



## Processing Impact on Nutritional, Antinutritional, and Phytochemical of Fenugreek Seeds (*Trigonella Foenum-Graecum L.*)

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### Abstract

Phytate and tannin are more pronounced antinutrients limiting the nutritional quality of fenugreek. The impact of roasting ( $130\pm 5^\circ\text{C}$  for 7 minutes), soaking (12 hrs. at room temperature) and germination (72 hrs. at  $25^\circ\text{C}$ ) on bioactive compounds (polyphenol and antioxidant activity), nutritional and anti-nutritional compounds (phytate and tannin) in Fenugreek were studied. Experimental data were analyzed using the software GenStat 12th Edition. Protein increased significantly ( $p < 0.05$ ) during roasting, soaking, and germination whereas fat decreased significantly. Iron and calcium increased during roasting whereas decreased during soaking and germination. Maximum reduction of phytate (54.55%) and tannin (66.73%) were found when fenugreek seeds were germinated (72 hours). The reduction percentage of antinutrients by soaking (12 hours) and roasting ( $130\pm 5^\circ\text{C}$  for 7 mins) was found to be a lesser effective method compared to germination. All the treatments had a significant ( $p < 0.05$ ) impact on their bioactive components. Phenolic content increased significantly ( $p < 0.05$ ) during roasting, soaking, and germination. Antioxidant activity ( $\text{IC}_{50} = 1.28 \text{ mg dm/ml}$ ) was found to be high in the germinated samples as compared to raw, roasted, and soaked samples. Hence, Germination of fenugreek seeds for 72 hours is concluded as the most effective and promising method for the reduction of antinutrients and increasing the nutritional components, phytochemicals, and antioxidant properties.

### Article Info

Article history:

Received date: 10 October 2021

Accepted date: 20 December 2021

Keywords:

Antioxidant Phytochemical Antinutrients  
Anti-Oxidant Activity Phenolic Content

### 1. Introduction

Fenugreek (*Trigonella foenum-graecum L.*) is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family Leguminaceae (Fabaceae) with trifoliate leaves, branched stem, white flowers, roots bearing nodules, and golden yellow seeds (Acharya. et al., 2008). It can be a good supplement to cereals because of its high protein (25%), lysine, soluble (20%), and insoluble dietary fiber (28%) besides being rich in calcium, iron, and beta-carotene (Shalini and Sudesh, 2002). It has been reported to exhibit pharmacological properties such as anti-tumor/anti-cancer, anti-diabetic, antibacterial, hypo-cholesterolemic, hypolipidemic, Hepato-protective, lactation aid paralysis, gout, edema, chronic cough, antioxidant activity and cures digestive problems and anorexia.

The undesirable taste of fenugreek seeds is due to components like coumarin, diosgenin, saponin, and steroids. Antinutrients (Phytate, Tannin, Enzyme and Protease inhibitors, Saponin, alkaloids, Oxalates, Haemagglutinins (lectin), Cyanogen,) are present in fenugreek seeds which hinders the digestion, absorption, and utilization of vitamins, minerals (Iron, Calcium, Zinc), and nutrients (Protein, Carbohydrate) and also inhibit the activity of digestive enzymes ( $\alpha$ -amylase, Pepsin, Trypsin, and Pancreatin). Fenugreek seeds reduce the amount of calcium oxalate in the kidneys which often contributes to kidney stones and have been applied to relieve muscle aches and gout pain (Charles and Soetan, 2014). However, the seeds are bitter due to the presence of bitter saponins, which limit their acceptability in foods (Pandey and Awasthi, 2015). Anti-nutrients are compounds that reduce the nutrient utilization and food intake of plants or plant products

used as human foods. These anti-nutritional factors must be inactivated or removed if values of food substances are to be fully maintained (Thakur et al., 2019). They are potentially harmful and give rise to a genuine concern for human health in that they prevent digestion and absorption of vitamins, minerals, and other nutrients. They can reduce the nutritional value of a plant by causing a deficiency in an essential nutrient or preventing digestion when consumed by humans or animals (McEwan, 2008). It has been possible to debitter and reduce the antinutritional factors of fenugreek seeds by employing various processing methods such as soaking, germination, roasting, etc. (Pandey and Awasthi, 2015).

