Analysis of Fatty Acid Composition and Physicochemical Characteristic of *Trigonella foenum-graecum* Linn Ripe Seed by Gas Liquid Chromatography

Mohammad Mizanur Rahman[1, 2*], Md. Oli Ullah[1], Md. Enamul Huq[1] and Md. Wahab Khan[1]

[1]Department of Chemistry, Bangladesh University of Engineering and Technology, Dhaka-1000, Bangladesh

[2]Concord Pharmaceutical Ltd., Department of Product Development, S-18, BSCIC, Narayanganj, Dhaka, Bangladesh

*****Corresponding author (e-mail: pmizan@gmail.com)

Fenugreek is an annual herb. It is often cultivated as a cold weather crop. It's a medicinal crop. The yield of the seed oil was found to be 6.45% on extraction with pet-ether (40^0C – 60^0C). The fatty acid composition of the ripe seed oil of *Trigonella foenum-graecum Linn* was determined by gas-liquid chromatography. The ripe seed was successively extracted by light petroleum ether which was analyzed for fatty acid composition. The major saturated and unsaturated fatty acids methyl ester of seed extract was found to be C16:0, palmitic acid (5.6%) and 18:3, linolenic acid (49.5%), respectively. The lower amount of caprilic acid (1.5%), lauric acid (2.1%), palmitic acid, stearic acid, arachidic acid, and behenic acid was found in the fenugreek ripe seed.

Key words: Fenugreek; ripe seed; saturated fatty acid; unsaturated fatty acid; *Trigonella foenum-graecum* Linn

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Trigonella foenum-graecum Linn, fenugreek is one of the 70 species of the genus Trigonella belonging to the family Fabaceae. It is an annual herb, a native of the Mediterranean region, Europe, Asia, South Africa and Australia [1]. It is also found to grow in the Sub-tropical region of India and Bangladesh. Fenugreek (*Trigonella foenum-graecum* L.) (Methi) herb is cultivated for culinary and medicinal purposes and also for fodder. It is also called in others name e.g. Sans-Methika; Hindi-Methi (seeds); Kasoorimethi; Bangla-Methi; and English-fenugreek etc.

Methi (fenugreek) seed is compressed, obliquely rhomboid, nearly ¼ inch long, pale orange, slightly rough, with a deep oblique furrow across the upper part, plane-convex cotyledons, very large radicles, doubled down on the edges of the cotyledons (accumbent) and no endosperm.

Fenugreek seed oil has a pungent odour and bitter taste and is often used as an insect repellent for grains and fabrics [2, 3]. Fenugreek (*Trigonella foenum-graecum* L.) seed (Figure 1) is traditionally used for condiments, artificial flavouring and hormone production, a tea made from fenugreek seeds is equal into quinine for reducing fevers. Fenugreek seeds are useful in the removal of dandruff [1, 2, 3, 4, 5, 6, 7, 8]. They may be eaten raw or cooked. Fenugreek is often used as an additive in the preparation of curry powder, a popular spice in many countries [3]. The seeds of fenugreek are known to have hypoglycaemic [9], hypocho-lesterolemic [10], gastro- and hepato-protective [11], antioxidant properties [12], and also it stimulates lactation in women [13]. The aqueous extract of the fenugreek seed has been reported to show antibiotic activity against *Micrococcus pyogenes var. aureus* bacteria [14].

Many Investigations have been carried out in different countries on various aspects, but no systematic research has been carried out on Bangladeshi fenugreek. Since the chemical composition of the sample varies with the Agro-climatic conditions [15,16], hence we have taken fenugreek samples from Bangladesh. Some disagreement about the presence of its constituents was observed. Therefore the present work was undertaken to carry out a fatty acids analysis of the fenugreek seed.

MATERIALS AND METHODS

Seed Materials

The ripe seeds were collected from the matured fenugreek plants during March 2014, at the place of Mymensingh District (24^0 38′ 3″ N, 90^0 16′ 4″ E), Bangladesh. The plant was identified by Bangladesh National Herbarium, Dhaka, Bangladesh. A voucher specimen (DACB Accession Number-42999) of this plant was deposited in the

Figure 1. Fenugreek (*Trigonella foenum-graecum* L.) seed.

Bangladesh National Herbarium, Dhaka, Bangladesh. The collected seeds were cleaned, air dried and crushed mechanically.

Physico-chemical Studies

Physico-chemical characteristics of fenugreek ripe seed such as moisture, ash, refractive index, acid value, iodine value, saponification value, and unsaponification matter were determined by following the standard procedures [17, 18, 19].

Isolation of Fatty Acids and Preparation of Methyl Ester

The neutral lipid from the air-dry powder about 450 g of ripe seeds was extracted with light petroleum ether (40-60^{0}C) in a Soxhlet apparatus for 22 h. The solvent was distilled off, and the extract was concentrated and dried under reduced pressure. The extract was preserved at 2-4^{0}C in the refrigerator to examine for fatty acid analysis.

