MeOH fractions from LH20 column chromatography were divided into three groups consisting of fractions 23−30, 31−35, and 36−40. The fractions 43−50 eluted by 70% acetone were also combined. Each lyophilized sample was dissolved in acetone (ca. 500 mg/L) and mixed with the same volume of a *trans*-3-indoleacrylic acid matrix solution (10 g/L).

MALDI−TOF mass spectra were acquired on a REFLEX II (Bruker Daltonik GmbH, Bremen, Germany) equipped with a pulsed ion extraction accessory, in which samples were irradiated with a nitrogen laser (wavelength 337 nm; 3-ns pulse). The reflectron mode of operation used 25 kV ion acceleration and 28.7 kV post-acceleration. Spectra obtained in the linear mode were measured using 25 kV ion acceleration without post-acceleration. All spectra were recorded with a detector voltage of 1.5−1.7 kV and were the averaged result of at least 100 laser shots. Laser intensity was variable, providing maximum flexibility in collecting spectra. The singly charged molecular ions of peptides or proteins, whose molecular weights were suitable for the calibration of the mass range, were used as external standards.

RESULTS AND DISCUSSION

DPPH Radical-Scavenging Activity and Total Polyphenol Content in Seed Coat of Brown Soybean. Table 1 shows the DPPH radical-scavenging activity and total polyphenol content in the seed coat of each variety. Because activities are expressed as the seed-coat weight required to achieve a 50% decrease in absorbance at 520 nm, a smaller seed-coat weight indicates a higher DPPH radical-scavenging activity. The brown soybean variety Akita-Zairai had a high radical-scavenging activity at 0.128 g seed coat. The black soybean variety Shinanoguro had the highest radical-scavenging activity (0.048), and another black variety, Shin-Tanbaguro, also had a high activity (0.055), which was comparable to that of Shinanoguro. Although the activity in the brown soybean seed coat was 2.33−

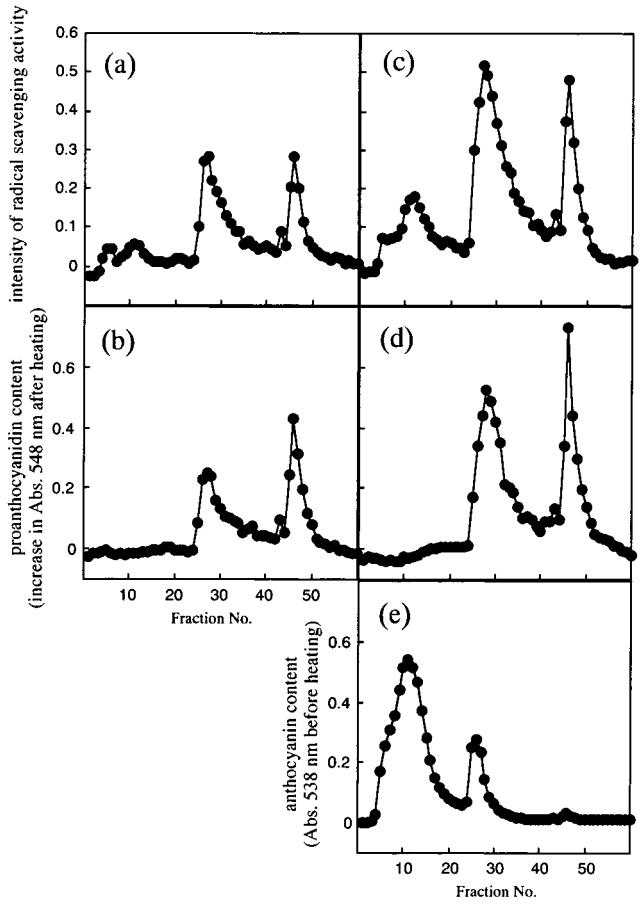

Figure 1. LH20 chromatographic profiles of the seed coat extract of brown or black soybean varieties. Left: relative intensity of DPPH radical-scavenging activity (a) and proanthocyanidin content (b) in the brown variety, Akita-Zairai. Right: relative intensity of DPPH radical-scavenging activity (c), proanthocyanidin content (d), and anthocyanin content (e) in the black variety, Shin-Tanbaguro.

2.67-fold less than that in the seed coat of the black soybean varieties, the brown soybean seed coat clearly had a significant amount of radical-scavenging activity. The other three reddish-brown varieties also had radical-scavenging activities, but to a much lesser degree than the Akita-Zairai. Among all the varieties used in this experiment, the radical-scavenging activity in the brown soybeans was closer to that in the black soybeans than that in reddish-brown soybeans. The highest total polyphenol content was observed in the black variety Shinanoguro, which had the highest DPPH radical-scavenging activity, at 94.8 mg/g seed coat. Shin-Tanbaguro was the second highest at 81.3 mg/g, and Akita-Zairai was the third at 41.6 mg/g. The other three reddish-brown varieties showed low polyphenol contents at 6.5−8.6 mg/g. The ranking in radical-scavenging activities among the varieties paralleled that of total polyphenol content. The radical-scavenging activity and the total polyphenol content were closely related, as the correlation coefficient was −0.880.

