Estimation of Lipid Composition in Fenugreek Seed by GC/MS

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(Received 24 / 2 /2009 , Accepted 25 / 10 /2009)

Abstract
This study concerns the estimation of the lipid composition and some chemical components using GC-Ms in fenugreek seed. Fenugreek seeds are rich in protein (24.6%), fat (7.9%), total fiber (5.76%), and also contains high concentrations of minerals (Ca, P, Mg, Fe and Zn) in addition to other minerals like (Cu, Mn, Na, K and Cr) at low concentrations. The unsaturated acid linoleic and olic acid were found in the seeds oil, also saturated fatty acid palmitic and steric acid were detected and the results showed that the percentage of unsaturated and saturated acids were 83% and 17% respectively.
Keywords: Lipid, Fenugreek, GC/MS, Saturated and Unsaturated acids.

Introduction
Fenugreek seed (Trigonella foenum graecum) is a commonly used condiment in Indian homes and also in Arabic countries. The plant has many culinary uses .Its tender leaves are used as vegetables and its seeds as condiments In Egypt the seed powder is mixed with corn flour to bake bread [(1)].

It has been reported that a defated fraction of fenugreek seeds lowers the blood glucose, this means that fenugreek seeds possesses hypoglycemic effect in both insulin and non insulin dependent diabetic patients [(2)]. It has been reported that a defated fraction of fenugreek seeds decreased the serum total cholesterol, LDL, VLDL cholesterol, and triglyceride levels with out any alteration in HDL fraction [(3)]. It is found also that galactomannan which extracted from fenugreek seed is reduced the plasma glucose [(4)].

Recently the corp has attracted much interest as a source good protein [(5)]. The proteins of seeds have been separated among which the enzyme amylase has been detected, purified, and characterized using ion-exchange chromatography and hydrophobic interaction chromatography [(6)].

Fenugreek seeds contain 7.5% of oil and its physical-chemical characteristics have been reported [(7)]. Another study was carried out to clarify the effect of fenugreek in persian folklore medicine as beneficial in the treatment of diabetes, on blood glucose and their possible effect on pancreatic tissue [(8).] Fenugreek is one of the richest sources of phytoestrogens and thus a very useful spice for women who have low estrogen levels, fenugreek is one of the richest sources of selenium, and which is among the most important antioxidant micronutrients [(9)]. However little information is available on the lipid composition of fenugreek seeds .The purpose of this study was to determine some chemical and lipid composition of fenugreek seed using GC-Maas.

Materials and Methods
The seeds (Trigonella foenum graecum L.) were obtained from local market of the Erbil north Iraq during 2007 .They were cleaned by hand and grounded in a mill.

Analytical methods
Moisture, ash and crud fiber were determined according to the AOAC method[(10)]. The total nitrogen and crud protein were determined by micro-kyeldahl[(11)].

Determination of minerals
Elements (Na , K) were determined according to Golterman procedure [(12)],using Gallenkamp Sc2753 flame emission photometer. Other elements including (P , Mg ,Ca ,Fe ,Zn ,Cu ,Mn ,Cr) were determined according to Whiteside procedure [(13)], using unicam SP9 atomic absorption spectrophotometer, suitable hallo cathode lamps (HCL) was used.

Total Lipid Analysis
Dried and crushed seeds were extracted with petroleum ether (60-80C) using soxhlet for 8 hours then the seeds oil weighted after the evaporation. Fatty acid methyl esters were prepared with KOH/MeOH at room temperature for 2 hours following by 78C^{o} for 3 hours [(14)].

The analysis of fatty acid methyl esters were performed by a gas chromatograph (shimadzu GC-14A) on a TC-WAX (GL sciences Inc.) column (30mx 0.25µm i.d.,0.25 mm film thickness) using nitrogen (1Kg/cm2) ,equipped with a FID (260)ess injector (250C^{o},split 1:20). The temperature was programmed from 170-225C^{o} at 1C min-1 followed by a final hold of 25 min. Identification of the methyl esters were made by comparison of reaction times of standard fatty acid methyl esters and a normalization technique was used for quantitation with computer program class CR-10 (shimadzu co.).

