SHORT COMMUNICATION

Tocopherol and Fatty Acid Composition of Some Indian Pulses

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ABSTRACT: The tocopherol and tocotrienol composition of the Indian pulses Bengal gram (*Cicer arietinum* L.), blackgram (*Vigna mungo* L.), green gram (*Vigna radiata* L.), and horse gram (*Dolichos biflorus* L.) were studied. The total tocopherol content ranged from 230 to 1567 mg/100 g fat, while the tocopherol content of the pulses as a whole ranged from 6.76 to 12.54 mg/100 g seed. Presence of such a high amount of tocopherol, both in the oil fraction of Indian pulses and in an oil fraction of a food material, is being reported for the first time. The fatty acid composition of the fat extracted from these pulses showed substantial amounts of unsaturated fatty acids (Bengal gram, 88.7%; black gram, 82.9%; green gram, 64.3%; and horse gram, 66.9%). These pulses contained 3.8 to 49.1% linolenic acid in the fat.

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KEY WORDS: Bengal gram, black gram, fatty acids, green gram, horse gram, Indian pulses, legumes, tocopherols, tocotrienols.

Indian pulses were examined as potential sources of natural tocopherols and tocotrienols. The tocopherol content of some foods is known (1–4). Depletion of tocopherols in the diet may have harmful biological effects (5). The antioxidant properties of tocotrienols are equal to or better than those of tocopherols and may have biologically important properties, such as inhibition of cholesterol biosynthesis (6–8). Per capita consumption of legumes in the form of pulses accounts for 40% of all food grains consumed in India (9). Therefore, tocopherols and fatty acid composition of Indian pulses are reported in this paper.

EXPERIMENTAL PROCEDURES

Materials. Bengal gram (*Cicer arietinum* L.), black gram (*Vigna mungo* L.), green gram (*Vigna radiata* L.), and horse gram (*Dolichos biflorus* L.) (local varieties and whole gram) were purchased from the market of Mysore City, India. Standard α-, β-, γ-, and Δ-tocopherols were obtained from Merck (Darmstadt, Germany).

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Methods. Moisture and fat contents of the samples were determined by AOCS methods (10). Fats from finely ground materials were extracted with petroleum ether (40–60°C) for 4 h, followed by removal of solvent under reduced pressure in a rotary flash evaporator. Tocopherols were determined by high-performance liquid chromatography (HPLC) with the procedure of Balz *et al.* (2). The HPLC system, manufactured by Merck Hitachi (Darmstadt, Germany), consisted of pump L-6000; F-1000 fluorescence spectrophotometer set to excitation wavelength 295 nm and emission wavelength 330 nm; D-2000 integrator; Lichrospher 100 Diol precolumn with particle size 5 μm, length 4 mm, and internal diameter 4 mm; Lichrospher 100 Diol column with particle size 5 μm, length 250 mm, and internal diameter 4 mm; Rheodyne 7125 injector; and a 20-μL sample loop. The system was operated at 21 ± 1°C, with mobile phase *n*-hexane/*t*-butyl methyl ether 94.4:5.6, vol/vol, at a flow rate of 1.2 mL/min. The sample size ranged from 5 to 20 μL of extracted fat in hexane (0.1 to 5.0% solutions). A calibration curve was prepared by injecting known concentrations of standard α-, β-, γ-, and Δ-tocopherols. The concentrations of tocopherols and tocotrienols in the samples were calculated from equations derived from individual tocopherol standards.

Fatty acid analysis. The fatty acid compositions of the oils extracted from Bengal gram, black gram, green gram, and horse gram were determined by capillary column gas chromatography. A Perkin-Elmer F22 gas chromatograph (Bodenseewerk, Perkin Elmer and Co., GmbH, Friedrichshafen, Germany), equipped with a flame-ionization detector and a BPX-70 capillary column (SGE, Weiterstadt, Germany; length 60 m, internal diameter 0.22 mm, film thickness 0.25 μm), was used under the following conditions. The temperature was programmed from 100 to 240°C at 2°C per min and then maintained at 240°C; flow rate of nitrogen used as a carrier gas was 0.89 mL/min; and data were integrated with a Shimadzu CR 3A chromatopac integrator (Shimadzu Corporation, Kyoto, Japan). The fatty acid methyl esters of each oil were prepared with sodium methoxide in methanol (11). The fatty acids were identified from relative retention times with respect to palmitic acid, and the results were expressed as relative area percentage of individual fatty acids.

