JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

HPLC Analysis of Sesaminol Glucosides in Sesame Seeds

ALI A. MOAZZAMI,*,† ROLF E. ANDERSSON,‡ AND AFAF KAMAL-ELDIN†

Department of Food Sciences and Department of Chemistry, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

An HPLC method was developed and validated for the quantification of sesaminol triglucoside and a sesaminol diglucoside in sesame seeds. These two lignans were isolated, and their structures were characterized by mass and nuclear magnetic resonance spectroscopy. Defatted sesame flour was extracted first with 85% ethanol for 5 h followed by 70% ethanol for 10 h at room temperature using naringenin as internal standard. Analysis of 65 different samples of sesame seeds indicated that the content of sesaminol triglucoside ranged from 36 to 1560 mg/100 g of seed (mean 637 ± 312) and that of sesaminol diglucoside ranged from 0 to 493 mg/100 g of seed (mean 75 ± 95). No significant difference was found between sesaminol glucoside contents in black and white seeds.

KEYWORDS: Sesame seed; lignan; sesaminol triglucoside; sesaminol diglucoside; HPLC analysis

INTRODUCTION

Sesame (*Sesamum indicum* L.), considered to be an important oilseed since ancient times, is composed of 50% oil and 20% protein (*1*). The most outstanding characteristic of sesame oil is its content of two major lignans, sesamin and sesamolin, which have attracted a lot of interest during last years because of health-promoting effects (*1*). For example, it was shown that sesamin reduces the absorption and biosynthesis of cholesterol, resulting in reduction of plasma and liver cholesterol in rats (*2*) and plasma cholesterol in humans (*3*). Sesamin also elevates γ-tocopherol in plasma and liver of rats (*4*). In addition, sesame seed contains large quantities of oil-insoluble lignan glucosides including pinoresinol glucosides (*5*) and sesaminol glucosides (*6*). Their aglycones possess peroxyl radical scavenging activities after being liberated in the gastrointestinal tract by β-glucosidase (*7*).

Kang et al. (*8*) showed that lipid peroxidation, measured as 2-thiobarbituric acid reactive substance (TBARS), was lower in the liver and serum and that liver α- and γ-tocopherol concentrations were significantly higher in rabbits fed defatted sesame flour (DSF) containing 1% sesaminol glucoside. In their experiment, sesaminol was detected in both serum and liver of rabbits. Sesaminol and its steroisomers, through their phenolic moiety, are potent inhibitors of the oxidation of low-density lipoprotein induced by 2,2′-azobis(2,4-dimethylvaleronitrile) (*9*). It was also shown that sesaminol glucosides inhibit allergen absorption *in vitro* (*10*).

It is important to understand the variation in the content of physiologically active constituents in raw materials for any potential incorporation in functional foods. This knowledge is of great interest for agronomist and plant breeders. However, despite the diversity of lignan glucosides in sesame seeds and considering their biological activities and potential use in functional foods, no validated method is available for their analysis. The only previous report (*11*) just reported range and mean concentrations of sesaminol triglucoside and a sesaminol diglucoside in 25 sesame seed samples. The aim of this study was to isolate, characterize, and develop a validated method to analyze sesaminol glucosides in 65 different varieties of sesame seed.

MATERIALS AND METHODS

Chemicals and Reagents. Sodium dihydrogen phosphate monohydrate, HPLC-grade acetonitrile, methanol, pre-coated silica gel 60 TLC plates (5 × 20 cm^2 or 10 × 20 cm^2, 0.25 mm layer thickness), and silica gel 60 for column chromatography (0.04−0.063 mm) were purchased from Merck (Darmstadt, Germany). Naringenin (4′,5,7-trihydroxyflavanone), used as internal standard, was purchased from Aldrich (Milwaukee, WI). Bondesil-C18 (40 μm) was purchased from Varian Inc. (Harbor City, CA). Absolute ethanol was from Kemetyl (Haninge, Sweden). All other solvents and reagents used in this study were of analytical grade and were used without further purification.

Sesame Seeds. Sixty-five different sesame seed samples were bred, grown, and harvested in Sesaco corporation nurseries in Texas. The sesame seeds analyzed in this study had white (n = 47), black (n = 11), and brown and yellow colors (n = 7). Seeds from semishattering (n = 8) and nondehiscent (n = 28) plants were harvested when the plants were dry, and the dehiscent types (n = 29) were harvested when half of the plant was dry. The plants were hand-selected, hand cut, put through a plot thresher, and carefully cleaned to remove all foreign materials.

