PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 14 (12): 907-912, 2015
ISSN 1680-5194

Content of Crude Protein, Fixed Oil and Lecithin in Sudanese Seed Accessions of Fenugreek (*Trigonella foenum-graecum* L.)

Nour Ahmed Osman Bashir[1], Salah Ahmed-Ali Elhussein[2] and Ahmed Abdurrahman Taha[3]
[1]Department of Chemistry, University College at Alwajh, University of Tabuk, Tabuk-71491, Saudi Arabia
[2]National Oilseed Processing Research Institute, University of Gezira, Wad-Medani, Sudan
[3]Department of Chemistry, Faculty of Science, University of Bahrain, Bahrain

Abstract: We have recently reported on the variability of α and β-sapogenins in several Sudanese accessions of fenugreek seeds. Results were discussed in terms of the importance of these chemical constituents as potential raw materials for steroid drug production and their reported biological activities. This report deals with variability in protein and seed fixed oil content (particularly its lecithin fraction) in 12 selected Sudanese fenugreek seed accessions. The protein content was high, compared to other pulses and varied between 35.2 and 42.2%, on an oven-dry basis. However, the oil content was low, below 10%, in accord with published reports. The oil fatty acid composition of one accession (A1) was dominated by 18:1, 18:2, 18:0 and 16:0. Linolenic acid (18:3) was present at low relative level (below 1.0%), in contrast to some other reports. The lecithin fraction, prepared from the seed oil, amounted to a high value of 3.5% of the seed oil weight of accession A1 and contained the phospholipids phosphatidic acid, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, in addition to appreciable amounts of phosphatidylglycerol. The major fatty acids of the crude lecithin fraction were 18:2, 18:1 and 16:0, in addition to palmitoleic acid (16:1). The latter is likely associated with plastidic phosphatidylglycerol.

Key words: Fenugreek accessions, sapogenins, fixed oil, protein, lecithin, fatty acids, palmitoleic acid

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume herb cultivated in several parts of the world for its edible seeds and as a forage crop. The pungent and slightly bitter seeds constitute an important commodity in the international spice trade market. The seeds are consumed as a food, a nutraceutical, a food flavor and color as well as a folk medicine.

Fenugreek seeds contain several important chemical constituents including essential oils, phenolics, mucilages, fixed oil and proteins, among others. Steroidal sapogenins of the seeds are of interest to the pharmaceutical industry as precursors for the partial synthesis of steroid drugs. A number of potentially useful biological activities were attributed to the seeds or their isolates, particularly their saponin. Examples include uses as anticancer (Amin *et al*., 2005; Chiang *et al*., 2007; Shabbeer *et al*., 2009; Lee *et al*., 2007; Raju and Bird, 2007; Raju and Mehta, 2009), antidiabetic (Hamza *et al*., 2012), anti-obesity (Valette *et al*., 1984) and hypo-cholesterolaemic (Petit *et al*., 1995) agents.

Fenugreek, as a legume crop, is gaining importance as human needs for more proteins increase (Duranti, 2006). The plant was also reported to be quite suitable for cultivation as a forage crop (Basu *et al*., 2009).

Sudanese fenugreek seeds offered in local retail markets were observed to display marked apparent morphological variability as far as seed shape, size and seed color are concerned and we wondered whether this variability is reflected on their economic chemical constituents. We have recently reported on the high potential of the seeds as a commercial source of steroidal sapogenins and the remarkable variability in these constituents among 30 indigenous seed accessions of fenugreek (Osman *et al*., 2014). A simple, selective infra-red spectrophotometric method was used which enabled the quantification of both 25α and 25β-epimers as well as total seed sapogenin.

The present report is concerned with fenugreek seed constituents other than sapogenins, which promise economic potential, namely, seed protein, fixed oil and its component lecithin.