## 2. Materials and Method

Fenugreek seeds (*Trigonella foenum graecum* L.), collected from the local market of Biratnagar, were cleaned, screened, and processed (Roasting, Soaking, and Germination).

### 2.1 Processing methods

#### 2.1.1 Roasting

Fenugreek seeds (50 g) were roasted in an open pan at  $130\pm 5^\circ\text{C}$  for 7 minutes with continuous stirring by ladle for proper and uniform roasting until it became slightly brown with a peculiar aroma.

#### 2.1.2 Soaking and germination

Fenugreek seeds soaked in distilled water (seed to water ration 1:5) for 12 h at room temperature (Ojha et al., 2018), drained and then dipped in KMS solution for 10 minutes to prevent mold growth (Jood et al., 1987). The soaked seeds were germinated in sterile Petriplates lined with wet Whatman no. 1 filter paper for 72 hours at  $25^\circ\text{C}$  at 90% RH. The drying out was prevented by moistening the muslin cloth and spraying the potable water (Ojha et al., 2018).

#### 2.1.3 Drying

Drying of soaked and germinated samples was carried out in a cabinet drier at  $50\pm 5^\circ\text{C}$  until the moisture content (12 % db) was obtained.

#### 2.1.4 Grinding

Raw and processed (soaked, germinated, and roasted) fenugreek seeds were ground, sieved through 0.5 mm size mesh, and stored in LDPE (40 micrometers) bag at room ( $30\text{-}35^\circ\text{C}$ ).

### 2.2 Preparation of methanolic extract of the samples

Briefly, to 1 g of each processed Fenugreek flour, 30 ml of methanol (80%) was added in mortar and pestle for homogenization. Then 15 ml methanol (80%) was used to wash the mortar and pestle and then pooled with the first homogenate. The mixture was refrigerated for half an hour and allowed to centrifuge at 4,500 rpm for 15 min at room temperature ( $27^\circ\text{C}$ ). The clear supernatant solution was filtrated by Whatman filter paper No. 42 and the volume was made up of 50 ml with 80% methanol, transferred to brown colored glass bottles, sealed, and stored in the refrigerator at temperature  $<7^\circ\text{C}$  (Karakaya, 2004).

### 2.3 Analytical Methods

#### 2.3.1 Physical parameter of seeds

The length, width, and height of seeds were measured by screw gauge according to Digvir and Stefan (2006) whereas the bulk density was determined by the Balasubramanium (1985) followed by Narvani and Panwar (1993).

#### 2.3.2 Determination of moisture content

It was determined by the hot air oven method (Ranganna, 2001).

#### 2.3.3 Determination of crude protein

It was determined by the Kjeldahl method (Rangana, 2001).

#### 2.3.4 Determination of ash content

The ash content was determined by incinerating the fenugreek seeds (5 g) in a muffle furnace at  $525^\circ\text{C}$  for 4-6 hours (Rangana, 2001)

#### 2.3.4 Determination of crude fat

It was determined as described in Rangana (2001).

#### 2.3.5 Determination of carbohydrates

It was determined by the different methods.

Carbohydrate (%) =  $100 - [\text{sum of moisture, protein, total ash, fiber, and fat}]$ .

#### 2.3.6 Determination of energy value

It was determined by the methods described by Basse et al., 2013.

Energy value per 100g =  $[\text{carbohydrate} * 4 + \text{protein} * 4 + \text{Fat} * 9]$  Kcal

### 2.3.7 Determination of iron

It was measured calorimetrically at 480 nm (Rangana, 2001).

### 2.3.8 Determination of calcium

It was determined as described by Rangana, 2001.