RESULTS AND DISCUSSION

The results of analysis for moisture and fat contents of the pulses are given in Table 1. The data are in partial agreement with literature values (12–15). However, the fat content determined in this study was slightly lower than values reported for ether-extractive materials for the pulses studied. This is expected because diethyl ether extracts not only the free fat (neutral lipids) but also some phospholipids (polar lipids). These pulses are known to have >50% polar lipids (13). Petroleum ether gave complete extraction of the tocopherols in the pulses.

The results for total tocopherol and tocotrienol contents of the pulses are given in Table 2. The oils extracted from Bengal gram, black gram, horse gram, and green gram showed high amounts of tocopherols (230, 742, 1132, and 1567 mg/100 g) while the pulses (whole gram) themselves contained low amounts of tocopherols (Table 3). The tocotrienol content of all these pulses was low. Bengal gram contained only γ-tocotrienol at 1.6% of the total tocols (3.67 mg/100 g of extracted fat, 0.18 mg/100 g of the pulse). Green gram contained tocotrienols (all isomers) at 6.19 mg/100 g fat, amounting to 0.4% of the total tocols.

The total tocopherol content, determined (as total reducing substances) by the vitamin E panel method (16), also showed high values for fat from these pulses. The unsaponifiable matter of the fat from these pulses showed an unusually large amount of tocopherol as examined by qualitative thin-layer chromatography (TLC) (17). The spots on a TLC plate that correspond to standard tocopherols produced a pink color when sprayed with a mixture of 0.2% ferric chloride and 0.5% dipyridyl in ethanol. Tocopherols also were separated by HPLC with fluorescence detection. Tocopherols have a strong native fluorescence; the excitation(E_{ex}^{max}, 295 nm) and emission (E_{em}^{max}, 330 nm) wavelength maxima of fluorescence signals of the individual tocopherol/tocotrienol peaks in the HPLC chromatogram were typical of tocopherols. The excitation and emission wavelength maxima for the fluorescence spectra for reported tocopherols confirmed that these components from pulses were tocopherols (2,18).

Among the pulses, γ-tocopherol was the major tocopherol accounting for 80.8 to 97.3% of the total tocopherols (Table 2). Horse gram and green gram contained Δ-tocopherol in substantial amounts (98 to 107 mg/100 g oil). Such a high content of tocopherols in the plant or animal kingdom has not been reported (1–4). The content of tocopherols generally does not exceed 250 mg/100 g oil. The richest source seems to be wheat germ oil (1).

The fatty acid composition of the oil extracted from the pulses studied is shown in Table 4. The fatty acid composition of these pulses agreed with the results of previous investigations (12–15,19–21).

During 1991, 14.26 million tons of pulses were consumed in India (9), accounting for a per capita consumption of about 40 g per day. The oil fraction of Indian pulses contained high amounts of tocopherols (especially γ- and Δ-tocopherols) and unsaturated fatty acids (especially linolenic). This is the first

TABLE 1
Moisture and Fat Content of the Studied Pulses[a]

Raw materials	Moisture content (% wet basis)	Fat content (% wet basis)	
		Petroleum ether extract[b]	Diethyl ether extract[c]
Bengal gram	11.50 ± 0.12	4.95 ± 0.03	5.20 (12)
Black gram	11.63 ± 0.15	0.91 ± 0.01	2.00 (13)
Green gram	11.32 ± 0.13	0.80 ± 0.03	1.40 (14)
Horse gram	11.39 ± 0.15	0.65 ± 0.05	0.50 (15)

[a]All determinations are averages for triplicate samples ± standard deviation.
[b]As determined by Soxhlet extraction with petroleum ether as the solvent.
[c]Numbers in parentheses refer to cited references.