The term *lecithin* refers to the fraction commercially obtained by degumming crude vegetable oils. Lecithins are important natural emulsifiers that find a number of applications in medicine as well as in the food and other industries (Scholfield, 1981). The main components of lecithin are phospholipids. Minor non-phospholipid constituents include steroid derivatives, glycolipids and pigments (Cherry and Kramer, 1989). Following their extraction, lecithins could be modified, to improve their functional (emulsifying) properties, by physical, chemical or biochemical (enzymic) methods (Garcia *et al*., 2008). Soybean is so far the most important commercial

Corresponding Author: Salah A.A. Elhussein, National Oilseed Processing Research Institute, University of Gezira, Wad-Medani, Sudan

source of lecithin, however, new lecithin plant sources have for long been receiving considerable research attention (Cherry and Kramer, 1989).

For the purpose of this study, 12 seed accessions were selected from among fenugreek seed accessions of our previous study (Osman *et al*., 2014) to represent different morphological characteristics and different sapogenin content.

MATERIALS AND METHODS

Seed material: Fenugreek seed accessions were procured from local markets or from the Gene Bank of the Agricultural Research Corporation, Sudan Government, Wad-medani, as previously stated (Osman *et al*., 2014). Data on the twelve seed accessions selected for this study are shown in Table 1, keeping the accession reference code as in the previous report (Osman *et al*., 2014).

Chemicals: All solvents and chemicals used were of analytical laboratory grade. Phospholipid standards (phosphatidic acid, phosphatidyl - choline, - inositol, - ethanolamine and phosphatidyl - glycerol) were obtained from Sigma Aldrich Co. (USA).

Fixed oil and crude protein determination: Fenugreek seed fixed oil was prepared by extracting powdered seed material in a soxhlet apparatus for 6 h, using n-hexane as solvent. The seed residue left was used for crude protein determination by the Kjeldahl sulfuric acid digestion method, multiplying the figure obtained for total nitrogen content by a factor of 6.25 (AOAC, 1990). Both fixed oil and crude protein contents were expressed on an oven-dry seed-weight basis.

Isolation of the lecithin fraction from fenugreek seed oil: The method used was that outlined by Cherry and Kramer (1989), Wiedermann (1981) and Eshratabadi *et al*. (2008) which consisted of mixing the oil with a little amount of water to hydrate the phospholipids, rendering them insoluble in the oil, followed by separating the precipitate (lecithin). Typically, to 5 ml of fenugreek oil 0.1 ml of water (2% of oil volume) was added. The mixture was heated on a water bath at 75°C with thorough stirring (magnetic rod) for 15 min. The sedimented lecithin fraction was separated from the rest of the oil by centrifugation (3000 rpm: 15 min) and carefully washed with acetone which was evaporated off before further analyses.

Thin-layer chromatography (TLC): Plates (20 x 20 cm) precoated with silica gel G 60, 0.2 mm thickness, Merck Co. (Germany), were used for 1 and 2-dimensional separations. Phospholipid spots or bands were detected on TLC by spraying with Dittmer's (Molybdenum blue) reagent (Dittmer and Lester, 1964). The reagent consisted of solutions A and B. Solution A was prepared by boiling 4.01 g MoO3 in 100 ml 12.5 25 N sulfuric acid for 3-4 h until the oxide was completely dissolved, the yellow solution left to cool overnight at room temperature (now blue in color). Solution B was prepared by boiling 0.18 g of molybdenum powder and 50 ml of solution A for 15 min followed by cooling and decanting from the remaining residue. The final spray reagent (light to dark green in color) was prepared by diluting an equal volumes of solutions A and B with 2 volumes of water. Phospholipids appear as intense blue spots on a white or light blue background.

Gas liquid chromatography: To prepare fatty acid methyl esters, fenugreek oil or phospholipid samples were dissolved in the methylation mixture methanol/benzene/ conc. Sulfuric acid (20: 10: 1) in Teflon capped sample tubes and heated at 75-80°C for 90 min. After allowing to cool, 4 ml of anhydrous sodium bicarbonate solution (5%) were added and the mixture twice extracted with hexane. Anhydrous sodium sulfate granules were added to the combined hexane layers containing the methyl esters before filtration and subsequent GLC analysis. A Varian Instrument Group Series 00-997140-01 gas chromatograph equipped with a computing integrator was used for the analysis of fatty acid methyl esters. Analysis was carried out isothermally at column oven temperature of 170°C, column inlet and detector oven temperatures of 180°C and the carrier gas flow rate was 50 ml/min.

