

## Correlation of Lignan Contents with Protein and Oil Contents in the Seeds of *Sesamum indicum* L.

Jin Hwan Lee<sup>1\*</sup>, In-Youl Baek, Jong-Min Ko, Kang-Bo Shim, Nam Suk Kang, Hyun-Tae Kim, Churl-Hwan Kang, Keum-Yong Park, Ki Hun Park<sup>2</sup>, and Tae Joung Ha\*

*Yeongnam Agricultural Research Institute, National Institute of Crop Science, Rural Development Administration, Miryang, 627-803, Korea*

*<sup>2</sup>Division of Applied Life Science (BK21 Program), EB-NCRC, Institute of Agriculture & Life Science, Gyeongsang National University, Jinju, 660-701, Korea*

Received November 21, 2007; Accepted December 26, 2007

The contents of lignan, protein, and oil of nine *Sesamum indicum* cultivars were analyzed. To determine the lignan contents, sesamin 1 and sesamolin 2 were isolated from the ethylacetate extracts of the Suwon cultivar, and the structures were fully characterized by spectral and physical methods. The seeds of nine cultivars were screened for two lignans, which were determined by HPLC using a C<sub>18</sub> reversed phase column coupled with a photodiode array detector. Suwon cultivar showed the highest concentration (1: 6.24±0.04 and 2: 3.58±0.01 mg/g), whereas Soonheuk displayed the lowest (1: 0.91±0.01 and 2: 0.73±0.01 mg/g). The average content ratio of sesamin 1 (3.64 mg/g) was significantly higher than that of sesamolin 2 (2.57 mg/g). The protein content ranged from 21.52±0.35 to 31.22±0.25%, Suwon containing the highest level and Soonheuk had the lowest. Kwangbaek showed the highest oil level (49.84±0.40%), while Soonheuk exhibited the lowest (42.52±0.05%). Sesamin 1 exhibited a stronger radical-scavenging activity in the ABTS (IC<sub>50</sub>: 63.2±2.4 μM) than its DPPH radical-scavenging activity (IC<sub>50</sub>>200 μM). These results lead to the conclusion that lignan content is affected by protein and oil contents. Concentration of the chemical components in the cultivars could be a key factor in the selection process of a high quality species.

**Key words :** ABTS, antioxidant, cultivar, lignan, oil, protein, *S. indicum*, seed

*Sesamum indicum* L (Sesame) belonging to the Pedaliaceae family has been categorized as one of the traditional health foods [Sugano *et al.*, 1993]. In Korea and other Asian countries, the sesame is considered to be the most important oil seed crop due to its high oil content as well as its medicinal effects and resistance to the

oxidative deterioration [Hu *et al.*, 2004; Kang *et al.*, 2000]. Several studies have well demonstrated that lignans in the sesame are responsible for various physiological activities including antioxidative [Yamashita *et al.*, 2000], anticarcinogenic [Hirose *et al.*, 1992], blood pressure-lowering [Matsumura *et al.*, 1998], serum lipid-lowering [Hirata *et al.*, 1996; Hirose *et al.*, 1991; Ashakumary *et al.*, 1999], anti-inflammatory [Hsu *et al.*, 2000], and estrogenic [Wu *et al.*, 2006] activities. The sesame protein and oil are also important nutritional sources [Abou-Gharbia *et al.*, 1997]. In particular, the sesame oil is fast becoming popular due to its low cholesterol level and its ability to reduce the incidences of hypertension and certain cancers [Lemcke-Norojarvi *et al.*, 2001; Hibasami *et al.*, 2000].

Antioxidants such as flavonoids, tannins, coumarins, xanthenes, and phenolics are found in various plant products [Larson RA, 1988]. For this reason, there is a growing interest in the extraction of these plant antioxidants for use as natural antioxidants. The ABTS

---

\*Both corresponding authors contributed equally to the work.

---

<sup>1</sup>Present address: Ministry of Environment, NAKDONG River Basin Environmental Office, Changwon, 641-722, Korea

---

\*Corresponding authors

Phone: 82-55-211-1695; Fax: 82-55-211-1709

E-mail: hwanletter08@hanmail.net (Jin Hwan Lee)

Phone: 82-55-350-1239; Fax: 82-55-352-3059

E-mail: taejoung@rda.go.kr (Tae Joung Ha)

---

**Abbreviations:** ABTS, 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid); BHA, butylated hydroxyl anisol; HPLC, high-performance liquid chromatography; NIRS, near-infrared reflectance spectroscopy; TFA, trifluoro acetic acid; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

and DPPH systems have both been commonly used to measure the total antioxidative status of various biological specimens due to their good reproducibility and easy quality control [Brand-williams *et al.*, 1995; Re *et al.*, 1999]. Although many secondary metabolites from the sesame exhibit antioxidant activities [Osawa *et al.*, 1985; Fukuda *et al.*, 1985], these activities have not yet been evaluated clearly through the ABTS and DPPH radical systems, which is of great importance to the enhancement of the value of sesame not only as a functional material but also as a dietary supplement. In spite of the many reports published on the sesame [Wu *et al.*, 2007; Were *et al.*, 2006; Elleuch *et al.*, 2007], studies on the exact characterization of the composition and contents of lignans, protein, and oil from different sesame cultivars are limited. The objective of current study was to determine the contents of lignan, protein, and oil from sesame seeds to provide the basic information on their compositional components for the development of high quality sesame cultivars.

In this study, we isolated two lignans **1** and **2** from Suwon one of the *S. indicum* cultivars, and their structures were identified using 2D-NMR together with other spectroscopic data. The contents of the lignans **1** and **2** obtained from nine Korean sesame cultivars were determined by HPLC using a C<sub>18</sub> reversed phase column. We also investigated the primary constituents such as protein and oil concentrations as well as demonstrated the selective ABTS radical-scavenging activity compared to the DPPH radical of sesamol **2**.

