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Chemical 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 chemical composition of fenugreek seed and Galactomannan Depleted Fenugreek Residue (GDFR). The evaluation was based on series of chemical analyses. 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 chemical composition. The proximate composition and mineral composition were analysed as per the methods of AOAC. Total phenolics content in the samples were analysed by the Folin-Ciocalteu method. The composition (%) of fenugreek seed and GDFR samples were crude protein (26.65 and 33.79), crude fibre (7.54 and 6.82), nitrogen free extract (57.10 and 46.48), neutral detergent fibre (38.52 and 18.47), acid detergent fibre (13.13 and 10.07), hemicellulose (25.38 and 8.40), calcium (0.33 and 0.37%), phosphorus (0.40 and 0.49%), zinc (39.94 and 49.36 mg/kg) and iron (30.96 and 43.52 mg/kg). The total phenolic content of fenugreek seed was 10.3 and GDFR was 9.86 mg/g.

Keywords: Composition, fenugreek, fibre, GDFR, minerals, phenolics

Introduction
There are products in nature like phytobiotic substances obtained from the plants and herbs having wide range of medicinal and growth promotional properties [1]. Medicinal and aromatic plants have been used for many years in human nutrition as spices and medical additives in animals to increase dietary energy utilization, improve the performance efficiency and as a new source of protein.

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 [2].

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 [3-6], anti-hyperthyroid effects [7], against thyroxine-induced hyperglycemia [8], anti-cancer effects [9], gastro-protective effects [10], antioxidant property [11], antinociceptive property [12], antimicrobial property [13], anthelmintic property [14], anti-sterility and anti-androgenic effects [15], wound healing property [16] and also anti-inflammatory and antipyretic actions [17].

Galactomannan in fenugreek, due to its viscous property, is effective in inhibiting the intestinal glucose uptake and lower blood glucose [18], 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 [19]. The remaining 50% of the material from fenugreek galactomannan extraction industry is available as galactomannan depleted fenugreek residue

(GDFR). Livestock and Poultry scientists today are challenged to find out new alternative growth promoters possessing "gut health promotion, improving nutrient utilization and enhance immunity with no deleterious effect on animal welfare, environment impact and provide a safe livestock product to the consumer and at the same time the material should be from natural sources".

Nutrient contents of GDFR for livestock and poultry has not been studied earlier. There is limited evidence on the use of fenugreek seed in livestock and poultry ration and the use of GDFR as feed ingredient for poultry is lacking. Therefore, the present experiment was designed to evaluate the chemical composition of fenugreek seed and Galactomannan Depleted Fenugreek Residue (GDFR) for livestock and poultry.

Methodology

A total of six fenugreek seed samples were collected from different areas of Tamil Nadu. 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, Tamil Nadu. These six samples in each of fenugreek seed and GDFR were analysed for chemical composition.

Proximate composition

The proximate composition *viz*., crude protein (CP), crude fibre (CF), ether extract (EE) and total ash (TA) were analysed as per the methods of AOAC [20]. The nitrogen free extract (NFE) was calculated by difference. All the values were expressed as percent on dry matter basis.

Fibre fractions

The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were quantified as per the method of Goering and Van Soest [21] and the hemicellulose was calculated.

Macro minerals

Calcium and total phosphorus contents were estimated according to the method of AOAC [20].

The level of sodium was quantified using flame photometer as per AOAC [20] and chloride using mercuric nitrate as per Eaton *et al*. [22]

Extraction of sodium from the feed ingredient samples was done by heat digestion using 2:1 ratio solution of concentrated nitric acid and perchloric acid, followed by filtration and dilution to 100 ml. The sample size taken was 0.5 to 2 grams such that the concentration of sodium was in the ranges of 100 to 25000 ppm.

According to the concentration of sodium the change in the colour of the flame was recorded in photo detector and the result was obtained in ppm. The concentration of sodium (ppm) of the sample was quantified as Na^+ (ppm)/ sample weight (g) × 100 (dilution factor).

For quantification of chloride, 10 grams of feed ingredient sample was dissolved in 100 ml of water to extract the chloride, 10 ml of the extract was titrated against standard mercuric nitrate (0.0141N) using mixed indicator (diphenylcarbazone and bromphenol blue).