RESULTS AND DISCUSSION

Protein content of the twelve accessions of fenugreek seeds: Table 1 shows values obtained for crude protein, expressed as a percentage of the oven-dry seed weight. Also included, for comparative reasons, are data on dominant outer seed coat color and total seed sapogenin content of each accession, reproduced from our previous report (Osman *et al*., 2014). Crude protein values ranged between 35.2% (in accession A10) and 42.2% (accession A21). This qualifies fenugreek seed as a rich source of proteins, taking into account that the protein content of the major legume seeds in current world cultivation were reported to range in average between 15 and 40% (Monti and Grillo, 1983). However, variation in protein content among the twelve Sudanese accessions was not as marked as that reported (Monti and Grillo, 1983) for pea (15.5-39.7%), faba bean (22.0-37.0%), lupins (17.0-38.7%) and other legume seeds (Monti and Grillo, 1983).

Fixed oil content of 12 fenugreek seed accessions: Table 1 also shows the oil content, on an oven-dry seed basis, of the twelve selected fenugreek seed accessions as well as the corresponding oil refractive index. The oil content values ranged between 4.9% (in accession A16) to 7.8% (in accession A1). A number of

Table 1: Content, on an oven-dry basis, of fixed oil, oil refractive index, crude protein and total steroidal sapogenin of 12 Sudanese seed accessions of fenugreek

Seed protein content (% dry wt.)	Oil refractive index (30°C)	Seed oil content (% dry wt.)	Total seed sapogenin (% of seed dry wt.)	100-seed wt.	Dominant seed-coat colour*	Accession code
35.5	1.4751	7.8	3.0	2.12	GB	A1
37.6	1.4747	5.1	2.0	0.78	GB	A2
36.6	1.4771	7.0	1.7	1.30	C	A5
35.2	1.4748	6.3	2.5	1.05	GB	A10
37.6	1.4747	6.6	1.5	1.32	YG	A14
35.6	1.4748	4.9	1.2	1.15	YB	A16
42.0	1.4771	6.8	2.4	1.47	GB	A18
39.1	1.4765	5.6	2.3	1.23	YG	A20
42.2	1.4758	7.0	1.1	2.00	B	A21
39.1	1.4769	6.1	2.7	2.04	YB	A24
35.4	1.4767	5.8	2.4	1.01	GB	A27
35.4	1.4740	6.0	2.4	1.88	YG	A30

G: Green, B: Brown, Y: Yellow, C: Cream, GB: Greenish-brown, etc

Table 2: Some physic-chemical characteristics of the fixed oil of seed accession A1

Oil characteristic	Value
Oil colour	yellow
Acid value	4.63
Free fatty acids (%), as oleic acid	2.33
Unsaponifiable matter, % w/w	4.66
Saponification value	196.20
Iodine value	86.30

Table 3: Fatty acid composition of fenugreek Seed oil (seed accession A1)

Fatty acid	Percent
Myristic (14:0)	0.9
Palmitic (16:0)	12.8
Stearic (18:0)	21.5
Oleic (18:1)	34.0
Linoleic (18:2)	30.0
Linolenic acid (18:3)	0.8

Table 4: Some physic-chemical characteristics of fenugreek lecithin (accession no. A1)

Value/Description	Parameter
seed oil of (%)	3.5
Consistency	Gummy
Colour	Light brown
Free fatty acids, (%)	3.6
Iodine value	166.0

Table 5: Fatty acid composition of the lecithin fraction isolated from the oil of fenugreek seed accession A1

