

Nutritional Composition, Microbial Load and Sensory Properties of Fenugreek (*Trigonella foenum-graecum* L.) Flour Substituted Injera

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Abstract

Injera, a staple food in Ethiopia, is large pancake-like bread prepared from cereals such as teff and sorghum. Fenugreek (*Trigonella foenum-graecum* L.) provides tremendous amount of active ingredients for health promotion, disease prevention and food preservation. It is rich in mucilaginous fiber and other dietary essentials; their use can be exploited as functional and nutritional foods as well as therapeutic agent.

The 5% germinated fenugreek-substituted *Injera* showed the highest crude protein ($15.90 \pm 0.14\%$), crude fiber ($3.42 \pm 0.11\%$) and ash ($2.86 \pm 0.06\%$) contents on dry weight basis; but the highest crude fat content ($11.90 \pm 0.14\%$) was obtained in 5% raw fenugreek-substituted *Injera*. Also, 5% roasted fenugreek-substituted *Injera* had highest Ca (168.7 ± 1.8 mg/100 g), Mg (16.3 ± 1.06 mg/100 g), Zn (2.0 ± 0.10 mg/100 g) and Fe (2.45 ± 0.21 mg/100 g). In sensory evaluation *Injera* samples substituted with 1% fenugreek rated as more acceptable than that of 5% substitution. The lowest total microbial load was recorded in 5% roasted fenugreek substituted *Injera* through all samples.

In conclusion, substitution of roasted and germinated fenugreek flour with teff flour showed more improvement, in nutritional composition, microbial load than that of raw fenugreek flour-substituted *Injera*

Keywords: Microbial load; Nutritional composition; Sensory acceptance

Introduction

Injera, a staple food in Ethiopia, is large pancake-like bread prepared from cereals such as teff and sorghum. It is characterized by having 'eyes' (honeycomb-like holes) in its top surface, which are produced due to the production of carbon-di-oxide during fermentation and baking [1]. It is a cultural food of some East African countries particularly Ethiopia, Eritrea and to some extent of Somalia [2]. Unfortunately mould spoilage is a serious problem that affects shelf life of *Injera* [3].

Fenugreek has anti-microbial activity and seasoning type sweet and highly spicy flavor. It is a good source of dietary fiber, fat, protein, and minerals [4]. However the fenugreek seeds have bitter taste due to the presence of saponins (tannin and other anti-nutritional factors) which limit their acceptability in foods. It has been possible to debitter fenugreek seeds by employing various processing methods such as soaking, germination or roasting. Also roasting or germination of fenugreek seeds has important effects on chemical composition, nutritive value and acceptability characteristics of products for human consumption [5].

In some part of Ethiopia, people use the raw fenugreek substituted *Injera*, but little is known about processed (germinated and roasted) fenugreek-substituted *Injera*. Also in our society, there has been no exact acceptable substitution level of fenugreek in *Injera* preparation. Therefore, this study was intended to evaluate fenugreek (roasted, germinated and raw) substituted *Injera* for its microbial load, nutritional composition and acceptable sensory substitution level of fenugreek flour in *Injera* preparation.

Materials and Methods

Experimental site and area of sample collection

The experiment was conducted at Hawassa University, College of Agriculture, School of Nutrition, Food Science and Technology. *Hunda'ol* fenugreek seed variety was obtained from Sinana Agricultural

Research Centre (SARC) found in Oromia National State, Bale Robe Sinana district, Ethiopia (Figure 1A). The *cross-37* teff variety was also obtained from Ethiopian Seed Enterprise of South Nation Nationality and People's Region, Hawassa Branch Office, Ethiopia (Figure 1B).

Experimental design

Completely Randomized Design (CRD) was used to evaluate proximate composition, mineral content and microbial load analysis of *Injera* samples which were done in duplicates; as well as Randomized Complete Block Design (RCBD) was used for sensory score with 9-point Hedonic scale.

Sample preparation

Roasting of fenugreek: Fenugreek seeds were cleaned, graded, sorted and roasted at 90°C in an uncovered pan for 4 minutes. It was continuously stirred with ladle for proper and uniform roasting until it became slight brown and left a peculiar aroma [6].

Germination of fenugreek: The seeds were cleaned and washed thrice by using potable water. Then the seeds were soaked in potable water for 24 h at room temperature with a ratio of seeds: water (1:5 w/v). The soaked seeds were germinated in plastic sieves by covering with the sterilized cloth for 48 h at room temperature with frequent watering in 12 h. Then the germinated fenugreek seeds were dried in open air [7].