Micro minerals

The micro minerals (Cu, Mn, Zn and Fe) were estimated by wet ashing process. Fenugreek seed or GDFR, 2.5 g was taken to which 25 ml of concentrated nitric acid was added and boiled gently for 30 - 45 minutes then slowly added 10 ml of 72% perchloric acid and boiled gently to nearly colourless solution. The soluble minerals were filtered through Whatman filter paper No.42 and made up to 100 ml using distilled water. Sub-dilution was made with 0.1 - 0.5N HCl. The micro minerals were analysed using Perkin Elmer Atomic Absorption Spectrophotometer Model 3110 equipped with hollow cathode lamp, air supply at 28 litres/minute at 51-65 psi and acetylene flow at 4 litres/minute at 12-14 psi and the temperature adjusted as per the recommendation of manufacturer.

Total phenolics

Total phenolics content in the samples of fenugreek seed and GDFR was analysed by the Folin-Ciocalteu method [23]. The total phenolics in 0.2 g of fenugreek seed or GDFR was extracted using 10 ml of 70% acetone maintained at 37°C for 2 hours then filtered through Whatman filter paper No.1 and 0.1 ml of the filtrate was reacted with 0.5 ml of 1N Folin-Ciocalteu reagent and 2.5 ml of 20% Na_2CO_3 solution for the development of colour. The optical density was measured at 725 nm using spectrophotometer.

Results and Discussion

Proximate composition

The proximate composition of fenugreek seed and GDFR collected from different areas of Tamilnadu are presented in Table 1. The mean proximate composition (in per cent) of fenugreek seed analysed in this study was dry matter - 91.32, crude protein - 26.65, crude fibre - 7.54, ether extract - 5.52, total ash - 3.19 and nitrogen free extract - 57.10. The various nutrients in the sample studied were within the range reported by earlier workers [2, 24-28].

The mean proximate composition (in per cent) of GDFR analysed in this study was dry matter - 91.37, crude protein - 33.79, crude fibre - 6.82, ether extract - 8.90, total ash - 4.01 and nitrogen free extract - 46.48. The levels of protein and ether extract were comparable, but the content of total ash in samples studied was higher than the values reported earlier [29].

Since galactomannan which is a component of carbohydrate fraction is separated from fenugreek seed, the level of fibre and NFE in GDFR were lower and correspondingly the levels of protein and ether extract were higher than FS.

Fibre Fractions

The fibre fractions *viz*., NDF, ADF and hemicelluloses in fenugreek seed and GDFR are presented in Table 2.

In fenugreek seed, the mean NDF, ADF and hemicellulose content (in per cent) were 38.52, 13.13 and 25.38 and in GDFR 18.47, 10.07 and 8.40 respectively. The fibre fractions evaluated in fenugreek seed were comparable to the reported value of Kochhar *et al*. [30] and Naidu *et al*. [25], but higher than Ribes *et al*. [31]. This might be due to the genetic factors or environmental conditions (e.g. harvest season, climate and stress condition) during fruit development and maturity [26], plant species and growth stage, agricultural practices (e.g. plant density per cultivated area, fertilization, irrigation level) and growing region [32, 33].

The NDF, ADF and hemicellulose content in GDFR was lower than fenugreek seed due to the removal of galactomannan. The NDF, ADF and hemicellulose content of GDFR was similar to that of maize [34].

Macro Minerals

The calcium, phosphorus, sodium and chlorine content in fenugreek seed and GDFR are presented in Table 3. Fenugreek seed contained calcium - 0.33, phosphorus - 0.40, sodium - 0.14 and chlorine - 0.21 per cent; the corresponding values for GDFR were 0.37, 0.49, 0.06 and 0.09 per cent respectively. The calcium, phosphorus and sodium content of fenugreek seed are within the range of earlier reports by Nabey and Damir [35], Leela and Shafeekh [36], Abaza [37], Ziwar [38] and Ali *et al*. [26]. Calcium, phosphorus, sodium and chlorine content in fenugreek seed and GDFR were higher than maize [39]. These minerals in GDFR were comparable to fenugreek seed.

