Effect of Germination and Roasting on Nutritive Composition and Anti-Nutrients in Fenugreek Seeds

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Abstract

Fenugreek seeds were processed by germination and roasting. Raw and processed fenugreek seeds were analyzed for total polyphenolic content, antioxidant activity and tannins in 80% methanolic extract and absolute methanolic extract. Total polyphenolic content, antioxidant activity and tannins were significantly (P ≤ 0.05) different in 80% methanolic extract and absolute methanolic extract. Germination process significantly (P ≤ 0.05) increased total polyphenolic content and antioxidant activity. The maximum level of phenolic content (93.27 mg/g) was found in fenugreek seeds germinated for 48 hrs. Total polyphenolic content, antioxidant activity and tannins were also significantly (P ≤ 0.05) different in roasted fenugreek seeds.

Keywords: Fenugreek seeds, Germination process, Estimation of phytic acid

I. INTRODUCTION

Herb and spices have been used in food as a food additives due to their natural antioxidant property. Fenugreek (Trigonella foenum-gracum) is an annual leguminous bean member of Fabaceae family. Its seeds and green leaves are having many medicinal applications (Thomas et al., 2011; Paridar et al., 2011; Vaidya et al., 2013). In India, it is extensively used as Ayurvedic medicine and in China as traditional medicine (Prasad et al., 2014). Interestingly, in herbal medicine, it is used in the treatment of diabetes (Leela and Shafeekh, 2008). Fenugreek leaves provide a good amount of various minerals and vitamins. They are rich in choline.

Medicinally seeds are the most important and useful part of fenugreek plant. Seeds are aromatic, bitter, carminative, galactagogue and have antibacterial properties. These seeds are golden-yellow in colour, small in size, hard, having four-faced stone like structure. Fenugreek seeds have high protein (25 %), lysine (5.7 g/16 g N), soluble (20 %) and insoluble (28 %) dietary fiber besides being rich in calcium, iron and betacarotene. The biological and pharmacological actions of fenugreek seeds are mostly attributed to the variety of its bioactive chemical constituents that serve as raw materials for the manufacturing of various hormonal and therapeutic drugs (Mehrafarin et al., 2010; Priya et al., 2011). Their application are limited due to bitter taste (Sharma 1986). However these days various processing techniques are applied for debittering of fenugreek seeds.

Secondary metabolites flavonoids and phenolic compounds are widely distributed in plant and it exert multiple biological effects including free radical scavenging, anti-inflammatory, and anticarcinogenic (Miller A. L.,1996). T. foenum-graecum contain high phenolic contents having high antioxidant activity (Kaur.C.et al., 2002). Among the plethora of bioactive compounds found in fenugreek seeds the major chemical constituents are polyphenolic compounds, galactomannan (fiber), diosgenin (saponin), quercetin (flavonoid), trigonelline (alkaloid) and 4-hydroxyisoleucine (unusual amino acid). Germinated fenugreek seed are rich in polyphenolic compounds. Being a rich source of mucilaginous fiber and other dietary essential, fenugreek seeds used as a functional and nutritional food.

Therefore, the present study was planned to evaluate raw, germinated and roasted fenugreek seed extracts as a source of natural antioxidant. In this study, the effect of germination and roasting on their chemical composition was also investigated.

II. MATERIALS AND METHODS

Fenugreek seeds were purchased from local market of Hissar, Haryana. 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 temp.
**A. Germination**

Fenugreek seeds (20 g) were soaked overnight in water at the ratio of 1:5 (w/v). The excess water was drained and seeds were germinated (tied in a muslin cloth) at room temperature for 24 h, 36 h and 48 h. The germinated seeds were dried in an oven at 50 °C til the constant weight.

**B. Roasting**

Fenugreek seeds (20 g) were roasted in an open pan at 130±5 °C, 150±5 °C and 200±5°C for 7 min, 5 min and 2 min respectively. Continuous stirring was done with laddle for proper and uniform roasting.

**C. Preparation of Fenugreek Seeds Extract**

Raw and processed (germination and roasting) fenugreek seeds were ground in Wall Mill. Ground sample were collected in air tight container separately for further analysis at room temperature. The prepared sample were extracted in absolute methanol and 80% methanol using orbital shaker for 4 hrs at 45°C. In extraction process, 3 g of prepared sample were weighed in universal bottle and 75 ml solvent was added. After extraction process supernatant were collected for further analysis.