Gas chromatography-mass spectrometric analyses were made by GC-MS equipment (HP 6890 series GC system) with mass selective detection. AJ & W WAX (GL sciences Inc.) column (30 mx 0.25 mm i. d. , 0.25 µm film thickness) was used, and the temperature was programmed from 150-250 C^{o} at 2 C^{o} min^{-1} with an initial hold of 4 min and a final hold of 36 min. the carrier gas was helium (1mil min $^{-1}$) and the split ratio was 1: 50 . The injection port was held at 250 C^{o} and the detector at 300 C^{o}.

Results and Discussion
Table (1) shows same constituents chemical composition of the fenugreek seeds. The concentration of major mineral of fenugreek seeds falls within the range of values reported for other varieties grown in the other parts of the world [(15)]. The seed contains higher proportions of minerals (Ca ,P ,Mg ,F and Zn) compared with other grain legumes [(16)].

Chemical analysis indicates that the seeds are rich source of protein (24.6%) and crude fiber (5.76%) which is responsible for lowering plasma cholesterol concentration and glucose levels in diabetes [(4)].

Table (1): Analytical data for fenugreek seeds.

Parameter	Value
Moisture	2.4 g / 100 g
Total Nitrogen %	3.94
Crude Protein (N*6.25)	24.6 g/ 100 g
Fat	7.9 g/ 100 g
Total fiber	5.76 g/ 100 g
Ash	3.9 g/ 100 g
P	368 mg/ 100 g
Mg	140 mg/ 100 g
Ca	64.4 mg/ 100 g
Fe	12 mg/ 100 g
Zn	7 mg/ 100 g
Cu	1.3 mg/ 100 g
Mn	1 mg / 100 g
Na	1.1 mg/ 100 g
K	0.35 mg/ 100 g
Cr	0.1 mg/ 100 g

The seed of fenugreek contained 7.9% total lipids as a golden-yellow oil. It seems that the percentage of total oils differs according to the location and the condition of cultivation of plant [17]. The fatty acids composition of the seed oil was determined by GC-Ms and the results are shown in Table (2).

Table (2): Fatty acid composition of the fenugreek seeds.

Fatty Acid	***Short Illustration***	***Total Fatty Acid Content (%)***	***Mg/ 100 g Seed***
Palmitic acid	C16:0	11	534
Linoleic acid	C18:2 Δ 9.12	47	22860
Oleic acid	C18:1 Δ 9	36	1741
Steric acid	C18:0	6	279
Total Saturated %		17	813
Total Unsaturated %		83	24601

Four fatty acids were identified by comparison with fatty acid methylester standards. The structure of unknown fatty acids was further supported by GC-MS spectrometry Fig (1).

Total saturated fatty acids amounted to (17%)that is palmitic acid (11%)(fig 2A) and steric acid (6%)(fig 2B).Total unsaturated fatty acid amounted to (83%) linoleic acid is the highest (47%)(fig 2C) followed by olic acid (36%)(fig2D).

The percentage of linoleic acid differs significantly from the results previously given by shahat [18] of (13.8%) for Egyptian fenugreek oil as well those of Badami &Kalburgr [19] of (13%) and of Zafaret [20] of (7%) for Indian grains. Hilditch&Williams[21] found that temperature and atmosphere are principal factors accounting for variations especilly in linoleic acid and also found that linolenic acid is either entirely absent from or present in only very small amounts in , most Leguminosae seed oils .

Thus as far as unsaturated fatty acid content is concerned the present work is supported by previous work by Balogun &Fetuga[22] and Sengupt &Basu [23] both groups of workers contended that the unsaturated fatty acid contents of leguminose seed fats resemble each other closely and that the chief components are oleic and linoleic acids .A deficiency of essential fatty acids is characterized by scaly dermatitis ,hair loss and poor wound healing and the synthetic diets should include about 2% of calories as linoleic acid [24] .It seems that C18:2w6 is the dominant fatty acid (linoleic acid).

