Variations in the Composition of Sterols, Tocopherols and Lignans in Seed Oils from Four *Sesamum* Species

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Seeds from different collections of cultivated *Sesamum indicum* Linn and three related wild species [specifically, *S. alatum* Thonn., *S. radiatum* Schum & Thonn. and *S. angustifolium* (Oliv.) Engl.] were studied for their oil contents and fatty acid composition of the total lipids. The oils from wild seeds were characterized by higher percentages of unsaponifiables (4.9, 2.6 and 3.7%, respectively) compared to *S. indicum* (1.4–1.8%), mainly due to their high contents of lignans. Total sterols accounted for *ca.* 40, 22, 20 and 16% of the unsaponifiables of the four species, respectively. The four species were different in the relative percentages of the three sterol fractions (the desmethyl, monomethyl and dimethyl sterols) and in the percentage composition of each fraction. Campesterol, stigmasterol, sitosterol and Δ^5-avenasterol were the major desmethyl sterols, whereas obtusifoliol, gramisterol, cycloeucalenol and citrostandienol were the major monomethyl sterols, and α-amyrin, β-amyrin, cycloartenol and 24-methylene cycloartanol were the main dimethyl sterols in all species. Differences were also observed among the four species in sterol patterns of the free sterols compared to the sterol esters. *Sesamum alatum* contained less tocopherols (210–320 mg/kg oil), and *S. radiatum* and *S. angustifolium* contained more tocopherols (*ca.* 750 and 800 mg/kg oil, respectively) than did *S. indicum* (490–680 mg/kg oil). The four species were comparable in tocopherol composition, with γ-tocopherol representing 96–99% of the total tocopherols. The four species varied widely in the identity and levels of the different lignans. The percentages of these lignans in the oils of *S. indicum* were sesamin (0.55%) and sesamolin (0.50%). *Sesamum alatum* showed 1.37% of 2-episesalatin and minor amounts of sesamin and sesamolin (0.01% each). *Sesamum radiatum* was rich in sesamin (2.40%) and contained minor amounts of sesamolin (0.02%), where *S. angustifolium* was rich in sesangolin (3.15%) and also contained considerable amounts of sesamin (0.32%) and sesamolin (0.16%).

KEY WORDS: Lignans, sesame, *Sesamum, S. alatum, S. angustifolium, S. indicum, S. radiatum,* sterols, tocopherols, unsaponifiables.

Sesame oil, obtainable from the seeds of *Sesamum indicum* Linn., is characterized by high oxidative stability compared to other vegetable oils (1,2). The Food and Agriculture Organization of the United Nations/World Health Organization Codex Alimentarius ranges for fatty acid composition of sesame oil are: palmitic (7–12%), stearic (3.5–6%), oleic (35–50%) and linoleic acid (35–50%) (3). Crude sesame oils contain *ca.* 500 mg tocopherols, mainly γ-tocopherol, per kg oil (4–6). The fatty acid composition and the tocopherol levels do not completely explain the high stability of sesame oil when compared to other oils. As an example, a sample of groundnut oil of comparable fatty acid composition and tocopherol level was less stable than a sample of sesame oil when heated at 195°C for three hours, as substantiated by a larger decrease in iodine value and an increase in peroxide and carbonyl values (7).

Sesamol (3,4-methylene-dioxy phenol) is a potent phenolic antioxidant (8) and is present in crude sesame oil in small amounts (9). Kikugawa *et al.* (10) found these levels insufficient to explain the high stability of the oil and suggested the presence of other antioxidants. Sesamolin {2-(3,4-methylenedioxy phenyl)-6-(3,4-methylenedioxyphenoxy)-*cis*-3,7-dioxabicyclo[3.3.0]octane} is a major constituent of the unsaponifiable fraction of sesame oil, and it liberates sesamol both during industrial refining (9) and frying (11). Sesamolin also transforms by the bleaching process to another phenolic antioxidant, sesaminol {2-(3,4-methylenedioxy-6-hydroxy phenyl)-6)3,4-methylenedioxy phenyl)-*cis*-3,7-dioxabicyclo-[3.3.0]octane}, which is the major antioxidant factor in refined sesame oils (9). Thus sesamolin, although not having any antioxidant properties in itself, is a precursor to these two phenolic antioxidants. Sesamin {2,6-*bis*-(3,4-methylenedioxy phenyl)-*cis*-3,7-dioxabicyclo-[3.3.0]octane} also is a major constituent in sesame oil unsaponifiables. Structurally, it is a lignan, similar to sesamolin, but it does not have any potential as an antioxidant or antioxidant precursor.

While the tocopherols and phenolics are antioxidants at typical storage temperatures (4–30°C), sterols with the $\Delta^{24,28}$ ethylidene side chain (*e.g.*, Δ^5- and Δ^7-avenasterols and citrostandienol) have antipolymerization effects that could protect vegetable oils from oxidation at high temperatures (12,13).