**D. Determination of Total Phenolic Content**

The total phenols in extracts was measured by UV spectrophotometry based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was Folin-Ciocalteu reagent (AOCS,1990). To 0.2 ml of extract, 7.5 ml water was added in a test tube and after that 0.5 ml of 1 N Folin-Ciocaltel reagent was added and, then, 1 ml of Na₂CO₃ were added. The sample was incubated for 30 min at room temperature. For a control sample, 0.2 ml of distilled water was used. The absorbance of the resulting blue-colored solutions was measured at 760 nm. Quantitative measurements were performed, based on a standard calibration curve of gallic acid in water. The results were expressed as gallic acid equivalents (GAE) in milligrams per gram of sample.

**E. Determination of Total Flavonoid Content**

Total flavonoid content was determined using a method by (Liu et al., 2008). Briefly, 2 ml of the extracts was taken in a test tube and 0.2 ml of 5% sodium nitrite was mixed. After 5 min 0.2 ml of 10% aluminium chloride was added and after 6 min 1 ml of 1M sodium hydroxide was added. The absorbance was measured at 510 nm. The results were expressed as Quarcetin equivalents (QE) in milligrams per gram of sample.

**F. Total Dietary Fiber**

The dietary fiber content in processed fenugreek seeds was estimated by rapid enzymatic assay method. Briefly, 1 g processed fenugreek seeds sample was subjected to sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglucosidase. Insoluble dietary fiber (IDF) filtered, and then residue were washed with warm distilled water. Combined solution of filtrate and water washings was precipitated with 4 volumes of 95% ethanol (EtOH) for soluble dietary fiber (SDF) determination. Precipitates was filtered and dried. Both SDF and IDF residues were estimated for protein, ash and blank, for the final calculation of SDF and IDF values. SDF was precipitated with EtOH and residue was filtered, dried and weighed. Total dietary fiber (TDF) value was calculated for protein and ash content (AOAC, 1991).

**G. Total Antioxident Activity**

Antioxidant activity was estimated by DPPH free radical scavenging method (Maizura et al.,2011). To 0.2 ml of extract taken, 2 ml DPPH in methanol was added and volume was made 3 ml. After that sample was incubated for 30 min in dark. Absorbance was measured at 517 nm.

\[
\% \text{ Antioxidant activity} = \left( \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \right) \times 100
\]

**H. Estimation of phytic acid**

Phytic acid was estimated by the method of Davies and Reid (1979). One g material was ground and extracted with 0.2 N HCl. To 0.2 ml of the extract, distilled water to make volume 1.4 ml was added. After that 1 ml of ferric ammonium sulphate solution was added, mixed and kept in a boiling water bath for 20 min. The contents were cooled and 5 ml of isooamyl alcohol was added and mixed. To this, 0.1 ml ammonia solution was added, shaken thoroughly and centrifuged at 3000 rpm for 10 min. The alcoholic layer was separated and the colour intensity was read at 465 nm against amyl alcohol blank after 15 min. Sodium phytate standards were run along with the sample. The results were expressed as mg phytic acid/100 g sample.
I. Estimation of tannins

Tannins were estimated by Vanillin-HCl method (Price et al., 1978). One ml of suitably diluted extract was taken in a test tube and 5 ml of freshly prepared vanillin-HCl reagent was added slowly with mixing and colour developed was read at 525 nm. Catechin standards were run simultaneously along with sample. The results were expressed as mg/100 g.

J. Statistical analysis

Each experiment was conducted in triplicates. The results were expressed as mean±SD. Analysis of variance was applied to analyze data and significance was accepted at p≤0.05 level.

