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Lipid Composition of Fenugreek (*Trigonella foenum-graecum* L.) Seeds

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ABSTRACT

*Total lipids extracted from fenugreek (*Trigonella foenum-graecum *L., Leguminosae) seeds amounted to 7·5% of the dry seeds. The total lipids consisted of 84·1% neutral lipids, 5·4% glycolipids and 10·5% phospholipids. Neutral lipids consisted mostly of triacylglycerols (86%), diacylglycerols (6·3%) and small quantities of monoacylglycerols, free fatty acids and sterols. At least five glycolipids and seven phospholipids were identified. Acyl-monogalactosyldiacylglycerol and acylatedsterylglycoside were the major glycolipids, while sterylglucoside, monogalactosylmonoacylglycerol and digalactosyldiacylglycerol were present in small amounts. The phospholipids consisted of phosphatidylcholine, and phosphatidylethanolamine as major phospholipids and phosphatidylserine, lysophosphatidylcholine, phosphatidylinositol, phosphatidylglycerol, and phosphatidic acid as minor phospholipids. The fatty acid composition of these different neutral lipids, glycolipids and phospholipids was determined.*

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L., Leguminosae) is mainly cultivated in India, North Africa and Mediterranean countries (Rosengarten, 1969). The tender leaves of fenugreek are used as a vegetable, while the seeds are used as condiments. Recently, the crop has attracted much interest as a source of good protein (Sauvaire & Baccou, 1976; Udayasekhara Rao & Sharma, 1987), and as a protein supplement to jowar (*Sorghum*

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vulgare) (Talwalker & Patel, 1970) or corn flour (Taha El-Katib, 1947; El-Madfa & Kuhl, 1976; Gorafi, 1983). Fenugreek seeds contain 7·5% of oil, and its physico-chemical characteristics have been reported (Shahat, 1947; Hilditch & Williams, 1964; Badami & Kalburgi, 1969; Baccou *et al.*, 1978; El-Sebaiy & El-Mahdy, 1983). However, little information is available on the lipid composition of the fenugreek seeds. The purpose of this study was to determine the composition of lipids of fenugreek seeds.

MATERIALS AND METHODS

Fenugreek seeds were purchased locally. Authentic neutral lipids, glycolipids, phospholipids, and fatty acid methyl esters were purchased from Sigma Chemical Co. (St. Louis, Missouri), for use as standards. Solvents used were of analytical grade, and were distilled before use.

Lipid extraction

The total lipids from triplicate, 20 g samples of fenugreek seeds were extracted and purified, following the established procedure of Folch *et al.* (1957). A measured portion of the purified lipid extract was used for gravimetric estimation of total lipids. Unsaponifiable matter and free fatty acid content of the lipids were determined according to AOCS methods (1973).

Lipid classes and fatty acid analysis

The total lipids (TL) were fractionated into neutral lipids (NL), glycolipids (GL), and phospholipids (PL), on a silicic acid column (Rouser *et al.*, 1967) using chloroform, acetone, and methanol successively. NL were estimated gravimetrically. GL and PL were quantitated by estimation of total sugars (Dubois *et al.*, 1956) and phosphorus (Marinetti, 1962), respectively, using appropriate factors (Williams *et al.*, 1966). NL were separated by thin-layer chromatography (TLC) using hexane: diethyl ether: acetic acid (80:20:1 v/v). Individual components of NL were identified by comparison with authentic standards and quantified by photodensitometry (Blank *et al.*, 1964). GL and PL were separated on TLC using chloroform:methanol:acetic acid:water (65:15:10:4 v/v). The different components of GL and PL were identified by comparison with authentic standards and by specific spray reagents (Rosenberg *et al.*, 1966; Vaskovsky & Kostetsky, 1968). Individual GL and PL separated on preparative TLC were scraped and extracted with chloroform:methanol:water (1:2:0·8 v/v) and quantitated by estimation of

total sugars (Dubois *et al.*, 1956) and phosphorus (Marinetti, 1962), respectively, using appropriate factors (Williams *et al.*, 1966).

Fatty acid methyl esters (FAME) were prepared by acid catalyzed transmethylation (Christie, 1982) of the lipids. The FAME were analyzed on a Shimadzu GC 9A gas chromatograph equipped with a flame ionization detector (FID), stainless steel column (152·4 cm × 3·17 mm i.d.) packed with 20% diethyleneglycol succinate on 80–100 mesh Chromosorb W support, at a column temperature of 180°C, the injection port and FID at 210°C, under a nitrogen flow rate of 40 ml/min. The peak area and relative percentage of FAME were obtained with a Shimadzu integrator. The component of each peak was identified by retention time data with those of authentic standards. All determinations were performed in triplicate and the mean values were reported.

RESULTS AND DISCUSSION

The seeds of fenugreek contained 7·5% total lipids (dry basis). The purified lipids had the following physico-chemical characteristics: appearance, golden-yellow liquid at ambient temperature (25–30°C); taste, bitter; odour, disagreeable; free fatty acid content, 1·7 g oleic acid/100 g oil; unsaponifiable matter, 9·1%. Fractionation of the lipids by silicic acid column chromatography showed the TL to consist of 84·1% NL, 5·4% GL and 10·5% PL (Table 1). The fatty acid composition of TL showed that oleic was the predominant fatty acid, followed by linoleic acid, while the other fatty acids were present to the extent of $\leq$2·7% (Table 1). The fatty acid profile of NL reflected largely that of TL, while GL and PL had the highest contents of palmitic, linoleic and oleic acids compared to TL and NL.

