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Biochemical Evaluation of *Trigonella foenum graecum* (Fenugreek) With Special Reference to Phenolic Acids

Imran Pasha, Muhammad Asim Shabbir, Muhammad Adnan Haider, Bahzad Afzal, Muhammad Farhan Jahangir Chughtai*, Shabbir Ahmad and Muhammad Sajid Manzoor
National Institute of Food Science & Technology, Faculty of Food, Nutrition & Home Sciences, University of Agriculture, Faisalabad, Pakistan

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Abstract. In current study nutritional constituents of fenugreek seeds *Trigonella foenum graecum* and antioxidant potential was determined. Rheological aspects of fenugreek supplemented flour were evaluated. Total phenolic contents (TPC) were quantified by using spectrophotometer. TPC content was higher in ethanol extract as compared to methanol extract, accounted for 9.11mg GAE/g, and 7.82mg GAE/g, respectively. High pressure liquid chromatography was used to analyze the individual phenolic acids. Chlorogenic acid was found in higher quantity accounted for 167. 9 µg/g and sinapic acid with lowest amount 8.6 µg/g. Composite flours with treatments T_1, T_2, T_3 and T_4 of fenugreek seed powder with wheat flour were prepared and their rheological properties revealed the T_4 with best and healthy results. Physicochemical and sensory analysis of cookies depicted that T_1 was best as compared to rest of the treatments.

Keywords: fenugreek, physicochemical analysis, phenolic acids, antioxidant, rheology, cookies.

Introduction

Fenugreek (*Trigonella foenum graecum*) is an annual medicinal herb of family Leguminosae. It is mainly produced in Pakistan, Iran, India and Middle eastern countries. Feenugreek grown in Pakistan and everywhere in Punjab, has a typical aromatic fragrance and due to its abundance, in Kasur, it is famous in the region as a Kasuri methi. Its seeds are consumed as a spice in diet (Kandhare *et al*., 2015; Shazia, 2011).

Fenugreek seeds have wide range of characteristics such as aromatic smell, bitter taste, carminative properties, antioxidant and antibacterial benefits (Bahmani *et al*., 2016). Major constituent of bulk seed is carbohydrate that accounts for 50%. Other chemical constituents of seed are 3 to 4% ash, 3 to 5% moisture, 25 to 30% protein, 7 to 9% lipids, 20 to 25% insoluble fibre, 20 to 30% galactomannans and 5 to 7% saponins that include lysine rich protein, mucilaginous fibre, flavonoids and volatile oils (Goswami, 2012). Seeds have 7.5% lipids that are usually in the form of triglycerides (6.3%) and 450 mg/100g phospholipids (Nayak *et al*., 2015; Srinivasan, 2006). Fenugreek fibre acknowledged widely for its health benefits by increasing HDL and decreasing LDL cholesterol level (Huang *et al*., 2016).

*Author for correspondence;
E-mail: m.farhan.chughtai@ hotmail.com

Antioxidants obtained from fenugreek seeds protect cellular structures from oxidative damage. Hypocholesterolemic effect through increased secretion of bile acids, lipid peroxidation instigate by H_2O_2 and oxidative hemolysis was prohibited by fenugreek seed (Mukthamba and Srinivasan, 2016; Arshadi *et al*., 2015).

Extract of fenugreek seeds can be obtained by using different solvents like hexane, ethanol and methanol. Fenugreek seeds contain phenolic acids such as vanillic acid, coumaric acid, ferulic acid and gallic acid. These acids have higher antioxidant potential and can be determined using HPLC analysis (Roberts *et al*., 2015; Dixit *et al*., 2005). Total phenolic content of fenugreek seed extract can be analyzed by Folin Ciocalteu method.

Fenugreek seeds powder can be applied to all kinds of food formulation. However, scientific research have showed its more use in functional foods and nutraceuticals. Flour fortified with 8-10% fenugreek has been used for preparing bakery items like pizza, bread, cakes and muffins with suitable sensory attributes (Huang *et al*., 2016; Srinivasan, 2005). Hooda and Jood (2005) formed biscuits from different combinations (0, 5, 10, 15 and 20%) of raw, dripping and sprouted fenugreek seed flours. Bread made from wheat flour supplemented with fenugreek seed powder flour has improved physicochemical, nutritional and rheological properties (Kumar *et al*., 2016; Sharma, 1986).

