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ORIGINAL PAPER

Characterization of Fenugreek (*Trigonella foenum-graecum*) Seed Lipids

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Abstract The composition and content of lipids, fatty acids, triacylglycerols, tocopherols and sterols in nine fenugreek genotypes were analyzed. Lipid content in fenugreek seeds ranged from 5.8 to 15.2%. Major fatty acids were: linoleic acid (45.1–47.5%), α-linolenic (18.3–22.8%), oleic (12.4–17.0%), palmitic (9.8–11.2%) and stearic (3.8–4.2%) acids. The ratios of n-6 to n-3 fatty acids were between 2.1 and 2.7. Similar fatty acid distribution was observed in all analyzed samples with some deviations. α-Tocopherol was the predominant component found in the fenugreek lipid antioxidants, and it constituted over 84% of the total amounts of tocopherols. It amounts ranged from 620 to 910 mg/kg lipids. *β*-Sitosterol was the major sterol in all samples, varying from 14,203 to 18,833 mg/kg of lipids. Campesterol and cycloartenol were other major sterols, and these compounds including *β*-sitosterol constituted 56–72% of all sterols. Fenugreek seed lipids consisted predominantly triunsaturated (56.9–66.5%) and diunsaturated (32.2–41.6%) triacylglycerides. Among these components trilinolein (LLL; 12.9–20.5%) dominated followed by PLL (14.0–20.4%), LnLnO (7.8–17.7%), PLO (5.7–11.6%), OLL (6.9–10.6%), LLLn (3.2–9.6%), and LnLnL (3.5–7.6%). Results of the study show that fenugreek seed lipids may be a source of a nutraceutical ingredient for food applications.

Keywords Fenugreek · Seed Lipids · Fatty Acids · Tocopherols · α-Tocopherol · Phytosterols · Triacylglycerols

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Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb from Papilionaceae Leguminosae family, and is extensively cultivated in Southern Europe, Northern Africa, Central Asia, North America, and some parts of Australia [1]. It is generally cultivated as a forage crop because it contains high amounts of protein, vitamins, essential amino acids, and offers good digestibility to cattle [2]. It has been reported that fenugreek seed when supplemented to a dairy cattle diet significantly increased the amount of polyunsaturated fatty acids in the milk, and decreased the level of milk cholesterol [3].

Fenugreek seed oil has a pungent odor and bitter taste and is often used as an insect repellent for grains and fabrics [4, 5]. Fenugreek seeds are also widely used as a spice and food colorant in food preparations. Distinctive and pungent aromatic compounds in the fenugreek seed add flavor and color pigments to the foods, so it is a desirable ingredient in food preparation [6]. Fenugreek is often used as an additive in the preparation of curry powder, a popular spice in many countries [4].

Fenugreek has also many medicinal properties and is one of the oldest medicinal plants used in many Asian and African countries [7]. Many of the claims made for its nutraceutical properties and health benefits have been supported by scientific studies. This has led to a growing interest in the production and marketing of fenugreek. The seeds of fenugreek are known to have hypoglycaemic [8], hypocholesterolemic [9], gastro- and hepato-protective [10], antioxidant properties [7], and also it stimulates lactation in women [11]. The aqueous extract of the fenugreek seed has been reported to show antibiotic activity against *Micrococcus pyogenes* var. *aureus* bacteria [12].

To the best of our knowledge, no detailed data are available about the lipid components of fenugreek seed. The main objective of this study was to widen the knowledge of the composition of fenugreek seed lipid. Therefore, the content and composition of fatty acids, triacylglycerols, tocopherols, and sterols in different fenugreek genotypes were investigated.

Materials and Methods

Materials

Fenugreek Seeds

A collection of the following genotypes of fenugreek seeds: Tristar, Quadro MP 30 (Quadro), AC Amber (Amber), F17, F75, F96, X92, Indian Temple (Temple) and L3312, were assessed in this study (in parentheses are the abbreviations used in this paper). The collection was a group of diverse geographic origin cultivars used to assess lipid distribution among them. All fenugreek genotypes were grown in three different experimental fields in Lethbridge, Alberta, Canada, this region is considered to be semi-arid and fenugreek was grown with irrigation. All plots were irrigated and for all locations cumulative growing conditions were not statistically different at $P \leq 0.05$. Taking into account the similarity of growing conditions, seeds from a particular genotype grown at different locations were combined and representative samples collected for lipids analysis.

Chemicals

All reagents of analytical or HPLC grade and sterol standards were purchased from Sigma-Aldrich (St Louis, MO, USA). Standards of tocopherols were obtained from Calbiochem–Novabiochem (San Diego, CA, USA). Standards of fatty acid methyl esters and triacylglycerides (TAG) were purchased from Nu-Chek-Prep (Elysian, MN, USA). Pyridine and Sylon BTZ were obtained from Supelco (Bellefonte, PA, USA).

