Lipid Composition of Ten Edible Seed Species from North Vietnam

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ABSTRACT: The lipid composition and oil content of ten edible seed species from North Vietnam *(Cassia tora, Ipomoea aquatica, Raphanus sativus, Citrullus lanatus, Cucumis melo, Cucurbita pepo, Luffa cylindrica, Phaseolus vulgaris, Vigna aurea, Sesamum orientale)* have been investigated. The contents of hydrocarbon, triacylglycerol, free fatty acid, sterol, di- and monoglycerol, and polar lipid fractions have been determined with a thin-layer chromatography (TLC)/flame-ionization detection analyzer. Molecular species of hydrogenated triacylglycerols and the fatty acid composition of total lipids also have been analyzed by capillary gas–liquid chromatography. The quantities of major phospholipid classes of four seed species *(C. tora, I. aquatica, R. sativus, V. aurea)* have been determined by two-dimentional TLC and the spectrophotometrical phosphorus analysis. The fatty acid compositions of nonpolar and polar lipid fractions of these four species also have been analyzed.
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KEY WORDS: Fatty acids, gas chromatography, phospholipids, seed lipids, TLC/FID, triacylglycerols.

Detailed study of the lipid composition of several seed species from one region and a comparison with that of plant seeds from other regions is of great interest (1,2). We selected the major plant species of North Vietnam whose seeds are traditionally used as food or as a prophylactic remedy against various diseases. We have conducted a comprehensive comparative study of lipids of these species with special attention to the quantitative characteristics of various lipid classes. This study covers both well-known and less studied species and is part of a series of research on the lipid composition of the seeds of Vietnamese plants.

EXPERIMENTAL PROCEDURES

Lipid preparation. Plant seeds were collected in May 1992 in Hanoi central market (Vietnam). Seed samples were stored at 4°C and constant humidity. Total lipids were extracted by the method of Bligh and Dyer (3). Total lipid contents in the seeds were determined gravimetrically. The total lipids were separated into polar and neutral fractions by silica gel column chromatography by chloroform and methanol elution (4). Triacylglycerol (TG) fractions of total lipids were obtained by preparative thin-layer chromatography (TLC) on silica gel plates K6 (Whatman, Maidstone, England) with hexane/diethyl ether/acetic acid (80:20:1, vol/vol/vol) eluent. The silica gel was scraped from the plate (R_f 0.7–0.8) and eluted with chloroform. Catalytic hydrogenation of the obtained TG was carried out in methanol over Adams's catalyst (PtO_2) for 2 h. TLC on silica gel plates impregnated with $AgNO_3$ was used to check completion of the reaction.

Total lipid analysis. The content of main lipid classes in total seed lipids was determined on a Iatroscan TH-10 TLC/flame-ionization detection (FID) analyzer (Iatron Laboratories, Kyoto, Japan). TLC separation of lipid classes was performed on Chromarod-SII (Iatron) silica gel stationary phase with hexane/diethyl ether/acetic acid (95:5:0.3, vol/vol/vol) as mobile phase. FID scanning speed was 5 mm/s. Lipids were identified by comparison with known standards. Chromatographic data were evaluated with a Shimadzu Chromatopac C-R4A integrator (Kyoto, Japan).

Fatty acid (FA) analysis. FA methyl esters (FAME) were prepared by the consecutive treatment of lipids with 1% sodium methylate in methanol and 5% HCl in methanol according to Carreau and Dubacq.(5) and were purified by TLC in benzene. Gas–liquid chromatography (GLC) of FAME was performed on a Shimadzu GC-9A gas chromatograph (FID detector) on a fused-quartz capillary column (30 × 0.25 mm i.d.) coated with Supelcowax 10M (Supelco, Bellefonte, PA). The column and detector temperature was 210°C; the injector temperature was 240°C. Helium was used as a carrier gas. Identification of FAME was confirmed by chromatographic comparison with authentic standards and calculation of equivalent chainlengths (ECL) (6). Chromatographic data were calculated with a Shimadzu Chromatopac C-R3A integrator.

TG analysis. A GLC analysis of hydrogenated TG was performed on the same chromatograph, on a short fused-quartz capillary column (1.2 × 0.32 mm i.d.) coated with OV-1 (bonded); carrier gas was helium. The injector temperature was 370°C. The column and detector temperatures were programmed from 290 to 330°C at 2°C/min and maintained

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at 330°C for 10 min. The TG were identified by comparison to authentic standards.