Isolation and Structural Characterization of Sesaminol Glucosides. Sesame seeds (ca. 250 g) were ground and defatted with *n*-hexane to obtain defatted sesame flour (DSF, ca. 125 g), which was extracted with 1 L of 80% ethanol for 24 h to obtain a crude extract containing sesaminol glucosides. Part of the crude extract (1 g) was dissolved in methanol (3 mL) and used for thin-layer chromatography (TLC). A portion of the solution (5 μL) was applied manually on informative

* Corresponding author. Telephone: +46 18 67 20 63. Fax: +46 18 67 29 95. E-mail: Ali.Moazzami@lmv.slu.se.
† Department of Food Sciences.
‡ Department of Chemistry.

10.1021/jf051541g CCC: $33.50
Published on Web 01/10/2006

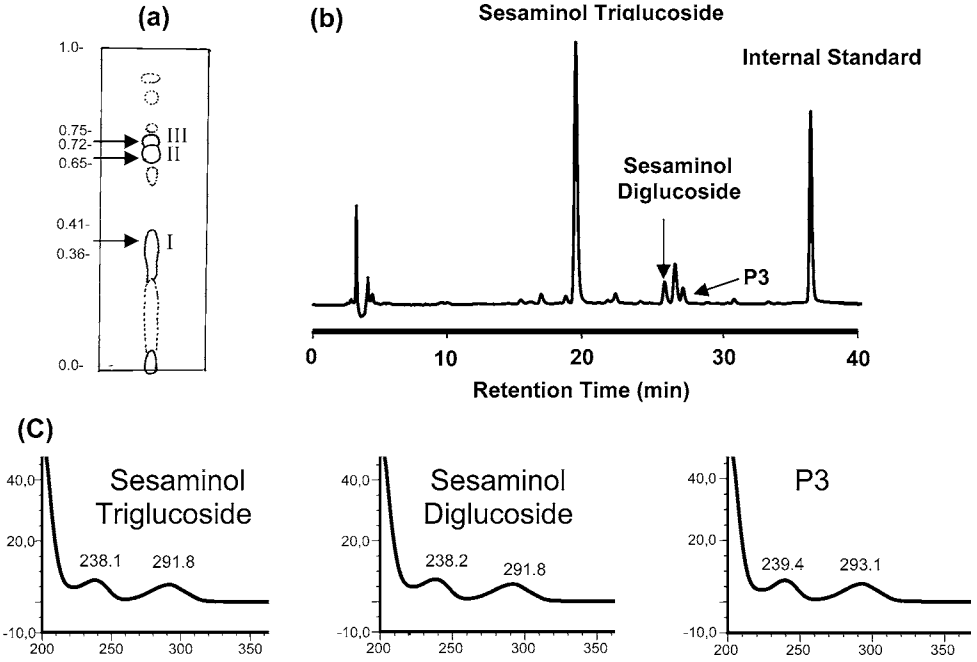


Figure 1. Chromatographic separation and UV spectra of sesaminol glucosides in sesame seeds: (a) TLC separation of defatted sesame flour extract (silica gel 60, chlorophorm/methanol, 1:1, v/v); (b) a typical reversed phase HPLC chromatogram of defatted sesame flour (detection 290 nm); (c) UV spectra of major peaks of defatted sesame flour extract.

Table 1. Structural Study of Defatted Sesame Flour Extract by TLC, HPLC-DAD, and LC-MS

compound	TLC R_f	HPLC t_R (min)	UV absorption (λ, nm)	LC-MS positive mode	LC-MS negative mode
sesaminol triglucoside	0.36–0.41	19.0	238, 291	$[S_3G + Na]^+ = 879.2$	$[S_3G - H]^- = 885.2$
sesaminol diglucoside	0.65–0.72	25.6	238, 292	$[S_2G + Na]^+ = 717.1$	$[S_2G + CH_3COO]^- = 753.2$
sesaminol diglucoside?[a]	0.72–0.75	25.6	239, 293	$[S_2G + Na]^+ = 717.1$	$[S_2G + CH_3COO]^- = 753.2$

[a] In the extract of the TLC spot (R_f = 0.72–0.75), there is a mixture of sesaminol diglucoside and P3.

TLC plates (5 × 20 cm^2), which were developed twice (in one dimension) using chloroform/methanol (1:1, v/v) as the mobile phase. The spots were visualized by spraying with 50% sulfuric acid in ethanol/diethyl ether (1:1, v/v) and R_f values for the different spots on the TLC plates were calculated **(Figure 1**a).