Lipid Extraction

The fenugreek seeds were ground in a coffee grinder before lipid extraction. Ground seeds (20 g) were homogenized with 200 mL of chloroform–methanol (2:1, v/v) following the Folch procedure [13]. Extraction was repeated three times. The lipid extract was concentrated under vacuum in a rotary evaporator (Buchi Labortechnik, Switzerland) at 35 °C. Solvent free lipids were weighed to determine the lipid content and then transferred to brown glass vials with iso-octane, flushed with nitrogen, and stored at −20 °C until further analysis.

Fatty Acid Composition

Methyl esters of fatty acids (FAME) were prepared according to AOCS Method Ce 1-62 [14]. Diluted FAME were separated on a HP 5980 series GC (Hewlett Packard, Palo Alto, USA) equipped with an SP-2560 fused silica capillary column (100 m × 0.25 mm, 0.25 μm; Supelco, Bellafonte, PA, USA) and a flame ionization detector (FID). A sample of 1 μL was injected into the GC injector operated in splitless mode with an injection time of 2 min. Hydrogen was used as the carrier gas at a flow rate of 2.0 mL/min. The column temperature was programmed from 70 °C to 160 °C at 25 °C/min, held for 30 min, then further programmed to 210 °C at 3 °C/min. Initial and final temperatures were held for 5 and 30 min, respectively. Detector and injector temperatures were set at 250 °C. Fatty acids were identified by comparison of the retention times with authentic standards and the results are reported as weight percentages after integration and calculation using ChemStation (Agilent Technologies, Mississauga, Canada).

Tocopherol Analysis

Tocopherols were analyzed according to the ISO 9936 method [15]. Briefly, lipids were dissolved in hexane (5 mg/mL) and separated using a Finnigan Surveyor Plus HPLC System (Thermo Electron, Waltham, MA, USA) with a Finnigan Surveyor model FL Plus fluorescence detector set for an excitation at 295 nm and an emission at 325 nm. A sample of 10 μL was injected onto a diol column (250 × 4.6 mm, 5 μm; Monochrom, Varian, Palo Alto, CA). The mobile phase was 7% *tert*-butyl methyl ether in hexane at a flow rate of 0.6 mL/min. Standards of tocopherol isomers were used for identification by comparing of the retention data. Quantification was based on

external calibration for each isomer separately and all results are expressed as mg tocopherols per kg of lipids.

Triacylglycerols Composition

Triacylglycerol's of fenugreek seed lipids were analyzed according to AOCS Method Ce 5b-89 using a Finnigan Surveyor Plus HPLC system (Thermo Electron, Waltham, MA, USA) equipped with a Sedex 75 evaporative light scattering detector (ELSD; Sedere, Alfortville, France) operated at 30 °C and an air pressure of 2.5 bar [14]. Lipids were dissolved in hexane (5 mg/mL), and 10 µL of the sample was injected onto two C18 columns connected in series (Gemini 110A, 250 × 3 mm, 5 µm; Phenomenex, Torrance, CA, USA). Temperature of the columns was set at 30 °C. A binary gradient system of acetonitrile and dichloromethane was used at flow rate of 0.4 mL/min. Gradient elution started with 70% acetonitrile and 30% dichloromethane and held for 15 min, changed to 60% acetonitrile/40% dichloromethane in 10 min and held for 10 min, programmed to 40% acetonitrile/60% dichloromethane in 15 min and held for 5 min, then to 30% acetonitrile/70% dichloromethane in 5 min and held for 5 min, and finally to 70% acetonitrile/30% dichloromethane in 20 min and held for 5 min. Triacylglycerols were identified by comparison of the retention data with authentic standards including relative retention time calculated using triolein as reference.

Sterol Analysis

Sterol content and composition were determined by GC following the procedure described by Rudzinska et al. [16]. Briefly, lipids (50 mg) were saponified with 1 M KOH in methanol for 18 h at room temperature, then water was added and the unsaponifiables extracted three times with hexane/methyl *tert*-butyl ether (1:1, v/v). The solvent was evaporated under a stream of nitrogen. Dry residues were dissolved in 0.3 mL pyridine and silylated with 1 mL of Sylon BTZ (Supelco, Bellefonte, PA, USA). Derivatives of the sterols were separated on a Trace GC Ultra (Thermo Electron, Rodano, Italy) equipped with DB-35MS capillary column (25 m × 0.20 mm, 0.33 µm; J&W Scientific, Folsom, CA). Sample of 0.5 µL was injected with an AS 3000 autosampler (Thermo Electron, Rodano, Italy) into a temperature-programmed injector (PTV) in splitless mode with an injection time of 5 min. The column temperature was held at 100 °C for 5 min, then programmed to 250 °C at 25 °C/min, held for 1 min, then further programmed to 290 °C at 3 °C/min and held for 20 min. The detector temperature was set at 300 °C. Hydrogen was used as the carrier gas at a flow rate of 1.5 mL/min. An internal standard, 5α-cholestane, was used for sterols quantification. Phytosterols were identified by comparison of retention data and the identity were confirmed using a Finnigan Trace 2000 GC coupled to a Finnigan Polaris Q quadrupole ion-trap MS after separation on a DB-5 capillary column (50 m × 0.2 mm, 0.32 mm; J&W). Helium was used as the carrier gas at a flow rate of 0.6 mL/min. All mass spectra were recorded using electron-impact ionization mode at 70 eV and scanning mass in the range of 100–650 Da. The ion source was held at 200 °C and the injector at 300 °C. A combination of the NIST Mass Spectra Library and laboratory sterols spectra library was used for identification.