### 2.3.9 Determination of Phytate

Young and Greaves (1940) method was used for the determination of Phytic acid content. Briefly, 0.2 g of the sample was soaked in 100 ml of 20% concentrated HCL for 3 hours. To 50 ml of the filtrate, 100 ml distilled water, 10 ml of 0.3% ammonium thiocyanate solution were added and titrated with standard iron (III) chloride solution that contained 0.00195 g iron per ml. Percentage of phytic acid is calculated by the formula:

$$\% \text{ Phytic acid} = \frac{\text{Titer value} \times 0.00195 \times 100}{2}$$

### 2.3.10 Determination of tannin

It was determined by the Folin-Dennis method. Briefly, to 0.5 g powdered sample, 75ml distilled water was added and boiled for 30 min. To 1ml of the sample extract, 75 ml distilled water, 5 ml of Folin-Denis reagent, and 10 ml of sodium carbonate were added. After well mixing, it was kept at room temperature for 30min. A set of reference standard solutions of tannic acid was prepared in the same manner. It was then measured in a spectrophotometer at 700nm (Sadasivam and Manickam, 2016).

### 2.3.11 Determination of DPPH radical scavenging activity

Antioxidant activity was measured utilizing 2, 2-diphenyl-1-1 picrylhydrazyl (DPPH) radical scavenging capacity. Different concentrations of the methanolic extract were taken in different test tubes, the volume made to 1 ml with methanol and then 4 ml of 0.1 mM methanolic solution of DPPH was added. After vigorously shaking, tubes were stand for 20 min at room temperature. Changes in absorbance of samples were measured at 517 nm. Free radical scavenging activity was expressed as inhibition percentage in Ranganna, 2001.

$$\% \text{ Free radical Scavenging Capacity} = \frac{\text{Control}_{OD} - \text{Sample}_{OD} * 100}{\text{Control}_{OD}}$$

## 2.4 Data analysis

Triplicate samples were used and the mean values

with standard deviation were computed and graphically represented using Microsoft excel-2016. Data were subjected to analysis of variance (ANOVA) at 95% confidence level using statistical software GenStat (Twelfth Edition developed by VSN International Limited). Means were compared by Fisher's Unprotected LSD method.

## 3. Results and Discussion

All the parameters are measured on a dry basis(db).

### 3.1 Physical parameters of raw fenugreek seeds

The length, width, and height were 0.41 cm, 0.24 cm, and 1.7 mm respectively. The bulk density was ranged from 682.56 to 691.62 kg/m<sup>3</sup>. The average bulk density of fenugreek seeds was found to be 687.62 kg/m<sup>3</sup>. Similar results have been reported by Altuntas et al. (2005) and Mabrouki et al. (2015).

### 3.2 Impact of processing methods on Proximate composition

#### 3.2.1 Moisture

The moisture content of roasted seed was 5.56% which is similar to 5.96% (Dwivedi et al., 2019); higher to 4.25% (Saini et al., 2016); Pandey and Awasthi (2015) but significantly lower than raw seed (9.39%) by 40.74%. In soaked dried seed it was 9.64% which is higher than the raw seed (2.72% ) by 9.39% but lower than 13.96% (Hooda and Jood, 2003). Higher might be due to hydration of seeds during soaking by Saini et al. (2016). Similarly, in germinated dried seeds it was 8.15%. which is lower than 7.5%. (Kavitha et al., 2015) and 7.71% (Atlaw and Kumar, 2018) and raw seed (9.39%) by 31.17%. Statistically, roasting and germination significantly decreased whereas soaking had no significant impact on the moisture content of the dried seed.

#### 3.2.2 Protein

The protein content of roasted seed (23.07%) was higher than raw seed (22.05%) by 4.62%. but is lower than 27.48% (Dwivedi et al., 2019). In soaked dried seed it was 24.95% which is higher than a raw seed by 13.15%. but lower than 26% (Hooda and Jood, 2003). While soaking, biological breakdown of various complex compounds into simpler compounds takes place as suggested by Narsih et al. (2012). Similarly, in germinated dried seed it was 31.47% which is higher than a raw seed by 42.71%, but lower than 32.04% (Mahmoud et al., 2012); 37.4% (Atlaw and Kumar,

2018), and closer to 29.89% (Kavitha et al., 2015). The reduction of seed nitrates into plant protein or ammonium compounds occurs during germination (Pandey and Awasthi, 2015). Sprouting causes the mobilization of proteins with the help of activated proteases, leading to the formation of polypeptides, oligopeptides, and amino acids. Some amino acids and peptides can be released, and the synthesis or utilization of others, to form new proteins, can occur. (Atlaw and Kumar, 2018). Statistically, soaking and germination significantly ( $p < 0.05$ ) and roasting do not significantly impact the protein content of raw seed.