Phospholipid analysis. The polar lipids were separated by two-dimensional TLC with solvent mixtures of chloroform/methanol/25% water NH_3/benzene (65:30:6:10, by vol) in the first dimension and chloroform/methanol/acetone/acetic acid/water/benzene (70:30:5:4:1:10, by vol) in the second dimension (7). After development, spots were located by spraying with 10% H_2SO_4 in methanol and subsequent heating. Phospholipid classes were identified by cochromatography with authentic samples and the specific spray reagents: Dragendorff reagent for choline lipids (8), 0.25% ninhydrine in acetone for amino-containing lipids, and molybdate reagent for phospholipids (9). Bands corresponding to the identified lipids were scraped from the plate and analyzed for lipid phosphorus by wet ashing with $HClO_4$ and subsequent reaction with molybdate reagent as described by Vaskovsky *et al.* (9).

RESULTS AND DISCUSSION

We investigated the lipid composition of ten species of edible plant seeds that are widespread in North Vietnam; these species belong to six families (Table 1). Some seeds, such as *Phaseolus vulgaris*, *Sesamun orientale* or *Cucurbita pepo*, have been studied extensively, and we wanted to compare our data and previously published data for the same seed species grown elsewhere.

Table 1 shows the content of total lipids in the seeds relative to the total seed weight. (We used the ordinal numbers from Table 1 to designate seed species throughout the text below.) The total seed lipids were separated into hydrocarbon, steryl ester, TG, free fatty acid (FFA), sterol, glyceride, and polar fractions and quantitated by means of the Iatroscan TLC/FID analyzer (Table 2). Quantitative determination of the phospholipid fraction in the total lipids was performed with molybdate reagent relative to inorganic phosphate.

The FA composition of total seed lipids was analyzed by GLC (Table 3).

The distribution of TG molecular species according to their molecular weights after full catalytic hydrogenation is given in Table 4.

The phospholipid compositions were determined for four seed species (*Cassia tora, Ipomoea aquatica, P. vulgaris,*

TABLE 1
Systematic Names and Total Lipid Content of Ten Seed Species Investigated

Number	Botanical name	Family	% Total lipids
1	*Cassia tora* L.	Caesalpiniaceae	7.1
2	*Ipomoea aquatica* (Forsk.)	Convolvulaceae	8.3
3	*Raphanus sativus* L.	Cruciferae	23.6
4	*Citrullus lanatus* (Thunb.)	Cucurbitaceae	26.0
5	*Cucumis melo* L.	Cucurbitaceae	25.0
6	*Cucurbita pepo* L.	Cucurbitaceae	34.2
7	*Luffa cylindrica* (Roem)	Cucurbitaceae	32.4
8	*Phaseolus vulgaris* L.	Fabaceae	28.0
9	*Vigna aurea* (Roxb.)	Fabaceae	2.0
10	*Sesamum orientale* L.	Pedaliaceae	42.5

TABLE 2
Total Lipid Composition (% ± SD) of Ten Seed Species (n = 3)

	Species number[a]				
Lipid class	1	2	3	4	5
Hydrocarbons + waxes	—	0.7 ± 0.2	—	0.3 ± 0.1	—
Steryl esters	0.7 ± 0.1	0.8 ± 0.5	0.5 ± 0.2	0.5 ± 0.3	0.2 ± 0.1
Triacylglycerols	72.9 ± 7.8	87.3 ± 2.4	97.0 ± 0.5	95.8 ± 0.6	96.2 ± 0.6
Free fatty acids	0.7 ± 0.3	0.6 ± 0.1	0.7 ± 0.4	—	0.2 ± 0.1
Sterols	2.4 ± 0.5	—	0.4 ± 0.1	0.5 ± 0.1	0.8 ± 0.1
Diglycerols	0.5 ± 0.3	—	0.5 ± 0.3	0.7 ± 0.2	0.7 ± 0.2
Monoglycerols	—	0.9 ± 0.6	—	—	—
Polar lipids	21.2 ± 3.5	7.8 ± 2.1	1.0 ± 0.1	1.1 ± 0.1	1.6 ± 0.2
Phospholipids[b]	16.5 ± 0.7	5.1 ± 0.4	1.0 ± 0.1	1.1 ± 0.1	1.6 ± 0.3
	Species number[a]				
Lipid class	6	7	8	9	10
Hydrocarbons + waxes	0.3 ± 0.1	—	0.5 ± 0.2	0.4 ± 0.2	—
Steryl esters	0.3 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.8 ± 0.2	—
Triacylglycerols	90.5 ± 0.4	94.8 ± 1.6	32.6 ± 5.2	24.5 ± 5.6	97.2 ± 0.8
Free fatty acids	—	—	—	8.9 ± 1.8	0.5 ± 0.1
Sterols	3.0 ± 0.6	1.9 ± 0.5	3.5 ± 1.3	10.2 ± 0.8	0.5 ± 0.3
Diglycerols	2.5 ± 0.7	—	—	1.9 ± 0.5	0.4 ± 0.1
Monoglycerols	2.0 ± 1.1	—	—	—	—
Polar lipids	1.2 ± 0.2	2.4 ± 0.6	63.0 ± 6.0	52.5 ± 5.6	1.4 ± 0.4
Phospholipids[b]	1.0 ± 0.1	1.8 ± 0.2	41.9 ± 4.6	25.0 ± 0.5	1.4 ± 0.1