Statistical Analysis

Data are presented as means ± standard deviation (SD) from triplicate experiments. Statistical analysis of the data was performed using SPSS package (version 10.0) applying 95% confidence interval.

Results and Discussion

Lipid Content

Lipid content in the analyzed fenugreek seeds ranged from 5.8 to 15.2% (Table 1). Seeds from varieties X92 and L3312 contained the highest amounts of lipids whereas in F17 and Quadro the lowest contents were observed (Table 1). The lipid contents in Tristar, X92 and L3312 were twice as high as in the other varieties analyzed. The lipid contents in the analyzed fenugreek varieties, were in agreement with the data reported by Hemavathy and Prabhakar [17] with the exceptions of Tristar, X92 and L3312.

Fatty Acids

Eighteen fatty acids were identified in fenugreek seed lipids (Table 1). Linoleic acid was the major one the contribution of which ranged from 45.0% in Amber to 47.5% in L3312. Linoleic acid was followed by linolenic, oleic, palmitic and stearic acids. The series of minor fatty acids was also observed with contributions below 1%. The same pattern of fatty acids was observed in all fenugreek samples analyzed (Table 1).

The saturated fatty acid (SAT) content ranged from 15.2% in X92 to 16.9% in Quadro. Palmitic acid was the major SAT and it contribution ranged from 9.8% in X92 to 11.2% in Quadro (Table 1). Monounsaturated Fatty Acids (MUFA) amounts ranged from 13.0 to 17.5% in fenugreek F75 and F96, respectively. Among MUFA, oleic acid was predominant and its content ranged from 12.4% in F75 to 17.0% in F96 (Table 1). PUFA were the dominant fatty