In previous investigations (14–19), oils from the seeds of three wild *Sesamum* species, specifically, *S. alatum* Thonn., *S. radiatum* Schum & Thonn and *S. angustifolium* (Oliv.) Engl., were analyzed along with oils from pure-line and mixed-line genotypes of *S. indicum* L. These species, all growing naturally in Sudan, were studied because they may contribute to favorable agronomic characters when used in plant breeding. Oils from the four species were fairly comparable in fatty acid composition (14,18) and triacylglycerol patterns (14). Oils from the wild seeds were, however, found to have significant differences in the composition of the three sterol fractions (des-, mono- and dimethyl sterols) as compared to *S. indicum* (16). Oils from the wild species contained high levels of the antipolymerization sterols compared to *S. indicum* (16). Results from qualitative analyses of the lignans present in the different species are described in the accompanying paper, where great variability was encountered (19).

In this study, the oils from additional samples of the four species were quantitatively analyzed for their content and composition of unsaponifiable matters, including sterols, tocopherols and lignans, to study the variability in these important minor constituents. Results are discussed in relation to oil stability.

MATERIALS AND METHODS

Seeds from different cultivars of *S. indicum* Linn. and from different collections of the three related wild species, *S. alatum* Thonn., *S. radiatum* Schum & Thonn. and *S. angustifolium* (Oliv.) Engl., were collected from different

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locations in Sudan. The samples analyzed and the codes used to identify them were presented elsewhere (18).

All solvents and reagents were of analytical grade (Merck, Darmstadt, Germany) and were used without further purification. Pre-coated silica gel 60 plates (20 × 20 cm, 0.25 mm layer thickness; Merck) were used for all thin-layer chromatography (TLC) separations. The TLC standard mixture, containing sterol, free fatty acid, triacylglycerol, methyl ester and steryl ester (SE), was from Larodan Fine Chemicals AB (Malmö, Sweden). Cholesterol (cholest-5-en-3β-ol), stigmasterol ([24S]-24-ethyl-cholest-5,22-dien-3β-ol), sitosterol ([24R]-24-ethyl-cholest-5-en-3β-ol) and cholestane, used as internal standard in the gas chromatography (GC) analysis of the sterols, were from Sigma Chemical Co. (St. Louis, MO). Cycloartenol (4,4,14-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol) was a gift from Prof. Takashi Kaneda (Faculty of Home Economics, Kohriyama Womens College, Fukushima, Japan). Tri-Sil (Pierce Chemical Co., Rockford, IL) was used for the preparation of the trimethylsilyl (TMS) derivatives of the sterols. The tocopherol standards α-tocopherol [2,5,7,8-tetramethyl-2-(4,8,12-trimethyl tridecyl)-6-chromanol], β-tocopherol [2,5,8-trimethyl-2-(4,8,12-trimethyl tridecyl)-6-chromanol], γ-tocopherol [2,7,8-trimethyl-2-(4,8,12-trimethyl tridecyl)-6-chromanol] and δ-tocopherol [2,8-dimethyl-2-(4,8,12-trimethyl tridecyl)-6-chromanol] were purchased as an isomer kit from Merck. Purified sesamol, sesamin and sesamolin standards were a gift from Dr. Yasuko Fukuda (Ichimura Gakuen Junior College, Inuyama, Japan). All determinations in this paper were carried out in duplicate, and mean values are reported.

Oil extraction and saponification. Crude lipids were extracted by vigorous shaking of duplicate samples (each 5 g of seeds) in stainless-steel tubes with four steel balls and 30 mL of heptane/isopropanol (HIP; 3:1, vol/vol) for 1 h, as described previously (18).

Total lipids were saponified by refluxing duplicate oil samples (*ca.* 5 g) for 1 h with ethanolic potassium hydroxide (50 mL, 1 M). The unsaponifiables were extracted twice with diethyl ether (40 mL). The ether extracts were washed with water and dried over anhydrous sodium sulfate according to IUPAC Method (20). The ether was evaporated *in vacuo* at *ca.* 25°C. The unsaponifiables were weighed to determine their percentages and then kept in chloroform/diethyl ether (4:1, vol/vol) solutions at −20°C for further analyses.

For the saponification of the free sterol bands [with some diacylglyceride (DAG)] and the sterol bands obtained from TLC (described later), the lipids were freed from solvents under nitrogen and then incubated at 70°C with 1M ethanolic KOH (5 mL) for 2–3 h. Water (5 mL) was then added, and the freed sterols were extracted in hexane (2 × 2 mL) and stored in solvents at −20°C until further analysis.

TLC. The crude lipid extracts were applied on TLC plates as 14-cm bands (*ca.* 70 mg/plate) with a Linomat-3 auto applicator (CAMAG, Muttenz, Switzerland). The TLC standards mixture was applied as a reference on one side of each plate, and the plates were developed in hexane/diethyl ether/acetic acid (HEA; 85:15:1, vol/vol/vol). Sample zones were covered with other glass plates, leaving the reference zone exposed to be visualized by iodine vapor. Bands corresponding to free sterol (R_f 0.82–0.97) were scraped and extracted (2 × 3 mL) as follows: free sterol (chloroform/diethyl ether (CDE); 4:1, vol/vol) and steryl ester (*n*-hexane). Because sesangolin coeluted with the free sterols when HEA was used for TLC, the free sterol fraction obtained from *S. angustifolium* was further purified by TLC with CDE (9:1, vol/vol) as the mobile phase (free sterol; R_f 0.25–0.46).

