

NUTRITIONAL POTENTIAL OF FENUGREEK SUPPLEMENTED BREAD WITH SPECIAL REFERENCE TO ANTIOXIDANT PROFILING

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Globally, fenugreek (*Trigonella foenum-graecum*) is well recognized for imparting flavor to several traditional foods. Besides, it provides tremendous amount of active ingredients for health promotion and disease prevention. Purposely, the present research work was designed to elucidate nutritional & antioxidant potential of fenugreek seeds and their incorporation in bread formulation @ 5, 10 & 15%. The supplementation of fenugreek seed powder @ 15% in designed bread increased total phenolic content from 99.00±4.45 (control) to 413.00±19.82 mg GAE/100g whereas, increment in flavonoid contents was up to 2.91±0.12 mg CE/g as compared to control (2.13±0.09). Alongside, it was found effective in increasing antioxidant activity *i.e.* evaluated through DPPH, FRAP and β -carotene assays as 51.00±2.39%, 450.00±21.60 μ mol Fe²⁺/g and 43.00±2.10%, respectively. However, 10% supplementation of fenugreek seed powder presented better sensory response of the prepared bread. All these outcomes have revealed that supplementation of fenugreek seed powder in bread serve as functional food accredited to rich nutritional, antioxidant & sensory quality.

Keywords: Nutritional profile, antioxidant, total phenolic content, flavonoids, bread

INTRODUCTION

The recent era has witnessed the coinage of lifestyle related malfunctions due to poor dietary habits, altering lifestyles and elevated consumption of refined and processed foods. The prevalence of metabolic dysfunctions has identified as a dominating factor for unhealthy living. In this regard, herbs and spices are in limelight to develop certain products that improve the health status besides provision of basic nutrition (Liu *et al.*, 2007; Sethi *et al.*, 2008). Fenugreek (*Trigonella foenum-graecum*) locally known as methi, is a member of legume family originated from Asia and South Eastern Europe while presently it is ubiquitous in Pakistan, India and Egypt (Betty, 2008). Globally, fenugreek leaves and seeds powder are recommended in food formulations and serve as medicinal herb, coffee alternative, insect repellent in grain storage & employed in fragrance manufacturing industries (Ahari *et al.*, 2009). The fenugreek seeds contain 25.2-30.1% protein, 7.2-9.3% lipids, 20.1-25.3% insoluble fiber, 20.4-30.2% galactomannan and 5.3-7.3% saponins along with ample amounts of volatile oils, free amino acids, mucilaginous fiber and flavonoids (Raju and Bird, 2006). It contains lysine & L-tryptophan rich protein, mucilaginous fiber and other important chemical constituents such as saponin, saponins, nicotinic acid, coumarin, phytic acid, fenugreekine, trigonelline and scopoletin that are supposed to be for its beneficial health effects. Phenolic compounds, for instance, through their antioxidant activity, are hypothesized to have cancer suppressing ability. It also comprises alkaloids

of pyridine-type, generally trigonelline (0.21-0.35%), choline (0.51%), carpaine and gentianine, the flavonoids; apigenins, orientin, quercetin, rutin, free amino acids; such as hydroxyisoleucine, histidine, arginine & lysine, minerals; iron & calcium and glycosides that yield steroids on hydrolysis *i.e.* tigogenin, yamogenin and neotigogenin (Mehrafarin *et al.*, 2010).

Flour containing fenugreek seeds powder has good attribute for bakery products and now a day's wheat flours have been supplemented with fenugreek seeds for achieving better results in developing number of food menus (Hooda and Jood, 2004). The present study was planned to evaluate the nutritional & antioxidant worth of fenugreek grown in Pakistan and to develop functional bread with the addition of fenugreek seeds powder.

MATERIALS AND METHODS

Procurement of raw materials: Wheat variety Lasani 2011 and fenugreek seeds (*Qasoori methi*) were procured from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan.

Preparation of raw materials: The wheat was cleaned by sieving followed by tempering. Afterwards, milling was carried out using Quadrumate Senior mill to get straight grade flour. Likewise, fenugreek seeds were cleaned by screening and washing, followed by drying and grinding to get fine powder. The resultant powder was stored in air tight containers prior to analysis. Composite flours were prepared

with the addition of 5, 10 & 15% of fenugreek seeds powder in wheat flour.