Grinding of samples: Fenugreek seeds which were raw, germinated

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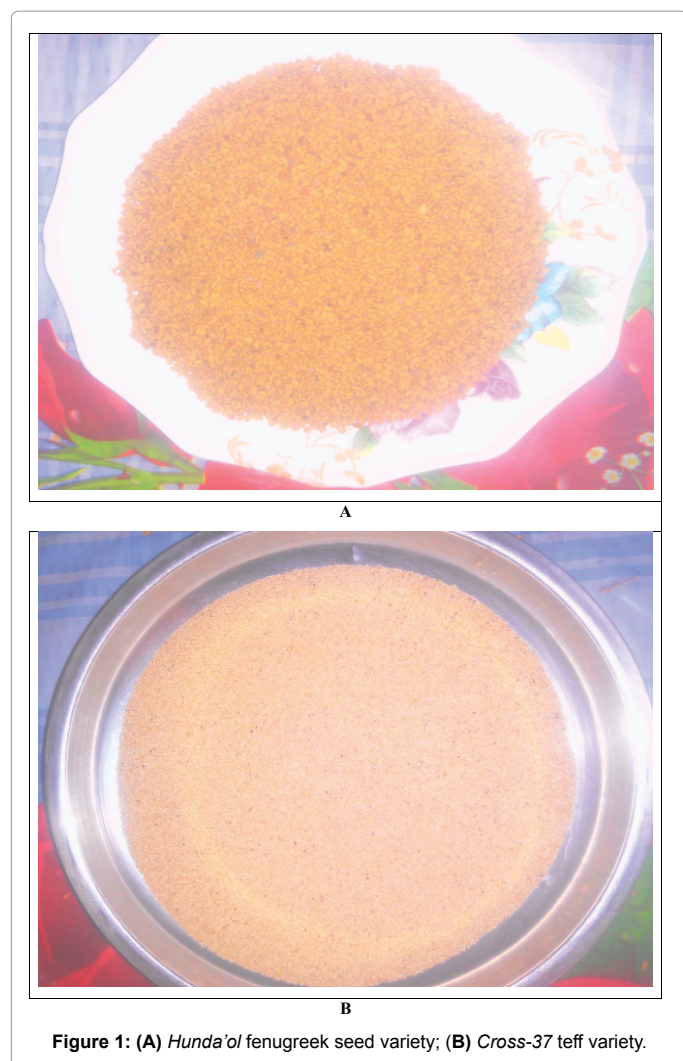


Figure 1: (A) *Hunda'ol* fenugreek seed variety; (B) *Cross-37* teff variety.

and roasted, grounded in grinder (JFSO-100) and passed through standard test sieve of 500 μm (ISO-3310-1 BODY S-STEEL, Made in Germany) to get uniform sized flour. The flours were collected and stored in polyethylene bags separately for further use at ambient temperature [7]. The cleaned teff was then grounded as home set level of *Injera* making [8]. The ground teff powder was collected, packed and stored in dry polyethylene bags to be used in further *Injera* making processes [8].

Substitution of fenugreek seed flour with teff flour: The findings of Hussein et al. [9], Nabila et al. [10], Atlaw and Jha [4] showed that the raw and germinated fenugreek seeds flour -substituted from 5-10% with cereals (maize and wheat) flour was improved nutritive values, acceptable sensory and reduced microbial load status of biscuit and bread. Based on these trends, we substituted the roasted, germinated and raw fenugreek seed flour at 1%, 5% and 10% with teff flour to make *Injera*. The substituted flour (fenugreek flour with teff flour), was homogenized, packed in polyethylene bags and stored at room temperature for further use of *Injera* making (Table 1).

Development of fenugreek substituted *Injera*: The fenugreek substituted *Injera* samples were prepared at laboratory in same way as done at home level in the form of teff flour (FSF) substituted at 1%, 5% and 10% flour of fenugreek + water + starter (*ersho*). Accordingly,

fenugreek substituted teff flour was mixed with clean water in the ratio 1:2 (w/v) and 16% of starter (*ersho*) by weight of flour and was kneaded by hand in a bowl. The resultant dough was allowed to ferment for 3 days at ambient temperature [3].

After this primary fermentation, surface water formed on top of dough was discarded. For every 1 kg of original flour, 200 mL of fermented mixture was mixed with about 400 mL of water and boiled (traditionally known as *absit* making). It was cooled before it was added into main part of dough [3]. The main dough was thinned by adding water equal to original weight of flour and stirred for 15 minutes. The batter was left covered for 2 h for secondary fermentation. After 2 h, the *absit* was added to thinned dough and mixed very well (known as batter making). The batter was left for about 30 minutes to rise before baking being started. Some more water was added to thin and form the right batter consistency.

Finally, about 500 mL of batter was poured onto hot clay griddle (*mitad*) in a circular motion from outside, working towards the centre. After 2-3 minutes of cooking, *Injera* was removed and stored in a traditional basket container (*messob*). Immediately, after cooling for a while (3 to 4 minutes), the baked *Injera* was stacked in a *messob* in which clean white polyethylene plastic was placed underneath of *Injera*. *Injera* samples were then wrapped with white polyethylene plastic before placing *messob* lid as practiced at home set. Then, *Injera* was packed in white polyethylene plastic bags and stored at room temperature until further study [2,3]. However, *Injera* containing 10% fenugreek flour was unacceptable in all sensory properties to panelists on preliminary sensory acceptance test as compared to the other samples. Therefore, it was excluded from this study, because of its unacceptability in all sensory properties.

Determining proximate and minerals of fenugreek-substituted *Injera*

Proximate composition (moisture content, total ash, crude protein, crude fat, crude fiber and total carbohydrate) and minerals (calcium, magnesium, iron, and zinc) content of *Injera* were analyzed using standard methods [11].

Evaluation of total microbial load of fenugreek substituted *Injera*

Yeast-mould, total aerobic mesophilic bacterial and total coli form counts were determined using pour plate technique as described by Tewodros et al. [12].