Column Fractionation of Radical-Scavenging Compounds in the Brown Soybean Seed Coat. To fractionate polyphenolic compounds, a seed coat extract derived from the brown soybean variety Akita-Zairai, which had a high degree of radical-scavenging activity, was applied to an LH20 column. Figure 1(a) shows a chromatographic profile of the relative intensity of DPPH radical-scavenging activity in the seed coat extract of Akita-Zairai. In the EtOH fractions, the DPPH radical-scavenging activities were very low. High DPPH radical-scavenging activities were observed in the fractions 25 and 45, eluted with MeOH and 70% acetone, respectively. The chromatographic profile of proanthocyanidin content is shown in Figure 1(b). Because we used the HCl−butanol hydrolysis method to evaluate proanthocyanidin content, data are expressed as an increase in absorbance at 548 nm after heating the reaction mixtures. We selected this wavelength because our preliminary TLC experiments with hydrolysates had indicated that procyanidins were the main components in the fractions (data not shown). In the brown soybean Akita-Zairai, proanthocyanidins were also detected in identical fractions showing high radical-scavenging activities. The chromatographic profile of proanthocyanidin content was analogous to that of the relative intensity of radical-scavenging activities, although the peak height of the MeOH fractions was lower than that of 70% acetone in proanthocyanidin contents, whereas the peak heights of DPPH radical-scavenging activities in both fractions were similar.

In the case of LH20 separation of antioxidants from buckwheat hulls (*7*), in which the chromatographic solvent conditions were basically the same as those in the present experiment, radical-scavenging activities were due to monomeric flavonoids, such as hyperin, rutin, and quercetin, which were detected in some EtOH fractions. However, in the present study, only negligible activity was detected in the EtOH fractions of the brown soybean seed coat, indicating that all of the radical-scavenging compounds were eluted in the MeOH and 70% acetone fractions.

To confirm that the radical-scavenging activities in the brown seed coat eluted entirely in MeOH and 70% acetone fractions, a recovery test was carried out with the Akita-Zairai soybean seed coat. The results of the recovery test indicated that the DPPH radical-scavenging activities were almost completely recovered in MeOH and 70% acetone fractions. The recovery rate compared to the original crude extract was 85 ± 4.9%. It is clear that most of the radical-scavenging activity of the brown seed coat was contained in the MeOH and 70% acetone fractions.

Column Fracionation of Radical-Scavenging Compounds in the Black Soybean Seed Coat. We also carried out LH20 column fractionation of the seed coat extract of the black soybean Shin-Tanbaguro (Figure 1(c), (d), and (e)). In the black soybean, DPPH radical-scavenging activities were detected in EtOH fractions containing anthocyanins rather than proanthocyanidins (Figure 1(c) and (e)). In the MeOH and 70% acetone fractions, both chromatographic profiles of relative intensities of DPPH radical-scavenging activity (Figure 1(c)) and proanthocyanidin contents (Figure 1(d)) were similar to those in the seed coat of Akita-Zairai. The chromatographic peaks of proanthocyanidin content in the black soybean were higher than those in the Akita-Zairai, indicating that the seed coat of the black soybean contained a larger amount of proanthocyanidin than the seed coat of Akita-Zairai, which might indicate a higher DPPH radical-scavenging activity in black soybean.

Degree of Polymerization of Procyanidins in the Brown or Black Seed Coat. The MALDI−TOF mass spectra of proanthocyanidin fractions from the brown soybean, Akita-Zairai, are shown in Figure 2. As