## Materials and Methods

**Plant material.** The seeds of nine sesame (*S. indicum*) cultivars were collected on September 10, 2005 at the experimental field of Yeongnam Agricultural Research Institute, Miryang, Korea, and air-dried in the shade for 7 days. The collected seeds were stored at 4°C until use.

**Reagents.** 2,2'-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium presulfate, and butylated hydroxyl anisol (BHA) were purchased from Sigma (St. Louis, MO). HPLC-grade water (99.9% v/v) and methanol (99.9% v/v) were from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA, 99.8% v/v) was purchased from Sigma. All reagents used in the experiments were of analytical grade.

**Instruments.** The purities of the two lignans were monitored by TLC (Merck), using the commercially available glass-backed plates and visualized under UV or sprayed with 10% H<sub>2</sub>SO<sub>4</sub> solution. The column

chromatography was carried out using a 230-400 mesh silica gel (kieselgel 60, Merck). The melting points were measured on a Thomas Scientific capillary melting point apparatus (Electrothermal 9300, Essex, London, UK) and are uncorrected. The IR spectra were recorded on a Bruker IFS66 (Bruker, Karlsruhe, Germany) infrared Fourier transform spectrophotometer (KBr), and the UV spectra were measured on a Beckman DU650 spectrophotometer (Beckman Coulter, Fullerton, CA). <sup>1</sup>H, <sup>13</sup>C, and 2D NMR data were obtained on a Bruker AM 500 (<sup>1</sup>H NMR at 500 MHz, <sup>13</sup>C NMR at 125 MHz) spectrometer (Bruker) in CDCl<sub>3</sub>. The mass spectrometer was a Bruker Daltonick GmbH (Bruker) equipped with an electrospray ionization (ESI) source and an ion trap mass analyzer. The chromatographic separation was achieved using an Agilent 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a quaternary HPLC pump, a vacuum degasser, an autosampler, and a wavelength UV detector. Analysis of the isolated lignans was carried out using the reverse phase separation on a C<sub>18</sub> column [LichroCART 125-4 HPLC-Cartridge (Lichrophore 100 RP-18e) (Merck)]. The NIR spectroscopic data were collected using a Foss NIRSystems spectrophotometer (model 6500, NIRSystem Inc., Silver Spring, MD) and the oil contents were determined using a BÜCHI B-811 Extraction System (Büchi, Schweiz, Germany).

**Extraction and isolation.** To evaluate the contents of the lignans, lignans **1** and **2** were isolated from the seeds of *S. indicum* (Suwon cultivar) using several silica gel column chromatography methods. The dried seeds of *S. indicum* (2.5 kg) were ground and extracted twice with EtOAc (5L×2) for 7 days at room temperature. The combined extracts were concentrated *in vacuo* to yield a brown gum (85.5 g), and the residue was sequentially partitioned with *n*-hexane (38.2 g), EtOAc (21.7 g), and MeOH (26.0 g). The EtOAc fraction was chromatographed on a silica gel (6×50 cm, 230-400 mesh, 420 g) using *n*-hexane/EtOAc (16 : 1→1 : 2) to give five fractions (A-E). Fraction C (3.8 g) was applied to a silica gel column (4.5 × 50 cm, 230-400 mesh, 110 g) chromatography with *n*-hexane/EtOAc (12 : 1→2 : 1) to afford 28 subfractions, among which subfractions 20-24 (0.8 g) were subjected to the silica gel column (2.5×40 cm, 230-400 mesh, 80 g) chromatography with hexane/acetone (10 : 1→4 : 1) to yield compound **2** (240 mg). Fraction D (4.9 g) was chromatographed over a silica gel as a stationary phase using *n*-hexane/EtOAc (12 : 1→1 : 2) as the mobile phase to afford 15 subfractions (1-15). Compound **1** (365 mg) was purified from the subfractions 8-12 by recrystallization in the *n*-hexane/acetone mixture (3 : 1).

**HPLC apparatus and measurements.** The pulverized seeds of *S. indicum* (1.0 g) were extracted with 20 mL

MeOH using a vortex mixer for 6 h at room temperature, followed by centrifugation at 3,000 rpm for 5 min (VS-6000CFN, VISION, Seoul, Korea). The extracts used for HPLC analysis were passed through 0.45- $\mu$ m filters (Millipore, MSI, Westboro, MA) before injection into a reverse phase LichroCART 125-4 HPLC-Cartridge (Lichrophore 100 RP-18e,  $\phi$  5  $\mu$ m) (Merck), and 20  $\mu$ L each of these solutions were injected into the HPLC system. The column temperature was set at 30°C, and the flow rate was set at 0.8 mL/min with isocratic elution, using 60% methanol-0.1% TFA with monitoring at 290 nm.

**Preparation of calibration curve from isolated lignans 1 and 2.** Approximately 2 mg each of the isolated lignans 1 and 2 were weighed and dissolved in a 10-mL volumetric flask containing MeOH to obtain the stock solutions. Each stock solution was diluted sequentially with 100, 80, 60, 40, 20, 10, and 5  $\mu$ g/mL MeOH. All stock solutions were stored at 4°C and brought to room temperature before use. Mean areas ( $n=3$ ) generated from the standard solution were plotted the concentrations to establish the calibration equation. A high linearity ( $r^2 > 0.999$ ) was obtained for each calibration curve. The two lignans were identified based on their retention times or by co-chromatography with other authentic examples, and their concentrations were calculated by comparing the peak areas of the samples with those of the standards.

