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ORIGINAL ARTICLE

Effect of processing techniques on nutritional composition and antioxidant activity of fenugreek (*Trigonella foenum-graecum*) seed flour

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Abstract Fenugreek (Pusa Early Bunching) seeds were processed by using different processing methods viz. soaking, germination and roasting. Raw and processed fenugreek seed flours were analyzed for nutritional composition, anti- nutritional, and antioxidant activity. Raw fenugreek seed flour contained higher amount of dietary fiber (45.4 %) followed by 41.7 % in soaked seed flour, 40.9 % in roasted fenugreek seed flour and 31.3 % in germinated fenugreek seed flour. Processing of fenugreek seeds improved in vitro starch digestibility and in vitro protein digestibility. Soaking, germination and roasting enhanced total phenolic content and the antioxidant activity of fenugreek seed flour as compared to raw fenugreek seed flour. The phenolic content of soaked, germinated and roasted fenugreek seed flours was 54.4, 80.8 and 48.5 mg of gallic acid equivalents/g of sample in contrast to raw fenugreek seed flour (45.4 mg of gallic acid equivalents/g of sample). The antioxidant activity of the extracts of soaked, germinated and roasted fenugreek seed flours was 60.7 %, 73.9 % and 32.0 % whereas as the raw fenugreek seed flour exhibited 18.1 % antioxidant activity. Processing of fenugreek seeds also decreased phytic acid content significantly ($P<0.05$) as compared to raw seeds.

Keywords Fenugreek seeds · Germination · Roasting · Antioxidant activity · In vitro starch digestibility · Phenolic content

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an old medicinal plant and has been commonly used as a traditional food and medicine. Fenugreek is one of the medicinal plants, originating in India and Northern Africa. Its seeds are used as condiment in most parts of India, as a supplement to wheat and maize flour in Egypt and in Yemen, they are one of the main constituents of the normal daily diet (Uhl 2000). Over 80 % of the total world's production of this seed is contributed by India, one of the major producers and exporters of fenugreek legume in the world. Fenugreek seeds can be a good supplement to cereals because of its high protein (25 %), lysine (5.7 g/16 g N), soluble (20 %) and insoluble (28 %) dietary fiber besides being rich in calcium, iron and beta-carotene (NIN 1987). In India seeds are used either boiled, pressure cooked, roasted or germinated, this basic processing is done to make seeds soft, palatable and to remove their bitterness (Mathur and Chaudhary 2009). The seeds of fenugreek contain lysine and L- tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarine, sapogenins and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and thought to help lower sugar levels (Bukhari et al. 2008). It is reported to have restorative and nutritive properties and to stimulate digestive processes (Khosla et al. 1995). Fenugreek seeds have also been reported to exhibit pharmacological properties such as anti-tumor, anti-viral and antioxidant activity.

However, the seeds are bitter in taste due to presence of saponins which limit their acceptability in foods (Sharma 1986). It has been possible to debitter fenugreek seeds by employing various processing methods such as soaking, germination, roasting etc. As fenugreek seeds are rich in mucilaginous fiber and other dietary essentials, their use can be exploited as functional and nutritional foods as well as therapeutic agent. Fenugreek has been used for centuries but few studies confirm its efficacy in treatment of diabetes. Traditional processing methods may affect reduction in the bitterness of the seeds and make possible its incorporation in various recipes. Earlier studies reported that sprouting or

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overnight soaking; washing of fenugreek seeds in running water and roasting removes the bitterness to a certain extent and makes possible its use in increased quantities for incorporation into various preparations which are commonly consumed. The beneficial effects of processing of fenugreek seeds may be attributed to an increase in low methoxy salts of calcium and magnesium as well as proto-pectin. It is probable that formation of these constituents has a role to play in reducing the blood sugar levels or it may also be likely that an active, hypoglycemic principle might have increased during processing like soaking and germination (Hooda and Jood 2003). Thus, in view of the aforesaid anti-diabetic and hypolipidemic role of processed fenugreek seeds the present study aimed at studying the effect of various processing treatments on the nutritional composition, anti-nutritional, phenolic content and antioxidant activity so that they could find potential as dietary supplement and health food for hyperlipidemic and diabetic subjects.

Materials and methods

Fenugreek seeds (Pusa Early Bunching) were procured from Vegetable Research Centre of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Seeds were cleaned to remove any extraneous material. Raw seeds were dried at 40±5 °C in a hot air oven to increase its keeping quality and stored in an airtight containers at ambient temperature.

Soaking

The cleaned seeds (50 g) were soaked in distilled water at the ratio of 1:5 (w/v) at room temperature for 12 h. The water was intermittently changed every 6 h. After 12 h, the excess water was discarded and seeds were dried in hot air oven at 40° C for 6 h.