For yeast and mould count, potato dextrose agar (PDA) was aseptically poured into plate and after incubating aerobically at 25°C for 3 to 5 days, yeast and mould was enumerated on plate bearing 30 to 300 colonies as colony forming units/g *Injera* (cfug^{-1}) according to ISO [13]. For total aerobic mesophilic bacterial count, the Nutrient Agar (NA) was used and after the plate incubated aerobically at 35°C for 48 h, the total aerobic bacteria was enumerated on plates bearing 30 to 300 colonies using colony counter as cfu/g according to ISO [14]. For coli forms count violet red bile lactose agar (VRBLA) was used and the planted plate was incubated aerobically at 35°C for 24 h examination for typical purplish red colonies signifying coli form as cfu/g according to ISO [15]. Finally, it was compared with maximum permissible microbial standard limits in ready-to-eat foods of baked products (cake, bread and biscuit) as described by Ambreen and Samina [16].

Evaluating sensory acceptances of fenugreek substituted Injera

The preliminary sensory acceptability test of *Injera* was carried out in order to optimize maximum acceptable limit of fenugreek substitution level in teff *Injera* making process. The sensory evaluations of *Injera*, quality attributes were carried out by panel's composed of 30 members selected from staff, undergraduate and graduating class students of Food Science and Post-Harvest Technology, Department of Hawassa University. The coded *Injera* samples were accompanied with answering sheet to panels. The scoring scheme of sensory attributes were established as described by Anil et al. [17] for (-ve) color, roll ability, sourness, aroma, taste, top eye distribution, bottom eye distribution and overall acceptance of *Injera* scores were evaluated using 9- point Hedonic scale.

Data analysis

The data were analyzed using one way analysis of variance (ANOVA) at 95% level of confidence with SAS Software Version 9. The means of each parameter was compared using Fischer's least significant differences (LSD) procedures.

Result and Discussion

Proximate composition and mineral content of fenugreek-substituted Injera

Proximate Composition: The highest moisture content was observed in RAF 5% followed by GEF 5% and ROF 5%. This was also in line with reports of Saini et al. [18] on proximate composition of raw, roasted and germinated fenugreek seeds. According to Tamiru and Kumar [5], the removal of moisture generally increased concentrations of nutrients and can make some nutrients more available. Moisture content of ROF 5%, GEF 5%, and RAF 1% were not significantly ($p>0.05$) different. However, there were significant ($p<0.05$) difference among RAF 5%, ROF 1% and GEF 1% in moisture content. As the amount of fenugreek substitution increased, there was slight increase in moisture content of *Injera*. This was in agreement with findings of

Atlaw and Jha [4] on moisture content of bread made from different blends of fenugreek and wheat flour. The moisture content of fenugreek substituted *Injera* was lower than that reported by Ashagrie and Dawit [3] in which the fresh baked *Injera* moisture content was 62-65%, probably due to varietal and processing difference (Table 2). The ash content of ROF 5%, RAF 5%, ROF 1% and GEF 1% were not significantly ($p>0.05$) different but, significantly ($p<0.05$) higher than that of control. Ash content was higher in roasted and germinated fenugreek-substituted *Injera* as compared to raw fenugreek substituted *Injera*. The observed increase of ash attributed from addition of macro and micro minerals may be due to the breaking down of bond between anti nutritional factors (tannin, phytate and oxalates) upon heating and germination of fenugreek seeds [19]. This was in line with reports of Atlaw and Jha [4] on ash content of bread supplemented fenugreek.

The crude protein of all fenugreek substituted *Injera* significantly ($p<0.05$) higher than that of control. There was also significant ($p<0.05$) difference among GEF 5%, RAF 5%, ROF 1%, GEF 1% and RAF 1% in crude protein. However, crude protein of ROF 5%, RAF 5% and GEF 1% were not significantly ($p>0.05$) different. The highest crude protein was obtained from GEF 5% and the lowest was in RAF 1%. This may due to synthesis of new proteins, some amino acids and peptides releasing, due to proteinases enzymes activation during germination of fenugreek [5].The increasing of crude protein in roasted fenugreek substituted *Injera* was also in agreement with that of reported by Magda [19] for crude protein composition of fenugreek seed flour.

The fat content of all *Injera* substituted with fenugreek significantly ($p<0.05$) higher than that of control. However, fat content of control and GEF 1% were not significantly ($p>0.05$) different. Similarly, there was no significant ($p>0.05$) difference among ROF 5%, ROF 1% and RAF 1% samples. The highest fat content was obtained in RAF 5% followed by ROF 5% and GEF 5%. The results of fat content, as given in Table 2, was decreased both in roasted and germinated fenugreek substituted *Injera* from that of raw fenugreek substituted may be due to loss of volatile oils upon open dry heat treatment [20] and loss of fat during germination may be due to its consumption as an energy source in the time of germination [18].