**Protein and oil analyses.** The seeds of *S. indicum* cultivars were finely ground using a coffee grinder (PHILIPS, HR2860, Amsterdam, Netherlands). The protein content was determined according to the data from the near-infrared reflectance spectroscopy (NIRS) [Kim *et al.*, 2006]. The NIR data were collected in the range of 400 to 2500 nm at 2-nm intervals using a Foss NIRSystems spectrophotometer (model 6500, NIRSystem Inc., Silver Spring, MD) and stored as the reciprocal logarithm ( $\log 1/R$ ) of the reflected energy. All statistics, regressions, and predictions were performed using the WINISI software (version 1.05) from Foss NIRSystem. Coefficient of determination ( $r^2$ ) and standard errors of calibration (SEC) were used to evaluate how well the calibration of the sample or multiple linear regressions fits the data. The oil content was measured by the Soxhlet method using the Buchi B-811 extracted system [Kim *et al.*, 2006]. Two grams of the pulverized seed was added to 200 mL of *n*-hexane in an extraction thimble and boiled for 2 h at 105°C. After cooling to room temperature in a desiccator, the extracted oil was weighted. Total oil contents were determined on a dry matter basis of the seeds.

**Measurement of Trolox equivalent antioxidant capacity (TEAC).** TEAC assay is based on the relative

ability of the antioxidants to scavenge the radical cation ABTS<sup>•+</sup> in comparison to a standard (Trolox) [Re *et al.*, 1999; Choi *et al.*, 2005]. 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 h until completely mixed and the absorbance stable. The ABTS<sup>•+</sup> solution was diluted with ethanol, and the absorbance was read at 734 nm. For the photometric assay 0.9 mL ABTS<sup>•+</sup> solution and 0.1 mL isolated compounds were mixed for 45 s, and the absorbance was measured immediately after 1 min at 734 nm. The antioxidant activities of the isolated compounds were calculated by determining the decrease in absorbance at different concentrations using the following equation:  $E = A_o - A_e / A_o \times 100$ , where  $A_e$  and  $A_o$  are absorbances of the samples with and without isolated compounds, respectively. The antioxidant activity was expressed as a TEAC value, which expresses the  $\mu$ M of Trolox having the antioxidant capacity corresponding to 1.0  $\mu$ M of the test substance.

**Measurement of DPPH radical-scavenging activity.** The antioxidant activities of the isolated compounds were measured on the basis of the scavenging activity of the stable DPPH free radical following the method described by Braca *et al.* [2001]. Various concentrations of the isolated compounds were added to EtOH to form a 0.15 mM mixture which was then shaken vigorously. The absorbance at 517 nm was determined after 30 min, and the radical-scavenging effect was calculated as  $A_o - A_e / A_o \times 100$ , where  $A_e$  and  $A_o$  were the absorbances of the samples with and without the isolated compounds, respectively.

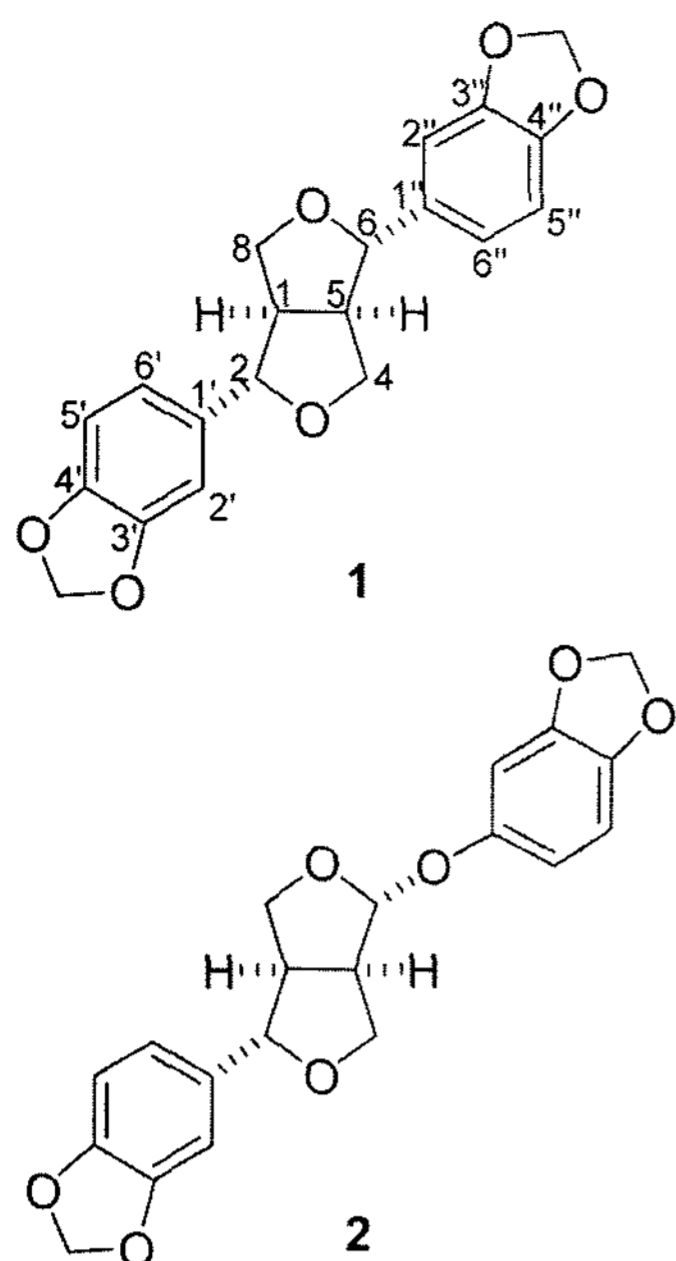
**Data analysis and curve fitting.** Each assay was conducted separately in triplicate. The data analysis was performed using the Sigma Plot 2001. The inhibitory concentration leading to 50% activity loss ( $IC_{50}$ ) was obtained by fitting the experimental data to the logistic curve using the following equation [Coperland, 2000].

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

## Results and Discussions

**Identification of the isolated compounds.** Through various chromatographic purifications of EtOAc fraction, two compounds 1 and 2 were isolated and analyzed using the physical and the spectral data to determine their structures (Fig. 1).

