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Chemical Composition of Some Varieties of Fenugreek Seeds (*Trigonella foenum gracecum*)

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Abstract

Proximate chemical composition and minerals contents of fenugreek whole seed samples from China, Sudan and Algeria were determined. The results showed that there was significant difference among the three varieties in their protein, fat and total minerals composition. The endosperm was separated from the embryo of the seed and the composition of each part was studied, most of the proteins are accumulated in the embryo fraction (39.87 %), while the gum is concentrated in the endosperm fraction (53.53 %). The major part of the saponin and lipids are found in the embryo fraction and easier to be separated from the embryo than from the whole seed, due to the presence of the gum which has disturbing effect on protein solubilisation. Amino acid profile and fatty acids composition were determined, the main amino acids are gultamic acid, aspartic acid, and arginin. The amino acid profile revealed that fenugreek proteins is similar to soybean proteins in their amino acid composition. The fatty acid composition of the total lipids indicated that lenoleic was the predominant fatty acid (61.29 %), followed by oleic acid (17.67 %).

Keywords: Fenugreek ; Gum ; Amino acid; Fatty acids; Chemical composition

Introduction

Fenugreek (*Trigonella foenum gracecum*) is an annual plant of legume

family (*Leguminosae, tribe Tritolieae*) This crop is native to an area extending from Iran to northern India, but is now widely cultivated in China, north and east Africa, Ukraine, and Greece (Petropoulos G A 2002). The plant was used for its medicinal value and for spring forage in India, Western Asia, and in the Nile Valley since remote antiquity. Trigonella from Latin indicates small triangular white flower, foenum- graecum means in greek hay, and fenugreek means cow horn. Fenugreek is known as *Hilba* in Sudan and called *Methi* in India, *Huluba* (Hulu Beans) in China and *Alholva* in Spain.

Fenugreek is an ancient plant indigenous to Sudan and Ethiopia. The area of production in Sudan is the northern region of the country along River Nile, where the climate is suitable for the production of the crop. In Sudan the seed powder is used by the lactating women as porridge (with sorghum or millet flour) or as dessert to increases the flow of milk, similar uses are reported in India (Mullaicharam et al 2013). It is also boiled with water and taken hot or cold to soothe stomach ailments. It is probably delays subsequent pregnancy due to the presence of diosgenin, the starting material used in the synthesis of sex hormones and oral contraceptives (Heintz 1959). Although Sudan is not one of the famous exporting countries of fenugreek, its production is very high and can be increased. In United State ground fenugreek seeds are widely used domestically in curry powder blend, mango chutney, stews, and hearty soups. It is sometimes used in picking brines for green beans and cauliflower, and added to mayonnaise for use salads. It blends particularly well milder flavored spices. Recently the crop attracted much interest (especially the seeds) as a cheap source of good protein, and as a supplement to jower- sorghum or corn flour. Ninety percent of fenugreek seed proteins are located in the cotyledon plus embryo. Fenugreek seed contains high level of protein between 25 and 30%. The amino acid profile of its protein revealed that lysine content in fenugreek seed protein is comparable to that of soybean protein (Sauvaire et al.1976) . Nur and Magboul (1986) found high values of leucine, lysine , and valine for fenugreek seed protein. Kochhar et al. (2006) reported that fenugreek seeds contain 11.8% moisture, 25.8% crude protein, 6.53% oil, 3.26% ash, and 6.28% crude fiber and 58.13% total carbohydrates on dry basis. However, El-Nasri and El-Tinay (2007) found that protein content of fenugreek was found to be 28.4%, crude fiber content was 9.3%, and crude fat was 7.1%. In general germination improves nutritional value of seeds, increasing their vitamins contents and reducing some of antinutritional factors, i.e. trypsin inhibitors, phytate content, and haemagglutinin activity (Rao and Sharma1987).

According to the US Federal Specifications (Rao and Sharma 1987) Fenugreek seed must contain no more than 5% total ash, 1% acid insoluble ash, and 8.5% moisture. Ninety five percent of

the ground shall pass through US standard No. 25 sieve.