### 3.2.3 Fat

The fat content of roasted seed was 4.15% which is lower than 9.01% (Dwivedi et al., 2019) and raw seed (4.33%) by 4.17% which may be due to reduction in actual weight with the shrinkage and loss of volatile oil on open dry heat treatment (Mathur. and Chaudhary., 2009). The fat content of soaked dried seed was 4.19% which is closer to 4.6% (Pandey and Awasthi, 2015); higher than 6.45% (Hooda and Jood, 2003), and lower than raw seed (4.33%). by 3.31%. Similarly, the fat content of germinated dried seed was 2.90%. which is quite similar to 2.3% (Kavitha et al., 2015) and lower than a raw seed by 33.04% which is due to its consumption as an energy source in the process of germination by Mansour and EL-Adway (1994) and due to the increased activities of the lipolytic enzymes during germination Mahmoud et al. (2012).

Statistically, there was no significant ( $p > 0.05$ ) impact of roasting and soaking but significantly affected by the germination process on the fat content of the raw seed.

### 3.3.3 Crude fiber

The crude fiber content of roasted seed was 8.31% which is lower than 13.71% (Dwivedi et al., (2019) and in raw seed (8.51%) by 2.31%. Similarly, in soaked dried seed it was 8.50% which was lower by 0.12% than in raw and is higher than 7.22% (Saini et al., 2016) and 6.95% (Hooda and Jood, 2003) in a soaked dried seed. The loss might be attributed to the enzymatic degradation of seeds during soaking (Mathur. and Chaudhary., 2009). In germinated dried seed it was 9.34% which is lower than 10.2% (Kavitha

et al., 2015) and 11.34% (Atlaw and Kumar, 2018). The 9.79% gain of crude fiber in germinated dried seed compared to raw was which might be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination (Anonymous, 2012). Statistically, roasting and soaking had no impact on the crude fiber content of raw seed but germination significantly ( $p > 0.05$ ) increased its value.

### 3.3.4 Ash

The ash content of roasted was found to be 3.94% which is higher than 3.8% as reported by Pandey and Awasthi (2015); lower than 5.57% as reported by Dwivedi et al. (2019) and higher than raw seed (3.89%) by 1.19%. Similarly, in soaked dried seed was 4.12% which is higher than a raw seed by 5.67% but closer to 4.17% (Saini et al., 2016) and 4% (Pandey and Awasthi, 2015) and higher than 2.92% (Hooda and Jood, 2003). Similarly, in germinated dried seed, it was 4.58% which is closer to 4.48% (Mahmoud et al., 2012); lower than 6.5% (Kavitha et al., 2015) but higher than 2.94% (Atlaw and Kumar, 2018), and raw seed by 17.52%. Statistically, there was no significant ( $p > 0.05$ ) impact of processing on ash content of the raw unprocessed seed.

### 3.3.5 Carbohydrate

Total carbohydrate contain in roasted seed was 54.95% which is greater by 6.06% than in raw seed. Similarly, in soaked dried seed it was 48.58% which is lower than raw seed (51.89%) by 6.22%, due to leaching of other nutrients leading to modification in structural components of the legume and thereby increasing the availability of starch, as reported by and due to the use of carbohydrate as a source of energy for embryonic growth (Vidal-Valverde et al., 2002).

In germinated dried seed it was 43.54% which is lower than a raw seed by 15.96% and higher than 41.31% (Mahmoud et al., 2012) due to an increase in amylase activity, which breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination (Inyang and Zakari, 2008).