**Compound 1:** amorphous white powder; mp 122-123°C; IR (KBr)  $\nu_{\max}$  2849, 1510, 1445, 1246, 1035  $\text{cm}^{-1}$ ;  $[\alpha]_D^{20} +65.0$  ( $c$  0.9,  $\text{CHCl}_3$ ); UV  $\lambda_{\max}$  290, 210 nm ( $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  3.07 (2H, m, H-1 and H-5), 3.90 (2H, dd,  $J=3.7, 9.2$  Hz, H-4 $\alpha$  and H-8 $\alpha$ ), 4.26



**Fig. 1.** Chemical structures of the isolated compounds **1** and **2** from seeds of *S. indicum*.

(2H, dd,  $J=6.9, 9.1$  Hz, H-4 $\beta$  and H-8 $\beta$ ), 4.75 (2H, d,  $J=4.4$  Hz, H-2 and H-6), 5.97 (4H, s, 2 $\times$ -OCH<sub>2</sub>O-), 6.79-6.88 (4H, m, H-5, H-6, H-5', and H-6'), and 7.29 (2H, s, H-2' and H-2''). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): see Table 1.

**Compound 2:** amorphous white powder; mp 90-90°C; IR (KBr)  $\nu_{\max}$  2860, 1510 cm<sup>-1</sup>;  $[\alpha]_D^{20}$  -198.0 ( $c$  0.5, CHCl<sub>3</sub>); UV  $\lambda_{\max}$  290, 280, 210 nm (CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  2.98 (1H, dd,  $J=1.3, 9.2$  Hz, H-1), 3.33 (1H, d,  $J=7.6$  Hz, H-5), 3.67 (1H, dd,  $J=7.4, 9.2$  Hz, H-4 $\alpha$ ), 4.00 (1H, d,  $J=9.1$  Hz, H-8 $\alpha$ ), 4.17 (1H, dd,  $J=6.0, 9.2$  Hz, H-8 $\beta$ ), 4.44 (1H, d,  $J=6.8$  Hz, H-2), 4.47 (1H, d,  $J=8.8$  Hz, H-4 $\beta$ ), 5.53 (1H, s, H-2'), 5.97 (2H, s, 4' and 5'-OCH<sub>2</sub>O), 5.98 (2H, s, 4'' and 5''-OCH<sub>2</sub>O), 6.54 (1H, dd,  $J=2.4, 8.4$  Hz, H-6'), 6.66 (1H, d,  $J=2.4$  Hz, H-6), 6.74 (1H, d,  $J=8.4$  Hz, H-5''), 6.82 (1H, d,  $J=7.9$  Hz, H-5'), 6.89 (1H, m, H-6''), and 6.90 (1H, s, H-2''). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): see Table 1.

Compound **1** was obtained as an amorphous white powder and in the LC-MS, the molecular ion peak [M+Na]<sup>+</sup> showed at  $m/z$  377. The UV spectrum analysis showed an absorption maximum at 290 nm and IR spectrum analysis showed strong absorption bands for CO (1035 cm<sup>-1</sup>). The <sup>1</sup>H NMR spectrum of compound **1** showed the presence of methylene dioxy groups at  $\delta$  5.97 (4H, s, 2 $\times$ -OCH<sub>2</sub>O-), furanofuran moieties [ $\delta$  3.07 (2H, H-1 and H-5), 4.75 (2H, H-2 and H-6), 3.90 (2H, H-4 $\alpha$  and H-8 $\alpha$ ), and 4.26 (2H, H-4 $\beta$  and H-8 $\beta$ )], and six aromatic protons [ $\delta$  6.79-6.88 (4H, H-5, H-6, H-5', and H-6') and 7.29 (2H, H-2' and H-2'')]. The H-1 appeared as a multiplet due to the couplings with H-8 $\alpha$ , H-8 $\beta$ , H-5,

**Table 1.** <sup>13</sup>C NMR of compounds **1** and **2** at 125 MHz (ppm, m)<sup>a</sup>

Position	Compound	
	<b>1</b>	<b>2</b>
1	54.8 (d)	53.1 (d)
2	86.2 (d)	87.5 (d)
4	72.1 (t)	71.7 (t)
5	54.8 (d)	53.7 (d)
6	86.2 (d)	100.6 (d)
8	72.1 (t)	70.3 (t)
1'	135.5 (s)	134.9 (s)
2'	107.0 (d)	107.4 (d)
3'	148.4 (d)	152.3 (d)
4'	147.5 (s)	148.5 (s)
5'	108.6 (s)	108.6 (s)
6'	119.7 (d)	109.5 (d)
1''	135.5 (s)	143.1 (s)
2''	107.0 (d)	107.0 (d)
3''	148.4 (s)	148.6 (s)
4''	147.5 (s)	147.8 (s)
5''	108.6 (d)	108.4 (d)
6''	119.7 (d)	120.1 (d)
2 $\times$ -OCH <sub>2</sub> O-	101.5 (t)	
3',4'-OCH <sub>2</sub> O-		101.5 (t)
3'',4''-OCH <sub>2</sub> O-		101.7 (t)

<sup>a</sup>The chemical shifts of compounds **1** and **2** were determined in CDCl<sub>3</sub>.