[a]Numbers as in Table 1.
[b]Phospholipid content was determined spectrophotometrically.

TABLE 3
Fatty Acid Composition (%) of Total Lipids of Ten Seed Species

	Species number									
Fatty acid	1	2	3	4	5	6	7	8	9	10
14:0	0.1	0.3	—	0.1	0.1	0.1	0.1	0.2	0.2	—
15:0	—	—	—	—	0.1	—	—	0.2	0.1	—
16:0	19.3	20.1	4.2	11.1	11.2	20.1	18.3	18.4	23.8	8.9
16:1n-7	0.3	0.2	0.1	0.1	0.1	0.1	0.2	0.5	—	0.1
17:0	0.1	0.1	—	—	0.1	0.1	0.1	0.2	0.2	—
18:0	6.4	9.8	1.7	8.3	6.4	8.2	6.7	1.4	5.9	5.7
18:1n-9	19.5	32.0	19.9	12.0	15.8	17.0	40.9	6.7	5.6	36.2
18:1n-7	0.9	—	1.0	0.7	—	0.6	0.9	2.0	0.5	1.4
18:2n-6	47.0	31.9	10.5	66.5	65.5	52.5	32.1	20.6	43.3	46.2
18:3n-3	2.0	1.1	9.2	0.1	0.3	0.4	0.2	48.9	18.3	0.4
20:0	2.1	2.3	1.2	0.3	0.2	0.6	0.4	0.2	0.9	0.7
20:1n-9	0.4	0.1	8.9	—	0.1	0.1	0.1	—	0.2	0.2
22:0	1.6	0.9	1.0	—	—	0.2	—	0.2	1.0	0.1
22:1n-9	—	—	37.8	—	—	—	—	—	—	—
Others	0.3	1.1[a]	4.7[b]	0.8	0.2	0.1	0.1	0.4	0.1	0.1
Saturated	29.5	34.0	8.9	19.8	18.0	29.3	25.6	20.6	32.1	15.4
Monoenoic	21.0	32.4	70.2	12.8	16.0	17.8	42.0	9.2	6.2	38.0
Polyenoic	49.0	33.4	20.5	66.6	65.8	52.9	32.3	69.6	61.6	46.5

[a]0.4% of 18:3n-6; 0.4% of 24:0.
[b]0.5% of 20:1n-7; 0.4% of 20:2n-6; 0.5% of 22:1n-7; 0.3% of 22:2n-6; 0.8% of 24:0; 1.6% of 24:1n-9.

Vigna aurea) that contained polar lipid fractions of more than 3% (Table 5). The major classes of phospholipids were phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). Phosphatidylserine, phosphatidylglycerol, and phosphatidic acid (PA) were present in smaller quantities; diphosphatidylglycerol and lysophosphatidylcholine (LPC) were present as minor components. An unidentified phosphorus-containing component, detected on the TLC-plate in the glycosphingolipid area, was present in small amounts in all four seed species. We did not detect LPC in *I. aquatica* seeds and no lysophosphatidylethanolamine in any of the investigated seed species.

Epoxy- and cyclopropenoic FA were earlier identified in the seed lipids of members of the genus *Casia* (10,11). Our study revealed that the total FA of *C. tora* from North Vietnam contain an insignificant quantity of 9,10-methylene-9-octadecenoic (sterculic) acid (about 0.2%). Our attempts to discover detectable amounts of 12,13-epoxy-9-octadecenoic (vernolic) acid or TG with acyl residues of vernolic acid in Vietnamese *C. tora* by the earlier described procedure (12) were unsuccessful. Comparison of our data concerning the phospholipid and TG composition of *C. tora* (Tables 2, 4, and 5) with those from other sources is not possible because the literature does not afford relevant information.

We found that the seeds of *I. aquatica* had a low oil content (8.3%, Table 1) and a significant proportion of polar lipids (Table 2); the oil content of *I. aquatica* was much lower than in the related species *I. horsfalliae* (19.8%) (13). The major FA were palmitic, oleic, and linoleic acids (Table 3); the major phospholipids were PC, PE, and PI (Table 5).