For the isolation of sesaminol diglucoside, ten TLC plates (10 × 20 cm^2) were used, leaving 1.5 cm on each side of the plate. Then the crude extract solution was applied as eight 5 μL spots separated by 1 cm distances along the central part of the plate. The plates were developed using the same method as above, allowed to dry, and exposed to iodine vapor for 20 min to stain reversibly. Separated spots were marked with a pencil, and then 2 cm was cut off from one side of each plate for visualization by spraying with 50% sulfuric acid in ethanol/diethyl ether (1:1, v/v). Areas corresponding to selected R_f values **(Table 1**) were scraped and extracted with chloroform/methanol (1:1, v/v). The extracts were dried and dissolved in 70% ethanol for further analysis with HPLC-DAD and LC-MS. The extract corresponding to R_f 0.65−0.72 was used for structural characterization of sesaminol diglucoside by NMR.

For the isolation of sesaminol triglucoside, the crude extract (4.5 g) was dissolved in 50 mL of 25% methanol and applied to 50 g of Bondesil-C18 (40 μm) silica packed in Buckner funnel (8 cm i.d., 4 cm) with slight suction **(Figure 2**). The column was eluted with 300 mL portions of 25, 50, 75, and 100% methanol in series, and four fractions were collected. After HPLC analysis (described later), the 50 and 75% methanol fractions were pooled together, dried, and dissolved in 10 mL of chloroform/methanol (1:1, v/v). This solution was then applied to an open column (50 cm, 2.5 cm i.d.) packed with 95 g of activated silica gel 60 (0.04−0.063 mm). This normal-phase column was eluted with chloroform/methanol (1:1, v/v), and fractions were collected in 10 mL tubes. After monitoring by HPLC-DAD, tubes numbered 35−39, which had high concentration and purity of sesaminol triglucoside, were pooled.

The identities of the compounds were confirmed by mass and nuclear magnetic resonance spectroscopy. Proton (^{1}H NMR), carbon-13 (^{13}C NMR), correlation (COSY), total correlated (TOCSY), heteronuclear multiple bond correlation (HMBC), heteronuclear multiple quantum correlation (HSQC), rotating Overhauser effect (ROESY), and nuclear Overhauser effect (NOESY) spectroscopies were performed using a Bruker DRX 400 instrument (Karlsruhe, Germany). The measurements were performed at frequencies of 400 (^{1}H) and 100 (^{13}C) MHz in CD_3-OD (D, 99.8%) at 30 °C. ^{1}H NMR chemical shifts were referenced to TMS while ^{13}C chemical shifts were referenced to the methyl group signals from residual methanol (δ 49.0). A sequence with presaturation of the water peak was used for ^{1}H NMR. Mixing times for different

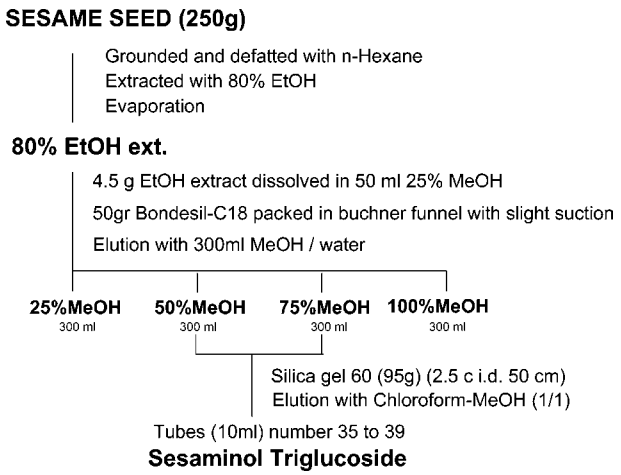


Figure 2. Isolation of Sesaminol Triglucoside.

NMR techniques were as follows: TOCSY (60 ms), HMBC (65 ms), ROESY (150 ms), and NOESY (300 ms).

HPLC Analysis of Sesaminol Glucosides. To obtain defatted sesame flour (DSF) for analytical purposes, sesame seeds were defatted by vigorous shaking of triplicate samples (each of 5 g of the seeds) in stainless steel tubes with four steel balls and 30 mL of hexane/2-propanol (3:1, v/v) for 1 h as described elsewhere (*12*).

Dried DSF samples were weighed (0.5 g) in glass tubes (35 mL) in triplicate and extracted for 5 h with 8.25 mL of 85% ethanol containing 100 μg/mL of naringenin as internal standard. Then, the extraction was continued overnight after adjusting the ethanol concentration to 70% by adding 1.75 mL of distilled water to the extraction tubes. Thereafter, the tubes were centrifuged for 10 min at 2000 rpm and the supernatants were filtered (0.45 μm PTEF membrane, Pall Acrodisc, Ann Arbor, MI).