Materials and Methods

Preparation of fenugreek seed powder and wheat flour. Wheat and fenugreek seeds were procured from Ayub Agricultural Research Institute, Faisalabad, Pakistan. After sieving and tempering, milling of wheat grains was carried out using Quardrumate senior mill to get straight grade flour. After screening and sun drying, fenugreek seeds were ground to a fine powder by passing through a 60 mesh sieve followed by storage in air tight containers prior to further analysis (Brummer *et al*., 2003) .

Chemical composition of wheat flour and fenugreek seed powder. The commercial white wheat flour and fenugreek seed powder were tested for their proximate composition and nitrogen free extract (NFE) according to the methods given by the American Association of Cereal Chemists (AACC, 2000).

Mineral profiling of wheat flour and fenugreek seed powder. Minerals like sodium and potassium were determined through flame photometer (Sherwood flame photometer 410, Sherwood Scientific Ltd. Cambridge, UK), while zinc, copper, iron and manganese were measured through atomic absorption spectrophotometer (Varian AA 240, Victoria, Australia) by following the procedure of AOAC (1995).

Antioxidant activity of fenugreek seed powder. ***Total phenolic contents***. Total phenolic content (TPC) was determined by using the method described by Bukhari *et al*. (2008). The total phenolic compounds were estimated from two different extracts of fenugreek seed powder namely ethanol and methanol extract by Folin-Ciocalteu method (FCM). TPC was done according to the method of Bukhari *et al*. (2008) with a minute modification. From a known concentration of the sample solution, 125 µL sample was taken in test tube. Then 500 µL distilled water was added in it. After that 125 µL of Folin-Ciocalteu reagent was added and given a standing time of 6 min. Then 1.25 mL of 7% sodium carbonate was poured in it. Final volume was made 3 mL by adding 1 mL of distilled water and the samples were given 90 min stay for completion of reaction. Absorbance of the sample was taken in triplicate at 760 nm by using a UV-Vis spectrophotometer (IRMECO Germany). Gallic acid was run as a standard along with the samples and its absorbance was taken at 760 nm. The solution was prepared by taking 25 mg and dissolved in 25 mL distilled water. Concentrations of gallic acid ranging from 0 to 450 µg/ mL were used and standard curve was used for the calculation of the total phenolic contents in the samples. Same procedure was followed for all other samples and preceded three replications.

TPC was calculated by the following formula:

$$C = c \times V/m$$

where:

C= total contents of phenolic compounds in mg/g GAE
c= concentration of gallic acid mg/mL
V= the volume of extract
m= weight of extract in g

Determination of individual phenolic acids using HPLC. Fenugreek seeds were dried in the shade and grounded to the powder form. This powder was used for our experiments. Methanol and ethanol extracts obtained by following the method of Dixit *et al*. (2005). For the determination of compositional differences, HPLC was used by making the samples from the fenugreek seed powder extracts. These extracts were centrifuged at 15000 rpm at 4 °C for 20 min. The supernatants were collected and filtered through a 2 µm filter. For the HPLC analysis; extract obtained and filtered. Various standards have been used for the determination and comparison with the samples. Some of the standards, ferulic acid, coumeric acid, gallic acid, syringic acid, sinapic acid, chlorogenic acid, vanilic acid and quercetin were used. The HPLC analysis was performed according to the method of Dixit *et al*. (2005) with some modification by using water HPLC system (water/ millipore, Shimadzu, 10-A, Japan). The separation of the polyphenols was conducted in a C_{18} column (Shim-pack CLC-ODS), 5 µm, 25 cm × 4.6 mm, 300 Å. The flow rate was set to 1 mL/min. A sample volume of 20 µL was injected to the column using a water 717 auto sampler. Gradients of mobile phase were A (H_2O:AA-94:6, pH=2.27), B (ACN-acetonitrile 100%). The solvent gradient in volumetric ratios of solvents A and B was as follows: 0-15 min= 15% B, 15-30 min= 45% B and 30-45 min= 100% B. UV visible detector of 280 nm was used to detect the eluent.