Table 1 Lipid content and fatty acid composition of fenugreek lipids *

	Fatty acids contribution (% w/w)								
	Tristar	X92	L3312	Amber	F17	F75	Quadro	F96	Temple
Fatty acids									
14:0	0.1 ± 0.01^{a}	0.1 ± 0.02^{ab}	0.1 ± 0.01^{b}	0.1 ± 0.01^{ab}	0.1 ± 0.02^{ab}	0.1 ± 0.01^{ab}	0.1 ± 0.01^{ab}	0.1 ± 0.01^{ab}	0.1 ± 0.02^{ab}
15:0	0.2 ± 0.02^{ab}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{ab}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{ab}	0.1 ± 0.02^{ab}	0.1 ± 0.02^{a}
16:0	11.2 ± 0.18^{e}	9.8 ± 0.18^{a}	10.7 ± 0.20^{cd}	10.3 ± 0.10^{bc}	10.9 ± 0.31^{de}	10.2 ± 0.20^{ab}	11.2 ± 0.24^{e}	10.4 ± 0.43^{bc}	9.9 ± 0.05^{a}
16:1	0.1 ± 0.02^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.02^{a}	0.2 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1 ± 0.02^{a}	0.1 ± 0.02^{a}
17:0	0.3 ± 0.02^{a}	0.3 ± 0.05^{a}	0.3 ± 0.08^{a}	0.3 ± 0.02^{a}	0.3 ± 0.10^{a}	0.3 ± 0.07^{a}	0.4 ± 0.15^{a}	0.3 ± 0.05^{a}	0.3 ± 0.05^{a}
17:1	0.2 ± 0.08^{a}	0.2 ± 0.04^{a}	0.2 ± 0.03^{a}	0.2 ± 0.05^{a}	0.2 ± 0.04^{a}	0.2 ± 0.05^{a}	0.2 ± 0.02^{a}	0.2 ± 0.03^{a}	0.2 ± 0.06^{a}
18:0	4.0 ± 0.15^{bc}	3.8 ± 0.15^{a}	3.9 ± 0.10^{ab}	4.1 ± 0.10^{bc}	4.1 ± 0.11^{bc}	3.8 ± 0.10^{a}	3.9 ± 0.10^{ab}	4.1 ± 0.11^{bc}	4.2 ± 0.15^{c}
18:1	16.2 ± 0.07^{de}	14.8 ± 0.16^{b}	16.3 ± 0.15^{de}	16.5 ± 0.09^{e}	15.4 ± 0.20^{bc}	12.6 ± 0.45^{a}	15.6 ± 0.47^{c}	17.1 ± 0.30^{f}	15.8 ± 0.60^{cd}
18:2	45.6 ± 0.29^{b}	46.7 ± 0.20^{c}	47.5 ± 0.19^{d}	45.1 ± 0.05^{a}	45.9 ± 0.40^{b}	47.2 ± 0.22^{cd}	47.3 ± 0.66^{cd}	45.9 ± 0.08^{b}	47.2 ± 0.15^{cd}
18:3 n-6	1.2 ± 0.09^{a}	1.2 ± 0.03^{a}	1.1 ± 0.05^{a}	1.2 ± 0.15^{a}	1.2 ± 0.12^{a}	1.2 ± 0.12^{a}	1.3 ± 0.18^{a}	1.2 ± 0.20^{a}	1.2 ± 0.11^{a}
18:3 n-3	19.6 ± 0.41^{bc}	21.6 ± 0.27^{e}	18.3 ± 0.30^{a}	20.7 ± 0.13^{d}	20.2 ± 0.22^{cd}	22.8 ± 0.64^{f}	18.5 ± 0.50^{a}	19.1 ± 0.10^{b}	19.8 ± 0.24^{c}
20:1	0.3 ± 0.05^{ab}	0.3 ± 0.03^{ab}	0.3 ± 0.03^{b}	0.3 ± 0.04^{a}	0.3 ± 0.03^{ab}	0.3 ± 0.04^{ab}	0.2 ± 0.03^{a}	0.3 ± 0.02^{ab}	0.3 ± 0.02^{a}
21:0	0.2 ± 0.03^{ab}	0.1 ± 0.02^{ab}	0.1 ± 0.02^{ab}	0.1 ± 0.04^{ab}	0.2 ± 0.02^{b}	0.1 ± 0.04^{ab}	0.1 ± 0.02^{ab}	0.1 ± 0.02^{ab}	0.1 ± 0.02^{a}
20:2	0.1 ± 0.01^{a}	0.1 ± 0.02^{a}	0.1 ± 0.03^{a}	0.1 ± 0.02^{a}	0.1 ± 0.01^{a}	0.1 ± 0.02^{a}	0.1 ± 0.01^{a}	0.1 ± 0.03^{a}	0.1 ± 0.02^{a}
22:0	0.5 ± 0.04^{a}	0.6 ± 0.04^{ab}	0.5 ± 0.10^{a}	0.6 ± 0.02^{ab}	0.7 ± 0.11^{b}	0.6 ± 0.15^{ab}	0.7 ± 0.07^{b}	0.5 ± 0.08^{a}	0.5 ± 0.05^{a}
22:1	0.2 ± 0.02^{a}	0.1 ± 0.01^{a}	0.1 ± 0.02^{a}	0.1 ± 0.04^{a}	0.2 ± 0.02^{a}	0.1 ± 0.04^{a}	0.1 ± 0.03^{a}	0.2 ± 0.02^{a}	0.1 ± 0.02^{a}
24:0	0.2 ± 0.08^{a}	0.2 ± 0.02^{a}	0.2 ± 0.02^{a}	0.2 ± 0.06^{a}	0.2 ± 0.05^{a}	0.2 ± 0.03^{a}	0.3 ± 0.05^{a}	0.2 ± 0.02^{a}	0.2 ± 0.01^{a}
Fatty acids groups									
SAT	16.8 ± 0.43^{d}	15.1 ± 0.33^{a}	16.1 ± 0.14^{c}	16.3 ± 0.20^{bc}	16.8 ± 0.19^{d}	15.7 ± 0.05^{abc}	16.9 ± 0.13^{d}	16.1 ± 0.61^{bc}	15.5 ± 0.20^{ab}
MUFA	16.9 ± 0.07^{d}	15.5 ± 0.15^{b}	16.1 ± 0.15^{d}	16.9 ± 0.04^{d}	15.7 ± 0.25^{bc}	13.1 ± 0.53^{b}	15.9 ± 0.43^{c}	17.5 ± 0.28^{f}	16.2 ± 0.70^{cd}
PUFA	66.4 ± 0.49^{ab}	69.4 ± 0.48^{e}	67.9 ± 0.26^{abc}	66.9 ± 0.23^{abc}	67.5 ± 0.37^{c}	71.4 ± 0.50^{f}	67.2 ± 0.38^{bc}	66.4 ± 0.39^{a}	68.3 ± 0.50^{d}
n-6/n-3	2.4 ± 0.05^{cd}	2.2 ± 0.02^{b}	2.7 ± 0.05^{e}	2.2 ± 0.01^{b}	2.4 ± 0.04^{c}	2.1 ± 0.07^{a}	2.6 ± 0.11^{e}	2.5 ± 0.00^{d}	2.6 ± 0.02^{d}
Lipids amount	12.2 ± 1.6^{c}	15.0 ± 1.7^{d}	15.2 ± 1.1^{d}	8.4 ± 0.8^{b}	5.8 ± 0.6^{a}	6.0 ± 0.6^{a}	5.8 ± 0.6^{a}	8.6 ± 0.9^{b}	6.9 ± 0.7^{ab}

Lipid amounts are expressed as percentages of the seed; fatty acid contributions expressed as weight percentages in lipids

SAT Saturated, *MUFA* Monounsaturated, *PUFA* Polyunsaturated fatty acids

Means within a column with different superscript letters are significantly different ($P < 0.05$)

* Values are reported as means ± SD of three replicate analyses ($n = 3$)

acids and their contribution ranged from 66.5% in F96 to 71.4% in F75. Among PUFA, gamma linolenic acid (GLA) contribution was at a similar level of 1.2% in L3312 and Quadro, while alpha linolenic acid (ALA) ranged from 18.3% in L3312 to 22.8% in F75 (Table 1). All fenugreek seed oils contained large amounts of linolenic acids (Table 1).