Samples were analyzed by HPLC (Dionex PDA-100, Dionex, Sunnyvale, CA) with a diode array detector (DAD), which also provided the UV spectra of the peaks, and an Econosil ODS column (5 μm, 250 mm × 4.6 mm, Alltech Co., Waukegan Road, Deerfield, IL). The eluents used were (A) 0.01 M phosphate buffer (pH 2.8) containing 5% acetonitrile and (B) acetonitrile. The elution conditions were 0−5 min (15% B), 30 min (30% B), and 40−50 (70% B), and the flow rate was 1.0 mL/min.

The quantities of sesaminol triglucoside and sesaminol diglucoside were calculated from the peak areas at 290 nm against the internal standard (naringenin). To calculate the relative response factors (RRFs) with reference to the internal standard, solutions including 14 different concentrations of sesaminol triglucoside and naringenin were analyzed by HPLC and peak areas were used to draw the calibration curves for sesaminol and naringenin. The response factor for sesaminol relative to naringenin was calculated as 0.345 and was used to calculate the concentrations of sesaminol triglucoside and sesaminol diglucoside in DSF, assuming equal response.

Mass Spectroscopy of Defatted Sesame Flour Extract. LC-MS (Agilent, 1100 series, Alpharetta, GA) with electrospray ionization (ESI) and a Genesis C18 column (4.6 mm × 150 mm, 4 m particle size, Jones Chromatography, Gengoed, U.K.) was used for further structural analysis of DSF extracts and the extracts obtained from TLC. The eluents used were (A) 10 mM acetic acid (pH 3) and (B) acetonitrile. The elution conditions were 0−5 min (20% B) and 30−50 min (70% B), and the flow rate was 0.4 mL/min. At the interface, the drying gas was set to 9 L/min, the nebulizer pressure was 30 psi, the drying gas temperature was 350 °C, and the scanning range was 300−1000 *m/z*.

Statistical Analysis. The analysis of variance was performed using MINITAB 14 software, and significant differences between samples means were determined using Tukey's test.

RESULTS AND DISCUSSION

In this paper, we report methods for the isolation and characterization of sesaminol diglucoside and sesaminol triglucoside from sesame seeds for the first time. Details of the methods used are given *vide infra*. The isolated standards were used to calibrate and validate an HPLC analytical method, which was then used to analyze 65 samples.

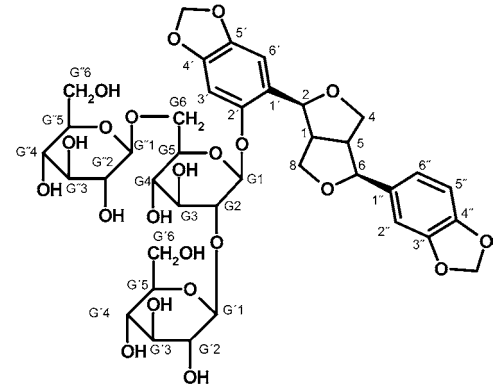
Isolation and Structural Characterization of Sesaminol Glucoside. Figure 1a shows the TLC separation of DSF lignans. The large TLC spot I (R_f 0.36−0.41) was assigned as sesaminol triglucoside by comparing its retention time and UV spectrum with the sesaminol triglucoside that was purified and characterized in this study. There were two other spots (R_f 0.65−0.72 and R_f 0.72−0.75) which were scraped and extracted with methanol/chloroform (1:1, v/v) for further analysis with HPLC-DAD and LC-MS. The extract from spot II (R_f 0.65−0.72) had an HPLC retention time of 25.6 min **(Figure 1b)** and a UV spectrum **(Figure 1c)** and mass spectrum **(Table 1)** similar to those of the peak eluting at R_f = 25.6 min on the HPLC chromatogram of the crude extract. This peak was characterized as sesaminol diglucoside (*vide infra*). The extracts from spot III (R_f = 0.72−0.75) gave two peaks in the HPLC chromatogram. The first peak had a retention time, UV spectrum **(Figure 1c),** and mass spectrum similar to those for the above-mentioned sesaminol diglucoside, and the second peak had a retention time (27 min) similar to that for an unknown, P3, on HPLC chromatogram. As shown in **Figure 1c,** sesaminol diglucoside and P3 have similar UV spectra**.** The detection of sesaminol diglucoside in the extract from TLC spot III (R_f = 0.72−0.75) can be because of contamination from TLC spot II (R_f = 0.65−0.72) or the presence of another isomer of sesaminol diglucoside eluting as P3.