Rheological properties of fenugreek composite flour. Composite flour was made with different fractions of wheat flour and fenugreek seed powder. The supplementation was made of wheat flour with five different levels (control, 2%, 4%, 6%, and 8%) of fenugreek seed powder. The fenugreek seed composite flour samples were evaluated for rheological properties

by using Farinograph and Mixograph by following the methods given in AACC (2000). The physical dough properties of different wheat flour blends containing fenugreek seed powder in different concentrations were determined by using Farinograph (Brabender D-4100 SEW; Germany) according to the procedure described in AACC (2000), the farinographs were interpreted for different characteristics like water absorption, dough stability, mixing tolerance index and dough development time. The fenugreek seed composite flour was run through Mixograph (National NSI-33R) to assess mixing property (peak height) of the dough by following the method described in AACC (2000).

Product development. Cookies were prepared using above mentioned percentage of composite flour according to the method no. 10-54 as mentioned in AACC (2000). Proximate analysis of cookies (triplicate) was estimated by the methods as mentioned above AACC (2000). Then, the cookies prepared were evaluated by a panel of trained judges for sensory evaluation (Lawless and Heymann, 2010).

Statistical analysis. Significant difference among the treatments of final data obtained was determined by using analysis of variance technique (ANOVA) under completely randomized design (CRD) on SPSS (Statistical Package for the Social Sciences, version 10.0.1, 1999). The mean of all treatments were also compared by using Tukey's (HSD) test adopting the method as described by Montgomery *et al*. (2008).

Results and Discussion

The present study was carried out to assess different characteristics of fenugreek seeds. Chemical composition and mineral determination of seed powder was analyzed along with wheat flour. Antioxidant activity was checked by total phenolic contents determination and individual phenolic acids detection through HPLC analysis. Moreover, rheological properties of composite flour were determined and cookies were prepared from different composite flour combinations. Proximate analysis and sensory analysis of the cookies were conducted.

Chemical composition of wheat flour and fenugreek seed powder. Table 1 and Fig. 1 shows the mean values of proximate analysis of wheat flour and fenugreek seed powder. Wheat flour was found to be higher in moisture content and protein content which accounted for 10.37 and 10.87, respectively. Ash, fat and fibre contents were found in least amount, while moisture and protein contents were found in higher amounts. It was found to NFE value of 73.25 and these results were in accordance with the previous study by Akhar *et al*. (2005) who has found the same nutritional composition. On the other hand, fenugreek seed powder was slightly higher in protein contents (27.55%) followed by moisture contents (10.17%) and fat contents (7.47%). Ash and crude fibre contents were notified in fewer quantities that were 2.67 and 3.79%, respectively. Nitrogen free extract (NFE) was accounted for 48.39%. Comparison of these values with that of total fenugreek seeds was in agreement with the results of Naidu *et al*. 2011) who found moisture content, ash, crude protein and fat content as 11.44%, 3.9%, 27.57%, 6.71% respectively.

Table 1. Chemical composition of fenugreek seed powder and wheat flour

Proximate analysis	Wheat flour	Fenugreek seed powder
Moisture	10.37±0.47	10.17±0.06
Ash	1.73±0.02	2.63±0.15
Fat	1.81±0.02	7.47±0.31
Protein	10.87±0.35	27.55±1.56
Fibre	1.98±0.08	3.79±0.31
NFE	73.25±0.62	48.39±1.33

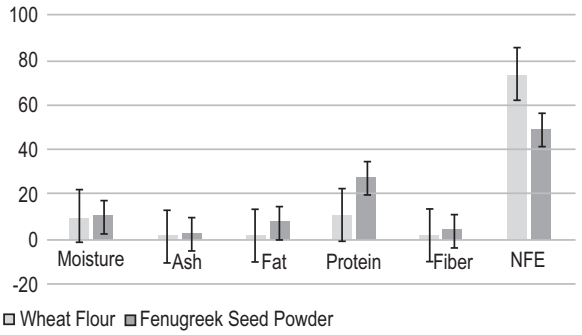

Fig. 1. Chemical composition of fenugreek seed powder and wheat flour

Mineral profiling of wheat flour and fenugreek seed powder. Mean values for minerals contents of wheat flour and fenugreek seed powder have been represented in Table 2 and Fig. 2. Both, wheat flour and fenugreek seed powder were rich in potassium with 124 mg/100 g and 533.33 mg/100 g, respectively. Wheat flour had lowest contents of Cu (0.17 mg/100 g) whereas fenugreek seed powder had the lowest value of Mn (1.53 mg/100 g). Other minerals like Na, Fe and Zn