The three sterol classes (desmethyl, monomethyl and dimethyl sterols) from the unsaponifiables of *S. indicum*, *S. radiatum* and *S. angustifolium* were prepared by one-dimensional TLC (CDE; 9:1, vol/vol). Those of *S. alatum* were obtained after two-dimensional TLC with hexane/diethyl ether (7:3, vol/vol) and CDE (9:1, vol/vol) as developing solvents in the first and second dimension, respectively, as previously described (15,16). The content of the sterols in each fraction was determined by weighing evaporated aliquots on a micro-balance. The recovery of the sterols from the TLC plate, previously determined as 96% (16), was used for these calculations.

GC analysis of the sterols. Cholestane was added as an internal standard to the sterols or the total unsaponifiables, and the TMS ether derivatives were prepared by heating with Tri-Sil at 60°C for 45 min. The reagent was then removed under nitrogen, and the TMS derivatives were dissolved in hexane (1 mL) and stored at −20°C if not directly used for injections.

The samples were analyzed in duplicate in a Varian Model 3700 gas chromatograph (Varian Analytical Instruments, Palo Alto, CA) equipped with a flame-ionization detector and a falling needle injector (Chrompack, Middelburg, The Netherlands). Each sample (1–2 μL) was injected into a fused-silica capillary column, HP-1 (25 m × 0.32 mm i.d., film thickness 0.35 μm) (Hewlett-Packard, Avondale, PA). The column and detector temperatures were 245 and 330°C, respectively. Helium was used as a carrier gas at a flow rate of 2.2 mL/min. Peaks were recorded and peak areas computed with an HP 3390 A integrator. Peaks were identified by comparison of their retention times with those of TMS derivatives of available standards and by comparison with previous chromatograms, in which peaks were identified by GC-mass spectrometry (MS) (16). The percentages of the sterols were calculated by direct internal normalization.

High-performance liquid chromatography (HPLC) analysis of tocopherols and lignans. HPLC analyses were performed on an SP 8700 HPLC system with SP 8750 organizer module (Spectra Physics, San Jose, CA). Samples (10 μL) of oil dissolved in hexane/chloroform (2:1, vol/vol) were injected in each case by using a manual valve injector.

The oils were analyzed for the tocopherols on two HPLC columns (10 cm × 3 mm i.d.; Chrompack), in series, packed with Chrom Spher silica (particle size 5 μm). The mobile phase was 6% diethyl ether in heptane at a flow rate of 0.7 mL/min for 15 min, and then 1.5 mL/min for 25 min. The effluent was monitored with a Perkin-Elmer (Norwalk, CT) LS-2 filter fluorimeter at an excitation wavelength of 295 nm and an emission wavelength of 320 nm. The peaks were recorded with an HP 3390 A integrator. Peaks were identified by comparison with standards and quantitated against β-tocopherol as an internal standard. The response factors of the different tocopherols under these conditions were determined as β-tocopherol (1.00), α-tocopherol (0.94), γ-tocopherol (0.98), δ-tocopherol (0.61), and these were used in the calculations.

For the analysis of the lignans, the oil solutions were injected into two Chrom Spher C 18 HPLC columns (10 cm × 3 mm i.d., particle size 5 μm; Chrompack) connected in series. The mobile phase was micropore-filtered 70% methanol in water at a flow rate of 0.4 mL/min. Peaks were detected at 290 nm with a Lambda-max model 480 ultraviolet (UV) detector (Waters, Milford, MA) and were recorded with an HP 3390 A integrator. Peaks were identified and quantitated by comparison with authentic samples used as external standards.

All lignans were quantitated after reversed-phase separations on this system, except sesamolin in *S. angustifolium*, which was quantitated against sesamolin as an external standard after normal-phase separation under the same conditions used for tocopherol analysis (19). The sesamolin content, thus determined, was subtracted from the sesamolin/sesangolin content to obtain the sesangolin content.

RESULTS AND DISCUSSION

Contents and composition of the unsaponifiable matter. *Sesamum indicum* samples contained 1.4–1.8% unsaponifiables. The wild species had higher unsaponifiable contents—*S. alatum* and 4.8 and 4.9%, *S. radiatum* had 2.5 and 2.7%, and *S. angustifolium* contained 3.6 and 3.7% (Table 1). The unsaponifiable percentages obtained in this study were higher than those in a previous study (16), where single samples of *S. alatum*, *S. radiatum* and *S. angustifolium* showed 4.2, 2.3 and 3.3%, respectively. It cannot be excluded that sample variations, year and place of seed production contributed to these differences. However, the fact that higher unsaponifiable percentages were obtained for all samples suggests that the differences may be due to the difference in extraction methods. The HIP mixture used in the extraction in this study was reported to extract larger amounts of chlorophyll and other pigments than *n*-hexane (21). Extractions followed by UV measurements (absorbances at maxima around *ca.* 288 nm) showed that HIP also extracted higher amounts of the lignan compounds. Thus, the high unsaponifiable percentages in this study are most probably related to high amounts of lignans and other constituents with similar or higher polarity.

Table 1 also shows the percentages of the total sterols in the unsaponifiables and in the oils, as well as the relative proportions of the three sterol classes (des-, mono- and dimethylated sterols). The percentages of total sterols in the unsaponifiables of the four species were: *S. indicum* (34–44%), *S. alatum* (19 and 24%), *S. radiatum* (18 and 22%) and *S. angustifolium* (16 and 16%). They were significantly lower than those obtained previously (16). The composition of each of the three sterol classes is shown in Tables 2–4. Table 5 shows the distribution of the major sterols between the free and esterified forms.