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (133 MHz) Spectral Data of Sesaminol Triglucoside and Sesaminol Diglucoside Isolated from Sesame Seeds

	sesaminol triglucoside		sesaminol diglucoside	
position	δ_H	δ_C	δ_H	δ_C
1	2.92 m	55.9	3.05	55.3
2	5.21 d, J = 5.2	82.5	5.21 d, J = 4.7	82.6
4	3.88 dd, J = 4.4, 9 (axial)	72.7	3.89 dd, J = 4.2, 9.1 (axial)	72.1
4	4.27 dd, J = 6.6, 9 (equatorial)		4.22 dd, J = 6.4, 9.1 (equatorial)	
5	3.03 m	55.6	3.00 m	55.5
6	4.7 d, J = 5.3	86.3	4.66 d, J = 5.4	86.6
8	4.23 d, J = 5.6 (axial)	73.6	4.09 dd, J = 4.4, 9.1 (axial)	73.9
8	4.23 d, J = 5.6 (equatorial)		4.31 dd, J = 7.3, 9.1 (equatorial)	
1′		125.5		125.6
2′		150.0		150.5
3′	6.91 s	99.03	6.93 s	99.5
4′		144.4		144.2
5′		149.0		148.6
6′	6.84 s	105.7	6.85 s	105.7
1″		136.6		136.4
2″	6.87 d, J = 1.5	107.3	6.87	107.1
3″		144.1		149.0
4″		148.6		148.6
5″	6.77 d, J = 7.8	108.7	6.77	108.8
6″	6.83 dd, J = 1.5, 7.6	120.4	6.84	120.4
–O–CH_2–O–′	5.9	102.6	5.91	101.9
–O–CH_2–O–″	5.91	102.4	5.90	102.0
G1	5.03 d, J = 7.6	101.05	4.85[a]	102.9
G2	3.81	81.6	3.46	74.86
G3	3.72	78.1	3.68	77.0
G4	3.45	71.0	3.44	71.1
G5	3.74	76.8	3.45	77.9
G6a	4.15 dd, J = 1.7, 11.7	70.11	4.15 dd, J = 1.9, 11.5	70.0
G6b	3.82 m		3.83 m	
G1′	4.88 d, J = 7.7	104.2	4.35 d, J = 7.7	104.5
G2′	3.26	75.7	3.24 dd, J = 8.1, 7.7	74.9
G3′	3.42	77.7	3.36	77.7
G4′	3.42	71.1	3.31	71.5
G5′	3.35	77.8	3.21	77.7
G6′a	3.74 m	62.1	3.85 m	62.7
G6′b	3.74 m		3.64 m	
G1″	4.35 d, J = 7.7	104.7		
G2″	3.24	75.1		
G3″	3.35	77.8		
G4″	3.21	71.9		
G5″	3.19	71.5		
G6″a	3.64 dd, J = 6.4, 11.5	62.7		
G6b″b	3.85 m			

[b] Because of virtual higher order, the coupling constant cannot be reported.

Table 2 presents the ^{1}H and ^{13}C NMR spectra of sesaminol triglucoside (sesaminol 2′-*O*-β-D-glucopyranosyl (1→2)-*O*-[β-D-glucopyranosyl (1→6)]-β-D-glucopyranoside) **(Figure 3**) isolated in this study. H-1 (δ 2.92) appeared as a multiplet due to coupling with H-8a, H-8e, and H-2. Similarly, H-5 (δ 3.03) appeared as a multiplet due to coupling with H-4a, H-4e, and H-6. These results were confirmed by COSY. On the other hand, HMBC confirmed the coupling between H-2 (δ 5.21) and C-8 (δ 73.6) and between H-6 (δ 4.70) and C-4 (δ 72.7). HMBC also confirmed coupling between H-1 and C-1′ (δ 125.5),



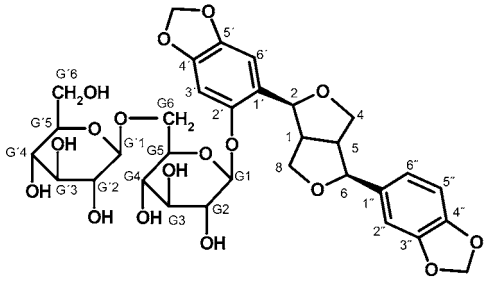


Figure 3. Structures of the two sesaminol glucosides analyzed in sesame seeds.