III. RESULT AND DISCUSSION

A. Total Phenolic Content

Data pertaining to total phenolic contents presented in Table 1. Total phenolic content of raw and processed fenugreek seeds was significantly (P ≤ 0.05) different in absolute methanolic extract and 80% methanolic extract. This difference might be due to difference in polarity of extracting solvent (Rostagno. M.A.et.al., 2003). Total phenolic content of raw fenugreek seeds was 67.32 mg/g and 59.72 mg/g in 80% methanolic extract and aqueous methanolic extract respectively. Total phenolic contents significantly (P ≤ 0.05) increase by germination process. In 80% methanolic extract phenolics content increased from 67.32 mg/g to 75.17 mg/g, 80.25 mg/g and 93.27 mg/g in samples germinated for 24 hr, 36 hr and 48 hr respectively. In absolute methanolic extract phenolic content increased from 59.72 mg/g to 89.54 mg/g in germinated fenugreek seeds. The highest level of phenolic content (93.27 mg/g) was observed in fenugreek seeds germinated for 48 hr in 80% methanolic extract. Increases might be due to the biosynthesis of phenolic compounds during germination process (Randhir et al., 2004). Total phenolic content in roasted fenugreek seeds increased significantly (P ≤ 0.05) from 73.12 mg/g to 86.43 mg/g in 80% methanolic extract as presented in Table 1.1. In roasting process TPC increased with roasting temp. and was observed maximum (86.43 mg/g) at 200°C temp (Jeong et al., 2004).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Raw</th>
<th>24 hr</th>
<th>36 hr</th>
<th>48 hr</th>
<th>130°C</th>
<th>150°C</th>
<th>200°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%Methanol</td>
<td>67.32±0.22a</td>
<td>75.17±0.06b</td>
<td>80.25±0.13b</td>
<td>93.27±0.33b</td>
<td>73.12±0.06b</td>
<td>81.30±0.44b</td>
<td>86.43±0.16b</td>
</tr>
<tr>
<td>Methanol</td>
<td>59.72±0.01a</td>
<td>66.64±0.14e</td>
<td>72.60±0.10e</td>
<td>89.54±0.05a</td>
<td>61.34±0.36e</td>
<td>64.12±0.09e</td>
<td>76.64±0.22e</td>
</tr>
</tbody>
</table>

Means in the same column with different superscripts differ significantly at (P < 0.05).

B. Antioxidant activity

Antioxidant activities of raw and germinated fenugreek seeds as shown in Table 2. DPPH radical-scavenging activity expressed in % inhibition of raw and germinated fenugreek seeds ranged from 39.11% to 73.65% in 80% methanolic extract. Antioxidant activity is significantly (P ≤ 0.05) different in both extracting solvent of raw and processed fenugreek seeds. In absolute methanolic extract antioxidant activity ranged from 16.47% to 63.72%. Germinated fenugreek seeds had significantly (P ≤ 0.05) higher DPPH radical scavenging activity compared to raw seeds. Polyphenols have high free radical scavenging activity. This increase might be due to the synthesis of compounds and tocopherols which are responsible for antioxidant activity (Sharma and Gujral, 2010).

Antioxidant activity increased from 39.11% to 49.03%, 54.19% and 61.29% in germinated and roasted at 130°C, 150°C and 200°C respectively in 80% methanolic extract. In absolute methanolic extract, antioxidant activity ranged from 29.11% to 35.37%.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Raw</th>
<th>24 hr</th>
<th>36 hr</th>
<th>48 hr</th>
<th>130°C</th>
<th>150°C</th>
<th>200°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%Methanol</td>
<td>39.11±0.08a</td>
<td>50.74±0.14a</td>
<td>53.87±0.12a</td>
<td>73.65±0.29a</td>
<td>49.03±0.14a</td>
<td>54.19±0.09a</td>
<td>61.29±0.04a</td>
</tr>
<tr>
<td>Methanol</td>
<td>16.47±0.19a</td>
<td>35.37±0.13a</td>
<td>53.41±0.21b</td>
<td>63.72±0.31b</td>
<td>29.11±0.11b</td>
<td>41.06±0.07b</td>
<td>44.31±0.23b</td>
</tr>
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Means in the same column with different superscripts differ significantly at (P < 0.05).

