Lignans and Tocopherols in Indian Sesame Cultivars

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ABSTRACT: Lignan (sesamol, sesamin, and sesamolin) profile was determined in different cultivars (botanically identified or market samples) of sesame seeds and commercial oils procured from different parts of India. The wide variation observed in total lignans from 21 sesame seed and 9 commercial oils was attributed to variations in sesamin and sesamolin contents. Lignan content was high (18 g sesamin/kg, 10 g sesamolin/kg) in sesame cultivars obtained from the northeastern states of India. In two of the commercial oils having the Agmark label, the total lignan content was ~12 g/kg (7.3 g sesamin, 4.7 g sesamolin), 50% of the maximum permissible levels of unsaponifiable matter. In both the seeds and commercial oils, γ-tocopherol was the only representative of tocopherol isomers identified. Sesamin and sesamolin were isolated and crystallized from high-lignan cultivars, and their purity was confirmed by HPLC and spectral (UV and fluorescence) analysis.

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KEY WORDS: Isolation and crytallization, sesame lignans, sesamin, sesamolin, γ-tocopherol.

The nonglyceride fraction of oilseeds contains a variety of biologically active components (1) including hydrocarbons, tocopherols, sterols, and triterpene alcohols. In addition, some oils have unique components: oryzanol and tocotrienols in rice bran oil, tocotrienols and carotenes in palm oil/red palm oil, and lignans (such as sesamol, sesamin, and sesamolin) in sesame oil. The role of tocopherols and tocotrienols as antioxidants is well established (2). Studies in experimental animals (3–6) have shown hypocholesterolemic effects of sterols, triterpene alcohols, oryzanol, and tocotrienols.

The high resistance of sesame oil to oxidative deterioration compared with other vegetable oils has been attributed to the presence of lignans. Sesame lignans also have antioxidant, hypocholesterolemic, and immunomodulatory effects (7–9). Sesame oil has been widely used as a domestic Ayurvedic remedy in India (10). Although sesame seeds and oils are used extensively in India, the lignan profile in Indian sesame cultivars has not been reported before. The present study was done (i) to determine the lignan profile in Indian sesame cultivars and (ii) to isolate and crystallize sesamin and sesamolin from high-lignan oils for evaluation of their biological activities.

MATERIALS AND METHODS

Materials. Sesamol and tocopherols (α, γ, δ) were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol, acetonitrile, *n*-hexane, and dichloromethane (HPLC grade) were procured from Qualigens India Ltd. (Mumbai, India). All other chemicals and reagents used were of analytical grade.

Sesame seeds/oils. Sesame seeds (market samples or botanically identified samples) and commercial oils were procured from different states of India. Oil was extracted with ether from 5 g of seeds using a Soxhlet apparatus. To the oil obtained was added 0.5 g of sodium sulfate to remove moisture, and ether was removed by using a rotary evaporator.

HPLC analysis of lignans and tocols. Lignan and tocol profiles in oils were determined by direct oil injections (1:100, wt/vol, oil/*n*-hexane for lignan profile; 1:20, wt/vol, oil/*n*-hexane for tocol profile) using a Shimadzu LC-10A reversed-phase HPLC system (Kyoto, Japan) equipped with a Shodex C18 column (4.6 mm i.d × 250 mm), connected to a manual valve injector and a Shimadzu SPD-10A UV-vis detector. The peaks were recorded using a Shimadzu C-R6A Chromatopac data processor. The mobile phase for lignan profile was methanol/water (75:25) at a flow rate of 0.8 mL/min (11) and acetonitrile/methanol/dichloromethane (60:35:5) for tocol profile at a flow rate of 2 mL/min (12). Peaks were identified and quantified by comparison with authentic lignans (sesamol procured from Sigma Chemical; sesamin and sesamolin isolated and crystallized in our laboratory) and tocols (α-, γ-, δ-tocopherol; α-tocotrienol) used as external standards. Sesamol and α-tocopherol were used as internal standards for calculating the percentage recovery of lignans and tocols.