and H-2, whereas H-5 appeared as a multiplet due to the couplings with H-4 $\alpha$ , H-4 $\beta$ , H-1, and H-6, as confirmed by the <sup>1</sup>H-<sup>1</sup>H COSY spectrum. The HMBC spectrum showed coupling between H-6''/H-2'' and C-6 as well as between the methylene dioxy group protons ( $\delta$  5.97) and C-4'' ( $\delta$  147.5), confirming the presence of the methylene dioxy group protons on this aromatic ring. On the other aromatic ring of the molecule, the HMBC spectrum showed the coupling between H-6'/H-2' and C-2. On the basis of these spectral data and careful analysis of the HMQC and HMBC data, the compound **1** was identified as a sesamin, one of the lignans [Kim *et al.*, 2006]. Compound **2** was obtained as an amorphous white powder, and in the LC-MS, the molecular ion peak [M+Na]<sup>+</sup> showed up at  $m/z$  393. The UV spectrum showed an absorption maximum at 290 nm. The <sup>1</sup>H NMR spectrum of **1** exhibited two methylene dioxy group protons ( $\delta$  5.97, s and 5.98, s), six aromatic protons [( $\delta$  6.54, H-2'), ( $\delta$  5.53, H-6'), ( $\delta$  6.74, H-5''), ( $\delta$  6.82, H-3'), ( $\delta$  6.89, H-6''), and ( $\delta$  6.90, H-2'')], and eight furanofuran protons [( $\delta$  2.98, H-1), ( $\delta$  3.33, H-5), ( $\delta$  3.67, H-4 $\alpha$ ), ( $\delta$  4.00, H-8 $\alpha$ ), ( $\delta$  4.17, H-8 $\beta$ ), ( $\delta$  4.44, H-2), ( $\delta$  4.47, H-4 $\beta$ ), and ( $\delta$  6.66, H-6)]. The <sup>13</sup>C NMR spectrum of **2** showed two methylene

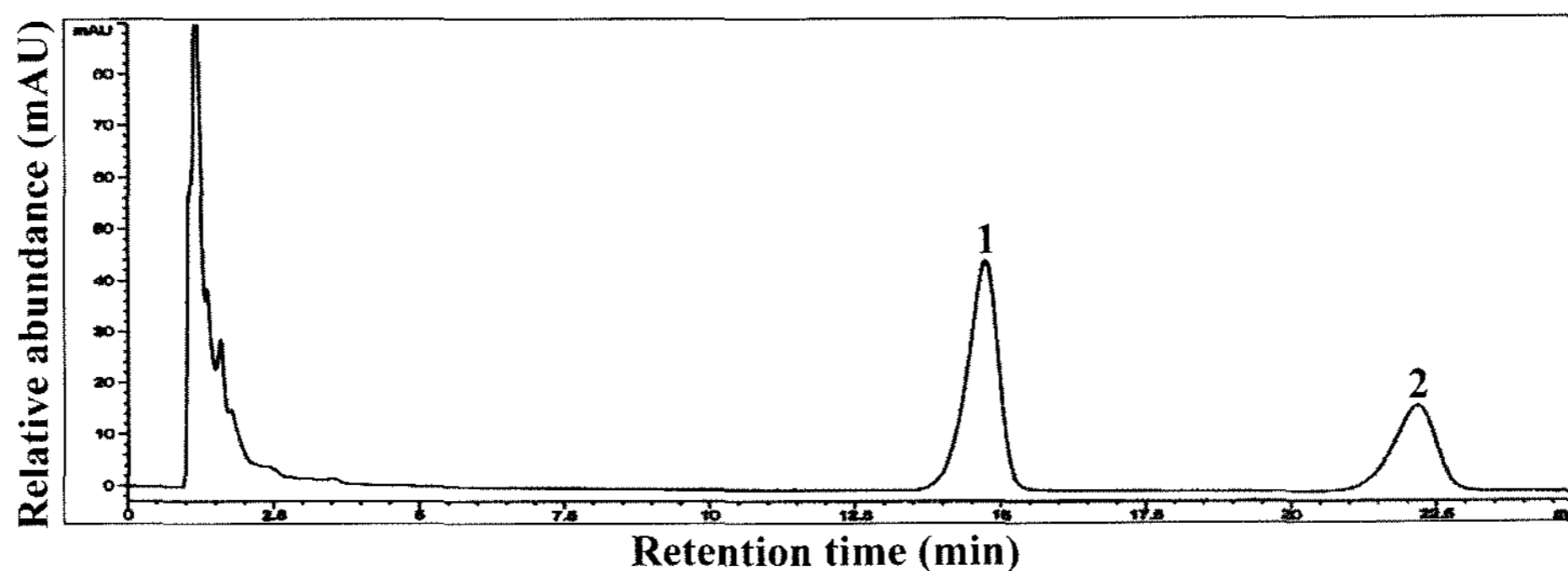


Fig. 2. HPLC chromatogram of the compounds isolated from the seeds of *S. indicum*.

dioxy group carbons ( $\delta$  101.5 and 101.7), six aromatic carbons [( $\delta$  107.4, C-2'), ( $\delta$  108.6, C-5'), ( $\delta$  109.5, C-6'), ( $\delta$  107.0, C-2''), ( $\delta$  108.4, C-5''), and ( $\delta$  120.1, C-6'')], and six furanofuran moieties carbons [( $\delta$  53.1, C-1), ( $\delta$  87.5, C-2), ( $\delta$  71.7, C-4), ( $\delta$  53.7, C-5), ( $\delta$  100.6, C-6), and ( $\delta$  70.3, C-8)]. The HMBC spectrum displayed  $^1\text{H}$ - $^{13}\text{C}$  long-range coupling from C-1' to H-1, H-2, H-2', H-3', H-5', and H-6', from C-1'' to H-6 and H-6''. Thus, these assignments and analysis of the HMBC spectra allowed the unequivocal assignment of all carbons. All data mentioned above indicated that the structure of compound 2 is sesamolol [Kato *et al.*, 1998].

**Lignan analysis of the seeds of sesame cultivars.** On the basis of the isolated lignans 1 and 2 (authentic compounds), a typical HPLC chromatogram of the lignans from the seeds of sesame was obtained (Fig. 2). The retention times were as follows: compound 1 (sesamin:  $t_R=14.7$  min) and compound 2 (sesamolol:  $t_R=22.1$  min).