Table 2. Minerals profile of fenugreek seed powder and wheat flour (mg/100 g)

Minerals	Wheat flour	FSP
Na	2.04±0.14	18.67±0.76
K	124±1	533.33±7.64
Cu	0.17±0.01	33.40±1.15
Mn	0.66±0.04	1.53±0.31
Fe	1.20±0.09	14.07±0.55
Zn	0.87±0.06	7.40±0.56

FSP= Fenugreek seed powder

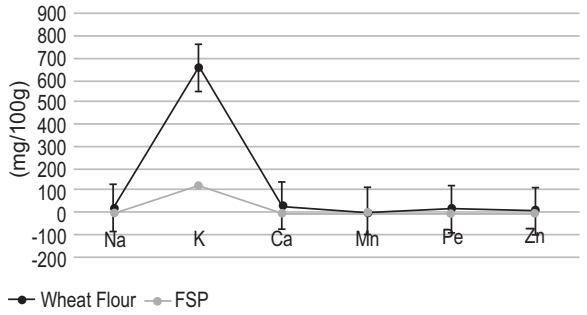

Fig. 2. Minerals profile of fenugreek seed powder and wheat flour

were also identified in sufficient amounts confirming fenugreek powder as a good source of minerals that helped in a number of physiological functions of body and maintains health status. These results were in line with the research outcomes of Goswami (2012) Srinivasan, 2006), who declared that iron, calcium, zinc ranged from: 0.33 mg to 3.73 mg, 4 mg to 64 mg and 0.35 mg to 2.99 mg/100, respectively.

Antioxidant potential of fenugreek seeds. ***Total phenolic contents***. Total phenolic contents of fenugreek seeds were determined by two types of extracts; methanol and ethanol and significant relation have been observed. Ethanol extract showed greater TPC contents (9.11 mg GAE/g) than methanol extract (7.82 mg GAE/g). These results were in accordance with the results of Bukhari *et al*. (2008) who reported 6.85 mg GAE/g for ethanol and 5.75 mg GAE/g for methanol extract of fenugreek seeds.

Phenolic acids analysis. Among the phenolic compounds and flavonoids of fenugreek seeds gallic, ferulic, vanillic, syringic, sinapic, chlorogenic, *p*-coumaric acid and quercetin were detected. Concentrations of individual phenolic acids and flavonoids in fenugreek seeds were represented in Table 3. Two types of extracts were used for HPLC analysis, namely methanol and ethanol extract. Results have revealed that methanol extract was efficient and more phenolic compounds were detected in methanol extract (Fig. 3) as compared to ethanol extract (Fig. 4).

Chlorogenic acid was the main phenolic acid detected

Table 3. Individual phenolic acids/flavonoids concentration in fenugreek seeds determined through HPLC

Phenolic acid/ Flavonoid (µg/g)	Solvents	
	Methanol extract	Ethanol extract
Gallic acid	17.13	40.03
Chlorogenic acid	167.9	-
p-coumeric acid	13.8	-
Ferulic acid	33.8	-
Sinapic acid	8.6	-
Vanillic acid	-	21.5
Syringic acid	-	61.8
Quercetin	42.9	113.57