between H-2 and C-5′ (δ 149.0), between H-5 and C-1″ (δ 136.6), and between H-6 and C-6″ (δ 120.4) and C-2″ (δ 107.3). ^{1}H NMR showed coupling between H-6″ and H-2″ (J = 1.5 Hz). The position of C-4″ (148.6 δ) was assigned from coupling with H-6″ in HMBC. The aromatic carbons were assigned from coupling to the corresponding hydrogen in HSQC. The glycosilation at C-2′ (δ 150) was confirmed by a cross-peak with the anomeric proton (G1, δ 5.03) and a downfield shift in comparison with C-2″ (δ 107.3). A downfield shift in C−G6 (δ 70.11) in comparison with C−G6′ (δ 62.1) and C−G6″ (δ 62.7) in addition to a cross-peak with the anomeric proton (G1′, δ 4.88) in HMBC confirmed the presence of a 1→6 bond between these sugars. Similarly, a downfield shift in C−G2 (δ 81.6) in comparison with C−G2′ (δ 75.7) and C−G2″ (δ 75.1) besides a cross-peak with the anomeric proton (G1″, δ 4.35) confirmed a 1→2 bond between these sugars. The coupling constant for the anomeric protons G1 (δ 5.03, J = 7.59 Hz), G1′ (δ 4.88, J = 7.47 Hz), and G-1″ (δ 4.35, J = 7.73 Hz) confirmed the β-configuration of these proton (6).

The structure of sesaminol diglucoside **(Figure 3)** extracted from TLC spot II (R_f = 0.65−0.72) was investigated by NMR, and its ^{1}H and ^{13}C NMR spectra are also presented in **Table 2**. The structure of the aglycone in sesaminol diglucoside was confirmed in the same way as for sesaminol triglucoside. HMBC showed a cross-peak between the anomeric proton (G1′, δ 4.35) and C−G6 (δ 70.0), indicating a 1→6 bond between sugars number two and one. Moreover, ROESY and NOESY spectra indicated a cross-peak between the anomeric proton (G1′, δ 4.35) and G6a (δ 3.83) and G6b (δ 4.15) which confirmed the result obtained by HMBC concerning the 1→6 bond between sugars. This sugar configuration (1→6) was different from that in the other sesaminol diglucoside isomer isolated and characterized by Katsuzaki et al. (*6*), in which the sugar residue had a 1→6 bond. The sesaminol diglucoside analyzed in this study is, thus, sesaminol 2′-*O*-β-D-glucopyranosyl (1→6)-*O*-β-D-glucopyranoside. It is possible that two isomers of sesaminol diglucoside exist in DSF since Katsuzaki et al. have isolated two pinoresinol diglucosides from defatted sesame flour having both 1→6 and 1→2 bonds between their sugars (*5*).

Development and Validation of the Analytical Method. The extraction efficiency of sesaminol triglucoside was investigated by taking different parameters including ethanol percentage, extraction time, cake-to-solvent ratio, and temperature into consideration. Among different ethanol concentrations, 70% (9.9 mg/g of DSF) and 80% (10 mg/g of DSF) provided higher recovery of sesaminol triglucoside compared with 50% (7.6 mg/g of DSF), 60% (9.3 mg/g of DSF), and 90% (3.3 mg/g of DSF) (n = 7, p < 0.01). The prolongation of the extraction time, from 15 h (9.8 mg/g of DSF) to 72 h (9.39 mg/g of DSF), caused a 4.2% decrease in the extractability of sesaminol triglucoside (n = 7, p < 0.001) possibly because of degradation by an enzyme. To gain the mutual benefit of higher recovery and less degradation, a method was developed based on extraction with 85% ethanol for 5 h followed by 70% ethanol for 10 h. This extraction method provided better extractability (10.4 mg/g of DSF) in comparison with extraction with either 70% (9.9 mg/g of DSF) or 85% ethanol (5.9 mg/g of DSF) (n = 6, p < 0.001). The recovery decreased significantly (n = 5, p < 0.01,) when the DSF to solvent ratio increased from 0.25 g/10 mL (10.47 mg/g of DSF) to 0.8 g/10 mL (10.04 mg/g of DSF), but no significant difference in recovery was found between 0.25 g/10 mL (10.47 mg/g of DSF) and 0.5 g/10 mL (10.40 mg/g of DSF). Therefore, considering the small coefficient of variation (CV = 0.6%) when 0.5 g/10 mL was used, this was chosen as a proper ratio. To investigate the effect of extraction temperature on the extractability of sesaminol triglucoside, DSF was extracted at 20, 37, and 55 °C. Statistical analysis revealed no difference between the different temperatures used, and room temperature was, therefore, chosen (n = 7).