Analysis of raw material and composite flours:

Chemical analysis: Chemical composition of wheat flour and fenugreek seeds powder was estimated by determining moisture, ash, crude protein, crude fat, crude fiber and nitrogen free extract (NFE) according to the prescribed methods of AACC (2000).

Extraction of polyphenols: The polyphenols from composite flours were extracted by following the protocol of Rusak *et al.* (2008) using ethanol at constant temperature of 60°C for 48 hours. Resultant extracts were filtered using vacuum filtration assembly and solvents were recovered by Rotary Evaporator (EYELA, N-N series, Japan) at a temperature of 40°C. The extraction yield was calculated and resultant extracts were stored at 4°C until used, afterwards the extracts were subjected to different assays.

Phytochemical Profiling:

Total phenolic content: Total phenolics of resultant extracts were estimated through spectrophotometer (Irmeco, Germany) following Folin-Ciocalteu method (Singleton *et al.*, 1999). The extract (1 mL) was mixed with 0.5 mL of Folin-Ciocalteu reagent along with 7.5 mL of distilled water and allowed to stand for 20 min. Following resting period, 1.5 mL of sodium bicarbonate solution (7%) was added to the mixture. Then stay for 20 min by placing sample tubes in water bath (40°C), took 0.5 ml from this solution and add 2 mL of distilled water and absorbance was measured at 755 nm using a UV/Vis Spectrophotometer (CECIL, CE7200) against control. Total polyphenols were calculated and expressed as Gallic acid equivalent (mg Gallic acid/100 g).

Total Flavonoids: Total flavonoids were determined following the procedure by Zhishen *et al.* (1999). Took two mL of aqueous extract in a 10 mL volumetric flask, then added 5 mL of distilled water followed by 0.3 mL of 5% NaNO₂ and after 5 min - 0.6 mL of 10% AlCl₃ was added. After another 5 min stay 2 mL of 1 M NaOH was added and volume was made upto 10 mL with distilled water. Take one mL from this solution after mixing this solution, add 9 mL distilled water and measured absorbance by using spectrophotometer at 510 nm. Total flavonoids were expressed as Catechin equivalents per dry matter.

Antioxidant Activity:

Free radical scavenging activity (DPPH assay): The DPPH radical scavenging activity of all extracts was measured according to the procedure of Oktay *et al.* (2003). Purposely, 1 mL of DPPH was added to each extract (4 mL) and incubated at room temperature for 30 min. The absorbance was noted at 520 nm using Spectrophotometer. Percent inhibition was calculated using the following formula;

$$\text{Reduction} = \frac{AB - AA}{AB} \times 100$$

AB = absorbance of blank sample (t = 0 min)

AA = absorbance of tested extract solution (t = 30 min)

β-carotene and linoleic acid assay: Total antioxidant activity of the extracts was monitored using assay based on coupled oxidation of β-carotene and linoleic acid (Adegoke *et al.*, 1998). Briefly, 2 mg of β-carotene was dissolved in 20 mL chloroform along with 40 mg linoleic acid and 400 mg Tween 20. After removing chloroform, 3 mL of the prepared emulsion was added in 0.10 mL sample and placed in a water bath for 120 min. Oxidation of β-carotene was determined through spectrophotometer at 470 nm.

Ferric reducing antioxidant power (FRAP assay): The ferric reducing ability of all the extracts was measured according to the procedures of Benzie and Strain (1996). Purposely, extract (0.5 mL) was mixed with phosphate buffer (1.25 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1.25 mL, 1%). After incubation, 1.25 mL of 10% trichloroacetic acid (TCA) was added along with 0.1% ferric chloride and then left at room temperature for 10 min. Sample absorbance was measured with spectrophotometer at 700 nm.

Bread preparation: For the purpose, respective flour, sugar, oil, yeast and water were used to make the bread (AACC, 2000; Method No. 10-09). After preparation, the bread was cooled and packed in polythene packages before further analyses.

Chemical analysis of bread: Chemical composition (moisture, ash, crude fat, crude fiber, crude protein and nitrogen free extract) of bread was determined by following the methods described in AACC (2000).

