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Fatty acid composition of fenugreek (*Trigonella foenum-graecum* L.) seed and Galactomannan depleted fenugreek residue

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Abstract
A study was undertaken to evaluate the fatty acid composition of fenugreek seed and Galactomannan Depleted Fenugreek Residue (GDFR). A total of six fenugreek seed samples collected from different parts of Tamil Nadu and six GDFR (Fenumax®) samples received from E.I.D. Parry (India) limited, Cuddalore, Tamil Nadu were analysed for fatty acid composition. The fatty acids content (g/100 g sample) of fenugreek seed and GDFR were palmitic acid (0.57 and 0.99), stearic acid (0.64 and 0.98), oleic acid (2.43 and 3.78), linoleic acid (1.59 and 2.92), linolenic acid (0.04 and 0.07), arachidic acid (0.03 and 0.05) and other fatty acids (0.22 and 0.13).

Keywords: Fenugreek, fatty acids, GDFR, composition

Introduction
India, the spice bowl of the world with more than 50 varieties of spices being produced. The total production of spices in India is estimated at 5.8 million tonnes and it accounts for over 45 percent of the world spice trade by volume and value. Fenugreek, an important spice was produced to the tune of 1.279 lakh tones in the year 2010-11. Rajasthan accounts for 74% of the fenugreek seed produced in India [1].

Fenugreek (*Trigonella foenum-graecum* L.) is known as methi in Hindi and Vendayam in Tamil. The largest producer of fenugreek in the world is India. In India, the seeds are used in curries (preparation of pickles, vegetable dishes, dhals and spice mixes such as Panch Phoron and sambar powder) and for its medicinal properties *viz*., anti-diabetic and cholesterol lowering properties [2-5], anti-hyperthyroid effects [6], against thyroxine-induced hyperglycemia [7], anti-cancer effects [8], gastro-protective effects [9], antioxidant property [10], antinociceptive property [11], antimicrobial property [12], anthelmintic property [13], anti-sterility and anti-androgenic effects [14], wound healing property [15] and also anti-inflammatory and antipyretic actions [16].

Galactomannan in fenugreek, due to its viscous property, is effective in inhibiting the intestinal glucose uptake and lower blood glucose [17], hence separation of Galactomannan are undertaken at industrial levels (eg. M/s. E.I.D. Parry (India) Limited, Bio Products Division, Cuddalore, Tamil Nadu, India) to produce anti-diabetic nutraceutical. The residue is designated as Galactomannan Depleted Fenugreek Residue (GDFR) and marketed as Fenumax®. With the increased incidence of diabetes in India and the clamour for using natural drugs for diabetes, the growth of Galactomannan separation from fenugreek is likely to increase resulting in more quantity of the GDFR available.

Galactomannan are the major polysaccharide found in fenugreek seed and represent approximately 50% of the seed weight [18]. The remaining 50% of the material from fenugreek Galactomannan extraction industry is available as Galactomannan depleted fenugreek residue (GDFR). Extractable oil from fenugreek represents about 6- 8% of the seed weight and contains ω-3(n-3), ω-6(n-6), and ω-9 (n-9) fatty acids along with many saponins, alkaloids, and sterols [19-20]. Shahat [21] studied Egyptian fenugreek oil and reported that it consists of 33.7% linoleic, 35.1% oleic and 13.8% linolenic acids. Baccou *et al.* [22] studied the fatty acid composition of fenugreek oil from different countries and found that the percentage of linoleic and linolenic acids differ according to place and conditions of cultivation of plant, and the oils had marked drying properties.

Dietary fatty acids modify the plasma lipoprotein profile and reduce the risk of cardiovascular disease, which has been shown in intervention studies [23-25] in particular for polyunsaturated and monounsaturated fatty acids. In this study, fatty acid composition of fenugreek seeds was investigated.

Methodology

A total of six fenugreek seed samples were collected from different areas of Tamilnadu. The samples were ground and used for analyses. The six samples of GDFR (Fenumax®) were received from E.I.D. Parry (India) limited, Bio Products Division, Cuddalore, Tamilnadu. These six samples in each of fenugreek seed and GDFR were analysed for fatty acid composition.