TABLE 2
Tocopherol and Tocotrienol Compositions of the Oils Extracted from the Studied Pulses (expressed on oil basis)

		Bengal gram		Black gram		Green gram		Horse gram	
	RRT[a]	A[a]	B[a]	A	B	A	B	A	B
Tocopherols									
α-	1.00	33.94 ± 1.43	14.7	3.01 ± 0.13	0.4	10.89 ± 0.13	0.7	5.03 ± 0.27	0.4
β-	1.59	1.87 ± 0.17	0.8	—	—	0.90 ± 0.09	0.1	—	—
γ-	1.73	186.17 ± 11.80	80.8	722.54 ± 31.8	97.3	1457.79 ± 77.19	93.0	1020.03 ± 30.91	90.1
Δ-	2.45	8.36 ± 1.40	3.6	16.74 ± 6.95	2.3	97.64 ± 2.12	6.2	106.75 ± 2.92	9.4
Total		230.34 ± 13.88	99.9	742.29 ± 36.44	100.0	1567.22 ± 79.53	100.0	1131.81 ± 33.86	99.9
Tocotrienols									
α-	1.26	—	—	—	—	2.74 ± 0.21	44.3	—	—
β-	2.04	—	—	—	—	0.85 ± 0.37	13.7	—	—
γ-	2.22	3.67 ± 0.19	100.0	—	—	1.92 ± 0.37	31.0	—	—
Δ-	3.20	—	—	—	—	0.68 ± 0.18	11.0	—	—
Total		3.67	100.0	—	—	6.19	100.0	—	—

[a]RRT = relative retention time; A = mg/100 g fat; B = percentage of total tocopherols/tocotrienols.

TABLE 3
Tocopherol and Tocotrienol Compositions (mg/100 g) of the Studied Pulses

	Bengal gram	Black gram	Green gram	Horse gram
Tocopherols				
α-	1.68	0.03	0.09	0.03
β-	0.09	—	0.01	—
γ-	9.22	6.58	11.66	6.63
Δ-	0.41	0.15	0.78	0.69
Total	11.40	6.76	12.54	7.35
Tocotrienols				
α-	—	—	0.02	—
β-	—	—	0.01	—
γ-	0.18	—	0.02	—
Δ-	—	—	0.01	—
Total	0.18	—	0.06	—
Total tocols	11.58	6.76	12.60	7.35
Coefficient of variation (%)	5.9	4.9	5.0	3.0

TABLE 4
Fatty Acid Composition (relative %) of the Oil Extracted from Pulses

Fatty acids[a]	Bengal gram	Black gram	Green gram	Horse gram
Saturated acids				
$C_{16:0}$	9.5	11.1	24.8	19.6
$C_{18:0}$	1.8	2.6	6.0	2.4
$C_{20:0}$	—	0.1	1.2	1.0
$C_{22:0}$	—	0.8	2.2	4.7
$C_{24:0}$	—	0.1	1.4	2.9
Total	11.3	14.7	35.6	30.6
Monounsaturated acids				
$C_{18:1n-9}$	19.0	26.1	5.4	14.9
$C_{20:1n-9}$	-—	0.1	—	0.4
$C_{22:1n-9}$	-—	0.1	—	—
Total	19.0	26.3	5.4	15.3
Diunsaturated acids				
$C_{18:2n-6}$	65.9	7.2	37.1	37.8
$C_{22:2n-6}$	-—	0.2	—	0.8
Total	65.9	7.4	37.1	38.6
Triunsaturated acids				
$C_{18:3n-3}$	3.8	49.1	21.8	13.0
$C_{22:3n-3}$	—	0.1	—	—
Total	3.8	49.2	21.8	13.0

[a]Coefficient of variation was <2.0% for all fatty acids.

report of high amounts of tocopherol in the oil fraction of these pulses.

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