The concentrations of compounds 1 and 2 were determined on the basis of the peak areas in the chromatogram as follows: 1,  $y=28.66x-7.00$ ,  $r^2=0.999$  and 2,  $y=18.92x-1.36$ ,  $r^2=0.999$ . The contents of the two lignans of the nine sesame cultivars grown in Milyang are shown in Table 2. Significant differences were observed in the lignan contents among the cultivars. The highest total lignan content was observed in the Suwon cultivar at 9.82 mg/g (1: 6.24 and 2: 3.58 mg/g) and the lowest was 1.64 mg/g (1: 0.91 and 2: 0.73 mg/g) found in the Soonheuk cultivar. The content of compound 1 ranged from  $0.91\pm 0.01$  to  $6.24\pm 0.04$  mg/g and that of compound 2 ranged from  $0.73\pm 0.01$  to  $3.58\pm 0.01$  mg/g (Table 2). In all nine Korean cultivars examined, the average content of compound 1 (3.64 mg/g) was significantly higher than that of compound 2 (2.57 mg/g).

The HPLC chromatograms of the isolated lignans 1 and 2 in the nine sesame cultivars are shown in Fig. 4A-4C.

The lignans 1 and 2 were found only in low amounts from Konheuk and Soonheuk cultivars, whereas Seodun,

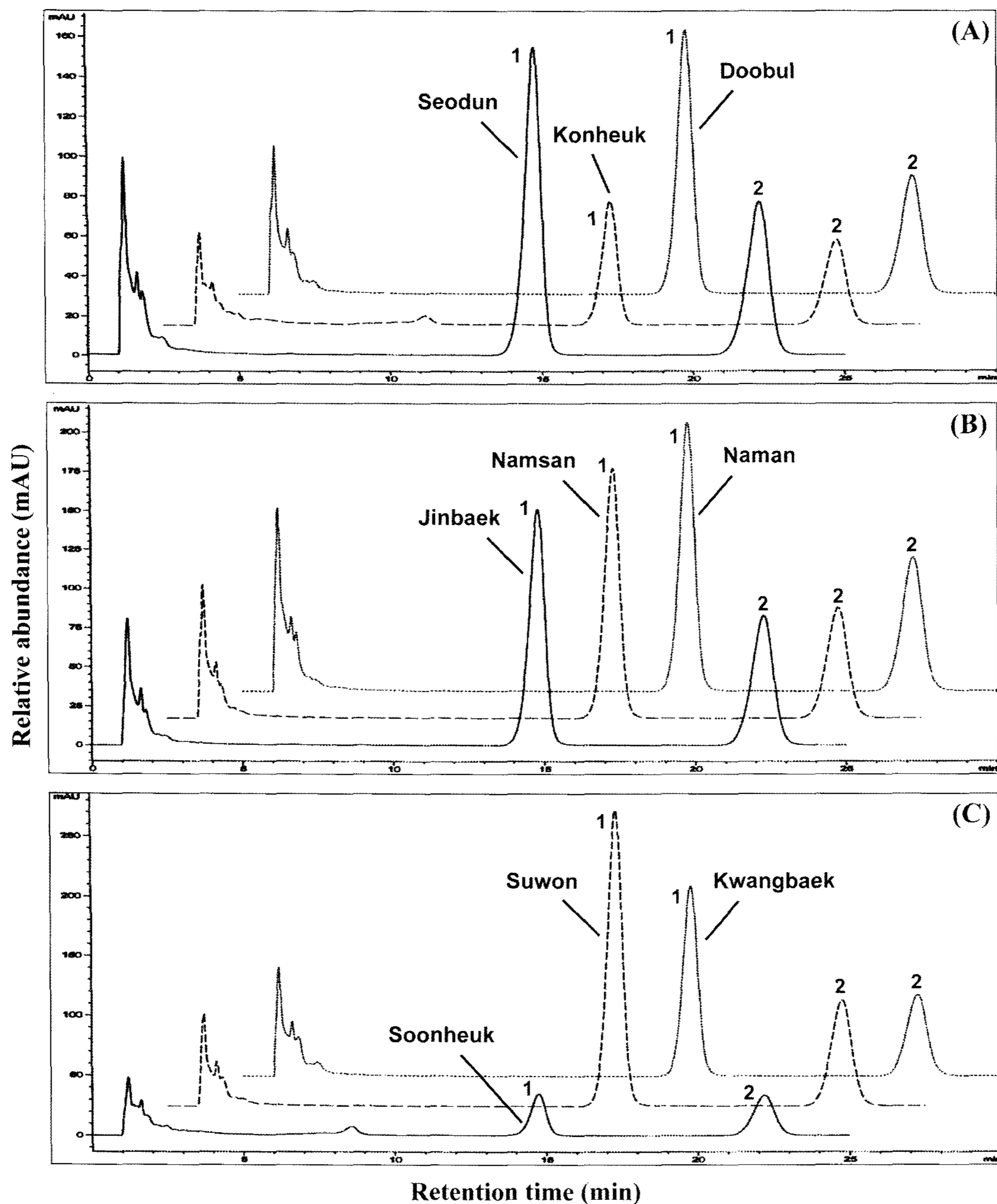
Table 2. Contents of compounds 1 and 2 from the seeds of *S. indicum* cultivars

Cultivar	Contents of two compounds (mg/g) <sup>a</sup>	
	Compound 1 (Sesamin)	Compound 2 (Sesamolol)
Seodun	4.16 $\pm$ 0.03	3.24 $\pm$ 0.02
Konheuk	1.64 $\pm$ 0.02	1.26 $\pm$ 0.01
Doobul	3.40 $\pm$ 0.03	2.12 $\pm$ 0.02
Jinbaek	3.99 $\pm$ 0.04	3.49 $\pm$ 0.04
Namsan	4.08 $\pm$ 0.03	2.70 $\pm$ 0.04
Naman	4.40 $\pm$ 0.02	3.53 $\pm$ 0.03
Soonheuk	0.91 $\pm$ 0.01	0.73 $\pm$ 0.01
Suwon	6.24 $\pm$ 0.04	3.58 $\pm$ 0.01
Kwangbaek	3.97 $\pm$ 0.03	2.48 $\pm$ 0.02

<sup>a</sup>Values indicate the means of three replications.