Phytochemical profiling of bread: Total phenolic content and flavonoids were performed for prepared bread by using procedures described by Singleton *et al.* (1999) and Zhishen *et al.* (1999), respectively as described above.

Antioxidant activity of bread: Free radical scavenging ability (Oktay *et al.*, 2003), β-carotene and linoleic acid assay (Adegoke *et al.*, 1998) and FRAP assay (Benzie and Strain, 1996) were also estimated for bread.

Sensory evaluation: The hedonic assessment of bread was carried out for sensory characteristics *i.e.* volume, color of crust, aroma, taste and overall acceptability by panel of six judges (Lawless and Heymann, 1998). The bread was examined for sensory evaluation using nine-point hedonic scale system ranged from extremely liking to disliking (9 = like extremely; 1 = dislike extremely) following the guidelines of Meilgaard *et al.* (2007). Panelists were provided separate booths equipped with white fluorescent light. To exclude any biasness from the experiment, samples were presented to the panel randomly and requested to assign scores for selected characteristics.

Table 1. Chemical composition (%) of raw material.

Parameter	Wheat flour	Fenugreek seeds powder
Moisture	13.5±0.25	10.65±0.53
Ash	0.41±0.05	4.14±0.21
Protein	10.5±0.13	22.86±1.14
Fat	1.13±0.04	6.98±0.35
Fiber	0.31±0.04	7.90±0.43
NFE	74.15±2.42	47.47±2.37

Values expressed are means ± standard deviation

Statistical analysis: The collected data from the current study was subjected to statistical analysis by applying completely randomized design (CRD) using Cohort version 6.1 (Costat-2003) according to guidelines of Steel *et al.* (1997).

RESULTS

Chemical composition of raw material given in Table 1, illustrated that fenugreek seed powder is substantial source of protein (22.86±1.14%) and crude fiber (7.90±0.43%) followed by ash content (4.14±0.21%). Total phenolic content (TPC) among different treatments with the addition of fenugreek seeds powder was found to be 305.00±14.03 mg GAE/100g (T₁), 410.00±19.27 mg GAE/100g (T₂) and 450.00±22.05mg GAE/100g (T₃). The flavonoids in different treatments were found in range of 2.38±0.08 mg CE/g (T₀) to 3.01±0.14 mg CE/g (T₃). The free radical scavenging activity (DPPH) of treatments increased with increasing percentage of fenugreek *i.e.* T₃ (58.00±2.84%) followed by T₂ (53.00±2.49%), T₁ (49.00±2.25%) and T₀ (35.00±1.57%). The ferric reducing ability (FRAP) values in different treatments as a function of fenugreek seeds powder addition in wheat flour ranged from 222.00±9.99 (T₀) to 501.00±24.54 µmol Fe²⁺/g (T₃). Different treatments depicted that β-carotene values ranged from 33.00±1.48% (T₀) to 51.00±2.49% (T₃).

The effect of supplementation of fenugreek seeds powder in bread illustrated chemical composition as 34.49±1.06 to 34.63±1.17% moisture, 1.39±0.07 to 1.72±0.06% ash, 11.74±0.99 to 12.79±1.06% protein, 6.25±0.23 to 6.81±0.34% fat, 1.65±0.08 to 2.29±0.11% fiber and 41.90±1.84 to 44.35±2.08% NFE (Table 3). The highest TPC (413.00±19.82 mg GAE/100g) was found in T₃ followed by T₂ (341.00±16.02 mg GAE/100g) and T₁ (238.00±10.94 mg GAE/100g). Total flavonoids for different treatments; T₀ (2.13±0.09 mg CE/g), T₁ (2.50±0.10 mg CE/g), T₂ (2.68±0.10 mg CE/g) and T₃ (2.91±0.12 mg CE/g), depicted maximum results for treatment with 15% fenugreek seeds powder supplementation (Table 4). The values for free radical scavenging activity of different treatments ranged from 31.00±1.27 to 51.00±2.39%. As indicated from values, highest reducing power (450.00±21.60 µmol Fe²⁺/g) was recorded in 15% seeds powder supplementation (T₃) while lowest (167.00±7.01 µmol Fe²⁺/g) in wheat flour (T₀). The

maximum β-carotene value 43.00±2.10% was recorded in T₃ followed by T₂ (40.00±1.88%) and T₁ (37.00±1.70%) while minimum 29.00±1.18% in T₀ (Table 4).