The percentages of the total sterols in the oils (Table 1) were slightly lower, but still comparable to previous results (16). The present oils also contained different levels of total tocopherols (Table 6) than previous samples (22). Differences might be due to differences in samples and/or extraction methods. The present samples contained significantly higher levels of sesamin and sesamolin (Table 7) than did previous samples (22). These high levels of sesamin and sesamolin may be the main reason behind the significantly low sterol percentages in the unsaponifiables of the present samples. While the sterols are the predominating constituents in the unsaponifiable fractions from most vegetable oils (23), the sesamin-type compounds are the major unsaponifiable constituents in the oils from the *Sesamum* species.

Values obtained for the relative proportions of the three sterol fractions in the various oils (Table 1) are comparable to previous findings (16). The different oils of *S. indicum* contained 85–89% desmethyl, 9–11% monomethyl and 2–4% dimethyl sterols. *Sesamum alatum* and *S. angustifolium* were comparable to *S. indicum* but with slightly lower percentages of monomethyl and dimethyl sterols. *Sesamum radiatum* had a significantly higher percentage of monomethyl sterols than did the other three species.

Sterol compositions. Sterols are present in vegetable oils in the free form or as SEs, sterol glucosides or esterified steryl glucosides. The percentage compositions of the total des-, mono- and dimethyl sterols released from all forms by saponification are presented in Tables 2–4. Little

TABLE 1

Content of the Total Unsaponifiables and Proportions of the Different Sterol Classes in the Oils from Four *Sesamum* Species

Sample	Unsaps[a] in oil (%)	Sterols (%)		Sterol fractions (%)		
		In unsaps	In oil	Desmethylate	Monomethylate	Dimethylate
Sesamum indicum						
GAS	1.7	38	0.65	87	10	3
GAB	1.5	34	0.51	85	11	4
HIR	1.8	42	0.76	89	9	2
ABS	1.4	44	0.62	88	10	2
S. alatum						
OB-2	4.9	19	0.93	93	3	4
UR	4.8	24	1.15	87	8	5
S. radiatum						
RDZ	2.5	18	0.45	76	19	5
RDK	2.7	22	0.59	75	20	5
S. angustifolium						
ANG-1	3.6	16	0.58	91	7	2
ANG-2	3.7	16	0.59	90	8	2

[a]Unsaps, unsaponifiables. GAS, GAB, OB-2, etc., are codes based on local names (Ref. 18).

TABLE 2

Composition of the Desmethyl Sterol Fractions of Oils from Four *Sesamum* Species

Species/sample[b]	Desmethyl sterol percentages (%, w/w)[a]							
	Cholesterol (1.81)	Campesterol (2.29)	Stigmasterol (2.44)	Sitosterol (2.78)	Δ^5-Avenasterol (2.88)	Δ^7-Stigmastenol (3.06)	Δ^7-Avenasterol (3.26)	Others[c]
Sesamum indicum								
GAS	0.2	12.5	6.7	61.5	10.8	3.1	0.3	4.9
GAB	0.2	16.5	8.7	62.0	8.1	0.4	0.5	3.6
HIR	0.1	14.8	6.0	61.1	11.5	0.5	0.8	5.2
ABS	0.1	16.9	6.2	57.5	11.3	0.6	1.3	6.1
S. alatum								
OB-2	0.2	18.8	14.0	35.2	23.5	0.1	0.9	7.3
UR	0.2	20.5	14.2	33.9	22.6	0.2	1.1	7.3
S. radiatum								
RDZ	0.2	11.8	5.7	60.2	12.4	2.2	2.3	5.2
RDK	0.3	11.9	4.4	59.6	12.5	3.0	3.7	4.6
S. angustifolium								
ANG-1	0.2	10.3	5.9	55.4	19.4	0.5	1.5	6.8
ANG-2	0.2	10.7	6.3	56.6	19.6	0.5	1.3	4.8

[a]Numbers in parentheses are relative retention times (RRT), to cholestane (Rt 9.84 min).
[b]GAS, GAB, OB-2, etc., are codes based on local names (Ref. 18).
[c]Other unknowns at RRTs 2.26, 2.56, 2.69 and 3.09.

TABLE 3

Composition of the Monomethyl Sterol Fractions of Oils from Four *Sesamum* Species

Species/Sample[b]	Monomethyl sterol percentages (%, w/w)[a]						
	Obtusifoliol (2.72)	Unknown (a) (2.77)	Unknown (b) (2.87)	Gramisterol (3.09)	Cycloeucalenol (3.16)	Citrostadienol (3.98)	Others[b,c]
Sesamum indicum							
GAS	25.2	4.8	5.5	22.6	6.0	28.7	7.2
GAB	21.1	4.4	5.1	23.4	8.1	22.2	15.7
HIR	21.0	4.4	5.2	22.1	7.7	26.3	13.0
ABS	24.6	4.9	6.3	20.3	9.7	20.9	13.3
S. alatum							
OB-2	32.4	5.8	5.4	24.8	3.0	15.9	12.7
UR	37.0	3.8	3.1	27.4	3.4	15.1	10.2
S. radiatum							
RDZ	19.8	1.6	0.8	13.2	11.5	46.9	6.2
RDK	18.9	2.4	1.8	10.3	10.2	47.8	8.6
S. angustifolium							
ANG-1	12.7	7.3	6.1	12.6	7.3	42.3	11.7
ANG-2	14.6	n.d.	3.7	13.9	9.4	46.6	11.8

[a]As in Table 2.
[b]As in Table 2.
[c]Other unknowns, RRTs 2.43, 3.02, 3.40, 3.58, 3.83, 4.22 and 4.43.

attention is generally paid to the proportions of the different types of sterol derivatives in seed oils, but free sterols and steryl esters are often the dominant forms. Steryl glucosides and esterified steryl glucosides are generally neglected in sterol compositional studies, likely due to their insignificant levels in mature seeds.