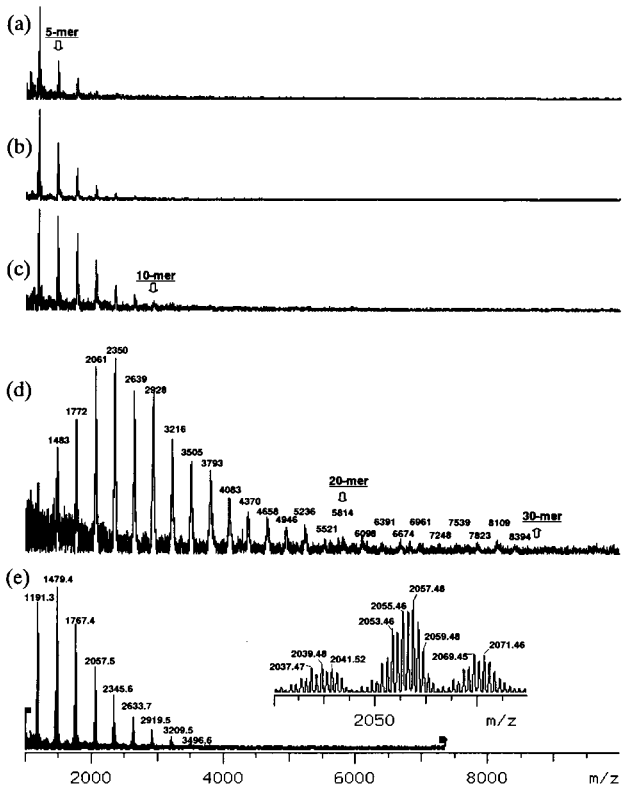

Figure 2. MALDI−TOF mass spectra of LH20 column fractions derived from the seed coat extract of Akita-Zairai. Numerals assigned to each peak correspond to *m*/*z*. Spectra in the linear mode are shown in (a) to (d). (a), MeOH fractions 23−30; (b), MeOH fractions 31−35; (c), MeOH fractions 36−40; (d) 70% acetone fractions, 43−50. The spectrum of 70% acetone fractions 43−50 in the reflectron mode is shown in (e).

the elution volume increased, the degree of polymerization (DP) also increased. Especially notable was the fact that 70% acetone fractions afforded oligomers with *m*/*z* values greater than 8000. Such a striking feature was also found in the spectra of the seed coat of the black soybean varieties Shin-Tanbaguro and Shinanoguro (data not shown).

The measurement in the linear mode at high acceleration voltage allowed intact detection of oligomers in the high-mass region. It gave a series of peaks separated by 288 amu suggesting a structure composed of catechin/epicatechin units without gallate or sugar substituents. Major procyanidin peaks were observed as the corresponding potassium adduct ions $[M + K]^+$, which were confirmed by shifting the *m*/*z* value in the presence of a silver ion. In the reflectron mode, superior spectra with high resolution and high accuracy were recorded, although the intensity was poor in the high-mass region (Figure 2(e)). The enlarged spectrum of the heptamer is depicted in the insert in Figure 2(e). The observed masses of $[M + K]^+$ or $[M + Na]^+$ were 2057.5 or 2041.5, which were almost equal to the calculated values for $C_{105}H_{86}O_{42}K_1$ or $C_{105}H_{86}O_{42}Na_1$ ([catechin unit]·n − 2·(n − 1)·[H] + [cation], where n was the number of DP, which was 7 in this case). In addition to these peaks, another series of peaks with 15 or 16 amu higher values than those of $[M + K]^+$ were found at low levels around *m*/*z* 2072. These might indicate that another type of proanthocyanidin, such as prodelphinidin, was present in the brown or black seed coats. Furthermore, 2 amu-smaller masses than those of the cation adduct ions were also detected. It was ascertained that these peaks did not originate from fragmentation or dehydration. Thus, although commercially available procyanidin C1 (epicatechin-(4β-8)-epicatechin-(4β-8)-epicatechin, Funakoshi, Tokyo, Japan) did not afford ions with masses 2 amu lower than the prospective mass, procyanidins in brown or black seed coat soybean did so, both in the linear and reflectron modes. This result suggests the presence of an A-type procyanidin, which had one additional linkage in each oligomer, in the seed coats of the brown or black soybean.

Although additional careful examination is needed to clarify the features of the minor components, MALDI−TOFMS has clearly shown that the principal constituents in the brown or black seed coats were procyanidin oligomers having catechin/epicatechin units up to a triacontamer.

Identity of the Radical-Scavenging Compound in the Brown Soybean Seed Coat. Proanthocyanidins are reported to possess a significant radical-scavenging capacity (*5, 9*), but the literature is scarce concerning proanthocyanidins in soybean. It has been reported that oligomeric and polymeric flavans are present in the black soybean (*5*) and that black and brown soybean cultivars contained proanthocyanidins even at the early stage of seed development (*10*); however, no descriptions of radical-scavenging activities have been reported. In the present study, we investigated the radical-scavenging activities in the seed coat of the brown soybean and proved that a high degree of activity is present.

We also demonstrated, using MALDI−TOFMS analysis, that the procyanidins in the brown soybean seed coat have a high degree of polymerization that may reach DP30. Many studies have reported a high degree of polymerization of proanthocyanidins in various plant materials using MS analysis. Condensed tannins in young apple fruits contained highly polymerized procyanidins with up to 15 catechin units (*11*). Values of DP22 have been reported for litchi pericarp (*12*) and DP17 in cider apple (*13*) using an electrospray ionization MS method, and DP11 in grape seed extract (*14*) using the MALDI−TOFMS method. In the present study, we detected a higher DP than those described. In addition, we have demonstrated that the seed coat of the black soybean, as well as the seed coat of the brown soybean, has highly polymerized procyanidins.