**Table 1:** Proximate composition of Raw / Roasted / Soaked / Germinated Fenugreek seeds.

Sample	Moisture (%)	Protein (% db)	Fat (% db)	Crude fibre (%db)	Ash (%db)	Carb (%db)
Raw	9.39 <sup>a</sup> ± 0.01	22.05 <sup>a</sup> ± 0.82	4.33 <sup>a</sup> ± 0.26	8.51 <sup>ab</sup> ± 0.46	3.89 <sup>a</sup> ± 0.01	51.81 <sup>a</sup> ± 0.39
Roasted	5.56 <sup>b</sup> ± 0.67	23.07 <sup>a</sup> ± 0.66	4.15 <sup>a</sup> ± 0.15	8.31 <sup>a</sup> ± 0.32	3.94 <sup>a</sup> ± 0.19	54.95 <sup>b</sup> ± 0.37
Soaked	9.64 <sup>a</sup> ± 0.15	24.95 <sup>b</sup> ± 0.31	4.19 <sup>a</sup> ± 0.10	8.50 <sup>ab</sup> ± 0.27	4.12 <sup>a</sup> ± 0.13	48.58 <sup>c</sup> ± 0.52
Germinated	8.15 <sup>c</sup> ± 0.18	31.47 <sup>c</sup> ± 0.72	2.90 <sup>b</sup> ± 0.10	9.34 <sup>b</sup> ± 0.76	4.58 <sup>a</sup> ± 0.85	43.54 <sup>d</sup> ± 0.89

\* Values presented are means of triplicate determination ± SD on a dry basis. This means having a similar superscript in a column are not significantly different at 5% significance.

### 3.3 Effect of processing methods on Mineral Composition

#### 3.3.1 Iron

Iron in roasted fenugreek was 12.79 mg/100gm which is greater than raw seed (11.45 mg/100gm) by 11.75%; 13.1 mg/100gm (Pandey and Awasthi, 2015) in roasted seed due to the breakdown of antinutritional compounds such as phytates and oxalates mineral (Pandey and Awasthi, 2015). Its value in soaked dried seed was 10.85 mg/100gm which is closer to 9.75 mg/100gm (Hooda and Jood, 2003) lesser than raw seed by 5.20% due to leaching out of some minerals into the soaking water ( Pandey and Awasthi, 2015). Similarly, in germinated samples, its value was 11.18 mg/100gm lower than a raw seed by 2.32%; close to 11.51 mg/100gm (Atlaw and Kumar, 2018); and 11.5mg/100gm (Pandey and Awasthi, 2015) in a germinated dried seed. Reduction indicated the transfer of nutrients from the seed material to the growing embryo reported by (Atlaw and Kumar, 2018). Statistically, soaking and germination behave no impact but roasting has a significant ( $p > 0.05$ ) impact on the iron content of the raw seed.

#### 3.3.2 Calcium

The calcium content in roasted fenugreek was 79.07 mg/100gm which is slower than 87.21 mg/100gm (Saini et al., 2016) and greater than 71.2 mg/100gm (Pandey and Awasthi, 2015) but higher than raw seed (78.29 mg/100gm) by 0.9. At high temperatures, anti-nutrient destruction is responsible for the increase of Ca concentration in the medium (Makinde and Akinoso, 2013). Its value in soaked dried seed was 74.97 mg/100gm which is higher than 70.60 mg/100gm (Hooda and Jood, 2003); 76.10 mg/ 100gm

(Saini et al., 2016); 68.2 mg/100gm (Pandey and Awasthi, 2015) but lower than a raw seed by 4.25%. Similarly, in germinated dried seed, its value was 72.01 mg/100gm which is closer to 71.22 mg/100gm (Hooda and Jood, 2003); higher than 70.7 mg/100gm (Pandey and Awasthi, 2015), and lower than 84.83 mg/100gm (Mahmoud et al., 2012); and raw seed by 8.03%. Statistically, the impact of roasting is not significant ( $p > 0.05$ ) but soaking and germination have a significant impact on the calcium content of the raw seed.