Our results for fatty acid composition are different from those reported by Hemavathy and Prabhakar [17], however, these authors used fenugreek from India and applied analytical techniques which may affect fatty acid composition due to limited separation. These authors found the following composition of fatty acids in fenugreek oil: 52.6% oleic, 40.0% linoleic, and 0.6% linolenic acid. When more efficient separation techniques were applied by Sulieman et al. [18], similar results were achieved to the data presented in this paper. These authors found the following composition of fatty acids in Sudan fenugreek: 43.2% linoleic, 22% linolenic, 16.7% oleic, 11% palmitic, and 4.5% stearic acid (Table 1). Similarly, El-Sebaiy and El-Mahdy [19] reported almost the same fatty acid composition in Egyptian fenugreek, however, found a higher content of erucic acid (9.7%). Elevated amounts of the latter fatty acid can be explained by differences in growing conditions, different varieties and the analytical system applied which may lead to incomplete separation of some fatty acids.

The ratios of n-6 to n-3 fatty acids in fenugreek seed lipids were between 2.1 (F75) and 2.7 (L3312). Results indicate that fenugreek seed lipids are a good source of PUFA and it has been found that it is one of the oils rich in ALA content (Table 1). Fatty acid intake, especially ALA, should be increased in the western diet to alleviate its deficiency. When the ratio of n-6 to n-3 is higher in the diet it lowers the synthesis of linolenic eicosanoids, and may stimulate inflammation among other processes [20]. A lower ratio of n-6/n-3 is desirable to reduce the risk of some chronic diseases, while the optimal ratio of n-6 to n-3 is presumed to be between 1 and 5 [21]. This ratio was reported as 1.8 by El-Sebaiy and El-Mahdy [18]. In the current western diet the ratio of n-6 to n-3 is in the range of 10 to 20, fenugreek seed lipids could be a useful ingredient to control it in the diet [22, 23].

Tocochromanols

Table 2 shows the composition and content of tocochromanols detected in fenugreek seed lipids. Among the analyzed samples, F17 contained the highest amount of total tocochromanols (1033 mg kg) while X92 contained the lowest (726 mg/kg). In the fenugreek seed oils, over 95% of the total tocochromanols content was composed of α-tocopherol and plastochromanol-8. It has been found that fenugreek seed lipids are an excellent source of α-tocopherol. α-Tocopherol was the predominant isomer found and it constituted over 84% the total tocochromanols (Table 2). The amounts of α-tocopherol ranged from 620 mg/kg in X92 to 910 mg/kg in F17 (Table 2). The level of α-tocopherol in fenugreek seed lipids was higher than those reported for commodity oils such as corn, cottonseed, palm, safflower, soybean, sunflower, olive, sesame [24]. This plant oil may be considered as a good source of vitamin E together with the excellent composition of fatty acids.

Plastochromanol-8, derivative of γ-tocotrienol, was found in fenugreek seed lipid, with amounts ranging from 63 mg/kg in F75 to 121 mg/kg in Amber. Other tocopherol isomers, β, γ, and δ, were found in small amounts, below 20 mg/kg lipids.

The high level of α-tocopherol and plastochromanol-8 may contribute to the health benefits offered by fenugreek seeds. It has been claimed that α-tocopherol protects cell membranes from oxidation by transferring lipid radicals into unreactive compounds [25].

To the best of our knowledge this is the first report of the tocopherols composition in fenugreek seed lipids and direct comparison is not possible.

Table 2 Tocopherol and tocotrienol composition of fenugreek lipids (mg kg^{-1} of lipids) *

Tocochromanols	Tristar	X92	L3312	Amber	F17	F75	Quadro	F96	Temple
α-Tocopherol	794 ± 8^{e}	620 ± 3^{a}	777 ± 1^{b}	768 ± 1^{d}	910 ± 2^{g}	762 ± 1^{c}	824 ± 1^{f}	736 ± 6^{b}	799 ± 1^{e}
β-Tocopherol	15 ± 1^{c}	12 ± 1^{a}	15 ± 1^{bc}	11 ± 1^{a}	16 ± 1^{c}	13 ± 1^{a}	12 ± 1^{a}	13 ± 1^{ab}	13 ± 1^{a}
γ-Tocopherol	18 ± 1^{d}	9 ± 1^{a}	17 ± 1^{c}	15 ± 1^{b}	15 ± 1^{b}	15 ± 1^{b}	18 ± 1^{d}	20 ± 1^{f}	19 ± 1^{e}
δ-Tocopherol	2 ± 1^{c}	ND	1 ± 1^{b}	ND	2 ± 1^{c}	ND	1 ± 1^{b}	2 ± 2^{c}	1 ± 1^{b}
Plastochromanol-8	99 ± 1^{f}	85 ± 1^{c}	91 ± 1^{d}	121 ± 1^{h}	90 ± 1^{d}	63 ± 1^{a}	102 ± 1^{g}	94 ± 1^{e}	79 ± 1^{ab}
Total amount	928 ± 8^{f}	726 ± 4^{a}	900 ± 1^{d}	915 ± 1^{e}	1033 ± 3^{h}	854 ± 1^{b}	957 ± 1^{g}	866 ± 9^{c}	911 ± 1^{e}