Per cent	Fatty acid
Palmitic (16:0)	29.1
Palmitoleic (16:1)	5.1
Stearic (18:0)	4.0
Oleic (18:1)	22.0
Linoleic (18:2)	39.0
Linolenic acid (18:3)	0.8

reports confirm the finding that fenugreek seeds contain a relatively low fixed oil value of below 10% (Rao and Sharma, 1987; Hemavathy and Prabhakar, 1989), for single accessions analyzed. One detailed study involving 46 genotypes of Indian fenugreek reported a range of 3.25-6.88% for the seed fixed oil. On the other hand, a relatively high value of 15.2% was reported for the seed oil of one Canadian fenugreek genotype (Ciftsi *et al*., 2011). In fact these authors found that three out of nine fenugreek genotypes studied had a seed lipid content of more than 10%. However, these authors used the mixed solvent (chloroform/methanol, 2:1 v/v) for oil extraction, a solvent more polar than the usually used nonpolar solvents such as hexane.

The frequently reported or claimed health beneficial effects of fenugreek seeds justify further work on the oil. Indeed a recent study (Al-Oqail *et al*., 2013) reported that isolated fenugreek seed oil had *in vitro* anticancer activity against three human cancer cell lines.

Physico-chemical parameters of fixed oil of accession A1 are given in Table 2 and the fatty acid composition of the oil is shown in Table 3. Oleic acid is the predominant acid (34.04%) followed by linoleic (30.62%), stearic (21.75%) and palmitic acid (13.59%). This fatty acid composition is different from compositions reported for fenugreek seed oils by Ciftsi *et al*. (2011), for Canadian seeds and by El-Sebaiy (1983), for an Egyptian seed, especially with respect to the relative proportion of linolenic acid. While the oil of the seed accession we studied contained less than 1.0% of linolenic acid (Table 3), this trienoic acid amounted to 12-21% of the oils of the nine Canadian-grown genotypes (Ciftsi *et al*., 2011) and to 23% of the total fatty acids of the seed oil of the Egyptian study (El-Sebaiy, 1983). However, our results agree closely with those of Hemavathy and Prabhakar (1989), who reported a similar value of less than 1.0% for linolenic acid in the seed oil of Indian fenugreek. These differences could be genetical or may be caused by environmental conditions prevailing during the growth of fenugreek plants, particularly temperature.

Fenugreek seed lecithin: The lecithin fraction was separated from the seed oil of accession A1 by hydration

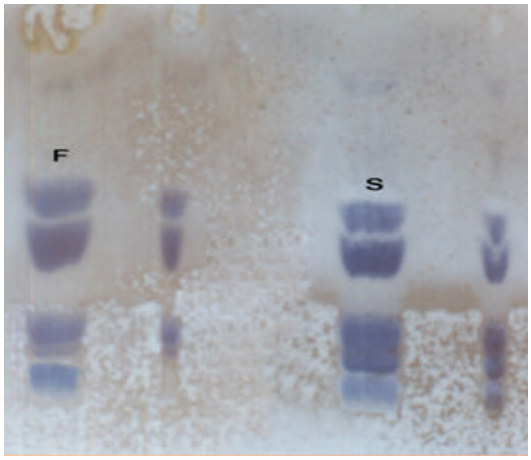

Fig. 1: One-dimensional TLC separation of components of the lecithin fraction prepared from the fixed oil of seed accession A1 of fenugreek (F) and of commercial soybean lecithin (S). TLC solvent: chloroform/methanol/7N ammonium hydroxide (130: 60: 8). Detection: Dittmer's spray reagent