The score for bread volume prepared with fenugreek seeds powder supplementation ranged from 7.02±0.09 to 7.39±0.04 (0 hours), 6.86±0.14 to 7.21±0.13 (24 hours), 6.74±0.09 to 7.02±0.16 (48 hours) and 5.57±0.04 to 6.90±0.12 (72 hours). The aroma of the bread ranged from 7.01±0.20 to 7.26±0.18 (0 hours), 6.77±0.12 to 7.02±0.15 (24 hours), 6.66±0.12 to 6.81±0.16 (48 hours) and 6.40±0.19 to 6.74±0.12 (72 hours) (Table 5). The sensory score for taste of bread varied from 6.91±0.15 to 7.62±0.06 (0 hours), 6.62±0.16 to 7.23±0.13 (24 hours), 6.36±0.13 to 6.90±0.21 (48 hours) and 6.24±0.11 to 6.80±0.18 (72 hours). The score for crust color of bread ranged from 7.01±0.15 to 7.52±0.03 (0 hours), 6.81±0.15 to 7.28±0.16 (24 hours), 6.66±0.12 to 7.06±0.14 (48 hours) and 6.62±0.14 to 6.92±0.20 (72 hours) (Table 6). The score for overall acceptability of bread ranged from 7.08±0.08 to 7.46±0.15 (0 hours), 6.83±0.18 to 7.22±0.17 (24 hours), 6.59±0.15 to 7.07±0.14 (48 hours) and 6.59±0.10 to 6.89±0.10 (72 hours) (Table 7).

DISCUSSION

The results of wheat flour analysis are in line with findings of Khan *et al.* (2009) and Mueen-ud-Din *et al.* (2009) who observed 11.78 to 13.42% moisture content, 0.41 to 0.55% ash content, 10.23 to 12.05% crude protein, 1.29 to 1.40% crude fat and 0.41 to 0.44% crude fiber in different Pakistani wheat varieties. The seeds of fenugreek had moisture content (8.30%), ash content (2.92%), crude protein (24.60%), crude fat (6.11%), crude fiber (7.72%) and nitrogen free extract (50.35%) (Mahfouz *et al.*, 2012). Similarly, Yaser *et al.* (2013) found that fenugreek seeds contain 6.57% moisture, 4.03% total ash, 26.78% proteins, 6.35% lipids, 6.75% crude fiber and 49.52% carbohydrates. Findings of the present study are also correlated with the results of Kasaye and Jha (2015) who found fenugreek powder had crude protein (29.89%), crude fat (7.91%) crude fiber (11.34%), carbohydrates (51.55%) and ash content (2.94%) on dry weight basis.

Fenugreek extract had polyphenols (9.47 mg GAE/g dry seeds) as major antioxidants (Dua *et al.*, 2013) and antioxidant activity directly related with polyphenol content of fenugreek (Naidu *et al.*, 2012). Similarly, total phenolic content in different fenugreek varieties were observed 127.8 to 139.2 mg GAE/100g (Ali *et al.*, 2015). Flavonoids are diverse group of polyphenolic components with exceptional strength to act as free radical scavenger, anti-inflammatory and antibacterial agent (Premanath *et al.*, 2011). Different solvents like methanol, ethanol, hexane, ethyl acetate and acetone were used for extraction and flavonoid content found as 607±3.6, 653±4.3, 208±4.2, 251±3.3 and 416±2.7

Table 2. Antioxidants in different fenugreek supplemented flours

Treatment	TPC (mg GAE / 100g)	Flavonoids (mg CE/g)	DPPH (%)	FRAP ($\mu\text{mol Fe}^{2+}/\text{g}$)	β -carotene & Linoleic assay (%)
T ₀	117.00±5.26c	2.38±0.08c	35.00±1.57c	222.00±9.99c	33.00±1.48c
T ₁	305.00±14.03b	2.57±0.10c	49.00±2.25b	421.00±19.36b	43.00±1.97b
T ₂	410.00±19.27a	2.79±0.09ab	53.00±2.49ab	465.00±21.85ab	45.00±2.11b
T ₃	450.00±22.05a	3.01±0.14a	58.00±2.84a	501.00±24.54a	51.00±2.49a

Values expressed are means \pm standard deviation; TPC: Total phenolic content, GAE: Gallic acid equivalents (Folin-Ciocalteu method), CE: Catechin equivalents, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant powder; T₀: Wheat Flour (Control); T₁: 5% fenugreek seeds powder supplemented wheat flour; T₂: 10% fenugreek seeds powder supplemented wheat flour; T₃: 15% fenugreek seeds powder supplemented wheat flour.