The effect of the solid matrix on the recovery of internal standard, naringenin, was investigated by spiking different amounts of DSF (0.25−0.8 g/10 mL) with different concentrations of internal standard (50−200 μg/mL). Analysis of variance indicated no significant effect of the DSF matrix on the recovery of naringenin under the investigated range (n = 6). The precision of the method was determined by analyzing sesaminol triglucoside in DSF in six different days. Each day, sesame cake was extracted and analyzed by HPLC (n = 5) using the method described *vide supra*. The intra-assay precision (within day variation) ranged from 0.6 to 2.8% with the average of 1.6%, and the inter-assay precision (between days variation) was 2.8%.

Using sesaminol triglucoside standard, the detection limits of the method were determined as 10 mg/100 g of DSF for sesaminol triglucoside and 8 mg/100 g of DSF for sesaminol diglucoside using a signal-to-noise ratio of 3. The concentration of sesaminol glucoside having a peak height 10 times higher than noise was considered as the limit of quantification (40 mg/100 g of DSF for sesaminol triglucoside and 32 mg/100 g of DSF for sesaminol diglucoside). Linearity was confirmed between the lowest and highest concentrations of sesaminol used in the standard curve (9 and 435 μg/mL, respectively).

Variation in Sesaminol Glucoside Contents in Defatted Sesame Flour. The method described above was used for the quantification of sesaminol triglucoside and sesaminol diglucoside in 65 different sesame seed samples **(Table 3)**. Sesaminol triglucoside had a range of 36−1560 mg/100 g of seeds (mean 640 ± 317 mg/100 g of seeds) that is broader than what Ryu et al. (*11*) had reported previously in 25 cultivars analyzed (mean 68.4 mg/100 g of seeds). The analysis of variance indicated no

Table 3. Variation of Sesaminol Triglucoside and Sesaminol Diglucoside Content (mg/100 g) in 65 Different Sesame Seeds[a]

sample number	seed color	sesaminol triglucoside	sesaminol diglucoside
1	white	444	26
2	white	176	tr
3	white	501	51
4	white	701	40
5	white	423	47
6	white	940	54
7	white	730	74
8	white	282	23
9	white	710	42
10	white	448	36
11	white	588	36
12	white	554	54
13	white	703	38
14	white	1559	86
15	white	549	494
16	white	119	17
17	white	410	35
18	white	260	50
19	white	460	51
20	white	1045	73
21	white	525	466
22	white	717	322
23	white	1030	53
24	white	1051	87
25	white	1009	71
26	white	931	63
27	white	808	45
28	white	452	31
29	white	501	420
30	white	1225	95
31	white	948	61
32	white	752	68
33	white	641	46
34	white	607	69
35	white	552	71
36	white	546	69
37	whitish	571	65
38	whitish	868	93
39	whitish	456	28
40	whitish	555	34
41	whitish	238	tr
42	whitish	937	52
43	whitish	1341	97
44	whitish	1253	82
45	whitish	635	67
46	whitish	955	55
47	whitish	662	101
48	whitish	775	52
49	yellow	284	35
50	yellow	750	60
51	yellow	677	62
52	yellow brown	653	52
53	brown	325	16
54	grey	73	ND
55	black	583	19
56	black	36	ND
57	black	241	30
58	black	1056	82
59	black	967	117
60	black	277	19
61	black	304	17
62	black	873	111
63	black	679	95
64	black	213	16
65	black	502	42
Range:		36–1560	ND–493
Mean ± SD:		640 ± 317	75 ± 95

[a] ND = not detected. The detection limit for sesaminol triglucoside was 10 mg/100 g of defatted sesame flour (DSF) (or 5 mg/100 g of seed assuming 50% oil content), and that for sesaminol diglucoside was 8 mg/100 g of DSF (or 4 mg/100 g of seed assuming 50% oil content). tr = trace amount. The quantification limit for sesaminol triglucoside was 40 mg/100 g of DSF (or 20 mg/100 g of seed assuming 50% oil content), and that for sesaminol diglucoside was 32 mg/100 g of DSF (or 16 mg/100 g of seed assuming 50% oil content).

significant difference between the sesaminol triglucoside content of black (mean 521 ± 339 mg/100 g of seeds) and white seeds (mean 691 ± 694 mg/100 g of seeds). Sesaminol diglucoside ranged from undetectable to 493 mg/100 g of seeds (mean 75 ± 95 mg/100 g of seeds). Four white sesame samples had high sesaminol diglucoside contents (322−493 mg/100 g of seeds). After excluding these four samples, there was a significant correlation between sesaminol triglucoside and sesaminol diglucoside ($R^2 = 0.6$, $p < 0.001$).