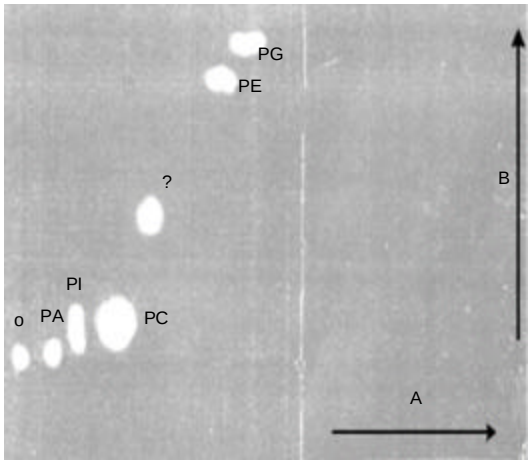

Fig. 2: Two-dimensional TLC separation of phospholipids of fenugreek lecithin (seed accession A1). Solvents: A = chloroform/ methanol/7 N ammonium hydroxide (130: 60: 8); B = chloroform/methanol/acetic acid/ water, (170: 25: 25: 6). Detection: Dittmer's reagent. Abbreviations: O = TLC origin, PA = Phosphatidic acid, PI = phosphatidyl inositol, PC = phosphatidyl choline, PE = Phosphatidyl ethanolamine, PG = phosphatidyl glycerol, ? = unidentified phopholipid. The photograph was taken 24 h after spraying with Dittmers reagent, when the originally intense blue phospholipid spots had changed color to white leaving a light blue background

of the oil followed by centrifugation. Table 4 shows some of the parameters determined for this crude lecithin fraction. Fig. 1 shows a one-dimensional TLC separation of the phospholipids of fenugreek lecithin compared with a commercial lecithin sample procured from a well-known local food manufacturing company. The general phospholipid pattern is not much different comparing the two lecithin samples. However, fenugreek oil seems to contain more neutral lipid (bands near the TLC solvent front in Fig. 1). Two-dimensional TLC separation of fenugreek accession A1 lecithin (Fig. 2) demonstrated the presence of phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphtidylethanolamine (PE), phosphatidylglycerol (PG) and an unidentified phospholipid (marked with ?). Authentic standards of PA, PI, PC. PE and PG were run separately in the 2-D Solvent system for identification purpose (results not given). A comparable two-dimensional TLC separation pattern was reported for sunflower phospholipids (Hollo *et al*., 1993). Glycolipids and other lecithin components devoid of phosphorus atoms are, of course, not detectable by the TLC spray reagent used (Dittmer's reagent). Generally, the phospholipid pattern of fenugreek lecithin was similar to that reported for lecithins of soybean, corn, sunflower and other seed oils (Scholfield, 1981; Cherry and Kramer, 1989). However, lysophospholipids and phosphatidylserine (PS) reported to occur as a minor constituent of soybean and corn lecithins (Cherry and Kramer, 1989) were not positively detected for fenugreek. On the other hand, PG reported to be present as a minor constituent of soybean and corn lecithins (Cherry and Kramer, 1989) and was absent in sunflower lecithin (Hollo *et al*., 1993) seems, merely judging from TLC spot area, to occur in more abundance in fenugreek's (Fig. 2).

The fatty acid composition of the total-lecithin fraction of the oil of fenugreek seed accession A1, shown in Table 5, fits the range of fatty acids reported for lecithins of soybean genotypes (Liu and Ma, 2011) and other seed lecithin sources (Cherry and Kramer, 1989) in that linoleic, oleic and palmitic acids were the major three fatty acids present. Of interest was the presence of considerable amounts of the unique fatty acid, palmitoleic acid (Table 5) which was barely detectable in fenugreek seed oil (Table 3). This fatty acid was also reported to occur in soybean lecithin in proportions varying from 7.0 to 8.6% of soy lecithin fatty acids (Cherry and Kramer, 1989). Palmitoleic acid was reported to occur exclusively in phosphatidylglycerol (PG) in clover leaves (Weenink and Shorland, 1964). PG is the only phospholipid present in photosynthetic membranes (Joyard *et al*., 1998) where PG and its esterified unique fatty acid play an essential structural and functional role in photosynthesis (Ohnishi and Thompson, 1991; Pineau *et al*., 2004). The presence of palmitoleic in

fenugreek lecithin is likely due to PG, which was present in appreciable amounts in fenugreek seed lecithin.