Table 3. Chemical composition (%) of different supplemented bread

Treatments	Moisture	Ash	Protein	Fat	Fiber	NFE
T ₀	34.46±1.09d	1.24±0.02d	11.12±0.55c	5.97±0.15c	1.27±0.01d	45.49±1.42a
T ₁	34.49±1.06c	1.39±0.07c	11.74±0.99b	6.25±0.23b	1.65±0.08c	44.35±2.08b
T ₂	34.56±1.35b	1.55±0.05b	12.32±1.20ab	6.54±0.44ab	1.99±0.11b	43.04±2.13c
T ₃	34.63±1.17a	1.72±0.06a	12.79±1.06a	6.81±0.34a	2.29±0.11a	41.90±1.84d

Values expressed are means \pm standard deviation; T₀: Wheat Flour (Control); T₁: 5% fenugreek seeds powder supplemented wheat flour; T₂: 10% fenugreek seeds powder supplemented wheat flour; T₃: 15% fenugreek seeds powder supplemented wheat flour.

Table 4. Antioxidants in different supplemented bread

Treatments	TPC (mg GAE/ 100g)	Flavonoids (mg CE/g)	DPPH (%)	FRAP ($\mu\text{mol Fe}^{2+}/\text{g}$)	β -carotene & Linoleic acid assay (%)
T ₀	99.00±4.45d	2.13±0.09c	31.00±1.27c	167.00±7.51c	29.00±1.18c
T ₁	238.00±10.94c	2.50±0.10b	45.00±1.93b	398.00±17.51b	37.00±1.70b
T ₂	341.00±16.02b	2.68±0.10ab	49.00±2.20ab	401.00±18.44b	40.00±1.88ab
T ₃	413.00±19.82a	2.91±0.12a	51.00±2.39a	450.00±21.60a	43.00±2.10a

Values expressed are means \pm standard deviation; T₀: Wheat Flour (Control); T₁: 5% fenugreek seeds powder supplemented wheat flour; T₂: 10% fenugreek seeds powder supplemented wheat flour; T₃: 15% fenugreek seeds powder supplemented wheat flour.

Table 5. Effect of treatments and storage on volume and aroma of supplemented bread

Treatments	Volume				Aroma			
	0 hours	24 hours	48 hours	72 hours	0 hours	24 hours	48 hours	72 hours
T ₀	7.39±0.04	7.21±0.13	7.02±0.16	6.90±0.12	7.26±0.18	7.02±0.15	6.81±0.16	6.74±0.12
T ₁	7.29±0.04	7.14±0.08	6.95±0.05	6.78±0.12	7.11±0.18	6.86±0.20	6.57±0.16	6.51±0.14
T ₂	7.19±0.12	7.06±0.10	6.86±0.07	6.68±0.10	7.08±0.12	6.85±0.18	6.54±0.19	6.48±0.17
T ₃	7.02±0.09	6.86±0.14	6.74±0.09	5.57±0.04	7.01±0.20	6.77±0.12	6.48±0.21	6.40±0.19

Values expressed are means \pm standard deviation; T₀: Wheat Flour (Control); T₁: 5% fenugreek seeds powder supplemented wheat flour; T₂: 10% fenugreek seeds powder supplemented wheat flour; T₃: 15% fenugreek seeds powder supplemented wheat flour.