There are several independent publications dealing with lipids of members of the genus *Vigna* (14–17), but there are no detailed data on the total lipid composition of *V. aurea*. This is probably connected with the low lipid content in the seeds of this species (Table 1). According to our data, the greater part of total lipids of *V. aurea* were polar lipids (52%),

TABLE 4
Triacylglycerol Composition (%) of Ten Seed Species[a]

	Species number									
TG[b]	1	2	3	4	5	6	7	8	9	10
C_{48}	0.4	0.4	—	—	—	—	—	—	0.4	—
C_{50}	17.2	17.0	1.0	3.1	2.1	11.4	8.9	2.0	14.5	2.9
C_{52}	42.0	36.4	11.0	32.0	30.4	41.7	40.1	24.3	42.0	26.6
C_{54}	33.4	35.2	22.6	63.9	66.9	46.3	49.1	69.7	26.8	69.6
C_{56}	4.2	8.6	11.4	0.5	0.5	0.2	1.7	1.4	7.9	0.9
C_{58}	1.8	1.2	20.9	0.5	—	0.2	—	1.2	4.8	—
C_{60}	0.8	0.7	13.7	—	—	—	—	0.9	1.8	—
C_{62}	—	—	17.0	—	—	—	—	—	—	—
C_{64}	—	—	2.2	—	—	—	—	—	—	—

[a]Fully hydrogenated oil. Components over 0.2%.
[b]TG, Triacylglycerol.

TABLE 5
Phospholipid Content (% ± SD) of Four Selected Seed Species (n = 3)

	Species number			
Phospholipid[a]	1	2	8	9
PC	53.1 ± 6.2	48.4 ± 2.3	52.0 ± 1.1	61.8 ± 0.4
PE	16.6 ± 1.1	27.1 ± 3.3	33.6 ± 1.9	12.9 ± 0.8
PI and PS	17.2 ± 3.2	20.9 ± 2.1	10.7 ± 0.7	18.0 ± 1.0
PA	10.7 ± 1.9	2.8 ± 0.3	1.5 ± 0.4	4.1 ± 0.7
PG	1.7 ± 1.2	—	1.8 ± 0.5	—
Unknown	0.8 ± 0.4	0.8 ± 0.3	0.5 ± 0.1	3.2 ± 0.1

[a]PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid; PG, phosphatidylglycerol.

with half being phospholipids (Tables 2 and 5). The major phospholipid was PC (61%). The total lipids had a fairly high percentage of sterols and free FA (Table 2). The total FA of *V. aurea* contained 32% of saturated FA; C_{56}–C_{60} glycerides made up 14.5% of total TG (Table 4).

Seeds of *Raphanus sativus* from North Vietnam had a moderate oil content (no more than 23%) and a high percentage of TG (97%). FA of *R. sativus* were notable for the high level (37.8%) of erucic acid (Table 3) common to seeds of the plants of the family Cruciferae (18,19). The presence of long-chain FA influenced the TG molecular species of *R. sativus*. Out of ten investigated Vietnamese species, the latter species had the greatest proportion of C_{56}–C_{64} TG (Table 4). It is worthy of note that we found no C66 glycerides or any other higher-molecular weight glycerides in the total TG fraction of *R. sativus* (Table 4), i.e., C_{22} and C_{24} acyl residues were incorporated into the TG molecules only in combination with shorter-chain FA, but not separately.

The next four species, *Citrullus lanatus*, *Cucumis melo*, *C. pepo*, and *Luffa cylindrica* belong to the family Cucurbitaceae. The predominant FA in seed lipids of this plant family were palmitic, oleic, and linoleic acids; small amounts of linolenic acid and long-chain FA were present (Table 3). The total lipids made up 23–34%, and the TG fraction accounted for over 90% of total lipids. The major TG were C_{52}–C_{54}. Total lipids had a small amount of polar lipids (less than 3%).

The total lipid composition of *L. cylindrica* from North Vietnam had not been examined earlier. Our study indicated that the lipid content in *L. cylindrica* is not high (32%), but seed oil has good nutritional parameters. The TG fraction was made up 95% of total lipids, the unsaturated FA fraction was 74% of total acids, and the ratio of oleic to linoleic acids was 1:3 (Tables 1–3).

We found that the seeds of *C. lanatus* from North Vietnam are rich in TG (95.8%), contrary to the value of 82% reported previously (20). Also we found a lower oil content in Vietnamese *C. pepo* (34.2%), opposite the data in a previous report (21).