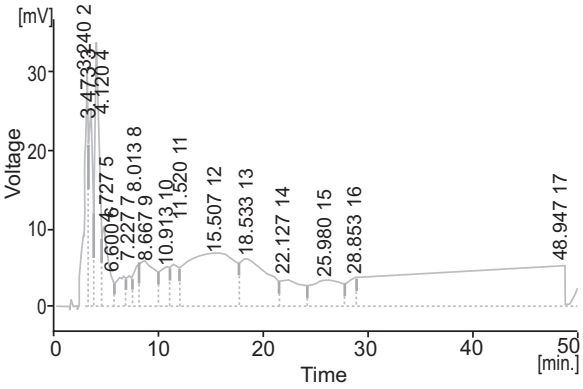

Fig. 3. Peak value of phenolic acids for methanol extract

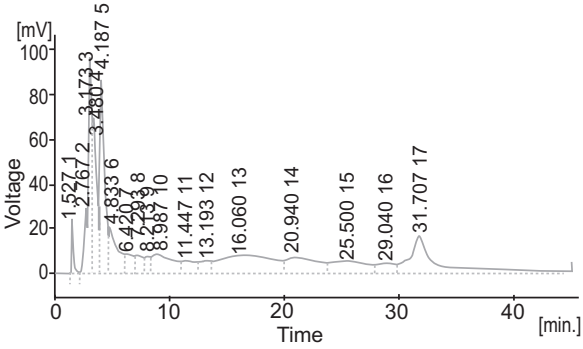

Fig. 4. Peak values of phenolic acids and flavonoid for ethanol extract

in methanol extract with a concentration of 167.7 µg/g followed by ferulic acid (33.8 µg/g), gallic acid (17.13 µg/g) and *p*-coumeric acid (13.8 µg/g). Sinapic acid was found in a low quantity (8.6 µg/g) than all other phenolics. Vanillic and syringic acid was not found in methanol extract of fenugreek seeds.

Only few phenolic acids were detected in ethanol extract named as gallic, vanillic and syringic acid. It is noted here that vanillic and syringic acid were not detected in methanol extract. Only ethanol extract showed the peaks of these phenolic acids. Gallic acid was the common acid detected in both extracts. Both ethanol and methanol extract gave the peaks of different acids and their results did not match with each other. It was mainly due to different characteristics of solvents. Syringic and gallic acid were found in higher quantity in ethanol extract and they had a concentration of 61.8 and 40.03 µg/g, respectively. Vanillic acid also notified in a sufficient amount that was 21.5 µg/g.

Quercetin is a flavonoid detected in both methanol and ethanol extracts. Ethanol extract of fenugreek seeds gave a very high concentration of quercetin that is 113.57 µg/g, whereas it had a low concentration of 42.9 µg/g in methanol extract. Both values of quercetin for methanol and ethanol extract were shown in Table 3. These all results were compared with the findings of Dixit *et al*. (2005) who checked the antioxidant activity of fenugreek seeds and determined few phenolic acids through HPLC analysis.

Rheological properties of fenugreek seed composite flour. ***Mixographic study***. Mixograph results are the best predictor for firmness and chewiness. The peak height percentage gives some indication of the strength and absorption of the flour. Table 4 illustrated the mean values of peak height among which the highest value was observed in T_4 with a mean of 87 at 8% level. Peak height increased as percentage of fenugreek seed powder in composite flour increased and it might be due to slow hydration characteristics of the supplementation material. There was highly significant effect of fenugreek seed powder on peak height percentage of straight grade flour. The values for peak height in control and fenugreek seed composite flour are in in comparison with the findings of Gajula *et al*. (2009) which declared it in the range of 13.4-45.8%.

Farinographic study. Farinographic characteristics of fenugreek seed composite flour varied significantly. Mean values for Farinographic studies were mentioned in Table 4 and Fig. 5. Water absorption went up from 55.50% in control to 60.66% in T_4 with 8% fenugreek seed composite flour. This was probably due to fenugreek interference with gluten formation by offering less resistance to the mixing blades of Farinograph and thus, making the dough less elastic and cohesive. Dough stability and dough development time increased with the increased level of substitution. Maximum dough development time (9.60 min) and dough stability (16.36 min) was recorded in T_4 with 8% level of substitution.

By increasing the substitution level, mixing tolerance index was decreased. Overall, fenugreek seed powder incorporation resulted in making the dough more stable. Further, fenugreek seed powder posed some resistance

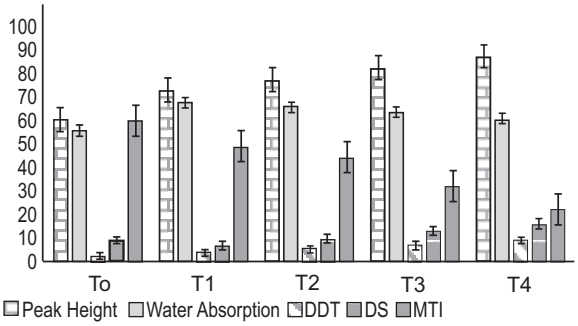

Fig. 5. Rheological properties of fenugreek seed composite flour

Table 4. Mean values for rheological properties of fenugreek seed composite flour