Table 6. Effect of treatments and storage on taste and crust color of supplemented bread

Treatments	Taste				Crust color			
	0 hours	24 hours	48 hours	72 hours	0 hours	24 hours	48 hours	72 hours
T ₀	7.62±0.06	7.23±0.13	6.90±0.21	6.80±0.18	7.52±0.03	7.28±0.16	7.06±0.14	6.92±0.20
T ₁	7.12±0.12	6.82±0.13	6.52±0.08	6.38±0.07	7.32±0.10	7.13±0.09	6.96±0.09	6.88±0.15
T ₂	7.02±0.08	6.67±0.10	6.40±0.05	6.30±0.16	7.23±0.12	7.07±0.13	7.00±0.07	6.83±0.17
T ₃	6.91±0.15	6.62±0.16	6.36±0.13	6.24±0.11	7.01±0.15	6.81±0.15	6.66±0.12	6.62±0.14

Values expressed are means \pm standard deviation; T₀: Wheat Flour (Control); T₁: 5% fenugreek seeds powder supplemented wheat flour; T₂: 10% fenugreek seeds powder supplemented wheat flour; T₃: 15% fenugreek seeds powder supplemented wheat flour.

Table 7. Effect of treatments and storage on overall acceptability of supplemented bread

Treatments	Time (Hours)			
	0	24	48	72
T ₀	7.46±0.15	7.22±0.17	7.07±0.14	6.89±0.10
T ₁	7.28±0.12	7.02±0.20	6.74±0.18	6.74±0.10
T ₂	7.23±0.18	6.97±0.22	6.74±0.12	6.74±0.15
T ₃	7.08±0.08	6.83±0.18	6.59±0.15	6.59±0.10

Values expressed are means \pm standard deviation; T₀: Wheat Flour (Control); T₁: 5% fenugreek seeds powder supplemented wheat flour; T₂: 10% fenugreek seeds powder supplemented wheat flour; T₃: 15% fenugreek seeds powder supplemented wheat flour.

QE $\mu\text{g/g}$ of fenugreek (Bukhari *et al.*, 2008). In an earlier study Ishtiaque *et al.* (2013) also found flavonoid content (5.80 mg QE/g) in fenugreek seeds and supplementation in wheat flour resulted in increased flavonoid content. Similarly, Dixit *et al.* (2005) observed that antioxidant activity in fenugreek seeds may be due to presence of polyphenols and flavonoids. The DPPH assay is being used to determine the free radical scavenging effect of natural antioxidant. The bleaching of DPPH absorption indicated the capacity of the test drugs to scavenge free radicals independently (Subhashini *et al.*, 2011; Dua *et al.*, 2013). Likewise, Belguith-Hadriche *et al.* (2010) observed antioxidant activity of fenugreek seeds extract by estimating its ability to scavenge DPPH and observed result *i.e.* 89.91 \pm 3.09%. Reducing power demonstrated the electron donor properties of fenugreek and then neutralized the free radicals by converting into stable products. A study was carried out by Khole *et al.* (2014) to separate and characterize bioactive molecules from germinated fenugreek seeds. Ethyl acetate, water and *n*-butanol were used for extraction purpose and the results for FRAP assay were obtained as 1.27 \pm 0.02, 131.027 \pm 11.05 and 0.14 \pm 0.007 mM AEAC, respectively. In a meta-analysis, fenugreek seeds powder extracted systematically to evaluate FRAP assay at ambient temperature by selecting solvents of varying polarity such as methanol, dichloromethane, hexane and water and found it in the range of 0.135 \pm 0.05 to 77.352 \pm 0.62 TE mg/g (Kenny *et al.*, 2013). The change in color of β -carotene has been widely used to determine the antioxidant potential of different plants extract. As β -carotene molecules by process of oxidation lose their double bonds resulted in loss of yellow color of compound. Likewise, antioxidant activity of fenugreek fractions as measured by the bleaching of β -carotene used different fenugreek fractions like fenugreek husk, endosperm and seeds extracts that exhibited 73%, 6% and 22% activity when compared with the corresponding values of 95% for BHA (Naidu *et al.*, 2011). Current results are in agreement with explorations of Subhashini *et al.* (2011) who determine the antioxidant activity of 70% ethanol extract of fenugreek by β -carotene bleaching method. The extract was employed in the range of 25-400 mg/mL and by adding more quantity of the extract, absorbance was decreased and the reason behind was inhibition of bleaching of the color β -carotene. Fenugreek extracts depicted inhibition at concentration of 0.202 mg/mL. The present findings of bread chemical composition are compatible with the previous studies like supplemented bread with fenugreek seeds increased total protein, fat, ash and fiber, while decreased moisture content and carbohydrates (Mahmoud, 2013). Similar findings were reported by Kasaye and Jha (2015) who found high fat, fiber and protein content in bread prepared with flour supplemented with fenugreek seeds powder. The shielding impact of fenugreek against chronic diseases has been