Germination

Fenugreek seeds (50 g) were soaked overnight in water at the ratio of 1:5 (w/v). The excess water was drained and seeds were kept in the dark for germination (tied in a muslin cloth) at 27°±2 °C temperature for 24 h. The germinated seeds were dried in an oven at 40 °C for 6 h.

Roasting

Fenugreek seeds (50 g) were roasted in an open pan at 130±5 °C for 7 min. It was continuously stirred with laddle for proper and uniform roasting until it became slight brown and left a peculiar aroma.

Raw and processed (soaked, germinated and roasted) fenugreek seeds were ground in grinder (make Phillips) and passed through standard test sieve (BSS 60) to get uniform sized flour. Flours were collected and stored in air tight food grade containers separately for further use at ambient temperature.

Analysis

Samples were analyzed for their chemical constituents namely, moisture, ash, crude protein, crude fat and crude fiber. They were also analyzed for minerals, dietary fiber (total/soluble/insoluble), phytic acid, in vitro starch digestibility, in vitro protein digestibility, total phenolic content and antioxidant activity.

Moisture,ash,crude protein, crude fat,crude fiber and dietary fiber

Methods of AOAC (1995) were used to determine the moisture, ash, crude protein, crude fat and crude fiber. For moisture, samples were accurately weighed in an aluminum dish and dried for 3 h in an oven at 103±2 °C. It was cooled in desiccator and then weighed. Samples was further dried for 1 h and reweighed until a constant weight was obtained. For ash estimation, 5 g sample was weighed accurately into a porcelain crucible and heated first over a low flame till it was completely charred and then incinerated in a muffle furnace (550 ° C for 16 h) to a grayish color ash. The crude fat was determined by extracting the sample in a Soxhlet apparatus for 16 h using petroleum ether (B.P. 60–80 °C). The solvent was evaporated and the residue was weighed to determine the fat content. The estimation of nitrogen was done by Kjeldahl method wherein the protein content was obtained by multiplying the nitrogen value with conversion factor of 6.25. For fiber estimation, defatted sample (2 g) was taken and digested with 1.25 % H_2SO_4 in spoutless beaker and boiled for 30 min. subsequently with 1.25 % NaOH solution. The contents were filtered through Whatman no. 54 filter paper using Buchner's funnel under the gentle suction. The filter paper with the residue was dried in oven at 105° C till constant weight was obtained. It was cooled in a desiccator and weighed. The loss in weight represented the crude fiber content.

The total dietary fiber, a measure of the sum of insoluble and soluble dietary fiber, based on digestion of food samples (1 g) with enzymes, was determined as described by Asp et al. (1983).

Minerals

The charred samples were incinerated at 550 °C in a muffle furnace and treated with 1 ml mixture of water and conc. HCL (1:5), filtered into 50 ml volumetric flask and made up

to volume with double distilled water, used for the estimation of Ca by Titrimetric method, AOAC (1995). Ash solution for AAS was prepared by wet digestion method described by Raghuramulu et al. (2003) and used for the estimation of P, Zn and Fe content of fenugreek seed flour using respective standard solutions, by Atomic Absorption Spectrophotometer model SensAA, GBC Scientific Equipment (USA) and measurements were made at 213.6 nm (P), 248.3 nm (Fe) and 213.8 nm (Zn).

In vitro starch digestibility and in vitro protein digestibility

In vitro starch digestibility was assessed by employing pancreatic amylase and incubating at 37 °C for 2 h liberated maltose was measured colorimetrically by using dinitrosalicylic acid reagent (Singh et al. 1982).

In vitro protein digestibility was assessed by employing pepsin and pancreatin method described by Akeson and Stahmann (1964). The nitrogen content was determined by the microkjeldahl method (AOAC 1995). Per cent protein digestibility of the sample was calculated by:

$$\% \ Digestibility = \frac{Protein \ in \ digested \ sample}{Crude \ protein} \times 100$$

Phytic acid

Phytic acid was determined by the method given by Haug and Lantzsch (1983). The sample extract (with 0.2 N HCl) was heated with an acidic iron solution of known iron content. The decrease in iron content (determined colorimetrically with 2, 2-bipyridine) in the supernatant was the measure of the phytate phosphorus.