Fatty acid composition

The fatty acids profile were analysed as per the method described by Sukhija and Palmquist [26]. Two grams of fenugreek seed or GDFR sample was weighed, twenty milliliters of Folch`s solution (containing chloroform, methanol 2:1 v/v) was added to the test tubes, homogenized with high speed homogenizer for 30 sec. The homogenate was left undisturbed overnight and then filtered through Whatman No. 42 filter paper into another test tube to which 10 ml of 0.88% sodium chloride solution was added. The filtrate was mixed well and left for 2 h [27].

The upper layer was siphoned off and the lower lipid layer was then extracted which was then transferred to brown bottles and evaporated. Three milliliter of 10 per cent methanolic HCl was added to the brown bottles, capped tightly, vortexed and heated in a water bath for 2 h at 65˚C. The samples were cooled and transferred to test tubes. To these test tubes, 5 ml of 6 per cent potassium carbonate solution and 2 ml of hexane were added carefully. The tubes were centrifuged at 6,000 rpm for 10 min to separate the solvent layer. The top hexane layer containing the fatty acid methyl esters was transferred to eppendorf tubes and sealed tightly with parafilm tape. Samples were stored at -20˚C until analysis.

The hexane layer (0.2 µl) was injected into the gas chromatograph (CERES 800 plus, Chemito, India) fitted with fused silica capillary column of 30 m × 0.25 mm internal diameter, 0.25 µ film thickness of stationary phase (SGE, Australia) connected to flame ionization detector of gas chromatograph. Ramped oven temperature condition (160˚C for 3 min and increased to 220˚C at the rate of 5˚C per 5 min and held for 5 min) was used. Temperature of both injector and detector was kept at 225˚C and 230˚C, respectively. The flow rates of nitrogen (as carrier gas), hydrogen and air were 1, 30 and 300 ml respectively. The output signal from gas chromatograph was analyzed based on area normalization by using Chemito software (IRIS 32 Lite). The fatty acids composition in the samples was estimated by comparing retention time of known authentic standards of methyl esters of fatty acids (Supelco, USA).

Results and Discussion

Fatty acid composition

The fatty acids content in fenugreek seed and GDFR are presented in Table 1. The fatty acids composition (g/100 g sample) of fenugreek seed and GDFR were 0.57 and 0.99 palmitic, 0.64 and 0.98 stearic, 2.43 and 3.78 oleic, 1.59 and 2.92 linoleic, 0.04 and 0.07 linolenic and 0.03 and 0.05 arachidic acids respectively.

Table 1: Fatty acid profile of fenugreek seed and GDFR (on DM)

Fatty acid (g/100g sample)	Fenugreek Seed	GDFR
Palmitic acid (16:0)	0.57 ± 0.04	0.99 ± 0.09
Stearic acid (18:0)	0.64 ± 0.04	0.98 ± 0.05
Oleic acid (18:1)	2.43 ± 0.15	3.78 ± 0.17
Linoleic acid (18:2)	1.59 ± 0.09	2.92 ± 0.10
Linolenic acid (18:3)	0.04 ± 0.01	0.07 ± 0.01
Arachidic acid (20:0)	0.03 ± 0.01	0.05 ± 0.01
Other fatty acids	0.22 ± 0.07	0.13 ± 0.04

Each value is a mean of six observations.

The samples in this study were having similar level of palmitic acid, higher stearic and oleic acids and lower linoleic, linolenic and arachidic acids in fenugreek seed than observed by earlier workers [28-34].

In GDFR, the palmitic, stearic and oleic acid contents were higher and linoleic, linolenic and arachidic acid contents were lower as evidenced by the results obtained in comparison to literature values [35] but the fatty acids content of GDFR is agreement with fenugreek seed.

The fatty acids evaluated in fenugreek seed and GDFR oils were different from the previously reported works that might be due to the genetic factors and environmental conditions during fruit development and maturity [33], place and conditions of cultivation of plant [22]. Hilditch and Williams [36] recorded that the temperature and atmosphere were the principal factors accounting for variations in fatty acids, especially in linolenic acid.

Conclusions

This study revealed the fatty acids content (g/100 g sample) of fenugreek seed and GDFR were palmitic acid (0.57 and 0.99), stearic acid (0.64 and 0.98), oleic acid (2.43 and 3.78), linoleic acid (1.59 and 2.92), linolenic acid (0.04 and 0.07), arachidic acid (0.03 and 0.05) and other fatty acids (0.22 and 0.13). In this study, Oleic acid and linoleic acid was indicated as the main source of fatty acid in fenugreek seed.

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