Namam, and Suwon cultivars, which have used as the standard cultivars for sesame breeding, showed 2-3 times higher lignan contents than those of the other cultivars (Fig 3, Table 2). These results demonstrate that Seodun, Namam, and Suwon cultivars are suitable for use as standard cultivars for the development of high quality sesame seeds.

**Analysis of protein and oil in sesame cultivars.** Protein and oil are also important nutritional sources found in the sesames [Abou-Gharbia *et al.*, 1997]. Contents of these components are shown in Table 3. The highest and the lowest protein content, determined according to the NIRS procedure were respectively found in Suwon cultivar (31.22 $\pm$ 0.25%) and Soonheuk cultivar (21.52 $\pm$ 0.35%). The protein contents of Konheuk (23.13 $\pm$ 0.54%) and Soonheuk (21.52 $\pm$ 0.35%) cultivars were lower than the average content of the total cultivars (28.46%), while those of the other seven cultivars were higher than the total average. The oil content was measured by a Soxhlet method. Kwangbaek cultivar exhibited the highest content (49.84 $\pm$ 0.40%), whereas that of Soonheuk cultivar was the lowest (42.52 $\pm$ 0.05%). The average oil



**Fig. 3.** HPLC chromatogram of 60% methanol-0.1% TFA extracts from the seeds of nine *S. indicum* cultivars. (A) Seodun, Konheuk, and Doobul cultivars, (B) Jinbaek, Namsan, and Naman cultivars, (C) Soonheuk, Suwon, and Kwangbaek cultivars.

content of the nine Korean sesame seeds was 46.32% (Table 3).

The protein and oil contents of the sesame seeds showed moderate positive correlations (Suwon cultivar) (Table 3). Moreover, the lignan and protein contents of Seodun, Jinbaek, Naman, and Suwon cultivars also showed significant positive correlations (Tables 2 and 3).

**ABTS and DPPH radical-scavenging activities of isolated compounds 1 and 2.** The systems ABTS and DPPH are both commonly used to measure the total

antioxidant status of various biological specimens due to their reproducibility and ease of quality control [Re *et al.*, 1999; Choi *et al.*, 2005; Braca *et al.*, 2001]. Moreover, the ABTS and DPPH radical-scavenging activities of the isolated compounds 1 and 2 have not yet been elucidated clearly. Therefore, the ABTS and DPPH radicals were chosen to evaluate the antioxidant activities of the isolated compounds. The change in the absorbance produced by the reduced DPPH was used to evaluate the abilities of the isolated compounds 1 and 2 to act as free radical

**Table 3. Contents of protein and oil from the seeds of *S. indicum* cultivars**

Cultivar	Contents of protein and oil	
	Protein (%) <sup>a</sup>	Oil (%) <sup>a</sup>
Seodun	30.37±0.95	46.44±0.51
Konheuk	23.13±0.54	43.08±0.10
Doobul	29.87±0.07	46.23±0.41
Jinbaek	31.19±0.28	46.17±0.49
Namsan	28.82±0.41	48.64±0.45
Naman	30.99±0.53	46.63±0.42
Soonheuk	21.52±0.35	42.52±0.05
Suwon	31.22±0.25	47.35±0.14
Kwangbaek	29.04±0.40	49.84±0.40

<sup>a</sup>Values indicate the means of three replications.

**Table 4. Antioxidant activities of isolated compounds 1 and 2 on ABTS and DPPH radicals**

Compound	IC <sub>50</sub> (μM) <sup>a</sup>	
	ABTS	DPPH
1	23.3% <sup>b</sup>	>200
2	63.2±2.4 <sup>a</sup>	>200
BHA/Trolox	19.4±1.3 <sup>a</sup> (Trolox)	35.4±2.2 <sup>a</sup> (BHA)

<sup>a</sup>Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate determinations.

<sup>b</sup>Percentage at 100 μM.

scavengers. At 200 μM, compounds **1** and **2** showed less than 10% scavenging activities (Table 4). The ABTS radical-scavenging activity was measured immediately after the addition of potassium persulfate to an ABTS solution. Compound **2** suppressed the absorbance of the ABTS radicals with an IC<sub>50</sub> value of 63.2±2.4 μM and, when Trolox was used as a positive control, exhibited IC<sub>50</sub> value of 19.4±1.3 μM. The scavenging activity of compound **2** against ABTS was higher than the DPPH radical-scavenging activity (Table 4). On the other hand, the scavenging activity of compound **1** against ABTS was 23.3% at 100 μM.

This study reports the compositional components including lignans, protein, and oil found in the Korean sesame cultivars. Two lignans **1** and **2** were isolated from the ethylacetate extract of Suwon cultivar and characterized using the physical and spectroscopic data. The seeds of nine sesame cultivars were screened for the two lignans. Suwon cultivar showed the highest concentration (**1**: 6.24±0.04 and **2**: 3.58±0.01 mg/g), while Soonheuk cultivar had the lowest (**1**: 0.91±0.01 and **2**: 0.73±0.01 mg/g). Suwon and Soonheuk cultivars respectively showed the highest and the lowest protein contents. In the oil content, Kwangbaek and Soonheuk respectively showed the

highest (49.84±0.40%) and the lowest (42.52±0.05%) contents. Moreover, compound **1** exhibited a strong radical-scavenging activity against the ABTS (IC<sub>50</sub>: 63.2±2.4 μM). After further processing for use as food ingredients, the Korean sesame cultivars may contribute to the enhancement of the human health. In addition, the evaluation of sesame quality in breeding aspect could be important in increasing the value of the functional materials. The relationships between lignans as the quality factors and the protein and oil contents will become increasingly important in improving the quality of the sesame seeds.

**Acknowledgements.** This study was supported by a Post Doctoral Course Program of Yeongnam Agricultural Research Institute (YARI), National Institute of Crop Science, Rural Development Administration, Republic of Korea.