Isolation and crystallization of sesamin and sesamolin. Sesamin and sesamolin were isolated and crystallized from Indian sesame cultivars having high lignan content according to the method of Budowski (13). Briefly, sesame oil (500 g) was dissolved in acetone (1:8, vol/vol) and cooled overnight at –70°C. The glyceride crystals were separated by filtration while the mixture was kept at –20°C. Removal of acetone from the filtrate gave a yellow oil that, when mixed with iso-octane and left at 4°C for 4–5 d, yielded sesamin crystals. The filtrate after evaporation of iso-octane was saponified with ethanolic potassium hydroxide, and the mixture diluted with water and extracted with ether. The filtrate after removal of ether was dissolved in chloroform and petroleum ether (b.p. 80–100°C). Upon cooling at 5°C, sesamolin crystallized from the solution. Sesamin and sesamolin were recrystallized in ethanol, and their physicochemical properties were determined by the HPLC elution pattern, on-line HPLC spectral characteristics using a stop-flow method, and absorption and emission characteristics in iso-octane and ethanol by spectrophotometry and spectrofluorimetry.

RESULTS AND DISCUSSION

The quantitative analysis of lignan and tocol profiles from 21 seed and 9 commercial oil samples is presented in Tables 1–3.

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Recoveries of lignans and tocols by the HPLC method was greater than 95%. The elution profile of sesame lignans in sesame oil to which sesamol (Sigma Chemical) was added is shown in Figure 1. Since a wide variation was observed in the total lignan content in seeds and in commercial oils, they were classified into low-, medium- and high-lignan oils (Table 3). The wide variations in the total lignans were due to variations in sesamin and sesamolin contents and resemble earlier reports (14–17). Sesamol was detected in neither seeds nor commercial oils. Sesamin and sesamolin contents were similar in seeds and commercial oils.

Out of the 21 seed samples, the oil extracted from two cultivars from the Indian State of Assam had high sesamin and sesamolin contents (Tables 1 and 3). Although a high sesamin content has been reported in *Sesamum radiatum* (16), the high levels of sesamolin observed here have not been reported elsewhere. According to the Prevention of Food Adulteration (PFA) Act 1954 (18), the maximum permissible level of unsaponfiable matter in oils is 10–15 g/kg oil, except for rice bran (<35 g/kg) and sesame (<25 g/kg) oils. Since sesame seeds grown in the northeastern states of India have high levels of unsaponifiable matter, sesame oil having high levels of unsaponifiable matter (15–25 g/kg) is permitted for human consumption only under the Agmark regulatory label (in accordance with the grade standards prescribed under the provisions of the Act). The data on lignan levels in two Agmark-labeled oils showed that about 50% of the maximum permissible levels of the unsaponifiable matter is contributed by lignans.

TABLE 1
Lignan Profile[a] in Sesame Seed Samples

Sample number	Location	Variety	Sesamin	Sesamolin	Total lignans
				g/kg oil	
1	Gujarat	White	6.6	4.5	11.1
2	Gujarat	Brown	7.6	8.2	15.8
3	Assam	Black	4.9	5.8	10.7
4	West Bengal	White	6.5	5.3	11.8
5	Bengal	Dehusked	8.1	4.8	12.9
6	Andhra Pradesh	Black	6.4	6.2	12.8
7	Assam	Black	7.5	7.7	15.3
8	Gujarat	Black	3.1	3.2	6.2
9	Andhra Pradesh	White	5.7	3.6	9.3
10	Orissa	White	3.6	2.3	5.9
11	Assam	White	18.6	10.0	28.6
12	Assam	Black	18.5	10.6	29.1
13	Phuletil[b]	White	7.0	3.7	10.7
14	Krishna[b]	Black	4.3	6.3	10.6
15	CO-1[b]	Black	7.5	6.2	13.7
16	Madhavi[b]	Brown	5.8	5.4	11.2
17	Rama[b]	Black	6.6	6.1	12.7
18	Gowri[b]	Black	5.0	4.0	9.5
19	TC-25[b]	White	3.6	2.3	5.9
20	T-85[b]	White	5.6	3.5	9.1
21	Rajeswari[b]	White	3.9	3.1	7.0

[a] The values are the average of at least two independent determinations. The difference between duplicates was 2–8%. Sesamol was not detected in any of the samples.
[b] Botanically identified sesame seeds procured from the Directorate of Oil Research (DOR), Hyderabad.