The major desmethyl sterols in the four species (Table 2) are campesterol ([24*R*]-24-methyl-cholest-5-en-3β-ol), stigmasterol ([24*S*]-24-ethyl-cholest-5,22-dien-3β-ol), sitosterol ([24*R*]-24-ethyl-cholest-5-en-3β-ol) and Δ^5-avenasterol {[24*Z*]-24(28)-Ethylidene-cholest-5-en-3β-ol}. Δ^7-Stigmastenol ([24*R*]-24-ethyl-5α-cholest-7-en-3β-ol) and Δ^7-avenasterol {[24*Z*]-24(28)-ethylidene-5α-cholest-7-en-3β-ol} were also present in small proportions. The wild species displayed a significantly different desmethyl sterol composition than *S. indicum*. Results were comparable to previous findings, with *S. radiatum* as the species most comparable to *S. indicum* in this respect. *Sesamum alatum* and *S. angustifolium* were both characterized by considerably higher percentages of Δ^5-avenasterol than the other two species. In *S. angustifolium*, this was at a slight expense of the percentages of sitosterol and campesterol. *Sesamum alatum*, on the other hand, had a markedly lower percentage of sitosterol and a markedly higher percentage of stigmasterol than the other three species.

The composition of the 4-monomethyl sterol fraction (Table 3) of the four species is also fairly comparable to previous results on single samples (16). Obtusifoliol (4α,14α-dimethyl-24-methylene-5α-cholest-8-en-3β-ol), gramisterol (4α-methyl-24-methylene-5α-cholest-7-en-3β-ol), cycloeucalenol (4α,14α,dimethyl-9β,19-cyclo-24-methylene-5α-cholestan-3β-ol) and citrostadienol

TABLE 4

Composition of the Dimethyl Sterol Fractions of Oils from Four *Sesamum* Species

	Dimethyl sterol percentages (%, w/w)[a]						
Sterol/sample[b]	β-amyrin (2.73)	Δ^8-sterol (2.87)	α-Amyrin (3.00)	Cycloartenol (3.16)	Δ^7-Sterol (3.42)	24-Methylenecycloartanol (3.70)	Others[c]
Sesamum indicum							
GAS	4.8	2.0	6.5	49.8	4.8	26.6	5.5
GAB	6.2	3.4	6.9	42.3	6.0	30.9	4.3
HIR	6.1	3.0	6.9	41.4	7.4	33.7	1.5
ABS	7.3	3.8	5.3	43.4	5.9	31.9	2.4
S. alatum							
OB-2	14.3	10.7	3.5	19.9	1.3	30.9	19.4
UR	11.7	8.0	2.8	25.8	2.9	23.3	25.5
S. radiatum							
RDZ	4.5	2.0	4.1	51.6	2.8	32.9	2.1
RDK	3.8	2.3	3.9	50.3	2.9	32.8	4.0
S. angustifolium							
ANG-1	4.2	8.7	5.1	45.8	6.6	28.0	1.6
ANG-2	7.1	8.4	4.5	42.5	5.9	27.1	4.5

[a]As in Table 2.
[b]As in Table 2.
[c]Other unknowns at RRT, 2.10, 2.28, 2.41, 3.02, 3.25, 3.59 and 3.75.

(4α-methyl-(24*Z*)-24-ethylidene-5α-cholest-7-en-3β-ol) predominated over many minor unknowns. *Sesamum indicum* contained 21.0–25.2% obtusifoliol, 20.3–23.4% gramisterol, 6.0–9.7% cycloeucalenol, 20.9–28.7% citrostadienol and 7.2–15.7% of some unknowns. The wild species were widely different from *S. indicum* in their monomethyl sterol composition. *Sesamum alatum* had a higher obtusifoliol content and a much lower citrostandienol content than did the *S. indicum* samples. The other two wild species, *S. radiatum* and *S. angustifolium*, were rather similar in the composition of their monomethyl sterols and contained lower percentages of obtusifoliol and gramisterol, but significantly higher percentages of citrostandienol than did *S. indicum*. The higher levels of citrostandienol and the other antipolymeriation sterols (Δ^5- and Δ^7-avenasterols) in the wild species, compared to *S. indicum*, are of great interest as they may add to the stability of the oils (13).