The seed of fenugreek contains 7.5% fat and it is physic-chemical properties have been reported (El-Mahdy and El-Sebaiy 1980). However, little information is available in lipids composition of fenugreek seeds. Hemavathy and Prabhaket (1989) have studied the composition of fenugreek oil and reported that, the total lipids consist of 84.1% neutral lipids, 5.4% glycolipids, and 10.5% phospholipids. The neutral lipids consist mostly of 86% triglycerides; oleic acid is the predominant fatty acid followed by lenoleic acid, while lenolenic is the least fatty acid. In contrast, Sidhu and Oakenfull (1990) found that the oil extracted from cotyledon contains 29% of lenolenic, and they suggested that the very low value for lenolenic acid reported by Hemavathy and Prabhakar (1989) would appear to be resulted from losses of lenolenic acid by autoxidation during extraction and analysis. In the analysis of unsaturated fatty acids, extreme care must be taken to avoid oxidation by adding antioxidant such as BHT. This finding is in agreement with Suchandra (2010) who was reported that the fatty acid profile of fenugreek seeds is dominated by unsaturated fatty acids namely oleic, lenolic and lenolenic acids accounting for 16.3%, 50% and 24.4% respectively of the total fatty acids. Rathore et al (2016) studied the genetic variation of in seventeen selected varieties of fenugreek, and reported that genetic diversity showed no relationship to geographical origin.

The volatile oil content of fenugreek seeds oil is very low; usually less than 0.02%, but it is extremely odorous. The oil has a very bitter taste, resulting from the presence of two alkaloids, trigonelline and choline. When ground fenugreek seeds are extracted with alcohol, it yields an aromatic essence, which when compounded with other aromatic chemical extracts may be used in the manufacturing of an imitation maple flavored syrup (Farrel et al.1985)

Madhava Maidu (2011) studied saponin content in fenugreek seeds and reported that saponin is concentrated in the endosperm (4.63g/100g). Sauvaire et al (1996) isolated eight saponins from fenugreek seeds using counter current chromatography. These bidesmosidicsaponins have two sugars with one bounded at C-3, and one attached through an ether linkage at C-26 with Glucose.

The objective of this study was to compare the three varieties of fenugreek seeds brought from different geographical regions in their chemical composition.

Materials and Methods

Materials

Three different fenugreek varieties were brought from three different countries. Chinese variety was brought from Wuxi town (Jiangsu Province, China), Sudanese variety was purchased from the local market of Khartoum North, Sudan,

and Achouri Allaoua (Agricultural University of Algeria) kindly donated the Algerian variety. The seeds were cleaned from dust and stones, and stored at 4^{o}C, Alpha-amylase (3.2.1.1), pullunase (3.2.1.41).

General Analysis

Moisture, crude protein (N x 6.25), fat, ash, and crude fiber were determined according to the approval methods of (AOAC 1990). The nitrogen free extract (NFE) was obtained by difference (100-moisture+ ash+ crude protein+ crude fiber +fat%). Total carbohydrate was determined by phenol/sulfuric acid method (Dubois et al.1956). Total saponin was performed by the modified specific spectrometric analysis after acid hydrolysis of saponin (Maier et al .1993). The endosperm and seed coat adjacent were separated from the embryo by hand. Total, soluble (gum), and insoluble dietary fiber were determined as non-starch polysaccharides using colorimetric method of Englyst (Dubois etal.1956), the starch was removed enzymatically from the defatted seed powder using alpha-amylase.

The amino acids samples were hydrolyzed with 6N HCI, duplicate aliquots were assayed in H835-50 HITACHI Amino Acid Analyzer. The total lipids were fractionated to neutral lipids, glycolipibs, and phospholipids in sillicic column using chloroform, acetone, and methanol successively (Ried et al.1977) . Neutral lipids were estimated graphimetricaly, glycolipids and phospholipids were quantitated by estimation of total sugar and phosphorus (Dubois et al.1956). respectively. Fatty acid composition was determined by 5880A GC with flame ionization detector and AC20 column under nitrogen flow rate of 3 ml/min.

Result and Discussion

Proximate composition of the Whole seed

The proximate chemical composition of fenugreek whole seed samples from China, Sudan, Algeria was studied; comparisons of the three samples are given in table (1), Sudanese sample is the highest in protein content (31.31 %) and the lowest in fat content (5.27 %). These results are similar to that reported by Nour and Magboul (1986) who studied the chemical composition of the Sudanese variety of fenugreek seeds, in the other hand El-Nasri and El-Tinay (2007) found protein and fat contents of fenugreek from Sudan were 28.4 and 7.1% respectively. Algeria sample was the lowest in protein (25.49%) and in agreement with finding of Kochhar (2006) who reported that protein content of fenugreek seeds is 25.8 in contrast Fahad et al (2013) found the protein content of Saudi fenugreek samples was only 12.91%. However, the protein values of the three samples are similar to the average value for twenty-four samples of Indian fenugreek seeds which was ranged between 25 and 32% (Bogacheva et al .1977). Moisture content of the three samples are higher

than the Federal Specifications (USA) for the good quality of fenugreek seeds which reported to be 8.5% while the ash content is lower (Rao and Sharma 1987). The significant difference in protein content among the three samples may be due to the different regions they come from, particularly when we consider the existing biotic and adapting factors in their respective places of origin. In general, there is no significant difference among the three samples of fenugreek seeds in their ash content, which encourages us to use only Sudanese sample for further investigation because of the its Availability.