TABLE 2
Lignan Profile in Commercial Sesame Oils

Sample number	Oil[a]	Sesamin	Sesamolin	Total lignans
			g/kg	
1	Vanraj	6.1	5.2	11.3
2	Virgin sesame oil	6.5	4.4	10.9
3	RMP	7.0	4.0	11.0
4	Anandam	7.85	5.3	13.1
5	AS brand[b]	9.0	5.1	14.2
6	Mansion[b]	8.4	4.8	13.2
7	Idhayam	6.3	3.9	10.2
8	Tilola	3.0	3.5	6.5
9	Tilsona	4.7	4.2	8.9

[a] Oils available in the Indian market.
[b] Agmark-labeled oils.

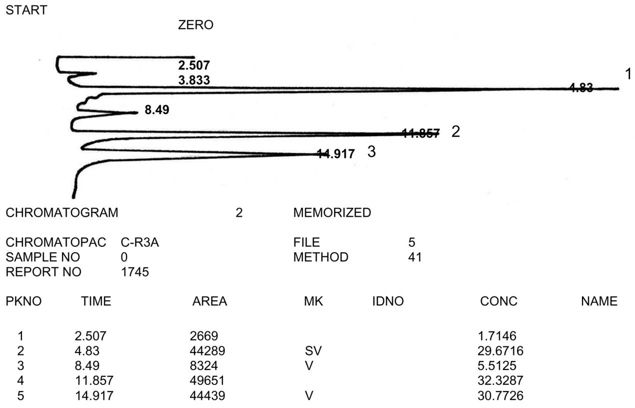
TABLE 3
Lignan Profiles in Sesame Seeds/Commercial Oils

	n^a	Sesamin	Sesamolin	Total lignans	γ-Tocopherol
		g/kg			
Sesame seeds					
Low lignans (<10 g/kg)[b]	7	4.4 ± 0.4	3.1 ± 0.24	7.6 ± 0.63	0.5 ± 0.01
Range		3–6	2–5	6–10	0.3–0.7
Medium lignans (10–20 g/kg)[b]	12	6.5 ± 0.32	5.8 ± 0.36	12.4 ± 0.5	0.5 ± 0.10
Range		4–8	4–8	10–16	0.3–0.7
High lignans (>20 g/kg)[b]	2	18.5 ± 0.05	10.3 ± 0.30	28.8 ± 0.21	0.19 ± 0.02
Commercial oils					
Low lignans (<10 g/kg)[b]	2	3.9 ± 0.85	3.9 ± 0.35	7.7 ± 1.2	0.8 ± 0.10
Range		3–5	3–4	6–9	0.7–0.9
Medium lignans (10–20 g/kg)[b]	7	7.3 ± 0.43	4.7 ± 0.22	12.0 ± 0.56	0.6 ± 0.05
Range		6–9	4–5	10–14	0.4–0.8

[a] Number of varieties analyzed in each group. Values are means ± SE.
[b] Classification as low, medium, and high lignans is based on the values indicated in parentheses as g/kg oil.

The sensitivity of the method used here for developing tocol profiles detects 10 ng (1×10^{-8} g/kg oil). Since γ-tocopherol was the only isomer detected in sesame seeds/commercial oils (Table 3), the level of other isomers could be less than 1×10^{-8} g/kg oil. Shahidi *et al.* (19) also reported the presence of only γ-tocopherol in sesame oil. However, other authors have found that γ-tocopherols contribute ~98% of total tocopherols (20,21) and the remaining 2% is α and δ isomers. Wide variations were observed in γ-tocopherol levels in seeds and commercial oils (Table 3). The variation in γ-tocopherol content observed in this study is higher than that reported by Kamal-Eldin and Appelqvist (16). That the two high-lignan oils have lower γ-tocopherol levels than the other oils is noteworthy (Table 3).

We isolated and crystallized sesame lignans from high-lignan sesame seeds. The yields of sesamin and sesamolin from 500 g sesame oil, which contained 9 g sesamin and 5 g sesamolin, was 5 and 0.5 g, respectively. These yields were comparable to those reported by Haslam and Haworth (22). In an RP-HPLC analysis, sesamol (Sigma Chemical) and sesamin (crystallized in our laboratory) eluted as single peaks, whereas sesamolin (crystallized) showed two peaks, one corresponding to sesamolin (93%) and the other to sesamin (7%). The identity of these peaks was confirmed by HPLC on-line spectral characteristics.