Cycloartenol (4,4,14-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol) and 24-methylene cycloartanol (4,4,14-trimethyl-24-methylene-9β, 19-cyclo-5α-cholestan-3β-ol) were the two major dimethyl sterols in the composition of the 4,4-dimethyl sterol fractions of the oils from the wild species and *S. indicum* varieties (Table 4). Other dimethyl sterols identified or partially identified by GC/MS were α-amyrin (5-olean-12-en-3β-ol), β-amyrin (5-urs-12-en-3β-ol), an unknown Δ^8-sterol [relative retention time (RRT) 2.87], and an unknown Δ^7-sterol (RRT 3.42). The present results for the composition of the dimethyl sterols in all species showed slight variations, but are still comparable to previous results (16). In this study, *S. indicum* contained 4.8–7.3% β-amyrin, 5.3–6.9% α-amyrin, 41.4–49.8% cycloartenol and 26.6–33.7% 24-methylene cycloartenol. *Sesamum radiatum* and *S. angustifolium* were comparable and related to *S. indicum* with regard to the dimethyl sterol composition. *Sesamum alatum*, on the other hand, contained a lower percentage of cycloartenol than the other three species. Variations between the different collections of each species may be due to a single factor or a combination of many factors, mainly differences in genotype, geographical location, soil, agronomic conditions and levels of maturity. *Sesamum radiatum* and *S. angustifolium* were the most comparable in detailed fatty acid compositions (14,18) and in sterol patterns, suggesting that these two species are rather closely related.

Table 5 presents the composition of the major total sterols and of the free sterol and steryl ester separated by TLC. The major (des-, mono- and di) methyl sterols in the four species were campesterol, stigmasterol, obtusifoliol, sitosterol, Δ^5-avenasterol, gramisterol, Δ^7-stigmastenol, Δ^7-avenastenol, 24-methylene cycloartanol and citrostadienol. Considerable differences were observed in the relative levels of these sterols in the steryl ester fraction, compared to the free sterol fraction, as specified below. The four species again showed a similar trend in the variations between the free and esterified sterols.

Although no biosynthetic studies on sesame seed sterols seem to have been published, a general biosynthetic pathway comparable to that for sterols in other oil seeds (24) can be assumed for *Sesamum* species. Based on this assumption, the following observations can be made. In general, the steryl ester fractions showed higher percentages of the "early" sterols in the biosynthetic pathway (the methyl sterols, the Δ^7 sterols and campesterol) compared to the free sterol fractions that were generally characterized by higher percentages of the "late" sterols (notably sitosterol and stigmasterol). These results are in line with those of Johansson (25) who previously found that the FS fractions of sunflower and poppy seed oils contained higher percentages of sitosterol and stigmasterol and lower percentages of campesterol than did the sterol ester fractions. Δ^5-Avenasterol showed the least relative differences between the two fraction. This sterol showed a higher percentage in the steryl ester fraction than in the free sterol fraction in *S. indicum* in opposition to the relative distribution between the two fractions in the three wild species. These observations may be a good basis for comparative and biosynthetic studies on free and esterified sterols in developing sesame seeds.

Direct GC analysis of the TMS ether derivatives of the

TABLE 5

Composition of the Major Free, Esterified and Total Sterols of Oils from Four *Sesamum* Species

Species (sample)	Sterol RRT[b]	Sterol percentages (w/w)[a]									
		Campesterol 2.29	Stigmasterol 2.44	Obtusifoliol 2.72	Sitosterol 2.78	Δ^5Avenasterol 2.88	Gramisterol 3.09	Δ^7Stigmastenol 3.06	Δ^7Avenasterol 3.26	24-Methylenecycloartanol 3.70	Citrostadienol 3.98	Others[c]
Sesamum indicum[d]	FS (65)	12.3	9.0	n.d.	68.3	6.4	n.d.	1.3	0.9	n.d.	n.d.	2.3
GAS	SE (35)	17.5	4.5	3.2	43.1	12.3	4.8	5.2	2.6	0.3	4.2	2.3
	TS (100)	13.7	7.3	0.2	59.6	8.4	3.3	2.6	1.2	n.d.	2.2	1.5
S. alatum	FS (79)	13.9	16.4	3.0	32.6	20.7	2.6	1.4	1.9	1.1	1.1	5.3
OB-2	SE (21)	19.8	6.2	6.3	25.1	15.7	1.3	2.4	3.3	12.5	0.7	6.7
	TS (100)	16.6	13.9	4.8	26.1	21.4	2.7	1.8	1.7	4.2	0.6	6.2
S. radiatum	FS (71)	7.1	5.0	1.5	54.9	10.1	1.3	6.8	3.1	n.d.	6.6	3.6
RDK	SE (29)	17.0	3.7	2.3	49.9	8.1	0.6	2.1	3.7	n.d.	3.0	9.6
	TS (100)	9.5	4.3	2.1	50.5	11.4	2.7	6.6	3.1	0.2	6.7	2.9
S. angustifolium	FS (69)	7.1	6.5	n.d.	56.8	20.1	0.9	0.9	1.2	n.d.	3.4	3.1
ANG-2	SE (31)	14.4	3.7	0.6	54.9	15.0	2.6	2.7	1.1	trace	2.2	2.8
	TS (100)	10.1	5.9	1.0	56.2	18.5	0.9	1.2	0.5	0.1	3.3	2.6

[a]Abbreviations: n.d., not detected, trace amount, <0.1%; FS, free sterols; SE, sterol esters; TS, total sterols; the percentage of each fraction is indicated in parentheses.
[b]RRT, relative retention times, to cholestane (Rt 9.84 min).
[c]Mostly cycloeucalenol + cycloartenol (RRT 3.16).
[d]GAS, OB-2, RDK and ANG-2 are codes based on local names.