Ash content of the three samples ranged between 3.1 and 3.29 %. Analysis of the ash composition revealed that, potassium and calcium are permanent minerals in concentration term in the three samples, followed in decreasing order by magnesium, sodium, iron, zinc, and copper (table 2). Zinc content of Chinese sample is higher than the earlier report of Abd EL-Aal and Rahma (1986) who studied the mineral composition of the Egyptian fenugreek seed sample. Iron and copper contents of the Sudanese sample are lower compared with other Sudanese samples studied by Nour and Magboul (1986). Algerian sample contains the least calcium and the highest iron value among the samples under study.

Table (1) proximate chemical composition of three different regional Fenugreek seeds (g / 100g)

Varieties	Proximate chemical composition							
	Moisture	Protein	Fat	Ash	T-NSP	S-NSP	I-NSP	T-CHO
Chinese	11.26[c]	29.00[b]	6.30[b]	3.12[a]	43.60[b]	23.55[b]	20.04[b]	50.32[b]
Sudanese	11.71[b]	31.31[a]	5.27[c]	3.29[a]	47.23[a]	27.14[a]	20.09[a]	48.43[a]
Algerian	11.97[a]	25.49[c]	8.14[a]	3.34[a]	40.98[c]	21.38[c]	19.59[c]	51.06[c]
S-E ±	0.006	0.02	0.04	0.13	0.02	0.006	0.006	0.15

Means within the column having similar letters are not significantly different at 0.05 level of probability according to DMRT, T-NSP = total non - starch polysaccharides, S-NSP= soluble non - starch polysaccharides, I-NSP = insoluble non - starch polysaccharides, T- CHO = total carbohydrate.

The variation in mineral composition observed in table (2) is probably not unusual as the mineral content of any crop is likely to vary due to various factors, such as trace metal content of the soil, mineral constituents of water used for irrigation, geographical location, and fertilizers and fungicides applied. In addition to the above mentioned factors the main factor that is likely responsible for increasing the level of minerals is various industrial activities carried out adjacent to the agricultural use of land.

Composition of different anatomical parts of the seed

The seed of fenugreek anatomically consists of: the seed coat and the embryo, the later is separated from the former by well-developed endosperm with an alleurone layer. The average weight for one thousand seeds is about 13. 01 g. The weight of the endosperm is about 47.69 % of the whole seed, while embryo is 39 and the seed coat is 13.1%.

Table (2) Mineral composition of three different regional Samples of fenugreek seeds *(mg / 100g)*

Varieties	Mineral composition						
	Fe	Cu	Zn	Ca	Mg	Na	K
Chinese	6.36 [b]	3.73 [b]	6.25 [a]	326.33 [a]	167.17 [b]	132.33 [a]	780.17 [a]
Sudanese	6.02 [c]	4.07 [a]	5.75 [b]	273.33 [b]	174.83 [a]	119.33 [b]	657.50 [b]
Algerian	9.75 [a]	3.62	3.38 [c]	200.67 [c]	137.33 [c]	103.33 [c]	537.33 [c]
S-E ±	0.006	0.006	0.006	0.55	1.76	1.80	0.76

Means within the column having similar letters are not significantly different at 0.05 level of probability according to DMRT

Table (3) Proximate chemical composition of endosperm, embryo, and the whole seed of fenugreek seeds from Sudan(g / 100g)

Seed part	Proximate chemical composition							
	Moisture	Protein	Fat	Ash	Fiber	NFE	Saponin	Gum
Whole seed	11.26 [a]	29.00 [b]	6.30 [b]	3.12 [b]	9.15 [b]	12.85	4.60 [b]	23.72 [b]
Endosperm	10.00 [b]	5.76 [c]	0.53 [c]	2.92 [c]	0.92 [c]	26.25 [a]	0.01 [c]	53.53 [a]
Embryo	9.57 [c]	39.86 [a]	12.12 [a]	4,60 [a]	10.10 [a]	16.98 [c]	6.77 [a]	0.01 [c]
S-E ±	0.06	0.03	0.03	0.0001	0.04	0.05	0.02	0.03