ND Not detected

Means within a column with different superscript letters are significantly different ($P < 0.05$)

* Values are reported as means ± SD of three replicate analyses ($n = 3$)

Table 3 TAG composition of fenugreek lipids (area %)*

TAG	ECN	Tristar	X92	L3312	Amber	F17	F75	Quadro	F96	Temple
LnLnLn	36	0.4 ± 0.1^{bc}	1.0 ± 0.1^{e}	0.5 ± 0.1^{bc}	0.6 ± 0.1^{d}	0.4 ± 0.1^{ab}	1.1 ± 0.1^{e}	0.3 ± 0.1^{a}	0.4 ± 0.1^{ab}	0.6 ± 0.1^{cd}
LnLnL	38	4.7 ± 0.2^{c}	5.8 ± 0.3^{e}	4.3 ± 0.2^{bc}	6.1 ± 0.3^{e}	4.2 ± 0.2^{b}	7.6 ± 0.4^{f}	3.5 ± 0.2^{a}	3.9 ± 0.2^{ab}	5.2 ± 0.3^{d}
LLLn	40	7.2 ± 0.7^{cd}	9.2 ± 0.8^{e}	7.7 ± 0.7^{d}	6.5 ± 0.6^{c}	6.9 ± 0.6^{cd}	9.6 ± 0.9^{e}	4.9 ± 0.4^{b}	3.2 ± 0.3^{a}	7.5 ± 0.7^{cd}
LnLnO	40	9.6 ± 1.2^{ab}	7.8 ± 0.9^{a}	10.2 ± 1.2^{abc}	17.7 ± 2.1^{e}	10.5 ± 1.3^{abc}	11.6 ± 1.4^{bcd}	12.6 ± 1.5^{cd}	14.2 ± 1.7^{d}	14.2 ± 1.7^{d}
LnLnP	40	1.1 ± 0.2^{bcd}	1.1 ± 0.2^{cd}	0.9 ± 0.2^{bc}	0.3 ± 0.1^{a}	0.8 ± 0.2^{bc}	1.4 ± 0.3^{d}	0.7 ± 0.1^{b}	0.8 ± 0.2^{bc}	1.0 ± 0.2^{bc}
LLL	42	18.0 ± 1.4^{cd}	17.7 ± 1.4^{c}	18.5 ± 1.5^{cd}	12.9 ± 1.0^{a}	17.3 ± 1.4^{c}	19.1 ± 1.5^{cd}	16.5 ± 1.3^{bc}	14.3 ± 1.1^{ab}	20.5 ± 1.6^{d}
LnLO	42	6.9 ± 0.7^{abc}	9.6 ± 1.0^{e}	5.9 ± 0.6^{ab}	8.2 ± 0.8^{cd}	5.7 ± 0.6^{a}	7.3 ± 0.7^{bc}	7.2 ± 0.7^{bc}	9.3 ± 0.9^{de}	6.3 ± 0.6^{ab}
PLLn	42	7.3 ± 1.1^{bc}	4.3 ± 0.7^{a}	6.7 ± 1.0^{b}	8.4 ± 1.3^{bcd}	10.5 ± 1.6^{d}	7.6 ± 1.2^{bc}	9.5 ± 1.4^{cd}	8.9 ± 1.3^{bcd}	8.6 ± 1.3^{bcd}
OLL	44	9.5 ± 0.5^{cd}	10.6 ± 0.5^{e}	9.1 ± 0.5^{c}	7.0 ± 0.4^{a}	8.9 ± 0.5^{bc}	6.9 ± 0.3^{a}	10.2 ± 0.5^{e}	10.6 ± 0.5^{de}	8.2 ± 0.4^{b}
PLL	44	19.4 ± 1.2^{c}	16.6 ± 1.0^{b}	16.6 ± 1.0^{b}	14.0 ± 0.8^{a}	20.4 ± 1.2^{c}	17.3 ± 1.0^{b}	20.2 ± 1.2^{c}	19.2 ± 1.2^{c}	15.9 ± 1.0^{b}
OOL	44	1.8 ± 0.2^{d}	2.2 ± 0.2^{e}	1.7 ± 0.2^{d}	1.6 ± 0.2^{cd}	1.3 ± 0.1^{bc}	0.9 ± 0.1^{a}	1.4 ± 0.1^{bc}	1.7 ± 0.2^{d}	1.2 ± 0.1^{b}
PLO	46	9.0 ± 0.8^{bc}	8.9 ± 0.8^{bc}	11.6 ± 1.0^{d}	10.2 ± 0.9^{cd}	8.5 ± 0.8^{b}	5.7 ± 0.5^{a}	8.2 ± 0.7^{b}	8.6 ± 0.8^{b}	9.1 ± 0.8^{bc}
PLP	46	0.8 ± 0.2^{ab}	0.7 ± 0.1^{a}	1.2 ± 0.2^{c}	0.9 ± 0.2^{abc}	1.0 ± 0.2^{abc}	0.7 ± 0.1^{a}	1.1 ± 0.2^{bc}	0.9 ± 0.2^{abc}	0.9 ± 0.2^{abc}
OOO	48	2.3 ± 0.1^{e}	2.7 ± 0.1^{f}	3.1 ± 0.2^{g}	3.6 ± 0.2^{h}	1.8 ± 0.1^{c}	1.1 ± 0.1^{b}	1.8 ± 0.1^{c}	2.1 ± 0.1^{d}	ND
POO	48	0.7 ± 0.1^{b}	0.7 ± 0.1^{b}	1.1 ± 0.1^{c}	0.6 ± 0.1^{b}	1.0 ± 0.1^{c}	0.7 ± 0.1^{b}	1.0 ± 0.1^{c}	0.9 ± 0.1^{c}	ND
SOO	50	0.4 ± 0.1^{de}	0.5 ± 0.1^{e}	0.3 ± 0.1^{b}	0.3 ± 0.1^{c}	0.4 ± 0.1^{cd}	0.2 ± 0.1^{b}	0.4 ± 0.1^{cd}	0.4 ± 0.1^{cd}	ND
POS	50	0.1 ± 0.1^{b}	ND	ND	ND	0.2 ± 0.1^{d}	ND	ND	0.2 ± 0.1^{c}	ND