TABLE 6

Tocopherol Contents and Composition of the Oils from Four *Sesamum* Species

Species/sample	Total tocopherols[a] (mg/kg oil) (RRT[b])	Tocopherol (T) composition (%, m/m)		
		α-T (0.74)	γ-T (1.22)	δ-T (1.84)
Sesamum indicum[c]				
GAS	540	n.d.	97.6	2.4
GAB	620	0.5	97.4	2.1
HIR	680	0.7	97.6	1.7
ABS	490	1.4	97.0	1.6
S. alatum				
OB-2	320	0.9	96.9	2.2
UR	210	2.5	97.3	0.2
S. radiatum				
RDZ	810	0.8	98.8	0.4
RDK	800	0.8	97.0	2.2
S. angustifolium				
ANG-1	760	n.d.	99.3	0.7
ANG-2	730	n.d.	98.8	1.2

[a]Total tocopherols calculated against β-tocopherol used as internal standard (see Materials and Methods section).
[b]Numbers in parentheses are RRT, relative retention times, to β-tocopherol (Rt 7.47 min).
[c]GAS, GAB, OB-2, etc., are codes based on local names, n.d., Not determined.

total unsaponifiables on HP-1 gave a satisfactory separation of the major sterols and the lignan compounds in *S. indicum* oils. This direct analysis has many benefits by showing the relative percentages of the major methyl sterols to the major desmethyl sterols. It is simple and avoids losses due to preparative TLC steps. In this direct analysis, sesangolin {2-(3,4-methylene dioxy phenyl)-6-(3,4-methylenedioxy-6-methoxy phenyl)-*cis*-3,7-dioxabicyclo-[3.3.0]octane} coeluted with sitosterol in *S. angustifolium* and 2-episesalatin {2-epi-(3,4,5-trimethoxy phenyl)-6-(3,4-methylene dioxy-5-methoxy phenyl)-*cis*-3,7-dioxabicyclo-[3.3.0]octane} coeluted with cycloeucalenol and cycloartenol (RRT 3.16) in *S. alatum*. To obtain the percentages of these sterols in the composition of total sterols (Table 5), calculations were made in relation to other sterols. Thus, the percentage of sitosterol in *S. angustifolium* was calculated from its relative percentage to campesterol (Table 2), and the percentages of cycloeucalenol and cycloartenol in *S. alatum* were calculated from their relative percentages to citrostandienol (Table 3) and 24-methylene cycloartanol (Table 4), respectively.

Tocopherol content and composition. All four species were comparable in their tocopherol composition (Table 6). The major tocopherol isomer in all species was γ-tocopherol (97–99%), which is in good agreement with literature data for *S. indicum* (6–8). γ-Tocopherol is a more potent antioxidant in oils (26), but it has lower vitamin E value in biological systems (27,28) than α-tocopherol.

The contents of the total tocopherols in *S. indicum* oils analyzed in this study were slightly higher than literature data (4–6). Beringer and Dompert (4) obtained 450 mg total tocopherols per kg of sesame oil (*S. indicum*), 99% was γ-tocopherol and 1% was γ-tocopherol. Coors and Montag (5) found 440–550 mg total tocopherols per kg in sesame oils from different sesame products (<1%

TABLE 7

Levels of Sesamin and Related Lignans in the Oils from Four *Sesamum* Species[a]

Species/sample	Lignan levels (% in oil)[b] 2-Episesalatin (0.66)	Sesamin (1.00)	Sesangolin (1.22)	Sesamolin (1.32)
Sesamum indicum				
GAS	n.d.	0.45	n.d.	0.54
GAB	n.d.	0.72	n.d.	0.41
HIR	n.d.	0.46	n.d.	0.66
PLM	n.d.	0.72	n.d.	0.50
YRO	n.d.	0.71	n.d.	0.51
BAB	n.d.	0.56	n.d.	0.47
ABS	n.d.	0.23	n.d.	0.39
Mean	—	0.55	—	0.50
SD	—	±0.18	—	±0.09
CV%	—	33.5	—	18.1
S. alatum				
OB-1	1.66	0.01	n.d.	0.01
OB-2	1.23	0.01	n.d.	0.02
UR	1.22	trace	n.d.	0.01
Mean	1.37	0.01	—	0.01
SD	±0.25	—	—	—
CV%	18.3	—	—	—
S. radiatum				
RDZ	n.d.	2.31	n.d.	0.02
RGD	n.d.	2.35	n.d.	0.02
RDK	n.d.	2.55	n.d.	0.02
Mean	—	2.40	—	0.02
SD	—	±0.13	—	—
CV%	—	5.4	—	—
S. angustifolium				
ANG-1	n.d.	0.28	2.92	0.17
ANG-2	n.d.	0.35	3.37	0.15
Mean	—	0.32	3.15	0.16
SD	—	±0.05	±0.32	±0.01
CV%	—	15.5	10.1	6.3

[a]GAS, GAB, OB-2, etc., are codes based on local names. SD, standard deviation, n.d., not detected; trace, trace amount, CV, coefficient of variation.
[b]Numbers in parentheses are relative retention times.

α-tocopherol, 97–98% γ-tocopherol and 2% δ-tocopherol). Speek *et al.* (6) found 510–550 mg/kg total tocopherols, which are reported as vitamin E in their paper. The composition of these tocopherols was 95.5–97.8% γ-tocopherol, 2.2–3.1% α-tocopherol and <0.5% of each of β- and γ-tocopherols.