Substitution level of FSF:TF	Raw FSF: TF	Roasted FSF:TF	Germinated FSF:TF
0:100	Control	Control	Control
1:99	RAF 1%	ROF 1%	GEF 1%
5:95	RAF 5%	ROF 5%	GEF 5%
10:95	RAF 10%	ROF 10%	GEF 10%

FSF: Fenugreek seed flour; TF : Teff flour; ROF 5% : 5% Roasted fenugreek substituted *Injera*; ROF 1%: 1% Roasted fenugreek substituted *Injera*; GEF 5%: 5% Germinated fenugreek substituted *Injera*; GEF 1%: 1% Germinated fenugreek substituted *Injera*; RAF 5%: 5% Raw fenugreek substituted *Injera*; RAF 1%: 1% Raw fenugreek substituted *Injera*

Table 1: Substitution of fenugreek seed flour with teff flour to make *Injera*.

<i>Injera</i>	Moisture	Ash	Protein	Fat	Fiber	Carbohydrate
Control	45.2 ± 1.13 ^c	2.60 ± 0.14 ^b	11.76 ± 0.37 ^d	6.67 ± 0.25 ^d	2.58 ± 0.12 ^d	31.19 ± 0.53 ^a
ROF 5%	52.2 ± 2.54 ^{ab}	2.78 ± 0.04 ^{ab}	14.47 ± 0.25 ^b	9.54 ± 0.23 ^b	3.35 ± 0.06 ^a	17.66 ± 2.62 ^d
GEF 5%	53.9 ± 3.04 ^{ab}	2.86 ± 0.06 ^a	15.90 ± 0.14 ^a	7.87 ± 0.18 ^c	3.42 ± 0.11 ^a	16.05 ± 3.54 ^{de}
RAF 5%	56.2 ± 1.20 ^a	2.73 ± 0.04 ^{ab}	14.07 ± 0.17 ^b	11.90 ± 0.14 ^a	3.18 ± 0.11 ^{ab}	11.92 ± 1.10 ^e
ROF 1%	45.7 ± 2.33 ^c	2.68 ± 0.11 ^{ab}	12.86 ± 0.20 ^c	9.17 ± 0.24 ^b	2.74 ± 0.28 ^{bcd}	26.85 ± 2.6 ^{ab}
GEF 1%	49.5 ± 0.77 ^{bc}	2.77 ± 0.05 ^{ab}	13.91 ± 0.37 ^b	7.07 ± 0.61 ^d	2.97 ± 0.33 ^{abc}	23.78 ± 2.02 ^{bc}
RAF 1%	51.6 ± 0.56 ^{ab}	2.63 ± 0.04 ^b	12.49 ± 0.05 ^d	9.66 ± 0.23 ^b	2.6 ± 0.10 ^{cd}	21.02 ± 1.0 ^{cd}

Values are expressed as mean ± SD (n=2) from duplicate experiments; Means with different letters in a column were significantly different at the level of $p<0.05$; ROF5%: 5% Roasted fenugreek substituted *Injera*; ROF 1%: 1% Roasted fenugreek substituted *Injera*; GEF 5%: 5% Germinated fenugreek substituted *Injera*; GEF1%: 1% Germinated fenugreek substituted *Injera*; RAF5%: 5% Raw fenugreek substituted *Injera*; RAF1%: 1% Raw fenugreek substituted *Injera*

Table 2: Proximate composition (%) of fenugreek-substituted *Injera*.

All fenugreek-substituted *Injera* was significantly ($p < 0.05$) higher in fiber content as compared to control. The highest crude fiber was observed in GEF 5% followed by ROF 5%. Fiber content of RAF 5%, ROF 1%, GEF 1% and RAF 1% samples were significantly ($p < 0.05$) different. However, there was no significant ($p > 0.05$) difference between ROF 5% and GEF 5%. Increase in crude fiber content observed both upon roasted and germinated fenugreek substituted *Injera*. This could be due a major constituent of cell walls, might be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination [6] and roasting of fenugreek [20]. Similarly, the amount of crude fiber of *Injera* was increasing while the amount of fenugreek flour increase. This could be due to the higher content of fiber in fenugreek flour [21]. According to Teferra et al. [22], fiber contents of *Injera* were important from nutritional point of view that facilitates digestion and absorption process in human body systems.

The total carbohydrate contents of all fenugreek substituted *Injera* were significantly ($p < 0.05$) decreased as compared to control. This could be due to teff has higher carbohydrate than that of fenugreek. Similar trend was reported by Hussein et al. [9] carbohydrate contents of gelatinized corn flour replaced by raw fenugreek, soaked fenugreek and germinated fenugreek flours to form biscuit. The increased fenugreek substitution showed lower carbohydrate content of *Injera*. This was also, in agreement with the result that reported by Atlaw and Jha [4] on carbohydrate profile of bread made from different blends of fenugreek and wheat flour. Fenugreek substituted *Injera* significantly ($p < 0.05$), highest carbohydrate was observed in ROF 1%, followed by GEF 1% and the lowest was observed in RAF 5%. This was in line with reports of Nacz and Shahidi [23] that the condensed tannin compounds (in raw fenugreek) tend to bind carbohydrates, thus decreasing the absorption and ingestion of nutrients.

Finally, substitution of processed fenugreek resulted in higher ash, protein and fiber content as compared to raw fenugreek substituted *Injera* while, higher fat was found in raw fenugreek as compared to processed fenugreek substituted *Injera*.