Total phenolic assay

Total phenolics were measured following the protocol developed by Chandler and Dodds (1983) and modified by Shetty et al. (1995). Phenolics were measured as gallic acid equivalents. Approximately 50 mg of the fenugreek seed flour was immersed in 2.5 ml of 95 % ethanol and kept in the freezer at −20 °C for 48 h. The sample was homogenized using a homogenizer (RQ 127 A/D, Remi Equipments) and centrifuged at 13,000 rpm for 10 min. One ml of the supernatant was transferred to a test tube and 1 ml of 95 % ethanol; 5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent were added. After an incubation period of 5 min. 1 ml of 5 % Na_2CO_3 was added, mixed well and the solution was kept in the dark for 1 h. Then the samples were vortexed and the absorbance measured at 725 nm using a UV spectrophotometer (Spectronic Genesys 10 UV; Spectro Unicam, Rochester, NY).

Total antioxidant activity

Preparation of extract: The antioxidant activity of fenugreek seed flours were estimated by the method given by Zhang and Hamauzu (2004). Ten gram of sample was homogenized with 15 ml of 80 % methanol. The homogenate was filtered through 4 layers of cheese cloth and the residue was treated, added with 15 ml of 80 % methanol for 2 successive extractions. The filtrates were combined and centrifuged at 4,000 rpm for 10 min. The supernatant of methanol extract was collected and diluted to various concentrations (1 %, 2.5 %, 5 %, 7.5 % and 10 %) for measurement of antioxidant activity. Several data indicated that the highest level of antioxidant activity was obtained with 5 % concentration, which was used for further study. After the samples at various concentrations were studied, the 5 % concentration was chosen as an appropriate concentration for assessing antioxidant activity.

Free radical scavenging activity-

Antioxidant activity was determined by the 1, 1-diphenyl-picryl- hydrazyl (DPPH·method of Brand-Williams et al. (1995)). Solution of DPPH·0.1 mM in methanol was prepared and 4 ml of this solution was treated with 0.2 ml of extract. A control was treated with 0.2 ml of distilled water instead of the extract. The mixture was left to stand for 60 min. and absorbance was taken at 517 nm.

Total antioxidant activity was expressed as the % of DPPH decrease using the equation:

$$\% \ Antioxidant \ activity = \frac{Control \ absorbance - sample \ absorbance}{Control \ absorbance} \times 100$$

Statistical analysis

Each experiment was replicated three times. The results were expressed as mean±SD. Analysis of variance was applied to analyze data and significance was accepted at $p \leq 0.05$ level.

Results and discussion

Raw fenugreek seed flour contained (Table 1) crude protein (32.7 %), crude fat (4.8 %), ash (3.7 %) and crude fiber (6.0 %). Raw fenugreek seed flour had higher amount of dietary fiber (45.4 %), phytic acid (552.3 mg/100 g) as compared to processed fenugreek seed flours. Significantly lower phenolic content (45.4 mg gallic acid equivalents/g of sample) and antioxidant activity (18.1 %) has been observed in raw fenugreek seed flour as compared to processed.

Table 1 Changes in nutritional composition, anti-nutritional, total phenolic content and antioxidant activity of fenugreek seed flour

	Raw	Soaked	Germinated	Roasted	CD ($P<0.05$)
Moisture %	6.3±0.34	5.2±1.03	5.5±0.30	4.2±0.14	1.07
Crude Protein %	32.7±0.43	35.1±0.70	41.2±1.10	36.8±1.00	1.60
Crude Fat %	4.8±0.16	4.6±0.15	3.7±0.12	4.5±0.22	0.32
Ash %	3.7±0.25	4.0±0.31	4.6±0.30	3.8±.22	0.52
Crude fiber %	6.0±0.07	6.0±0.10	8.8±0.6	6.2±0.11	0.58
Carbohydrates by difference %	46.1±1.14	44.8±1.30	35.7±0.56	43.9±1.23	2.07
Total dietary fiber (TDF) %	45.4±0.56	41.7±0.63	31.3±0.74	40.9±0.84	1.32
Insoluble dietary fiber (IDF) %	25.1±0.79	22.5±1.03	19.9±0.47	23.7±0.58	1.42
Soluble dietary fiber (SDF) %	20.2±0.25	19.2±1.39	11.3±0.46	17.1±0.26	1.42
Ca mg/100 g	70.5±0.68	68.2±0.86	70.7±0.62	71.2±0.77	1.27
P mg/100 g	544.5±1.26	612.8±0.90	632.3±0.92	611.9±0.27	1.76
Fe mg/100 g	11.6±0.59	10.7±0.19	11.5±0.31	13.1±0.25	0.70
Zn mg/100 g	5.7±0.34	5.2±0.20	5.5±0.02	6.2±0.12	0.35
In vitro starch digestibility (mg maltose released/g meal)	36.3±0.71	39.3±0.51	46.1±0.33	37.3±0.43	1.01
In vitro protein digestibility %	48.6 ±1.07	57.4±0.67	63.0±0.73	55.8±0.58	1.48
Total phenolic content (mg of gallic acid equivalents/g of sample)	45.4±0.02	54.4±0.01	80.8±0.02	48.5±0.01	0.14
Antioxidant activity %	18.1±0.70	60.7±0.57	73.9±0.45	32.0±0.76	1.19
Phytic acid mg/100 g	552.3 ±2.52	504.2±2.52	308.7±1.95	327.1±2.00	4.2