Thus, fenugreek seed oil is a promising source for a lecithin fraction that could be even further modified by physical or chemical means as described by Scholfield (1981); Cherry and Kramer (1989) Garcia *et al*. (2008); Liu and Ma (2011).

REFERENCES

Amin, A., A. Alkaabi, S. Al-Falasi and S.A. Daoud, 2005. Chemopreventive activities of *Trigonella foenum-graecum* (Fenugreek) against breast cancer. Cell Biol. Int., 29: 687-694.

Association of Official Analytical Chemists (AOAC), Official Methods of Analysis, 15th Edition (1990), AOAC, Washington, USA.

Al-Oqail, M.M., N.N. Farshori, E.S. El-Sheddi, J. Musarrat, A.A. Al-Khedhairy and M.A. Siddiqui, 2013. *In vitro* cytotoxic activity of seed oil of fenugreek against various cancer cell lines. Asian Pacific J. Cancer Prev., 14: 1829-1832.

Arivalagan, M., K.K. Gangopadhyay and G. Kumar, 2013. Determination of steroidal saponins and fixed oil content in fenugreek (*Trigonella foenum-graecum*) genotypes. Indian J. Pharmaceutical Sci., 75: 110-113.

Basu, S.K., S.N. Acharya, M.S. Bandara, D. Friebel and J.E. Thomas, 2009. Effects of genotype and environment on seed and forage yield in fenugreek (*Trigonellafoenum-graecum* L.) grown in Western Canada. Aust. J. Crop Sci., 3: 305-314.

Chiang, C.T., T.D. Way, S.J. Tsai and J.K. Lin, 2007. Diosgenin, a naturally occurring steroid, suppresses fatty acid synthase expression in HER 2-overexpressing breast cancer cells through modulating Akt, mTOR and JNK phosphorylation. FEBS Letters, 581: 5735-5742.

Cherry, J.P. and W.H. Kramer, 1989. Plant Sources of Lecithins. In B.F. Szuhaj (ed) Lecithins: sources, manufacture and uses, AOCS, Champaigne, Il., USA: pp: 16-31.

Ciftsi, O.N., R. Prezybylski, M. Rudzinska and S.N. Achyra, 2011. Characterization of fenugreek (Trigonella foenum-graecum) seed lipids. J. Am. Oil Chemists Soc. (JAOCS), 88: 1603-1610.

Duranti, M., 2006. Grain legume proteins and nutraceutical properties. Fitoterapia, 77: 67-82.

Dittmer, J.C. and R.L. Lester, 1964. A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. J. Lipid Res., 5: 126-127.

Eshratabadi, P., M.H. Sarrafzadeh, H. Fatemi, M. Ghavami and N. Gholipour-Zanjani, 2008. Enhanced degumming of soybean oil and its influences on degummed oil and lecithin. Iranian J. Chem. Engineering, 5: 65-73.

El-sebaiy, L.A., 1983. Lipid changes during germination of fenugreek seeds (*Trigonella foenum-graecum*). Food Chem., 10: 309-319.

Garcia, H.S., I.H. Kim, A. Lopez-Herviandez and C.G. Hill, 2008. Enrichment of lecithin with n-3 fatty acids by acidolysis using immobilized phospholipase A1. Grasas and Aceites, 59: 368-374.

Hamza, N., B. Berhe, Z. Cheze, R. Le Garrec, A. Umar, A.N. Agli, R. Lassalle, J. Jove, N. Gin and N. Moore, 2012. Preventive and curative effect of *Trigonellafoenum-graecum* L. seeds in C57BL/BJ models of type 2 diabetes induced by high-fat diet. J. Ethnopharmacol., 142: 516-522.

Hemavathy, J. and J.V. Prabhakar, 1989. Lipid composition of fenugreek (*Trigonella foenum-graecum* L.) seeds. Food Chem., 31: 1-7.

Hollo, J., J. Peredi, A. Ruzics, M. Jeranek and A. Erdelyi, 1993. Sunflower lecithin and possibilities for utilization. JAOCS, 70: 997-998.