Treatment	Peak height	Water absorption	DDT	DS	MTI
T_o	60.00±5^{d}	55.50±0.8^{d}	2.50±0.4^{d}	9.60±2.2^{bc}	59.66±2.5^{a}
T_1	72.66±3.21^{c}	67.40±0.7^{a}	3.93±0.9^{cd}	6.80±0.9^{c}	48.66±1.5^{b}
T_2	77.00±2^{bc}	65.66±0.8^{a}	5.53±0.6^{c}	9.80±1.36^{bc}	44.33±2.1^{b}
T_3	82.00±1.53^{ab}	63.53±0.9^{b}	7.36±0.7^{b}	13.13±1.0^{ab}	32.00±2.6^{c}
T_4	87.00±2^{a}	60.66±0.7^{c}	9.60±0.7^{a}	16.36±0.9^{a}	22.33±2.5^{d}

T_o = 100% wheat flour; T_1 = 98% wheat flour + 2% fenugreek seed powder; T_2 = 96% wheat flour + 4% fenugreek seed powder; T_3 = 94% wheat flour + 6% fenugreek seed powder; T_4 = 92% wheat flour + 8% fenugreek seed powder

to weaken the dough in mixing of Farinograph as if more than 10% of powder has been added than the mixing gets difficult to be done and overall dough structure disturbed, therefore it is recommended that up to 10% addition is fine enough to get the good dough characters (Hooda and Jood, 2003).

Chemical composition of fenugreek cookies. The mean values for proximate analysis (moisture, ash, fat, protein, fibre and NFE) of cookies prepared from different treatments (T_0, T_1, T_2, T_3 and T_4) have been expressed in Table 5 and Fig. 6. Highly significant results observed in case of protein, fibre and NFE, whereas remained non-significant for moisture, ash and fat. Mean values ranged between 3.11 to 3.12% for moisture content, 1.72 to 1.88% for ash content and 21.11 to 21.27% for fat content. Protein and fibre content increased by increasing level of substitution. Control biscuits contained 9.29% protein content which inclined to 10.22% in T_4 which may be due to considerable higher protein content of fenugreek seeds. Fibre content also went up to 2.38% in T_4 as compared to control. NFE value has a decreasing trend by increasing level of incorporation (Hooda and Jood, 2005).

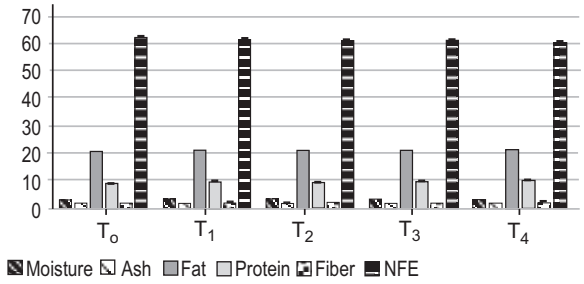

Fig. 6. Chemical composition of fenugreek cookies

Sensory characteristics of cookies. The sensory attributes i.e. colour (09), crispiness (09), flavour (09), overall acceptability (09), taste (09) and texture (09), were evaluated by a panel consisting of six members and the data was evaluated statistically. Cookies samples were arranged, coded in random manner and presented to the trained panelists for sensory evaluation (Rai *et al*., 2014; Krishnan *et al*., 2011; Okpala and Chinyelu, 2011).

The mean values for sensory attributes of cookies have been expressed in Table 6 and Fig 7. Colour, flavour and taste showed highly significant results by replacement of fenugreek seed flour. Texture was also affected significantly, whereas crispiness remained non-significant. The sensory score for control and T_1 was higher as compared to other treatments. It was observed that score decreased sharply for colour, taste and flavour and cookies derived from fenugreek seed composite flour did not show excellent palatability. Generally, the mouth feel of the cookies was affected by the presence of fenugreek seed flour which lead to a residual taste in the mouth.