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START
                    ZERO

CHROMATOGRAM                2        MEMORIZED

CHROMATOPAC   C-R3A                  FILE          5
SAMPLE NO     0                      METHOD        41
REPORT NO     1745

PKNO     TIME          AREA        MK      IDNO        CONC         NAME

  1      2.507         2669                            1.7146
  2      4.83          44289       SV                  29.6716
  3      8.49          8324        V                   5.5125
  4      11.857        49651                           32.3287
  5      14.917        44439       V                   30.7726
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FIG. 1. Elution profile of lignans from sesame oil with added sesamol (from Sigma Chemical Co., St. Louis, MO) on RP-HPLC. (1) Sesamol; (2) sesamin; (3) sesamolin.

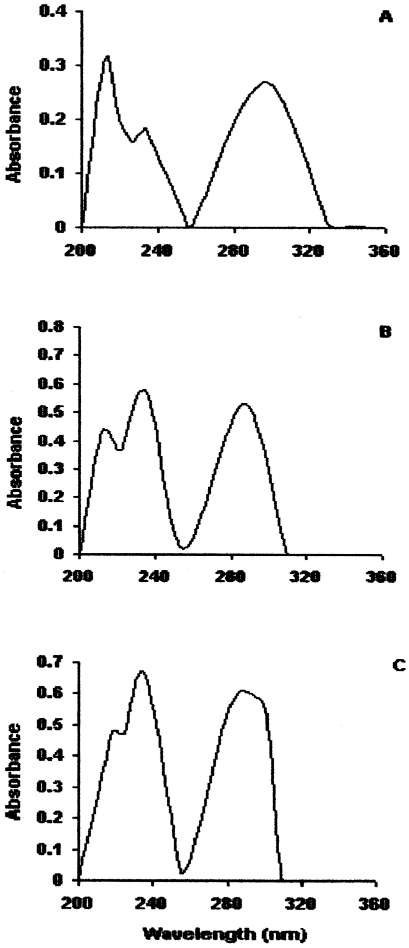

FIG. 2. UV absorption spectra of sesame lignans in isooctane. (A) Sesamol (from Sigma Chemical Co., St. Louis, MO), (B) sesamin, and (C) sesamolin (isolated and crystallized in our laboratory).

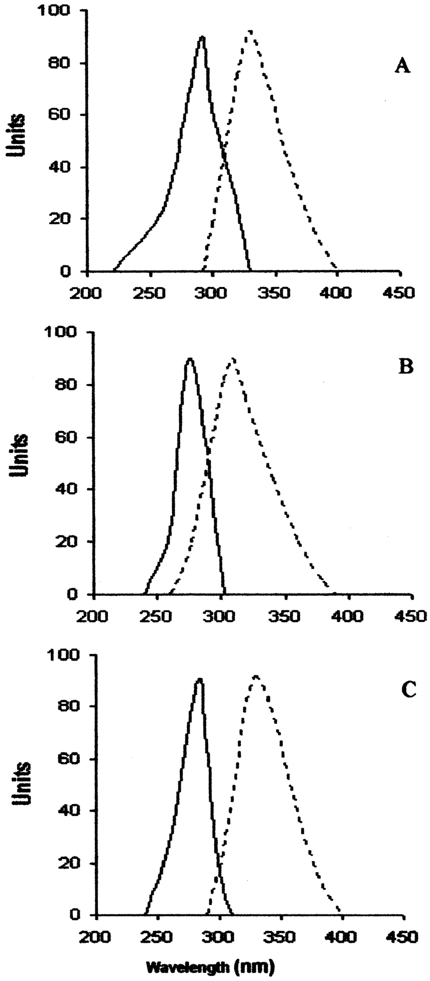

FIG. 3. Fluorescence spectra of sesame lignans in ethanol. (A) Sesamol (from Sigma Chemical Co., St. Louis, MO), (B) sesamin, and (C) sesamolin (isolated and crystallized in our laboratory). Solid line represents excitation spectra; dashed line represents emission spectra.

The UV absorption spectra of sesamin, sesamolin (isolated crystals), and sesamol (Sigma Chemical Co.) (presented in Fig. 2) are identical to published data (23). All three lignans (sesamol, sesamin, and sesamolin) exhibited weak fluorescence in the UV region (Fig. 3). The fluorescence spectrum of sesamol is similar to published data (24), but those of sesamin and sesamolin have not been reported previously.

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