*Means within the column having similar letters are not significantly different at 0.05 level of probability according to DMRT

The chemical composition of whole seed, endosperm and embryo are shown in table (3), most of the proteins are accumulated in the embryo fraction (39.87 %), while the gum is concentrated in the endosperm fraction (53.53 %). The major part of the saponin and lipids are found in the embryo fraction and easier to be separated from the embryo than from the whole seed, due to the presence of the gum which has disturbing effect on protein solubilisation (Sauvaire et al.1984). The gum is the major constituent of the endosperm, and contains a little amount of proteins (5.76 %) in the alleurone layer surrounding the endosperm cells and usually separated with endosperm fraction. Ried and Berley (1979) reported that the alleurone cells of *Trigonella foenumgraecum* seeds obtained from the outermost cell layer of the endosperm are small and thick walled and contains alleurone grains, while the rest of the endosperm is composed of large cells with thin primary walls which appear to be completely full of galactomannan.

Table (4) **Amino acid composition of fenugreek seeds (Sudanese sample)**

Amino acids	mg / g protein
Aspartic acid	94.83
Threonine	30.00
Serine	43.10
Glutamic acid	177.93
Glysine	42.41
Alanine	33.10
Cytosine	14.13
Valine	30.69

Results are expressed as average of three experiments

Methionine	8.97
Isoleucine	33.79
Leucine	57.93
Tyrosine	23.45
Phenylalanine	32.41
Lysine	54.83
Histidine	20.00
Arginine	74.14
Proline	35.17

Amino acid analysis

Amino acid composition of Sudanese sample was studied and the results are shown in table(4). The main amino acids are gultamic acid, aspartic acid, and arginin. These results are in line with the findings of Nour and Magboul (1986) and Sauvaire et al (1996). The amino acid profile revealed that fenugreek protein is similar to soybean proteins in their amino acid composition. Lysine content of fenugreek protein is very high compared with cereal proteins, because of that it can be used as supplement to the cereal products to improve their protein quality. Rao and Sharma (1987) found that addition of 5 - 10 g of fenugreek seed powder to the wheat or jwar diet supplemented the protein quality, probably the lysine content of defatted fenugreek powder plays an important role in elevating the nutritive value of cereal proteins.

Fatty acid analysis

Fenugreek seed is a low fat containing material, as shown in table (1) with the fat contents ranging from 5.27 % to 8.14 %. The purified lipid of fenugreek seeds had the following physico-chemical characteristics: appearance; golden-yellow liquid at ambient temperature (25 - 30 °C); taste; bitter; odour, disagreeable. The bitter taste of the fenugreek seed oil may be due to the presence of two alkaloids, trigonelline and cholline. Fractionation of the total lipids by cilicic acid column chromatography showed that the total lipid consist of 84.6 % neutral lipids, 5.8 % glycolipids, and 9.5 % phospholipids table (5). Similar finding was reported by Hemavathy and Prabhaket (1989) who were found that the total lipids of fenugreek seeds consist mostly of 84.1% neutral lipids, 5.4% glycolipids and 10.5% phospholipids

Table (5) **Fractions of fenugreek seed (Sudanese sample) lipids (g/ 100g)**

sample	Neutral lipids	glycolipids	phospholipids
Chinese	84.6	9.5	5.9

The fatty acid composition of the total lipids (table 6) indicated that lenoleic was the predominant fatty acid (61.29 %), followed by oleic acid (17.67 %). Hemavathy and Parbhakar (1989) reported that the predominant fatty acid in fenugreek seeds oil was lenoleic acid (52.6 %). However Suchandra et al (2010) reported that the fatty acid profile was dominated by unsaturated acids namely oleic, lenoleic and lenolenic acids accounting for 16.3%, 50% and 24.4% respectively of the total fatty acids. The very low values for the unsaturated fatty acids appear to have resulted from the loss of unsaturated fatty acids by autoxidation during extraction and analysis. Thus, in the analysis of the unsaturated fatty acids extreme care must be taken to avoid oxidation by adding antioxidants such as BHT.

Table 6 **. Fatty acid composition of fenugreek seeds (Sudanese sample)**

Fatty acids	g / 100g of fat
14 : 0	0.01
16 : 0	12.87
18 : 0	6.72
18 : 1	17.67
18 : 2	61.29

18 : 3	16.8
20 : 0	1.88
20 : 1	0.41

Conclusion:

Geographical location of fenugreek has significant effect on protein, fat and mineral content of the seeds. Defatted fenugreek power could be use as good supplement to bread making flour due to high lysine content.

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