Mineral content: The highest calcium (Ca) content was observed in ROF 5% followed by GEF 5%. The sample of ROF 5% was 1.56, 1.11 and 1.22 times greater than that of control, GEF 5% and RAF 5% in calcium content, respectively. The calcium content of all fenugreek substituted *Injera* were significantly ($p < 0.05$) higher than that of control (Table 3). However, RAF 1% and control were not significantly different. The *Injera* substituted with roasted and germinated fenugreek significantly ($p < 0.05$) higher as compared to raw fenugreek substituted *Injera* in calcium content. This was similar with results that reported by Tamiru and Kumar [5], on germinated fenugreek.

Germinated and raw fenugreek substituted *Injera* showed

significantly ($p < 0.05$) lower in magnesium content as compared to roasted fenugreek substituted *Injera*. The increase of magnesium in roasted fenugreek substituted *Injera* may be related to break down of anti-nutritional compounds which bind it and reduce its availability during roasting [18].

Significantly ($p < 0.05$) highest zinc content was observed in ROF 5% followed by GEF 5% and RAF 5%. There was, no significant ($p > 0.05$) difference among ROF 1%, GEF 1% and RAF 1%. However, zinc content of ROF 1%, GEF 1% and RAF 1% were gradually increased from control. Similar trend was reported by Hussein et al. [7] on zinc content fenugreek.

The iron (Fe) content, on germinated fenugreek substituted *Injera* was lower as compared to raw and roasted fenugreek substituted *Injera*. Decrease in iron content of germinated fenugreek substituted *Injera* may be due to leaching of iron to soaking medium [18]. There was no significant ($p > 0.05$) difference between ROF 1%, GEF 1% and RAF 1% in iron content. Increase in iron content of roasted fenugreek substituted *Injera* may be due to decrease in anti-nutritional factors that bind iron during roasting of fenugreek. Similarly, Magda [17] reported on iron content of raw, boiled and germinated fenugreek seeds. The iron content of *Injera* increased with an increased fenugreek substitution, because of high iron content in the fenugreek seed [4]. Similarly, Ibrahim and Hegazy [7] reported that a significant increase in the iron contents with increased fenugreek substitution as compared to control biscuit.

In general, substitution of germinated fenugreek resulted increase in Ca and reduction in Mg, Zn and Fe compared with the raw fenugreek substitution of *Injera*, meanwhile, roasted fenugreek substitution resulted in increase in most of minerals specially Ca, Mg, Zn and Fe compared with raw fenugreek substitution of *Injera*. The results obtained for mineral composition was in line with Magda [17] who stated that fenugreek seeds are found to contain high levels of minerals (Ca, Zn, Mg and Fe) in raw, boiled and germinated fenugreek seeds.

Total microbial load of fenugreek-substituted injera

As explained in Table 4, at 1st day no yeast-mould colony count was observed in all fenugreek substituted *Injera*. At 2nd day of storage the highest yeast-mould colonies count was obtained in control followed by RAF 1%, GEF 1% and ROF 1%. The lower colony count of yeast-mould was observed in roasted and germinated fenugreek substituted *Injera* than that of raw fenugreek substituted *Injera*. This could be roasting and germination of fenugreek provided more antimicrobial compound as compared to raw fenugreek substituted *Injera* [6]. The yeast-mould colonies counts of GEF 5%, RAF 1% and control were significantly ($p < 0.05$) different. However, RAF 5%, ROF 1% and GEF

<i>Injera</i>	Ca	Mg	Zn	Fe
Control	107.0 ± 2.8 ^e	6.4 ± 1.13 ^d	0.70 ± 0.2 ^c	0.95 ± 0.07 ^c
ROF 5%	168.7 ± 1.8 ^a	16.3 ± 1.06 ^a	2.0 ± 0.10 ^a	2.45 ± 0.21 ^a
GEF 5%	151.9 ± 4.0 ^b	12.4 ± 0.60 ^b	1.60 ± 0.14 ^b	1.60 ± 0.14 ^c
RAF 5%	137.8 ± 1.7 ^c	14.05 ± 0.35 ^b	1.83 ± 0.11 ^{ab}	2.30 ± 0.28 ^b
ROF 1%	140.9 ± 1.6 ^c	8.70 ± 0.42 ^c	1.1 ± 0.14 ^c	1.30 ± 0.14 ^d
GEF 1%	124.0 ± 1.6 ^d	6.65 ± 0.21 ^d	1.0 ± 0.14 ^c	1.25 ± 0.21 ^d
RAF 1%	108.2 ± 2.5 ^e	7.30 ± 0.42 ^{cd}	0.95 ± 0.07 ^c	1.29 ± 0.21 ^d

Values are expressed as mean ± SD (n=2) from duplicate experiments; Means with different letters in a column were significantly different at the level of $p < 0.05$; ROF 5%: 5% Roasted fenugreek substituted *Injera*; ROF1 %: 1% Roasted fenugreek substituted *Injera*; GEF 5%: 5% Germinated fenugreek substituted *Injera*; GEF 1%: 1% Germinated fenugreek substituted *Injera*; RAF5%: 5% Raw fenugreek substituted *Injera*; RAF1%: 1% Raw fenugreek substituted *Injera*

Table 3: Mineral content (mg/100 g) of fenugreek-substituted *Injera*.