In this study we analyzed fresh oils obtained from seeds stored at −20°C. The total tocopherol levels were higher in the oils from *S. indicum* (490–680 mg/kg oil), *S. radiatum* (800 and 810 mg/kg oil) and *S. angustifolium* (730 and 760 mg/kg oil), but lower in those from *S. alatum* (210 and 320 mg/kg oil) than in a previous study (22), where we obtained 420, 540, 540 and 510 (mg/kg oil) in the seed oils of the four species, respectively. Variations in tocopherol levels could be due to differences in genotype, maturity level and environmental temperature during seed development. Other factors, such as seed and oil storage and processing, are also known to affect tocopherol levels in vegetable oils (29).

Lignan contents. Table 7 demonstrates the identity and levels (% in oil) of sesamin and its related lignans in the oils from the four species as obtained by direct HPLC analysis of the oil solutions. The qualitative analysis of these lignans is reported in an accompanying paper (19), where risks for misinterpretation of sesangolin for sesamolin are discussed.

A wide range of variation in the levels of sesamin and sesamolin was observed in the *S. indicum* "cultivars" analyzed and also by previous investigators (5,30,31). In this study, sesamin ranged between 0.23 and 0.72%, and sesamolin ranged between 0.39 and 0.66%. Ranges previously reported for sesamin were 0.53–0.62% (5), 0.07–0.61% (30) and 0.16–0.89% (31). Sesamolin, on the other hand, had been reported at the following ranges: 0.28–0.36% (5) 0.02–0.48% (30) and 0.12–0.48% (31). Beroza and Kinman (32) studied the effects of strain and location on the levels of sesamin and sesamolin in *S. indicum* seed oils. In 33 strains, sesamin ranged from 0.34 to 1.13 (mean 0.70, SD ± 0.18%), and sesamolin ranged between 0.14 and 0.59% (mean 0.41, SD ± 0.08%). Analysis of variance showed that the sesamin levels [coefficient of variation (CV) = 26.3%] were more subject to variation than were the sesamolin levels (CV = 19.4%) (32). Their data indicated that sesamin and sesamolin levels were not significantly affected by location. These findings are in good agreement with our calculations of variance (Table 7).

Compared to *S. indicum*, the wild species varied widely in the types and levels of the lignans. *Sesamum alatum*

showed low sesamin (0.01%) and sesamolin (0.02%) contents but was characterized by a high content (1.22–1.66%) of the recently isolated 2-episesalatin (17). *Sesamum radiatum* was rich in sesamin (2.40%) and contained minor amounts of sesamolin (0.02%). *Sesamum angustifolium*, on the other hand, was characterized by its high concentrations of sesangolin (3.2%) and also contained considerable amounts of sesamin (0.32%) and sesamolin (0.16%). Analysis of variance of 2-episesalatin in three *S. alatum* samples collected at different locations showed a coefficient of variation of 18.3%, and the data for sesangolin in *S. angustifolium* gave a CV of 10.1%.

Whereas no interspecific variations were noted between the four species in fatty acid composition (18), significant variations in the percentage composition of the different sterols and in the levels, but not the percentage composition, of the tocopherols were found. Significant intraspecific variations in the levels of sesamin and sesamolin occurred among the different cultivars of *S. indicum* and large interspecific differences in the identity, and levels of the different lignans were observed among the different *Sesamum* species. Variations in lignan composition suggest that the biosynthesis of these compounds in the seeds is specifically controlled. The identity and levels of the lignans present in the wild *Sesamum* species are important factors to be determined in future genetical, phytochemical or taxonomic studies on the other "unexplored" *Sesamum* species.

Stability considerations. The induction periods of the HIP-extracted oils from the seeds of the four species, as measured in Rancimat equipment at 110°C under normal daylight conditions, were: *S. alatum*, 1.7 h; *S. radiatum*, 4.0 h; *S. angustifolium*, 10.2 h; and *S. indicum*, 13.4 h (unpublished observations). Obviously, oils from the seeds of *S. alatum* and *S. radiatum* were quite unstable compared to the oils from *S. indicum* and *S. angustifolium*. The order of stability seemed related to the chlorophyll contents of the oils (unpublished observations). However, the characteristic that *S. indicum* and *S. angustifolium* oils contain considerable amounts of sesamolin (Table 7), possibly acting as an antioxidant precursor under the Rancimat conditions at 110°C, might also be interesting. Extended work is needed to study the antioxidant properties of sesame oil and the significance of its different minor constituents.

As sesamolin is a precursor to two phenolic antioxidants, sesamol and sesaminol, during refining (9), and also generates sesamol during frying (11), the presence of high levels of this compound in sesame oil may be highly desirable. Structurally, sesamin, sesangolin and 2-episesalatin seem to have no antioxidant effect because they lack any phenolic function. However, if they are metabolized in the body to compounds with one or more phenolic groups, they could serve as antioxidants *in vivo*. Intensive research is also needed to understand the exact role of sesamolin and its derived phenolic antioxidants in the stabilization of sesame oil and to assess the health aspects of sesamin, sesamolin and other related lignans in *Sesamum* oils. Screening of the world collection of *S. indicum* seeds may be necessary to obtain strains with high sesamolin and, possibly, low sesamin levels.

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