The control and T_1 treatment had maximum overall acceptability whereas biscuits prepared from 6% and

Table 5. Mean values for proximate analysis of fenugreek cookies (%)

Treatment	Moisture	Ash	Fat	Protein	Fibre	NFE
T_o	3.11±0.10	1.7233±0.08	21.11±0.10	9.29±0.07^{d}	2.04±0.08^{c}	62.56±0.12^{a}
T_1	3.11±0.09	1.7567±0.09	21.14±0.09	9.49±0.06^{c}	2.12±0.09^{bc}	62.20±0.1^{b}
T_2	3.12±0.11	1.7800±0.10	21.17±0.09	9.66±0.05^{b}	2.21±0.10^{abc}	61.83±0.06^{c}
T_3	3.11±0.10	1.8367±0.07	21.22±0.10	9.79±0.03^{b}	2.31±0.10^{ab}	61.50±0.1^{d}
T_4	3.12±0.08	1.8867±0.10	21.27±0.12	10.22±0.08^{a}	2.38±0.10^{a}	60.90±0.10^{e}

Table 6. Mean values for sensory characteristics of cookies

Treatment	Colour	Crispness	Flavour	Taste	Texture	Overall
T_o	8.66±0.58^{a}	8.00±0	8.66±0.58^{a}	8.66±0.58^{a}	9.00±0^{a}	8.66±0.58^{a}
T_1	8.00±0^{ab}	7.66±0.58	7.66±0.58^{b}	7.00±0^{b}	7.66±0.58^{ab}	7.66±0.58^{ab}
T_2	7.66±0.58^{ab}	8.00±0	7.00±0^{b}	7.00±0^{b}	7.66±0.58^{ab}	7.33±0.58^{ab}
T_3	7.00±0^{ab}	7.33±1.15	7.00± 0^{b}	6.33±0.58^{bc}	7.33±1.15^{ab}	6.66±0.58^{b}
T_4	6.33±1.15^{b}	6.66±1.53	6.00±0^{c}	6.00±0^{c}	6.66±1.15^{b}	6.00±1^{b}

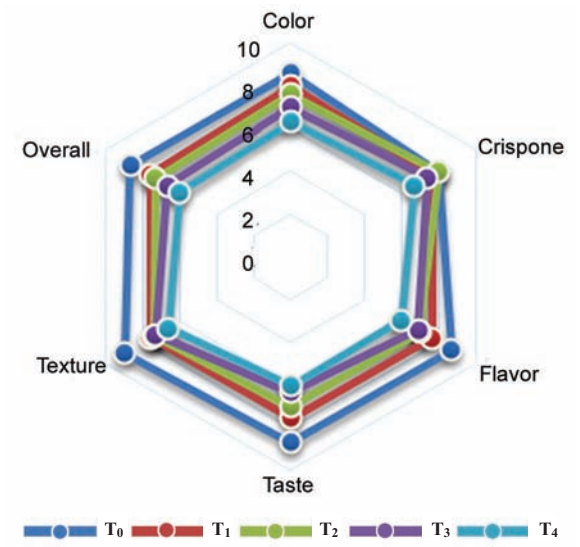

Fig. 7. Sensory characteristics of fenugreek cookies

8% fenugreek composite flour were not mainly acceptable by panelists. Cookies prepared from 6% and 8% fenugreek composite flour impaired the taste of biscuits significantly and the score decreased to 6.33 ± 0.58 and 6.0 ± 0, respectively. This decreasing trend was due to the bitter taste of fenugreek seeds. The overall acceptability score for control was 8.66 on a 9-point hedonic scale. Overall acceptability rate was poor for 6% and 8% level of substitution. Similar observations with supplementation of fenugreek seed flour and wheat flour were also reported by Hooda and Jood (2005). From overall acceptability, it was concluded that fenugreek seed flour was acceptable up to 5% level of substitution for the formation of cookies without affecting sensory quality.

Conclusion

Comprehensive analysis of chemical composition, antioxidant activities and rheological properties of fenugreek seed powder has proved its renowned health promoting potential. Low extent of fat and good quality protein explores the vitality of its chemical composition. Minerals are also present in minute quantity having potassium in more quantity than all other minerals. Moreover, phenolic acids have also been found in sufficient amount which exhibit great antioxidant activity. Cookies prepared from 2 to 4% level of substitution of fenugreek seed composite flour got the maximum sensory results as compared to 6-8% level of substitution. Thus present research work confirms healthy antioxidant potential of fenugreek seeds with the presence of requisite phenolic acids in it.

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