C. Total Flavonoid Content

Data pertaining total flavonoids content is presented in Table 2. Total flavonoids content of raw fenugreek seeds was 3.48 mg/g in 80% methanolic extract. Total flavonoids content in 80% methanolic extract was increased to 5.29 mg/g and in absolute methanolic extract it increased to 5.10 mg/g in processed fenugreek seeds.
Effect of Germination and Roasting on Nutritive Composition and Anti-Nutrients in Fenugreek Seeds (IJSTE/ Volume 3 / Issue 06 / 007)

Table 3

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Raw</th>
<th>Germination 24 hr</th>
<th>Germination 36 hr</th>
<th>Germination 48 hr</th>
<th>Roasting 130°C</th>
<th>Roasting 150°C</th>
<th>Roasting 200°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%Methanol Methanol</td>
<td>3.48±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.15±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.98±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.17±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.29±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.61±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.72±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
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Means in the same column with different superscripts differ significantly at (P < 0.05).

Total flavonoids content of raw and processed fenugreek seeds significantly (P ≤ 0.05) different in two different extracting solvent. Flavonoids content in raw fenugreek seeds was significantly (P ≤ 0.05) lower than germinated seeds. Significantly (P ≤ 0.05) increase in the total flavonoids content of fenugreek seeds was observed as a result of germination process. A gradual increase was observed in flavonoid content with increase in germination time from3.48 mg/g to 5.29 mg/g. The highest level (5.29 mg/g) was recorded for fenugreek seeds germinated for 48 hr. Roasting process also increased the flavonoids content from 3.48 mg/g to 3.92 mg/g in 80% methanolic extract.In absolute methanolic extract, flavonoids content also increased from 3.15 mg/g to 3.54 mg/g.

E. Antinutritional Factors

Significant (P ≤ 0.05) difference was observed in tannin content extracted in two solvents in as shown in Table 3. Tannins content was higher in absolute methanolic extract (41.76mg/100g) and reduced to 27.23 mg/100g during germination process. In germination process, tannins content was decreased from 12.52 mg/100g to 9.40mg/100g in fenugreek seed in 80% methanolic extract. As the germination period increased tannin content gradually decreased. The reduction might be due to enzymatic breakdown of the bond between phytate and tannins into water (Shimelis and Rakshit, 2007) and bonding of polyphenols with carbohydrate or protein (Saharan et al., 2002).

In roasting process, tannins were decreased from 12.52mg/100g to 8.42mg/100g in 80% methanolic extract and from 41.76 mg/100g to 20.37 mg/100g in absolute metabolic extract. During roasting breakdown of the bond between phytate and might takes place which results in destruction of phytates, tannins and oxalates (Reddy et al., 1978).

Table 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>TDF (%)</th>
<th>IDF (%)</th>
<th>SDF (%)</th>
<th>PA (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Germination 24 hr</td>
<td>50.20±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.70±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.40±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>317.18±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>36 hr</td>
<td>43.40±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.20±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.65±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>157.44±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>48 hr</td>
<td>40.20±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.45±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.50±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117.74±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Roasting 130°C</td>
<td>45.45±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.45±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.75±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>298.77±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>150°C</td>
<td>41.40±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.45±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.45±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>182.25±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>200°C</td>
<td>32.60±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.65±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.53±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>177.18±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Means in the same column with different superscripts differ significantly at (P < 0.05).

There was no significant (P ≤ 0.05) change in soluble dietary fiber germinated for 36 hr and 48 hr. Soluble dietary fibre also significantly (P ≤ 0.05) reduced from 17.60% to 11.50% upon germination process. Reduction in dietary fiber content during germination may be due to enzymatic breakdown of the galactomannan units. Shakuntala et al.(2011) concluded that decrease in soluble dietary fiber content on germination of fenugreek seeds. An enzyme α-galactosidase during germination of fenugreek seeds partially attacks galactomannan to produce galactose. Decrement in total dietary fiber, insoluble dietary fiber and soluble...
dietary fiber also noticed during roasting process of fenugreek seed as shown in Table 4. Total dietary fibre (TDF) reduced from 45.45% to 32.60% during processing. Significant (P ≤ 0.05) reduction in soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) also determined as listed in table 4. Reduction in IDF content during roasting might due to retrogradation of starch (Mathur and Chaudhary, 2009).

IV. CONCLUSION

It may be concluded from the present study that nutritional and functional quality of fenugreek seeds can be improved by various processing methods e.g germination and roasting. Antioxidant activity also increases significantly after processing which are found to be responsible for functional properties of processed fenugreek seeds. Therefore, the use of processed fenugreek seeds can be exploited in functional foods as well as a therapeutic agent.

REFERENCES