### 3.4 Effect of processing methods on Polyphenols composition

The total phenol content of the raw fenugreek seeds was 80.85 mg GAE/g as dry basis which is higher than 67.32 mg GAE/g (Manju et al., 2016); 72.99 mg GAE/g (Saxena et al., 2016) and lower than 85.88 mg GAE/g (Naidu et al., 2011). The difference in cultivar, geographical region, season, the polarity of the solvents used, extraction method and extraction time; chemical diversity of phenolic and flavonoid compounds, and the complexity of composition in plant sources may contribute to the variation of phenolic compounds profile (Wijekoon et al., 2011). The total phenol content of roasted seed was 85.70 mg GAE/g which is higher than in raw seed by 5.99%; 73.12 mg GAE/gm (Manju et al., 2016). The disruption of the cell wall through heating broke the covalently bound phenolic compound or by the breakdown of insoluble phenolic compounds to release it (Boateng et al., 2008) and the development of Maillard reaction products which leads to the release of bound polyphenols that have been reported to possess scavenging activity on reactive oxygen species (Segev et al., 2012). The thermal treatment applied to foods of plant origin by heat-

ing or roasting causes evaporation of intracellular water, triggering chemical reactions that can change the lignocellulosic structure and promotes protein denaturation, which may result in greater availability of plant phenolic compounds (Rizki et al., 2015). The phenolic content of the soaked dried seed was 83.38 mg GAE/gm which is higher by 3.12% in the raw seed; by 9.43 % in soaked dried seed (Ojha et al., 2018). Similarly, the Phenolic content of the germinated dried seed was 117.70 mg GAE/gm which greater than in raw seed by 45.56%. Phenolic content increased from 67.32 mg GAE/gm to 93.27 mg GAE/gm during 48-hour germination; 77.97% to 80.8 mg GAE/gm during 24-hour germination (Pandey and Awasthi, 2015); from 1186 mg GAE/100gm to 1815 mg GAE /100gm during 4 days germination (Sharara, 2017). Increases could be due to biosynthesis, bioaccumulation, and degradation of polymerized polyphenols, specifically hydrolyzable tannins, and the hydrolysis of other glycosylated flavonoids (Randhir et al., 2004b).

### 3.5 Effect of processing methods on the antinutritional factors

#### 3.5.1 Phytate

Phytate content of the raw fenugreek seed was found to be 85.09 mg/100gm which is higher than 64.22 mg/100gm (Atlaw and Kumar, 2018); and 23 mg/100gm (Kavitha et al., 2015) but lower than 152.91 mg/100gm (Burham, 2017) and 164 mg/100gm (El-Shimi et al., 1984). The variation in phytic acid contents is due to phosphorus utilization efficiency (Tesfaye et al., 2017) and the location and year of production, variety (cultivar), post-harvest treatment, and their interaction Oomah et al. (1996). The Phytate content of the roasted seed was 54.15 mg/100gm i.e., reduced by 36.37% than in raw seed. Similarly, El-Mahdy and Sebaiy (1982) show the reduction of the phytate by 39.85%; when roasted at 185 °C for 15 mins; by 40.77%. when roasted at 130±5 °C for 7 min of Fenugreek seed (Pandey and Awasthi, 2015). Soaking (12 hrs) reduced it by 9.03%. (Hooda and Jood, 2003) and by 8.7% (Pandey and Awasthi, 2015). Phytate is water-soluble and soaking enhances the naturally occurring phytase (Kumar et al., 2010). It, in germinated (72 hrs) dried seeds was 38.67 mg/100gm which it by 54.55%. Germination for 3 days loss 50% (Kavitha et al., 2015); 44% loss during 24 hours (Pandey and Awasthi, 2015); by 42.14% during 48 hours (Hooda

and Joo, (2003); 70.42% loss during 72 hours of germination (Atlaw and Kumar, 2018). Plant seed utilize phytate as a source of inorganic phosphate during germination and thus tend to increase palatability and nutritional value (Wang et al., 1997) and its hydrolysis following the activation of the enzyme phytase and phosphatase activity during the germinating process (Mabrouki et al., 2015). The phytase activity originates and increased in the germinated seeds which results in the reduction of phytic acid content in fenugreek seeds after germination and roasting (Pandey and Awasthi, 2015). Statistically, soaking do not significantly ( $p>0.05$ ) impact on phytate content of while roasting and germination had significantly impact on it.