Joyard, J., E. Marechal, C. Miege, M.A. Block, A. Dorne and R. Douce, 1998. Structure, distribution and biosynthesis of glycerolipids from higher plant chloroplasts. In P.A. Siegenthaler and N. Murata (ed) Lipids in Photosynthesis: Structure, Function and Genetics, pp: 21-52. Kluwer Academic Publishers, Dordrecht, Netherlands.

Lee, J., K. Jung, Y.S. Kim and D. Park, 2007. Diosgenin inhibits melanogenesis through the activation of phosphatidylinositol-3-kinase pathway (PI3K) signaling. Life Sci., 81: 249-54.

Liu, D. and F. Ma, 2011. Soybean Phospholipids. In D. Krezhava (ed) Recent Trends for Enhancing the Diversity and Quality of Soybean Products, ISBN 975-953-307-533-4 (2011), Publisher: InTech (Croatia/ China).

Monti, L.M. and S. Grillo, 1983. Legume seed improvement for protein content and quality. Plant Foods for Human Nutr., 32: 253-266.

Osman, N.A., S.A. Elhussein and A.D. Albalawi, 2014. Variability in the content of 25 alpha and 25 beta-steroidal sapogenins among thirty morphologically differen Sudanese seed accessions of fenugreek. J. Life Sci., 8: 744-757.

Ohnishi, M. and G.A. Thompson, 1991. Biosynthesis of the unique trans-delta 3-hexadecenoic acid component of chloroplast phosphatidylglycerol: evidence concerning its site and mechanism of formation. Arch. Biochem. Biophys, 288: 591-599.

Petit, P.R., Y.D. Sauvaire, D.M. Hillaire-Buys, O.M. Leconte, Y.G. Baissac, G.R. Ponsin and G.R. Ribes, 1995. Steroid saponins from fenugreek seeds: extraction, purification and pharmacological investigation on feeding behavior and plasma cholesterol. Steroids, 60: 674-680.

Pineau, B., J. Girard-Bascou, S. Eberhard, Y. Choquet, A. Tremolieres, C. Gerard-Hirne, A. Bennardo-Connan, P. Decottignies, S. Gillet and F-A. Wollman, 2004. A single mutation that causes phophatidylglycerol deficiency impairs synthesis of photosystem II cores in *Chlamydomonas reinhardtii*. Eur. J. Biochem., 271: 229-338.

Raju, J. and R.R. Bird, 2007. Diosgenin, a naturally occurring furostanolsaponin, suppresses 3-hydroxy-3-methylglutaryl CoA reductase expression and induces apoptosis in HCT-116 human colon carcinoma cells. Cancer Letters, 255: 194-204.

Raju, J. and R. Mehta, 2009. Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. Exp. Dermatol., 18: 232-237.

Rao, P.U. and R.D. Sharma, 1987. An evaluation of protein quqlity of fenugreek seeds (*Trigonella foenumgraecum*) and their supplementary effects. Food Chem., 24: 1-9.

Shabbeer, S., M. Sabolewski, R.K. Anchoori, S. Kachhap, M. Hidalgo, M. Jimeno, N. Davidson, M.A. Carducci and S.R. Khan, 2009. Fenugreek: a naturally occurring edible spice as an anticancer agent. Cancer Biol. Ther., 8: 272-278.

Scholfield, C.R., 1981. Composition of Soybean lecithin. J. Am. Oil Chem. Soc., 58: 889-892.

Valette, G., Y. Sauvaire, J.C. Baccou and G. Ribes, 1984. Hypocholesterolaemic effect of Fenugreek seed in dogs. Atherosclerosis, 50: 105-111.

Wiedermann, L.H., 1981. Degumming, refining and bleaching soybean oil. JAOCS, 58: 159-166.

Weenink, R.O. and F.B. Shorland, 1964. The isolation of trans-3-hexadecenoic acid from the lipids of red clover (*Trifolium pretense*) leaves. Biochim Biophys Acta, 84: 613-614.