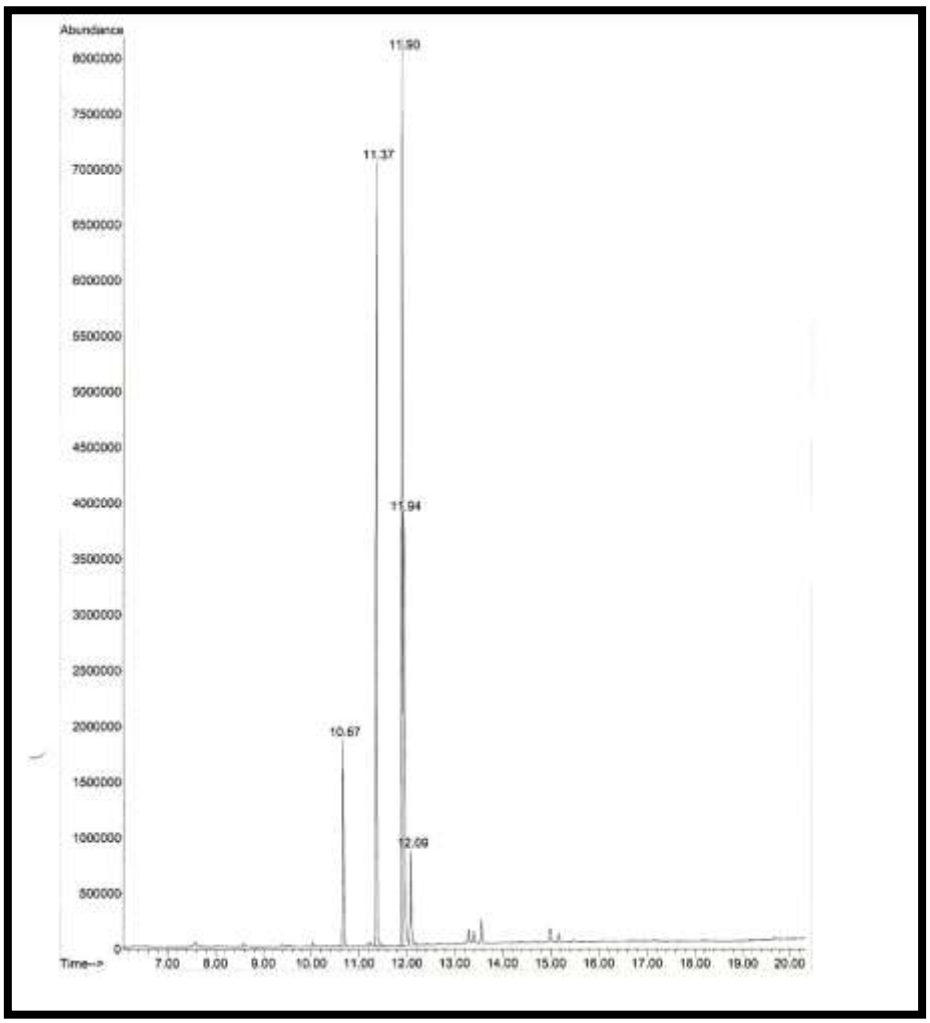

Fig. (1): Capillary GC/MS of isolated fatty acid from Fenugreek seed oil.