Mean±SD, $n=3$

Previous studies reported total phenolics 54.3 mg of CAE/g dw and 10 % antioxidant activity in raw fenugreek seeds. Lower in vitro starch digestibility (36.3 mg maltose released/g flour) and in vitro protein digestibility (48.6 %) have been estimated in raw fenugreek seed flour as against processed fenugreek seed flours.

Soaking

Soaking of fenugreek seeds decreased fat content to (4.6 %) whereas significant increase in protein content (35.1 %) was observed. A similar trend in decrease in fat content in fenugreek seeds after soaking has been observed by Hooda and Jood (2003). Total carbohydrates (44.8 %) also decreased but marginally. Significant decrease in total dietary fiber (45.4–41.7 %), insoluble dietary fiber (25.1–22.5 %) and soluble dietary fiber content (20.2–19.2 %) was observed. This decrease might be attributed to enzymatic degradation of seeds during soaking (Mathur and Chaudhary 2009). Decrease in dietary fiber content after soaking has also been previously reported by Hooda and Jood (2003). Phenolic content increased from 45.4–54.4 mg gallic acid equivalents/g of sample. In response to phenolic content, antioxidant activity increased significantly after soaking from 18.1–60.7 %. In general soaking did not show any significant changes in total ash (4.0 %). Decrease in Ca and Zn was observed while significant increase in P content has been found. Fe content also decreased but marginally. Comparatively lower contents of mineral when soaked in water might be due to leaching out of some amount in to soaking water (Nolan and Duffin 1987). Phytic acid content (552.3–504.2 mg/100 g) decreased significantly after soaking, which ultimately caused significant increase in in vitro starch digestibility and in vitro protein digestibility. Findings of Hooda and Jood (2003) are in agreement with the increase in vitro starch digestibility and in vitro protein digestibility.

Germination

Germination of fenugreek seeds caused decrease in fat content as compared to raw seeds. Loss of fat during germination may be due to its consumption as an energy source in the process of germination (Mansour and El-Adway 1994). El-Aal (1986) reported decrease in total fat content along with decrease in free fatty acids, monoglycerides and polar lipids upon germination. The protein content increased from 32.7–41.2 % after germination. This increase might be due to reduction of seed nitrates into protein or ammonium compound (Hooda and Jood 2003). Increase in protein content of germinated seeds might be attributed to enzymatic synthesis of protein, which is in consent with the findings of Mansour and EL-Adway (1994) and Mathur and Chaudhary (2009). Increase in crude fiber content upon germination, a major constituent of cell walls, might be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination (http://en.wikipedia.org/wiki/Sprouting, 2012). There was marked reduction in total dietary fiber (TDF) content, insoluble dietary fiber (IDF)