The esterification of the fenugreek ripe seed extract was done with BF_3 - MeOH (Bron trifluoride-methanol) complex [20]. Five mg of extract was taken in a reaction tube and BF_3CH_3OH reagent (5 ml) was added**.** The mixture was boiled for 5 min. Hexane (5 ml) was added to it and boiled for further 1 min. After cooling the tube, a solution of saturated salt was added and vortexed. Then the upper layer containing methyl esters was transferred to a vial with anhydrous sodium sulphate at the bottom. Then the ester was filtered through a syringe filter and transferred to a small vial (2 ml). The residual solvent was removed by blowing nitrogen gas and stored in a refrigerator before analysis by gas liquid chromatography (GLC).

Preparation of Standard Fatty Acid Methyl Ester

Nine of standard free fatty acids (C8:0, caprilic acid; C12:0, lauric acid; C14:0, myristic acid; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:3, linolenic acid; C20:0, arachidic acid and C22:0, behenic acid) were individually weighed. About 5 mg of each was taken in a reaction tube, and BF_3CH_3OH reagent (5 ml) was added11 to it. The mixture was boiled for 5 min. Hexane (5 ml) was added to it and boiled for a further 1 min. After cooling the tube a solution of saturated salt was added and vortexed. Then the upper layer containing methyl esters was transferred to a vial with anhydrous sodium sulphate at the bottom. Then the ester was filtered through syringe filter and transferred to a small vial (2 ml). The solvent was concentrated by blowing nitrogen gas and stored in a refrigerator before analysis by GLC [20]. The fatty acids in the experimental samples were identified by comparing their retention time and peak positions. The relative percentage of the fatty acids were also determined by comparing their peak areas (Peak area = ½ × peak height × peak width).

Gas Liquid Chromatography

The fatty acid methyl esters were quantified by gas-liquid chromatography method using a capillary column (2000 mm × 4 mm) equipped with a flame ionization detector (PyeUnicam 4500). Column packing was done with 10% diethylene glycol succinate on100 - 120 mesh diatomic CAW with column temperature 100^{0}C, detector temperature 220^{0}C, hydrogen flow rate 4 ml/min and samples volume of injected 0.1 ml.

RESULTS AND DISCUSSION

The amount of extractive was 6.45(%) percent from pet-ether extract off fenugreek ripe seed. Al-Jasass et al. [21] got about 4.51% from the fenugreek seeds. The variations of oil content may be due to soil composition, the maturity of the seeds and climatic differences in various geographical locations.

It has been seen in Table 1 that the fenugreek ripe seed fatty acid is reddish-yellow. It appeared as a translucent viscous oily liquid at room temperature of 29 – 30^{0}C. The reflective index of the ripe seed of fenugreek is 1.4809 at 26^{o}C which indicates that the

oil contains long chain fatty acids. The acid value of fenugreek lipid obtains 1.84(%). This value indicates the proportion of free fatty acid has in the fenugreek oils. Saponification value of fenugreek fatty acids obtained 185.13.

Iodine value was found 117.0 in fenugreek fatty acid. It was known that Iodine value measures the degree of unsaturation of fatty acids content in any fat or oil [22]. Therefore, the more the Iodine value, the more unsaturated the fat or oil will be. It may be mentioned that the refractive index of the fats or oils depends on to some extent on their unsaturation. So there is a relationship between refractive index and an iodine value of fat or oil. The results of iodine value and the refractive index of fenugreek seed oil reported by different researchers was depicted to give this idea [22].

The unsaponifiable matter obtained 3.98% in fenugreek fatty acid, which was higher than the 1% unsaponifiable matter standard suggested by Fontanel [23]. The high value of unsaponifiable matter might be due to the presence of colouring substances like tannin, sterol, tocopherols, and steroid etc. [24].

In Table 2, linolenic acid was the dominant unsaturated fatty acid in fenugreek seed. Major fatty acids of fenugreek ripe seed were found oleic acid (30.3%). The lower amount of caprilic acid (1.5%), lauric acid (2.1%), lyristic acid (2.5%), palmitic acid (5.6%), stearic acid (4.3%), arachidic acid (3.0%) and behenic acid (1.2%) was detected in the fenugreek ripe seed. Whereas Al-Jasass et al. [21] found 30.80% linolenic acid (C18:3), is the major fatty acid. On the other hand, myristic acid, 1.38%; palmitic acid, 3.85%; stearic acid, 1.78%; oleic acid (*cis*), 8.29% and oleic acid (*trans*), 10.76% was found by Al-Jasass et al. [21] in fenugreek seed fatty acid.