TABLE 1
Major Lipid Classes of Fenugreek Seeds and Their Fatty Acid Composition[a]

Lipid class[b]	*Wt* %	*Fatty acid composition* (%)									
		14:0	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*	*20:0*	*20:1*	*22:0*	*22:1*
TL	7·5	1·0	0·5	2·0	52·6	40·0	0·6	0·2	0·2	0·2	2·7
NL	84·1	1·3	0·4	1·5	52·2	39·8	0·6	0·3	0·1	0·2	3·6
GL	5·4	2·2	5·6	1·0	42·2	46·9	1·3	0·1	0·1	0·1	0·5
PL	10·5	1·2	4·4	2·6	59·4	31·4	0·4	0·1	0·1	0·1	0·3

[a] All values are means of three replicate analyses.
[b] TL, Total lipids; NL, Neutral lipids; GL, Glycolipids; PL, Phospholipids.

TABLE 2
Neutral Lipids of Fenugreek Seeds and Their Fatty Acid Composition[a]

Neutral lipid[b]	*Wt* %	*Fatty acid composition* (%)									
		14:0	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1
TG	86·6	1·2	1·5	1·0	54·9	36·4	0·8	0·6	0·1	0·4	3·5
Sn-1,3,DG	2·5	1·6	1·9	2·0	59·1	33·7	0·1	0·2	0·1	0·1	1·2
Sn-1,2 (2,3)-DG	3·8	1·4	1·3	2·0	61·3	33·8	0·2	—	—	—	—
MG	0·2	1·2	1·8	3·2	54·5	36·2	0·5	0·2	0·1	0·1	2·2
FFA	1·7	1·0	2·4	2·8	40·0	52·3	0·3	0·2	0·1	0·1	0·4
S,SE, H + P	5·1	—	—	—	—	—	—	—	—	—	—

[a] All values are means of three replicate analyses.
[b] TG, Triacylglycerols; DG, Diacylglycerols; MG, Monoacylglycerols; FFA, Free fatty acids, S, Sterols; SE, Sterolesters; H, Hydrocarbons; P, Pigments.

In regard to the neutral lipid fraction, triacylglycerols (TG) were found to be the major component (Table 2). *Sn*-1,2 (2,3)-DG together with *sn*-1,3-DG were the second largest component of NL. The quantity of *sn*-1,3 (2,3)-DG was slightly higher than *sn*-1,3-DG, and was similar to that observed in peanut oil (Sanders, 1980). The fatty acid composition of different components of NL, except hydrocarbons, pigments, sterol esters and sterols, is presented in Table 2. Oleic and linoleic acids were present in high amounts in different components of NL.

TABLE 3
Glycolipids of Fenugreek Seeds and Their Fatty Acid Composition[a]

Glycolipid[b]	*Wt* %	*Fatty acid composition* (%)									
		14:0	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*	*20:0*	*20:1*	*22:0*	*22:1*
A-MGDG	43·4	1·1	5·7	1·1	41·8	49·0	0·6	0·1	0·1	0·1	0·4
ASG	37·5	1·9	2·5	1·5	38·5	51·6	2·5	0·2	0·1	0·1	1·1
SG	9·6	0·4	2·4	0·2	41·5	52·6	1·6	0·5	0·2	0·4	0·2
DGDG	6·2	0·8	4·0	0·3	39·7	50·8	3·1	1·0	0·1	0·1	0·1
MGMG	3·3	0·1	3·9	0·4	42·2	50·3	2·1	0·7	0·1	0·1	0·1

[a] All values are means of three replicate analyses.
[b] A-MGDG, Acyl-monogalactosyldiacylglycerol; ASG, Acylatedsterylglycoside; SG, Sterylglycoside; DGDG, Digalactosyldiacylglycerol; MGMG, Monogalactosylmonoacylglycerol.

TABLE 4
Phospholipids of Fenugreek Seeds and Their Fatty Acid Composition[a]

Phospholipid[b]	*Wt* %	*Fatty acid composition* (%)									
		14:0	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1
PC	54·6	1·8	5·0	3·0	58·3	30·7	0·6	0·2	0·1	0·1	0·2
PE	29·5	0·9	4·8	2·0	65·5	25·8	0·4	0·2	0·2	0·1	0·1
PS	4·1	0·2	0·2	0·8	58·5	38·8	0·4	0·1	0·3	0·3	0·4
LPC	3·8	0·5	0·4	0·7	46·5	50·0	0·8	0·4	0·2	0·3	0·2
PI	3·2	0·4	0·3	0·3	43·0	55·0	0·5	0·1	0·2	0·1	0·1
PG	2·6	0·3	0·6	0·2	47·0	51·0	0·3	0·1	0·3	0·1	0·1
PA	2·2	0·3	0·9	0·1	40·8	57·0	0·2	0·1	0·2	0·2	0·2

[a] All values are means of three replicate analyses.
[b] PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PS, Phosphatidylserine; LPC, Lysophosphatidylcholine; PI, Phosphatidylinositol; PG, Phosphatidylglycerol; PA, Phosphatidic acid.

The glycolipid fraction was resolved into acyl-monogalactosyldiacylglycerol (A-MGDG), acylatedsterylglycoside (ASG), digalactosyldiacylglycerol (DGDG), sterylglycoside (SG) and monogalactosylmonoacylglycerol (MGMG) (Table 3). A-MGDG and ASG were present in the major quantities, while SG, DGDG and MGMG in small quantities ($\leq$9·6%). The fatty acid analysis of these different GL (Table 3) showed a high proportion of linoleic acid compared to oleic acid.

The phospholipid fraction was resolved into seven components by TLC (Table 4). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE), were present in large quantities, while phosphatidylserine (PS), lysophosphatidylcholine (LPC), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidic acid (PA) were present in small quantities ($\leq$4·1%). The fatty acid composition of different PL (Table 4) showed a high proportion of oleic acid in PC, PE, PS and linoleic acid in LPC, PI, PG and PA.

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