accredited to the antioxidant potential of its total phenolic and flavonoid content like alkaloids, flavonoids and saponins (Kumar *et al.*, 2013). The flavonoids contents in wheat-fenugreek supplemented bread were found higher when compared with control bread because fenugreek powder is a better source of flavonoid compounds than wheat flour. However, wheat-fenugreek flour bread has flavonoids content lower than supplemented flour. The possible reason of loss during processing conditions like baking, there might be loss of flavonoid compounds due to thermal process that leads to reduce antioxidant activities (Dietrych-Szostak and Oleszek, 1999).

The DPPH value for bread prepared from wheat flour found lower than fenugreek-wheat supplemented bread. The bread prepared from refined and whole wheat flour had DPPH values in the range of 2.83 to 3.90 $\mu\text{mol TE/g}$ and 2.79 to 4.05 $\mu\text{mol TE/g}$ (Yu *et al.*, 2013). However, average DPPH value was observed 64.2% in fenugreek supplemented chicken patties due to higher activity of fenugreek (Devatkal *et al.*, 2012). In a previous study, higher content of β -carotene (22.5mg/100 g) observed in low humidity air dried fenugreek compared to radiofrequency dryer dried sample (6.2 mg/100g; 76.2 mg/100g) and hot air dried fenugreek (6.0 mg/100 g; 148.1 mg/100g) (Naidu *et al.*, 2012). The supplementation of fenugreek seeds in the diet of rats prevented enzymatic leakage, alter lipid peroxidation and enhanced antioxidant potential (Thirunavukkarasu *et al.*, 2003). So fenugreek seeds can be a potential source of bioactive compounds and addition of fenugreek powder in rats diet @ of 1g/kg of body weight lowered lipid peroxidation and improved significant system (Annida and Prince, 2004). No bioactive components were detected in paratha prepared with wheat flour. However, β -carotene content found slightly higher when 25% of normal fenugreek incorporated in wheat flour (Sudha *et al.*, 2013).

The current findings of bread sensory evaluation are well supported by earlier studies of Hooda and Jood, (2004), Hefnawy *et al.* (2012), Rasool *et al.* (2013) and Kasaye and Jha, (2015). The supplementation of legume flours affects the qualitative parameters of baked products like bread volume. The other bakery product like pastries volume was found to be decreased mainly due to decrease in the amount of gluten caused by the addition of materials from which it is not possible to isolate gluten. By lowering the amount of gluten, the ability to keep ferment gas during the rising of dough is also lowered and consequently it influences as lower volume (Bojnanska *et al.*, 2010). When fenugreek was added in wheat flour it modified the sensory attributes like taste and mouth feel of the product. There was a decreasing trend for odor and taste, probably due to fenugreek flavor (Hooda and Jood, 2004). The desirable aroma of fenugreek seeds came from polysaccharides (galactomannan), volatile oils and alkaloids, such as choline and trigonelline (Rasool *et al.*, 2013). Color

of the baked product depends on physicochemical properties of the dough (pH, water content, amino acid content and reducing sugars) and processing conditions applied during baking process like relative humidity, temperature, air speed, modes of heat transfer (Zanoni *et al.*, 1995). Likewise, with increasing fenugreek seed flour in wheat flour, dull brown color of the bread was observed (Chauhan and Sharma, 2000).

Conclusion: The supplementation of fenugreek seeds powder in wheat flour resulted in improved nutritional and antioxidant status with acceptable quality of bread. It was observed that crude protein, crude fiber and ash content of bread increased with the addition of fenugreek powder. Similarly, polyphenol and flavonoid content and antioxidant activity of bread found increasing trend with increasing the supplementation level. It is concluded from the present study that fenugreek seeds powder can be added upto 15% in bread making flour that increased its nutritional profile and antioxidant activity without affecting sensory attributes adversely.

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