It is known that seed oil content of plants of the genus *Phaseolus* varies greatly (1,16). According to our data, the TG fraction of Vietnamese *P. vulgaris* seeds accounts for less than 40% of total lipids (Table 2). The proportion between the individual phospholipid classes of Vietnamese *P. vulgaris* (Table 5) was similar to that of species from other regions (22,23).

Sesamum orientale is a subspecies of widely distributed *S. indicum* from which sesame oil is extracted on a large scale. Our results for Vietnamese *S. orientale* lipids, obtained by a combination of GLC, TLC/FID analyses, and spectrophotometry (Tables 1–5), are in good agreement with the previous results (1).

As shown in Table 4, several investigated seed species contained a significant amount of TG with higher-molecular weight than in tristearylglycerol (C_{54}). This is associated with the presence of FA with 20 and 22 carbon atoms. However, the content of C_{20} and C_{22} FA in the total lipids (Table 3) of four species (*C. tora*, *I. aquatica*, *P. vulgaris*, *V. aurea*) was insufficient to explain the obtained quantity of C_{56}–C_{58} glycerides. We assumed that this fact may be explained by an irregular distribution of long-chain FA between polar and nonpolar lipids. Hence, we analyzed separately the FA composition of polar and nonpolar lipid fractions of four species (Figs. 1 and 2). GLC analysis showed that C_{20} and C_{22} FA are practically absent in the polar lipids and mostly concentrate in the nonpolar lipid fraction. Hence, the high content of C_{20} and C_{22} FA in the neutral lipid fraction is the cause of the relatively high percentage of C_{56}–C_{58} glycerides we obtained.

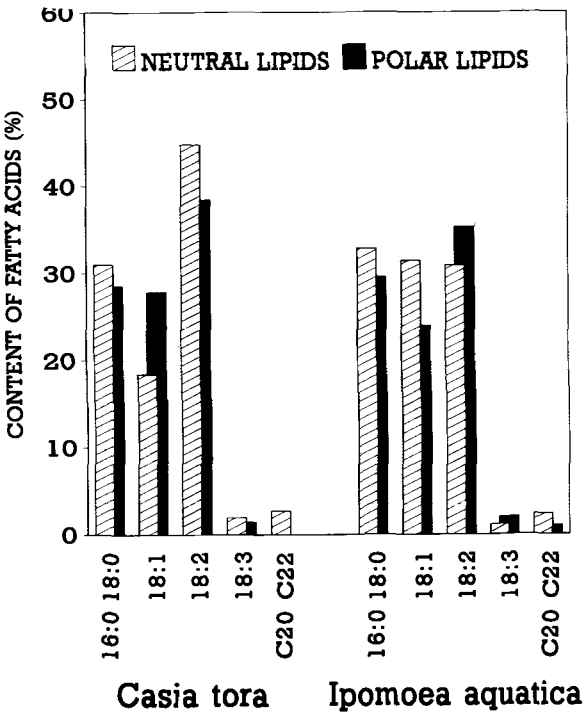

FIG. 1. Fatty acid composition of neutral and polar lipids of *Cassia tora* and *Ipomoea aquatica* seeds.

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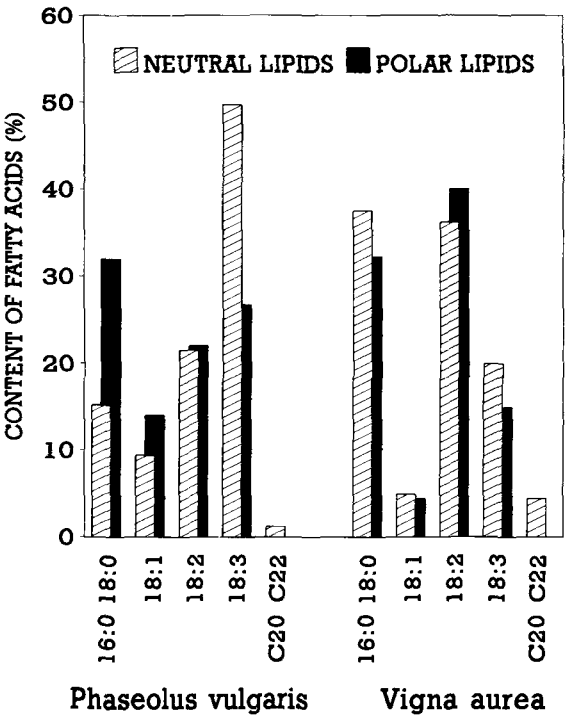

FIG. 2. Fatty acid composition of neutral and polar lipids of *Phaseolus vulgaris* and *Vigna aurea* seeds.

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