#### 3.5.2 Tannin

The tannin content of raw fenugreek was 674.84 mg/100gm which is lower than 718.92 mg/100gm (Atlaw and Kumar, 2018) higher than 353 mg/100gm (Mabrouki et al., 2015) and 394 mg/100gm (Saini et al., 2016). In roasted seed, it was 481.13 mg/100gm which was lesser by 28.7%. In soaked dried seeds (435.84 mg/100gm) it was reduced by 35.41%; by 39.64% (12 hr ) (Ojha et al., 2018). Similarly, in germinated dried seed it was 224.52 mg/100gm which was lesser by 66.73 %; by 60.52% (72 hrs) (Ojha et al., 2018); by 58.6% (48 hrs) (Mabrouki et al., 2015); by 63.41%. (Kavitha et al., 2015) and by 57.27% (Maria and Victoria, 2018). This might be due to the leaching of tannins into water (Shimelis. and Rakshit., 2008) and bonding of polyphenols with carbohydrate or protein (Saharan et al., 2002); formation of a hydrophobic association of tannin with seed protein and enzymes (Rusydi and Azlan, 2012) and is attributed to the presence of polyphenol oxidase and enzymatic hydrolysis (Rao and Deosthale, 2006). Statistically, tannin content was significantly ( $p>0.05$ ) affected by the roasting, soaking, and germination.

### 3.6 Effect of Processing Methods on Antioxidant Activity and DPPH

Free radical scavenging activity for DPPH radical, expressed as IC<sub>50</sub>. were 5.95 mg dm/ml, 3.38 mg dm/ml, 1.54 mg dm/ml 1.28 mg dm/ml and the antioxidant activity (AA) were 16.95%, 26.98%, 62.28% and 75.66% for raw, roasted, soaked and germinated dried seed respectively.

**Table 2:** IC50 concentration of methanolic extracts of plant samples for DPPH inhibition

Parameter	Total Antioxidant Activity		
	DPPH Radical Scavenging Activity (% inhibition)	IC <sub>50</sub> Value (mg dm/ml)	mg Ascorbic eqvt/mg dm
Raw	16.95±1.98 <sup>a</sup>	5.95	9.27
Roasted	26.98±1.39 <sup>b</sup>	3.38	16.29
Soaked	62.28±0.78 <sup>c</sup>	1.54	35.69
Germinated	75.66±1.45 <sup>d</sup>	1.28	42.91

\*IC<sub>50</sub> value from Ascorbic Acid standard curve = 55.18816 mg/ml

AA are 18.1%, 32%, 60.7% and 73.9% seed respectively (Pandey and Awasthi, 2015); 10.4%, 13.6% and 55.45% (Ojha et al., 2018) in raw, roasted, soaked and germinated respectively. Roasting may destroy bioactive compounds but it can also form antioxidant compounds through Maillard reaction like melanoidins at high temperatures (Wani et al., 2017) and due to higher release of bound phenolics from the roasted samples (Rashid et al., 2015) and due to heat inactivating the endogenous oxidative enzyme, preventing further oxidation of antioxidant compounds in the raw plant material. The many metabolic changes that take place upon the sprouting of seeds increase endogenous hydrolytic enzymes activity that modifies the endosperm and the synthesis of compounds like vitamin C and tocopherols which are responsible for the increase in AA (Sharma. and Gujral., 2010). Statistically, the DPPH activity of raw Fenugreek seed was significantly ( $p>0.05$ ) affected by the roasting, soaking, and germination.

#### 4. Conclusion

Germination of Fenugreek seeds for 72 hrs seems to be a promising and effective method for reduction of antinutrients; increasing nutritional composition, phytochemicals, and antioxidants.

#### Acknowledgments

We would like to sincerely thank the Central Campus of Technology, Hattishar, Dharan for all the help and coordination. We are indebted to every person who is directly or indirectly involved in the completion of this work.

#### Conflicts of Interest

The authors report no conflicts of interest for this work.

#### Funding

No funding resource.

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