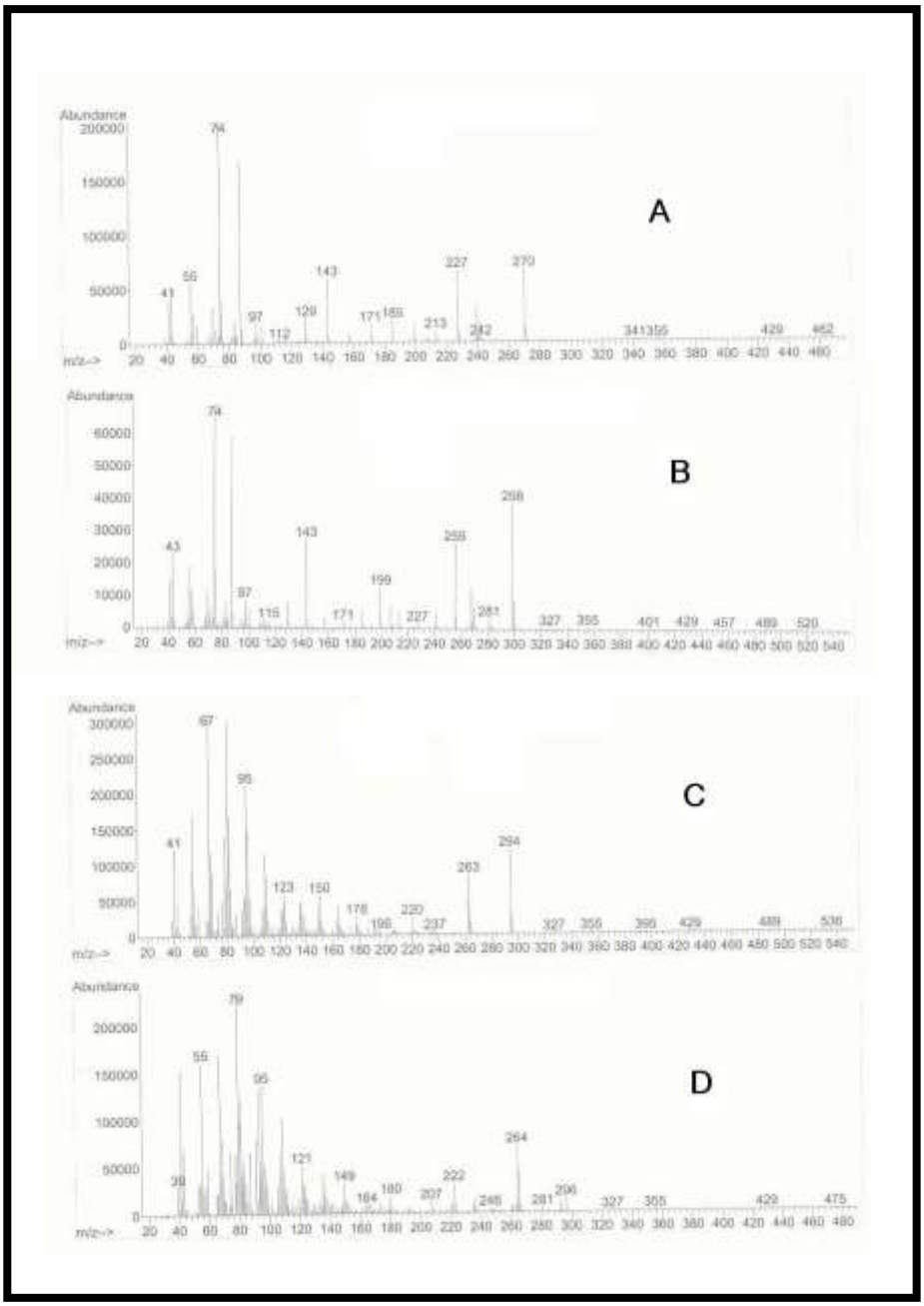

Fig. (2): Mass spectra of isolated fatty acid from fenugreek seed oil.

A: mass spectrum of peak No. 1, B: mass spectrum of peak No. 4,
C: mass spectrum of peak No. 2, D: mass spectrum of peak No. 3

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تقدير تركيب الاحماض الدهنية في بذور الحلبة باستخدام GS-MS

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(تاريخ الاستلام: ٢٤ / ٢ / ٢٠٠٩ ، تاريخ القبول: ٢٥ / ١٠ / ٢٠٠٩)

الملخص

تتناول البحث دراسة كيمياوية وتعين الاحماض الدهنية الموجودة فى بذور الحلبة بواسطة GS-MS حيث اظهرت النتائج احتواء بذور الحلبة على البروتين بنسبة (24.6%) والزيوت بنسبة (7.9%) والألياف بنسبة (5.76%) وكذالك يحتوى علي نسبة عالية من العناصر (Ca,P,Mg,Fe,Zn) ونسبة قليلة من (Cu,Mn,Na,K,Cr) .

تم اثبات وجود (Linoleic acid ,olic acid) كأحماض غير مشبعة ووجود (Palmaitic acid , Steric acid) كأحماض مشبعة ثم تم تحديد نسبتيها (83%) و(17%) الأحماض الدهنية غير المشبعة والمشبعة على التوالي.