ND Not detected, Fatty acids; *Ln* linolenic, *L* linoleic, *O* oleic, *P* palmitic *S,* stearic

Means within a column with different superscript letters are significantly different ($P < 0.05$)

* Values are reported as means ± SD of three replicate analyses ($n = 3$)

Triacylglyceride Composition

The distribution of the TAG in Table 3 is presented. The distribution patterns of the molecular species of TAG were very similar in all analysed fenugreek genotypes. The TAG predominantly consisted of the triunsaturated (UUU: 56.9–66.5%) and diunsaturated (SUU: 32.2–41.6%) components, similarly to many commodity vegetable oils [26, 27]. Trisaturated (SSS) TAG were present below 0.1% and are not included in the Table 3. Content of TAG composed of tri-PUFA ranged between 21.7% in F96 and 33.8% in Temple. The TAG with ECN (Equivalent Carbon Number) of 42 were predominant (29.4–35.5%), followed by 44 (22.6–31.8%), 40 (17.9–24.5%), 46 (6.5–12.8%), 48 (0–4.2%), and 50 (0–0.5%). ECN is affected by the fatty acid unsaturation and for the fenugreek seed oil majority of TAG were in the range of 38–46, whereas the most of the commodity oils have predominantly triacylglycerides with ECN in the range of 44–50 [26].

Among individual TAGs, LLL (12.9–20.5%) and PLL (14.0–20.4%) formed majority followed by LnLnO (7.8–17.7%), PLO (5.7–11.6%), OLL (6.9–10.6%), LLLn (3.2–9.6%), and LnLnL (3.5–7.6%) (Table 3). Genotype had a significant effect on the TAG composition, which is directly related to the differences in fatty acid composition. In the terms of uppermost TAG, fenugreek seed lipids are similar to safflower, thistle, walnut, grapeseed, corn, and sunflower oils [28, 29].

To the best of our knowledge, there are no published data on the quantification of TAG in fenugreek seed oil and a direct comparison is not possible.

Phytosterols

Among 22 sterols found in fenugreek lipids only one minor compound was not possible to identify (Table 4). All results represent the amount of sterols in fenugreek seed lipids. The genotype had a significant effect on the amount of individual sterols. It has been established that Quadro contains the highest amount of total sterols (18,833 mg/kg), while Temple has the lowest amount at 14,203 mg/kg.

Amongst individual sterols, β-sitosterol was the major component in all samples varying from 5,989 mg/kg (31.8%) in Quatro to 6,684 mg/kg (43.2%) in Tristar. Followed by campesterol and cycloartenol, and these three major sterols make up 56% in Quadro and 72% in Tristar of the total amounts of all sterols. Other sterols followed with the following contribution: cholesterol ranged from 270 mg/kg (1.9%) in Temple to 1,281 mg/kg (6.8%) in Quadro, stigmasterol from 307 mg/kg (1.8%) in X92 to 1032 (5.7%) in L3312, sitostanol from 31 mg/kg (0.2%) in Tristar to 272 mg/kg (1.5%) in L3312, Δ^5-avenasterol from 681 mg/kg (4.4%) in Tristar to 1,243 mg/kg (6.9%) in F96, Δ^7-stigmasterol from 200 mg/kg (1.1%) in F75 to 1,014 mg/kg (5.6%) L3312, Δ^7- avenasterol not detected in two varieties up to 199 mg/kg (1.1%) in L3312, and

Table 4 Composition of sterols identified in fenugreek seed lipids (%)