content, and soluble dietary fiber (SDF), content upon germination. Reduction in dietary fiber content after germination may be attributed to enzymatic breakdown of the galactomannan units during sprouting. Shakuntala et al. (2011) reported a decrease in soluble dietary fiber content upon germination of fenugreek seeds. This decrease is accompanied by drop in galactan content. An enzyme α-galactosidase from germinated fenugreek seeds partially attacks galactomannan to yield galactose. The decrease in polysaccharide and dietary fiber content may be attributed to their breakdown and utilization by the growing sprouts. Neeraja and Rajyalakshmi (1996) also reported reduction in TDF after germination of fenugreek seeds from 47.8–33.8 % . The breakdown of mucilage during germination was previously reported by El- Mahdy and El- Sebaiy (1982). Germination of fenugreek seeds for 24 h reduced the phytic acid content from 552.3–308.7 mg/100 g. El- Mahdy and El-Sebaiy (1982) found that phytase activity which was absent in the un-germinated seeds originates after germination and the phosphatase activity was increased in the germinated seeds which results in the reduction of phytic acid content in fenugreek seeds after germination. During sprouting enzymatic hydrolysis of phytate phosphorus takes place which decreases phytic acid content (Gupta et al. 2001). Germination resulted in perceptible increase in in vitro protein digestibility (63.0 %) and in vitro starch digestibility (46.1 mg maltose/g flour). Sprouting causes mobilization of protein with the help of protease leading to the formation of peptides, oligopepetides and free amino acids (Jood and Kapoor 1988). Germinated fenugreek seed flour extract exhibited good free-radical scavenging activity of about 73.9 % and total phenolic content of 80.8 mg gallic acid equivalents/g sample. Naidu et al. (2011) reported 50–70 % free radical scavenging activity in different concentrations of fenugreek extract. Randhir et al. (2004) reported higher antioxidant activity during early germination, which correlates to high phenolic content suggesting that initially phenolics are antioxidant in nature. Shakuntala et al. (2011) reported that sprouts of germinated fenugreek seeds were rich in polyphenols (97.55 mg/100 g). Cevallos-Casals and Cisneros-Zevallos (2010) reported that germinated edible seed spices are an excellent source of dietary phenolic content. They also observed that these phenolic compounds are responsible for the antioxidant properties of the sample. The antioxidant activity appears to be directly correlated to the polyphenol contents of fenugreek seeds. Germination caused significant changes in the phenolic composition of Lupin seeds due to mainly endogenous enzymes activation and the complex biochemical metabolism of seeds during the process (Duenas et al. 2009). Among the mineral content Zn and Fe decreased whereas other minerals (Ca and P) increased significantly. Decrease in Fe content in germinated fenugreek seed flour might be due to leaching of Fe in to soaking medium (Duhan et al. 2002). Decrease in Fe content during germination of fenugreek seeds was reported by El-Shimi et al. (1984). Lestienne et al. (2005) reported that reduction in Zn content in soaked and germinated seeds is due to leaching of Zn into soaking medium. Increase in Ca and P might be due to decrease in phytates, tannins and other anti-nutritional factors that bind the minerals as reported by El- Mahdy and El- Sebaiy (1982). Earlier reports also cite the overall differences in mineral composition of seeds on germination (Ahmad Rafik and Laila 1982).

Roasting

Roasting caused decrease in fat content (4.5 %) whereas there was perceptible increase in protein content (36.8 %). The effect of roasting upon the fat content of the beans is to reduce its actual weight with the shrinkage. Some of the more volatile fatty acids are driven off, and the fats break down to give a larger percentage of free fatty acids, some light esters, acrolein, and formic acid. The fat will come to the surface, through breaking of the fat cells, with a decided alteration in the chemical nature of the fat (Trigg 2012). Reduction in fat content upon roasting may be due to loss of volatile oils on open dry heat treatment (Mathur and Chaudhary 2009). Decrement in total dietary fiber, insoluble dietary fiber and soluble dietary fiber has been found. Reduction in IDF content after roasting probably might be due to retrogradation of starch during roasting (Mathur and Chaudhary 2009). Roasting of fenugreek seeds decreased phytic acid content from 552.3–327.1 mg/100 g. Decrease in phytic acid after roasting might be due to thermolability of phytic acid. Sprouting had more reducing effect on phytic acid when compared to roasting. Increase in in vitro starch digestibility (37.3 mg maltose released/g flour) and in vitro protein digestibility (55.8 %) after roasting of fenugreek seeds might be attributed to reduction in antinutritional factors such as phytates, tannins and oxalates. Roasting of fenugreek seeds also enhanced total phenolic content to 48.5 mg gallic acid equivalents/g sample and antioxidant activity to 32.0 %. Jeong et al. (2004) reported that antioxidant activities of defatted sesame meal extract increased as the roasting temperature of sesame seed increased, but the maximum antioxidant activity was achieved when the seeds were roasted at 200 °C for 60 min. Roasting of sesame seeds at 200 °C for 60 min significantly increased the total phenolic content, radical scavenging activity. Ca, P, Fe and Zn content increased slightly upon roasting. During roasting there is also breakdown of the bond between phytate and P. This increase may be attributed to the destruction of phytates, tannins and oxalates upon heating as shown by Reddy et al. (1978).

Nutritional composition, anti-nutritional and antioxidant activity compared well with the fenugreek seeds composition reported by Hooda and Jood (2003), Sharma (1986) and Gopalan et al. (2004). However, some values are much

different in studies of other countries viz. Sudan and Egypt. These differences may be attributed to the difference in climatic conditions, type of soil and cropping pattern of different countries.

Conclusion

It may be inferred from the present study that nutritional and therapeutic quality of fenugreek seeds can be improved through processing methods viz. soaking, germination and roasting. Antioxidant activity also increases significantly after processing which are found to be responsible for medicinal properties of processed fenugreek seed flour. Therefore, the use of processed fenugreek seed flour can be exploited in functional foods as well as a therapeutic agent on a regular basis.

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