1% were not significantly ($p>0.05$) different in yeast-mould colonies count. This implies higher availability of antimicrobial activity in processed fenugreek [5].

In addition, at 4th day, the yeast-mould colonies count of all fenugreek substituted *Injera* were significantly ($p<0.05$) lower than that of control sample. However, there was no significant ($p>0.05$) difference among RAF 5%, ROF 1% and GEF 1% in yeast-mould colonies count. Similarly, yeast-mould growth of ROF 5% and GEF 5% were not significantly ($p>0.05$) different. Significantly ($p<0.05$) maximum yeast-mould colonies counts were appeared in control through 1 to 4 day of storage while, in ROF 5% first colony was observed at 4th day of storage. This was correlated with results of Wagh et al. [24] who reported that the fenugreek seeds have very significant antimycotic activity against *Aspergillus niger* and *A. fumigates*.

The highest number of total aerobic mesophilic bacterial count was observed in control followed by RAF 1% and RAF 5%. The control was significantly ($p<0.05$) higher in total aerobic mesophilic bacterial count as compared to fenugreek substituted *Injera*. There was, no significant ($p>0.05$) difference between RAF 5% and RAF 1% while, no growth of total aerobic mesophilic bacterial colonies were found in samples of ROF 5%, GEF 5%, ROF 1% and GEF 1% at 1st day of storage. Similarly, no significant ($p>0.05$) growth of total aerobic mesophilic bacterial colonies were obtained among RAF 5%, ROF 1% and GEF 1% at 2nd day of storage. However, ROF 5%, GEF 5% and RAF 1% were significantly ($p<0.05$) different in total aerobic mesophilic bacterial growth. The lower number of total aerobic mesophilic bacterial colonies count was observed in ROF 5% and GEF 5% as compared to other fenugreek-substituted *Injera* at 4th day of storage. Samples of ROF 5%, GEF 5% and RAF 1% were significantly ($p<0.05$) different in total aerobic mesophilic bacterial colonies count. But, there was no significant ($p>0.05$) total aerobic mesophilic bacterial colonies among RAF 5%, ROF 1% and GEF 1% samples. However, all fenugreek substituted *Injera* of total aerobic mesophilic bacterial colonies count

at 4th day of storage were significantly ($p<0.05$) lower as compared to control sample.

Generally, the increment of fenugreek flour substitution in *Injera* was a cause for decreasing in microbial count. Both yeast-mould and total aerobic mesophilic bacterial count was highest in control sample as compared to fenugreek substituted *Injera*. This could be due to higher anti-microbial effect in fenugreek substituted *Injera* from fenugreek seed constituents of phenolic compound antimicrobial activity, which was in agreement with finding of Norziah et al. [25] who reported that the fenugreek seed exhibited highest antimicrobial activity. As it was observed the raw fenugreek substituted *Injera* appeared to have higher yeast-mould and total aerobic mesophilic bacterial colonies count as compared to roasted and germinated fenugreek substituted *Injera* (Tables 4 and 5). This might be due to lower phenolic contents in raw fenugreek substituted *Injera* than that of roasted and germinated fenugreek substituted *Injera* from fenugreek seed flour [26]. The roasted fenugreek substitution was most effective in inhibiting yeast-mould and total aerobic mesophilic bacterial spoilage in *Injera* followed by germinated and raw fenugreek substitution probably due to active constituents in fenugreek seed. This was also due to the low moisture content and more phenolic compound in roasted and germinated fenugreek substitution may be prevented microbial activity [18]. *Coli* forms were not detected in all *Injera* samples. Similar trend was reported by Atlaw and Jha [4], on microbiological analysis of bread and biscuit supplemented with fenugreek flour.

According to Ambreen and Samina [16] ; Atlaw and Jha [4] standard maximum permissible limits in ready-to-eat foods of baked products (cake, bread and biscuit) for total aerobic bacterial colony count (total aerobic mesophilic bacterial) is 2.0×10^5 cfu g⁻¹, coliforms bacteria is <20 cfu g⁻¹, yeast and mold is $<1.0 \times 10^4$ cfu g⁻¹, respectively. The developed fenugreek substituted *Injera* had lower microbial profile as compared to standard maximum permissible limits in ready-to-eat baked products. Therefore, the result suggests that the use of fenugreek

<i>Injera</i>	1 st day	2 nd day	4 th day
Control	0.39 ± 0.12 ^a	2.18 ± 0.04 ^a	2.35 ± 0.03 ^a
ROF 5%	ND	ND	1.98 ± 0.02 ^e
GEF 5%	ND	0.93 ± 0.04 ^d	2.05 ± 0.00 ^d
RAF 5%	ND	1.35 ± 0.05 ^c	2.09 ± 0.02 ^{cd}
ROF 1%	ND	1.39 ± 0.02 ^c	2.14 ± 0.01 ^c
GEF 1%	ND	1.41 ± 0.05 ^c	2.19 ± 0.04 ^c
RAF 1%	ND	1.63 ± 0.03 ^b	2.25 ± 0.01 ^b

Values are expressed as mean ± SD (n=2) from duplicate experiments; ND=Not detected; Means with different letters in a column were significantly different at the level of $p<0.05$; ROF 5%: 5% Roasted fenugreek substituted *Injera*; ROF 1%: 1% Roasted fenugreek substituted *Injera*; GEF 5%: 5% Germinated fenugreek substituted *Injera*; GEF 1%: 1% Germinated fenugreek substituted *Injera*; RAF 5%: 5% Raw fenugreek substituted *Injera*; RAF 1%: 1% Raw fenugreek substituted *Injera*

Table 4: Total yeast-mould count (log₁₀ cfug⁻¹) of fenugreek-substituted *Injera*.