Despite the high content of sesaminol triglucoside in sesame seeds and the fact that its aglycone has strong free radical scavenging properties (*13*), limited research has been performed in order to figure out its physiological activities. Moreover, nothing is known about the biosynthesis (*14*) and transformation of sesaminol glucosides during seeds maturation, although sesaminol triglucoside is apparently a major sesame seed lignan. The chromatographic and quantification parameters of the new HPLC method, which was developed and validated for the first time in this study, were stable and reliable, and the method was sensitive and linear in the reported range. The use of naringenin as internal standard made easy the analysis of different sesame seeds. A broad range of sesaminol glucosides was found in the defatted sesame flour that should be considered when breeding new sesame seeds or for any potential incorporation of the seeds into functional foods. Moreover, a new isomer of sesaminol diglucoside was isolated and characterized using NMR and LC-MS and quantified by the method developed and validated in this study. A method was also developed for isolation of sesaminol triglucoside which was simpler and faster than what Katsuzaki et al. have reported (*6*).

ACKNOWLEDGMENT

We are grateful to Dr. Jelena Jastrebova from our Department for kind help during LC-MS experiments.

LITERATURE CITED

(1) Namiki, M. The chemistry and physiological functions of sesame. *Food Rev. Int.* **1995**, *11*, 281−329.

(2) Hirose N.; Inoue T.; Nishihara K.; Sugano M.; Akimoto K.; Shimizu S.; Yamada S. Inhibition of cholesterol absorption and synthesis in rats by sesamin. *J. Lipid Res.* **1991**, *32*, 629−638.

(3) Hirata F.; Fujita K.; Ishikura Y.; Hosoda K.; Ishikawa T.; Nakamura H. Hypercholesterolemic effect of sesame lignan in human. *Atherosclerosis* **1996**, *122*, 135−136.

(4) Kamal-Eldin A.; Pettersson D.; Appelqvist L. Sesamin (a compound from sesame oil) increases tocopherol levels in rats fed ad libitum. *Lipids* **1995**, *30*, 499−505.

(5) Katsuzaki H.; Kawasumi M.; Kawakishi, S.; Osawa T. Structure of novel antioxidantive lignan glucosides isolated from sesame seed. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 2087−2088.

(6) Katsuzaki, H.; Kawakishi, S.; Osawa, T. Sesaminol glucosides in sesame seeds. *Phytochemistry* **1994**, *35*, 773−776.

(7) Tamura, G.; Gold, C.; Ferro-Luzzi, A.; Ames, B. N. Fecalase: A model for activation of dietary glycosides to mutagens by intestinal flora. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 4961−4965.

(8) Kang, M.; Kawai, Y.; Naito, M.; Osawa, T. Dietary defatted sesame flour decreases susceptibility to oxidative stress in hypercholesterolemic rabbits. *J. Nutr.* **1999**, *129*, 1885−1890.

(9) Kang, M.; Katsuzaki, H.; Osawa, T. Inhibition of 2, 2′-azobis (2, 2-dimethylvaleronitrile)-induced lipid peroxidation by sesaminols. *Lipids* **1998**, *33*, 1031−1036.

(10) Kobayashi, S.; Watanabe, J.; Kawabata, J.; Fukushi, E.; Shinmoto, H. A novel method for producing a foodstuff from defatted black sesame seed that inhibits allergen absoption. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 300−305.

(11) Ryu, S.; Ho, C. T.; Osawa, T. High performance liquid chromatographic determination of antioxidant lignan glycosides in some variations of sesame. *J. Food Lipids* **1998**, *5*, 17−28.
(12) Appelqvist, L-Å. Further studies on a multisequential method for determination of oil content in oilseeds. *J. Am. Oil Chem. Soc.* **1967**, *44*, 209−214.
(13) Suja, K.; Yayalekshmy, A.; Arumughan, C. Free radical scavenging behavior of antioxidant compounds of sesame (*Sesamum indicum*) in DPPH· System. *J. Agric. Food Chem.* **2004**, *52*, 912−915.
(14) Kato, M.; Chu, A.; Laurence, B.; Lewis, D.; Lewis, N. Biosynthesis of antioxidant lignans in *Sesamum indicum* seeds. *Phytochemistry* **1997**, *47*, 581−591.

Received for review June 28, 2005. Revised manuscript received December 2, 2005. Accepted December 8, 2005. The authors would like to thank Sesaco Corporation (Paris, TX) for sponsoring this project.

JF051541G