Compared to black soybean, the radical scavenging activity in the brown soybean seed coat was limited to MeOH and 70% acetone fractions from LH20 chromatography, indicating that anthocyanins were not present in the brown soybean seed coat. The synchronicity in the LH20 chromatographic profiles of the relative intensity of DPPH radical-scavenging activity and proanthocyanidin content indicates that procyanidin has a significant role in the radical-scavenging activity in the brown soybean seed coat. The results from MALDI−TOFMS analysis of MeOH and 70% acetone fractions are strong indicators that procyanidins were predominant compounds in those fractions showing significant radical-scavenging activity. These results, including that of the recovery test, showed that procyanidins are quite likely to be the predominant compounds responsible for the radical-scavenging activities in the brown soybean seed coat.

ACKNOWLEDGMENT

The authors are grateful to Drs. Tadahiro Nagata, Akio Yanagida, and Ryoichi Matsunaga for their helpful advice. We also thank Messrs. Michio Naraki and Masao Minamoto for managing the field-grown plants and Ms. Hiromi Hayase, Michiko Fukumori, and Mie Kato for technical support.

LITERATURE CITED

(1) Wei, H.; Bowen, R.; Cai, Q.; Barnes, S.; Wang, Y. Anitioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc. Soc. Exp. Biol. Med.* **1995**, *208*, 124−130.

(2) Miyazawa, M.; Sakano, K.; Nakamura, S.; Kosaka, H. Antimutagenic activity of isoflavones from soybean seeds (*Glycine max* Merrill). *J. Agric. Food Chem.* **1999**, *47*, 1346−1349.

(3) Yoshida, K.; Sato, Y.; Okuno, R.; Kameda, K.; Isobe, M.; Kondo, T. Structural analysis and measurement of anthocyanins from colored seed coats of Vigna, Phaseolus, and Glycine legumes. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 589−593.

(4) Furuta, S.; Suda, I.; Nishiba, Y. Radical-scavenging activities of boiled extracts from colored soybeans. *Kyushu Agric. Res.* **1998**, *60*, 26 (in Japanese).

(5) Ariga, T.; Asao, Y.; Sugimoto, H.; Yokotsuka, T. Occurrence of astringent oligomeric proanthocyanidins in legume seeds. *Agric. Biol. Chem.* **1981**, *45*, 2705−2708.

(6) Suda, I.; Ogata, H.; Mizuki, N.; Takahata, Y.; Nishiba, Y. Measurement of radical-scavenging activities of colored agricultural products and foods by DPPH/spectrophotomeric assay. *Kyushu Agric. Res.* **1999**, *61*, 32 (in Japanese).

(7) Watanabe, M.; Ohshita, Y.; Tsushida, T. Antioxidant compounds from buckwheat (*Fagopyrum esculentum* Möench) hulls. *J. Agric. Food. Chem.* **1997**, *45*, 1039−1044.

(8) Porter, L. J. Tannins. In *Methods in Plant Biochemistry, Vol. 1, Plant Phenolics*; Harborne, J. B., Ed.; Academic Press: London, 1989; pp 389−419.

(9) Ricardo da Silva, J. M.; Darmon, N.; Fernandez, Y.; Mitjavila, S. Oxygen free radical scavenger capacity in aqueous models of different procyanins from grape seeds. *J. Agric. Food Chem.* **1991**, *39*, 1549−1552.

(10) Todd, J. J.; Vodkin, L. O. Pigmented soybean (*Glycine max*) seed coats accumulate proanthocyanidins during development. *Plant Physiol.* **1993**, *102*, 663−670.

(11) Ohnishi-Kameyama, M.; Yanagida, A.; Kanda, T.; Nagata, T. Identification of catechin oligomers from apple (*Malus pumila* cv. Fuji) in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and fast-atom bombardment mass spectrometry. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 31−36.

(12) Roux, E. L.; Doco, T.; Sarni-Manchado, P.; Lozano, Y.; Cheynier, V. A-type proanthocyanidins from pericarp of *Litchi chinensis*. *Phytochemistry* **1998**, *48*, 1251−1258.

(13) Guyot, S.; Doco, T.; Souquet, J.-M.; Moutounet, M.; Drilleau, J.-F. Characterization of highly polymerized procyanidins in cider apple (*Malus sylvestris* var. Kermerrien) skin and pulp. *Phytochemistry* **1997**, *44*, 351−357.

(14) Krueger, C. G.; Dopke, N. C.; Treichel, P. M.; Folts, J.; Reed, J. D. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of polygalloyl polyflavan-3-ols in grape seed extract. *J. Agric. Food. Chem.* **2000**, *48*, 1663−1667.

Received for review March 6, 2001. Revised manuscript received August 28, 2001. Accepted September 3, 2001. This study was partially supported by grant-in-aid from the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan.

JF010307X