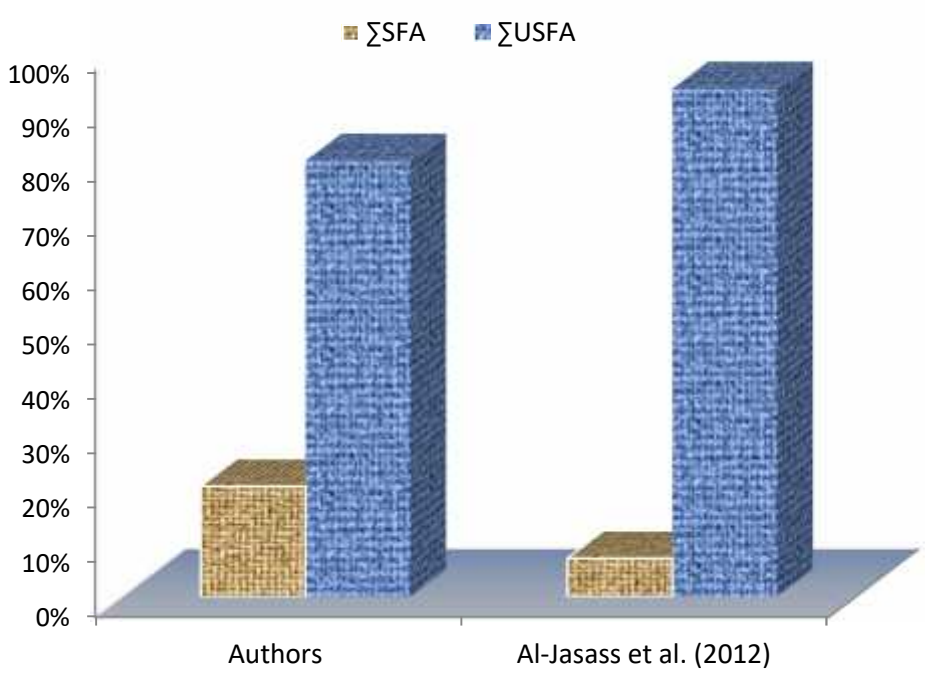
The fatty acid profiles of the fenugreek seed is shown in Figure 2. The total saturated fatty acid (ΣSFA) of ripe seed was detected higher (20.2%). On the other hand, total unsaturated fatty acid (ΣUFA), 79.8% was found in the ripe seed. However, FAME showed the higher a unsaturated/saturated ratio (USR) in the ripe seed (3.95). It is indicated that the amount of total unsaturated fatty acid iwas higher. Whereas Al-Jasass et al. [21] found 7.01% (ΣSFA) and 92.99% (ΣUSFA) in fenugreek seed oil.

Table 1. Physico-chemical characteristic of the fenugreek ripe seed and its fatty acid parameters (Dry matter basis).

Parameter	Fenugreek ripe seed
Moisture(%)	13.09
Taste	Bitter
Color	Reddish yellow
Odor	Coffee/ Maple syrup
Appearance	Opaque, viscous oily liquid
Specific gravity	0.9133
Refractive Index (26^0C)	1.4808
Acid Value	1.81
Iodine value	115.0
Saponification value	183.26
Unsaponifiable matter (%)	3.95

Table 2. Fatty acid composition (%w/w) of the fenugreek ripe seed (percentage composition).

Fatty Acids	Structure	Ripe seed (%)
Caprilic acid	C8:0	1.5
Lauric acid	C12:0	2.1
Myristic acid	C14:0	2.5
Palmitic acid	C16:0	5.6
Stearic acid	C18:0	4.3
Oleic acid	C18:1	30.3
Linolenic acid	C18:3	49.5
Arachidic acid	C20:0	3.0
Behenic acid	C22:0	1.2



(∑SFA: Total saturated fatty acid; ∑UFA: Total unsaturated fatty acid)

Figure 2. The fenugreek seed oil fatty acid profiles comparison of authors with Al-Jasass et al. [21].

The results for fatty acid composition are different from those reported by Al-Jasass et al. [21] and Hemavathy et al. [25]. The results were varying for different climate, environment and soil quality. Due to this, there is a variety of variations in the formation of chemicals in plants from regions to region. However, Hemavathy et al. [25] used fenugreek from India and applied analytical techniques which may affect fatty acid composition due to limited separation. These authors [25] found the following composition of fatty acids in fenugreek oil: 52.6% oleic, 40.0% linoleic, and 0.6% linolenic acid.

CONCLUSION

This study revealed the fatty acid composition of fenugreek ripe seed which was not previously studied in Bangladesh. Fenugreek ripe seed was detected in seven saturated fatty acids and two unsaturated fatty acids. In this study, Oleic acid and linolenic acid was indicated as the main source of the fatty acid in fenugreek ripe seed. An interesting finding of our study concluded that the highest total unsaturated fatty acid (79.8%) was identified in the fenugreek ripe seed. Fatty acids contents and their composition depended on the kinds of the plant seeds. Utilization of the seed oil in food products might enhance the profitability of seed production and the processing industries, and might be of benefit to consumers.

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