Sterol	Sterols composition (%)								
	Tristar	X92	L3312	Amber	F17	F75	Quadro	F96	Temple
Cholesterol	3.2	3.2	4.0	5.0	4.4	6.6	6.8	4.2	1.9
14α-Methyl-5α-cholest-8-en-3β-ol	1.2	1.2	1.4	1.9	1.1	1.2	1.8	1.9	1.2
Lathosterol	1.7	1.9	0.9	2.6	0.9	1.0	1.3	1.6	1.8
14α-Methyl-5α-cholest-8-en-3β-ol	1.3	1.4	0.6	ND	ND	1.8	2.0	1.5	1.9
Coprostanol	1.2	1.2	1.1	ND	ND	1.6	1.8	1.5	2.0
Pollinastanol	1.1	1.0	0.6	3.4	1.7	1.6	0.8	1.1	1.0
Campesterol	20.5	11.2	11.0	12.2	8.7	12.3	9.7	9.9	8.7
24-Methylene pollinastanol	1.3	1.5	1.2	0.8	1.9	1.8	1.3	1.1	1.0
4.4′-Dimethyl-9β,19-cyclo-5α-cholestan-3β-ol	1.1	1.0	0.7	1.1	0.7	1.0	2.0	1.1	1.3
Stigmasterol	6.0	1.8	5.7	3.4	2.5	2.5	5.5	2.2	2.5
β-Sitosterol	43.2	49.6	41.7	35.3	44.4	49.2	31.8	39.3	47.9
Sitostanol	0.2	1.0	1.5	0.6	1.0	0.9	0.7	0.7	0.6
Δ^5-Avenasterol	4.4	6.7	5.9	5.8	6.1	5.3	5.5	6.9	5.7
4α-Methyl-5α-stigmasta-7,24-dien-3β-ol	ND	2.4	1.9	ND	3.3	0.8	2.9	3.2	2.5
Δ^7-Stigmasterol	2.6	3.5	5.6	5.8	3.0	1.1	5.2	5.7	4.2
Cycloartenol	8.2	7.9	9.7	13.6	15.0	6.1	14.8	11.0	10.5
Cycloeucalenol	0.8	0.9	1.1	0.9	1.4	1.4	0.9	1.1	1.0
9,19-Cyclolanost-7-en-3-ol	ND	0	0.7	1.6	1.8	1.2	1.4	1.5	1.8
α-Sitosterol	2.0	2.6	2.2	3.8	1.0	1.0	1.5	2.6	ND
Δ^7-Avenasterol	ND	ND	1.1	0.6	1.1	0.5	0.8	1.9	0.7
24-Methylenecycloartanol	ND	ND	0.5	1.0	ND	0.6	0.5	ND	0.5
Unknown	ND	ND	0.9	0.6	ND	0.5	1.0	ND	1.3
Total sterol content (mg/kg lipids)	15,473	17,058	18,113	15,950	16,543	18,168	18,833	18,016	14,203

Unknown Minor unidentified sterol like compound

ND Not detected

α-sitosterol not found in Temple and up to 606 mg/kg (3.8%) in Amber. Among sterols, we observed the presence of cholesterol, the sterol mainly present in lipids of animal origin. The highest amount of cholesterol was found in Quadro at 1,281 mg/kg (6.8%), whereas the lowest in Temple at 270 mg/kg (1.9%), however, its contribution was below 7% of the total amounts of sterols.

When compared to other commodity oils such as rapeseed, safflower, sesame, cottonseed and sunflower, fenugreek seed lipids had a similar distribution of sterols, characterized by β-sitosterol as the major component followed by campesterol. It has been established that fenugreek seed lipids are one of the richest sterol sources among commodity oils such as extra virgin olive oil (3,280 mg/kg), crude borage oil (5,680 mg/kg), expeller pressed sunflower (6,920 mg/kg), canola (13,260 mg/kg), corn 15,320 mg/kg), and crude evening primrose (21,940 mg/kg) [30]. In commodity oils, the cholesterol contribution is usually below 1% of the total amount of sterols, significantly lower than detected in fenugreek oil [30]. It has been recognized that plant sterols could reduce plasma cholesterol levels in humans [31]. The mechanism of cholesterol reduction in the presence of phytosterols is based on the blocked absorption of it in the digestive tract. Since the amount of cholesterol in fenugreek is low and in the presence of excessive amounts of phytosterols it can be expected that it absorption will be minimal and the positive effect of phytosterols will overcome it [31]. Furthermore, fenugreek incorporated in the diet will be below or at the same amount of a typical portion of legumes, it means that the content of cholesterol available for absorption will be below the level of this component present in a typical egg. Fenugreek seed lipids could be helpful in decreasing the blood cholesterol levels when they are a part of the diet.

To the best of our knowledge, there are no published studies on the qualitative and quantitative determination of sterols of fenugreek seed oil, limiting the possibility of comparing data.

Conclusion

The results of the study demonstrate that fenugreek seed lipids contain reasonable amounts of nutraceuticals such as

α-tocopherol, ALA and sterols. Lipid content from this seed has a good ratio of n-6 to n-3 fatty acids which can be beneficial in the human diet. Thus, good quality seed lipids together with the other healthy components in them makes fenugreek seeds a potential source of nutraceuticals. Utilization of the seed oil in food products may enhance the profitability of seed production and the processing industries, and mainly may be of benefit to consumers.

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