<i>Injera</i>	1 st day	2 nd day	4 th day
Control	1.83 ± 0.20 ^a	2.26 ± 0.05 ^a	2.39 ± 0.2 ^a
ROF 5%	ND	1.04 ± 0.05 ^e	1.22 ± 0.05 ^d
GEF 5%	ND	1.25 ± 0.03 ^d	1.28 ± 0.14 ^d
RAF 5%	1.13 ± 0.02 ^b	1.30 ± 0.04 ^c	1.39 ± 0.5 ^c
ROF 1%	ND	1.32 ± 0.10 ^c	1.41 ± 0.04 ^c
GEF 1%	ND	1.34 ± 0.03 ^c	1.43 ± 0.07 ^c
RAF 1%	1.18 ± 0.06 ^b	1.42 ± 0.03 ^b	1.75 ± 0.03 ^b

Values are expressed as mean ± SD (n=2) from duplicate experiments; ND=not detected; Means with different letters in a column were significantly different at the level of $p < 0.05$; ROF 5%: 5% Roasted fenugreek substituted *Injera*; ROF 1%: 1% Roasted fenugreek substituted *Injera*; GEF 5%: 5% Germinated fenugreek substituted *Injera*; GEF 1% : 1% Germinated fenugreek substituted *Injera*; RAF 5%: 5% Raw fenugreek substituted *Injera*; RAF 1% : 1% Raw fenugreek substituted *Injera*

Table 5: Total aerobic mesophilic bacterial count (log₁₀ cfug⁻¹) on fenugreek-substituted *Injera*.

Injera	Color	Roll ability	Sourness	Aroma	Taste	TED	BED	OVA
Control	7.3 ± 0.1 ^a	6.9 ± 0.2 ^a	7.2 ± 0.2 ^a	4.6 ± 0.2 ^c	4.5 ± 0.3 ^b	5.2 ± 0.1 ^b	6.6 ± 0.2 ^a	6.0 ± 0.8 ^b
ROF 5%	3.7 ± 0.1 ^c	3.8 ± 0.3 ^d	3.6 ± 0.2 ^d	4.1 ± 0.1 ^c	3.6 ± 0.2 ^c	3.4 ± 0.2 ^c	3.8 ± 0.1 ^c	3.7 ± 0.2 ^c
GEF 5%	2.5 ± 0.1 ^c	2.4 ± 0.2 ^e	2.3 ± 0.3 ^c	3.3 ± 0.1 ^d	2.9 ± 0.1 ^d	2.9 ± 0.1 ^c	3.2 ± 0.3 ^d	2.8 ± 0.4 ^d
RAF 5%	4.8 ± 0.2 ^b	3.5 ± 0.1 ^d	3.6 ± 0.2 ^d	4.4 ± 0.1 ^c	3.7 ± 0.1 ^c	5.1 ± 0.2 ^b	4.3 ± 0.2 ^b	4.2 ± 0.6 ^e
ROF 1%	5.3 ± 0.1 ^b	5.7 ± 0.2 ^b	7.1 ± 0.1 ^a	5.1 ± 0.1 ^b	6.9 ± 0.2 ^a	4.9 ± 0.3 ^b	7.1 ± 0.1 ^a	6.0 ± 1.0 ^b
GEF 1%	5.2 ± 0.2 ^b	4.3 ± 0.03 ^e	4.5 ± 0.2 ^c	4.9 ± 0.1 ^c	4.8 ± 0.1 ^b	4.6 ± 0.1 ^b	4.7 ± 0.2 ^b	4.8 ± 0.5 ^e
RAF 1%	7.2 ± 0.2 ^a	7 ± 0.2 ^a	6.4 ± 0.2 ^b	6.4 ± 0.1 ^a	6.6 ± 0.1 ^a	6.9 ± 0.3 ^a	6.3 ± 0.2 ^a	6.7 ± 0.3 ^a

Values are expressed as mean ± SD (n=2) from duplicate experiments; ND=not detected; Means with different letters in a column were significantly different at the level of $p < 0.05$; ROF 5%: 5% Roasted fenugreek substituted *Injera*; ROF 1%: 1% Roasted fenugreek substituted *Injera*; GEF 5%: 5% Germinated fenugreek substituted *Injera*; GEF 1%: 1% Germinated fenugreek substituted *Injera*; RAF 5%: 5% Raw fenugreek substituted *Injera*; RAF 1%: 1% Raw fenugreek substituted *Injera*

Table 6: Sensory acceptance of fenugreek substituted *Injera*.

flour in *Injera* production is beneficial to improve and prevent yeast-mould, total aerobic bacterial and coli form spoilage [4].