## References

- Abou-Gharbia HA, Shahidi F, Shehata AAY, Youssef MM (1997) Effect of processing on oxidative stability of sesame oil extracted from intact and dehulled seed. *J Am Oil Chem Soc* **74**, 215-221.
- Ashakumary L, Rouyer I, Takahashi Y, Ide T, Fukuda N, Aoyama T, Hashimoto T, Mizugaki M, Sugano M (1999) Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation in the rat. *Metabolism* **48**, 1303-1313.
- Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I (2001) Antioxidant principles from *Bauhinia terapotensis*. *J Nat Prod* **64**, 892-895.
- Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol* **28**, 25-30.
- Choi YM, Ku JB, Chang HB, Lee JS (2005) Antioxidant activities and total phenolics of ethanol extracts from several edible mushrooms produced in Korea. *Food Sci Biotechnol* **14**, 700-703.
- Coperland RA (2000) Enzyme: A practical introduction to structure, mechanism, and data analysis. Wiley-VCH, New York, NY, USA. pp. 266-332.
- Elleuch M, Besbes S, Roiseux O, Blecker C, Attia H (2007) Quality characteristics of sesame seeds and by-products. *Food Chem* **103**, 641-650.
- Fukuda Y, Osawa T, Namiki M, Ozaki T (1985) Studies on antioxidant substances in sesame seed. *Agric Biol Chem* **49**, 301-306.
- Hibasami H, Fujikawa T, Takeda H, Nishibe S, Satoh T, Fujisawa T, Nakashima K (2000) Induction of apoptosis by *Acanthopanax senticosus* HARMS and its component, sesame in human stomach cancer KATO III cells. *Oncol Rep* **7**, 1213-1216.
- Hirata F, Fujita K, Ishikura Y, Hosoda K, Ishikawa T, Naka-

- mura H (1996) Hypocholesterolemic effect of sesame lignan in humans. *Atherosclerosis* **122**, 135-136.
- Hirose N, Doi F, Ueki T, Akazawa K, Chijiwa K, Sugano M, Akimoto K, Shimizu S, Yamada H (1992) Suppressive effect of sesamin against 7,12-dimethylbenz[a]anthracene induced rat mammary carcinogenesis. *Anti-cancer Res* **12**, 1259-1265.
- Hirose N, Inoue T, Nishihara K, Sugano M, Akimoto K, Shimizu S, Yamada H (1991) Inhibition of cholesterol absorption and synthesis in rats by sesamin. *J Lipid Res* **32**, 629-638.
- Hsu DZ, Su SB, Chien SP, Chiang PJ, Li YH, Lo YJ (2005) Effect of sesame oil on oxidative-stress-associated renal injury in endotoxemic rats: involvement of nitric oxide and proinflammatory cytokines. *Shock* **24**, 276-280.
- Hu Q, Xu J, Chen S, and Yang F (2004) Antioxidant activity of extracts of black sesame seed (*Sesamum indicum* L.) by supercritical carbon dioxide extraction. *J Agric Food Chem* **52**, 943-947.
- Kang MH, Naito M, Sakai K, Uchida K, and Osawa T (2000) Mode of action of sesame lignans in protecting low-density lipoprotein against oxidative damage in vitro. *Life Sci* **66**, 161-171.
- Kato MJ, Chu A, Davin LB, Lewis NG (1998) Biosynthesis of antioxidant lignans in *Sesamum indicum* seeds. *Phytochemistry* **47**, 583-591.
- Kim JC, Kim KH, Jung JC, Park OS (2006) An efficient asymmetric synthesis of furofuran lignans: (+)-sesamin and (-)-sesamin. *Tetrahedron:Asymmetry* **17**, 3-6.
- Kim KS, Park SH, Choung MG (2006) Nondestructive determination of lignans and lignan glycosides in sesame seeds by near infrared reflectance spectroscopy. *J Agric Food Chem* **54**, 4544-4550.
- Kim SL, Berhow MA, Kim JT, Chi HY, Lee SJ, and Chung IM (2006) Evaluation of soyasaponin, isoflavone, protein, lipid, and free sugar accumulation in developing soybean seeds. *J Agric Food Chem* **54**, 10003-10010.
- Larson RA (1988) The antioxidants of higher plants. *Phytochemistry* **27**, 969-978.
- Lemcke-Norojarvi M, Kamal-Eldin A, Appellqvist LA, Dimberg LH, Ohrvall M, Vessby B (2001) Corn and sesame oils increase serum gamma-tocopherol concentrations in healthy Swedish women. *J Nutr* **131**, 1195-1201.
- Matsumura Y, Kita S, Tanida Y, Taguchi Y, Morimoto S, Akimoto K, Tanaka T (1998) Antihypertensive effect of sesamin. III. Protection against development and maintenance of hypertension in strokeprone spontaneously hypertensive. *Biol Pharm Bull* **21**, 469-473.
- Osawa T, Nagata M, Namiki M, Fukuda Y (1985) Sesamolol, a novel antioxidant isolated from sesame seeds. *Agric Biol Chem* **49**, 3351-3352.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying improved ABTS radical cation decolorization assay. *Free Radical Bio Med* **26**, 1231-1237.
- Sugano M, Akinmoto KA (1993) Multifunctional gift from nature. *J Chin Anim Sci* **18**, 1-11.
- Were BA, Onkware AO, Guda S, Welander M, Carlsson AS (2006) Seed oil content and fatty acid composition in east African sesame (*Sesamum indicum* L.) accessions evaluated over 3 years. *Field Crop Res* **97**, 254-260.
- Wu WH (2007) The contents of lignans in commercial sesame oils of Taiwan and their changes during heating. *Food Chem* **104**, 341-344.
- Wu WH, Kang YP, Wang NH, Jou HJ, Wang TA (2006) Sesame ingestion affects sex hormones, antioxidant status and blood lipids in postmenopausal women. *J Nutr* **136**, 1270-1275.
- Yamashita K, Kagaya M, Higuti N, Kiso Y (2000) Sesamin and alpha-tocopherol synergistically suppress lipid-peroxide in rats fed a high docosahexaenoic acid. *Biofactors* **11**, 11-13.