Micro Minerals

The copper, manganese, zinc and iron content in fenugreek seed and GDFR are presented in Table 3. The mean copper, manganese, zinc and iron content (mg/kg) in fenugreek seed was 13.98, 9.69, 39.94 and 30.96 and in GDFR was 15.63, 11.66, 49.36 and 43.52 respectively.

The copper, manganese and zinc content of fenugreek seed are in agreement with the ranges of these mineral as observed by Abaza [37], Anonymous [2], Ziwar [38], Ali *et al*. [26], Al-Jasass and Al-Jasser [27] and Mahmoud *et al*. [40]. The lower level of iron content observed in this study might be due to the variety of fenugreek seed and availability of minerals in the soil. Copper, manganese, zinc and iron contents of GDFR are comparable with fenugreek seed.

The copper, manganese and zinc contents of fenugreek seed and GDFR were higher than maize and the iron content was lower in fenugreek seed and GDFR when compared to maize [39].

Total Phenolics

The total phenolic content in fenugreek seed and GDFR is presented in Table 4. The total phenolic content of fenugreek seed and GDFR was 10.30 and 9.86 mg/g as tannic acid equivalent.

The total phenolics content (mg/g) as tannic acid equivalent in fenugreek seed were higher than the reported level of 1.05 by Kochhar *et al*. [30] and 2.9 by Mahmoud *et al*. [40]. The variation in total phenolics content might be due to the different solvents used in the extraction procedure[41]. In GDFR the total phenolic content was similar to fenugreek seed.

Table 1: Proximate composition (%) of fenugreek seed and GDFR (on DM)

Composition	Fenugreek Seed	GDFR
Dry matter	91.32 ± 0.07	91.37 ± 0.10
Crude protein	26.65 ± 1.09	33.79 ± 0.83
Crude fibre	7.54 ± 0.24	6.82 ± 0.44
Ether extract	5.52 ± 0.31	8.90 ± 0.33
Mineral matter	3.19 ± 0.19	4.01 ± 0.29
Nitrogen free extract	57.10 ± 1.15	46.48 ± 0.82
Acid insoluble ash	0.83 ± 0.16	0.92 ± 0.18

Each value is a mean of six observations.

Table 2: Fibre fraction (%) of fenugreek seed and GDFR (on DM)

Composition	Fenugreek Seed	GDFR
Neutral detergent fibre	38.52 ± 2.05	18.47 ± 1.42
Acid detergent fibre	13.13 ± 0.54	10.07 ± 0.40
Hemicellulose	25.38 ± 1.90	8.40 ± 1.03

Each value is a mean of six observations.

Table 3: Mineral content of fenugreek seed and GDFR (on DM)

Composition	Fenugreek Seed	GDFR
Calcium (%)	0.33 ± 0.02	0.37 ± 0.02
Phosphorus (%)	0.40 ± 0.02	0.49 ± 0.01
Sodium (%)	0.14 ± 0.03	0.06 ± 0.02
Chlorine (%)	0.21 ± 0.04	0.09 ± 0.03
Copper (mg/kg)	13.98 ± 0.27	15.63 ± 0.54
Manganese (mg/kg)	9.69 ± 1.12	11.66 ± 1.32
Zinc (mg/kg)	39.94 ± 2.21	49.36 ± 1.49
Iron (mg/kg)	30.96 ± 5.56	43.52 ± 4.54

Each value is a mean of six observations.

Table 4: Total phenolics content of fenugreek seed and GDFR (on DM)

Composition	Fenugreek Seed	GDFR
Total phenolics (mg/g)	10.30 ± 0.19	9.86 ± 0.99

Each value is a mean of six observations.

Conclusions

The composition (%) of fenugreek seed and GDFR samples were crude protein (26.65 and 33.79), crude fibre (7.54 and 6.82), nitrogen free extract (57.10 and 46.48), neutral detergent fibre (38.52 and 18.47), acid detergent fibre (13.13 and 10.07), hemicellulose (25.38 and 8.40), calcium (0.33 and 0.37%), phosphorus (0.40 and 0.49%), zinc (39.94 and 49.36 mg/kg) and iron (30.96 and 43.52 mg/kg). The total phenolic content of fenugreek seed was 10.3 and GDFR was 9.86 mg/g. Hence, based on the nutrient composition of fenugreek seed and GDFR, it has been used in livestock and poultry feed industry.

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