Sensory acceptance of fenugreek-substituted *Injera*

Acceptance of color showed that the control has ranked at top due to excellent colour followed by raw and roasted fenugreek substituted *Injera* while, minimum colour acceptance was observed in germinated fenugreek substituted *Injera*. There was significant ($p < 0.05$) difference observed between control and fenugreek substituted *Injera* in color. However, no significant ($p > 0.05$) difference between control and RAF 1%. Similarly, colour acceptance of RAF 5%, ROF 1% and GEF 1% were not significantly ($p > 0.05$) different. There was a decrease in color intensity with increase in the level of substitution of fenugreek. Similar trend was reported by Sami and Abdelmoneim [27] that the effect of incorporation of different levels of raw and germinated fenugreek seed flour on colour acceptance evaluation of baladi bread samples.

The highest score of roll ability was observed on RAF 1% followed by control and ROF 1%. The roll ability of ROF 5% and RAF 5% samples were not significantly ($p > 0.05$) different. However, roll ability of GEF 5%, ROF 1%, GEF 1% and RAF 1% were significantly ($p < 0.05$) different. The roll ability of fenugreek substituted *Injera* was decreased as amount of fenugreek flour increased in the *Injera* substitution. It could be related with the gelatinization capacity of the cassava (fenugreek) which leads to decreasing the roll ability of *Injera* [28].

The highest acceptance of sourness was found in control followed by ROF 1% and RAF 1%. There was no significant ($p > 0.05$) difference observed between control and ROF 1% in sourness. Similarly, sourness of GEF 5% and GEF 1%; ROF 5% and RAF 5% were not significantly ($p > 0.05$) different. The increment in fenugreek substitution level showed decreased acceptance of sourness. This could be due to fenugreek has higher anti nutritional factors than that of teff. Similarly, Hemlata and Pratima [6] reported that the fenugreek has bitter taste due to presence of saponins (anti nutritional factors) which limit their acceptability in foods.

The highest acceptance of aroma was observed in RAF 1% followed by ROF 1%. The aroma of ROF 5% and RAF 5% were not significantly ($p > 0.05$) different but, aroma of GEF 5%, ROF 1% and RAF 1% were significantly ($p < 0.05$) different. However, there was no significant ($p > 0.05$) difference in aroma of control, ROF 5%, RAF 5% and GEF 1%. The score of taste decreased with increased level of fenugreek substitution. The ROF 1% was highest in taste mean value followed by RAF 1%. The taste of *Injera* was associated with sweet, sour and bitter sensations triggered in the mouth by contact with *Injera* [29]. The taste of ROF 1% (6.9), and RAF 1% (6.6) were significantly ($p < 0.05$) higher

than that of control (4.5). However, taste of control and GEF 1% were not significantly ($p > 0.05$) different.

The appearance (top eye distribution) of *Injera* was one of the most important parameters, which refers to quality eyes of honey comb-like structure of *Injera* top surface formed during cooking due to escaping carbon-di-oxide bubbles [29]. The RAF 1% had significantly ($p < 0.05$) highest top eye distribution (TED) followed by control *Injera*. The lower value of TED was observed in GEF 5%. The TED of control, RAF 5%, ROF 1% and GEF 1% were not significantly ($p > 0.05$) different. Similarly, TED of ROF 5% and GEF 5% were not significantly ($p > 0.05$) different. There was also highest score of bottom eye distribution (BED) observed in ROF 1% followed by RAF 1% and control without significant ($p > 0.05$) difference. Similarly, BED of RAF 5% and GEF 1% were not significantly ($p > 0.05$) different.

Overall acceptability (OVA) refers to combinations of evaluations by consumers or panelists to a product [29]. The highest score of OVA observed on RAF 1% followed by ROF 1% and control. There was no significant ($p > 0.05$) difference between control and ROF 1% in OVA. Similarly, OVA of ROF 5%, RAF 5% and GEF 1% were not significantly ($p > 0.05$) different. This could show less preference of germinated fenugreek substituted *Injera* to panels than that of roasted and raw fenugreek substituted *Injera*.

Generally the color, roll ability, sourness, aroma, taste, upper eye distribution, bottom eye distribution and over all acceptances of fenugreek substituted *Injera* were decreased while the amount of fenugreek substitution increased from 1 to 5% and this showed that the *Injera* samples made from 1% leveling of roasted, germinated and raw fenugreek were within more acceptable limits than that of 5% level of substitution (Table 6). These findings were well supported with findings of Sami and Abdelmoneim [27], who concluded that substitution of 2% fenugreek flour into wheat flour, gave bread with the best overall quality acceptance.

Conclusion

In conclusion, nutritional composition, microbial load and sensory properties of fenugreek (*Trigonella foenum-graecum* L.) flour substituted *Injera* were evaluated. The *Injera* substituted with processed (roasted and germinated) fenugreek showed more improvement in total ash, crude protein, crude fiber and minerals (Ca, Mg, Zn and Fe) contents with further reduced microbial load as compared to raw fenugreek substituted and control. Accordingly, the highest improvement was obtained in roasted fenugreek substituted *Injera*. However, *Injera* substituted with raw fenugreek was higher, in crude fat content than that of processed fenugreek substituted *Injera*. It may be concluded from present study that the nutritional quality of *Injera*

could be improved with substitution of fenugreek flour at 1% up to less than 5% without affecting sensory attributes of *Injera* adversely.

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