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Effect of teff-sorghum-fenugreek flour blending ratios on nutritional and sensory quality of injera

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Abstract

Foods which lack required amount of nutrients cause serious public health problems. Blending could improve the nutrient composition and hence alleviating associated problems. Injera is an Ethiopian fermented and leavened flatbread commonly made from teff flour. It could be also made from the blends of different cereal flours but lacks scientific investigation on the proper blending ratios of these cereal flours. This study was, therefore, conducted to investigate the effects of teff, sorghum and fenugreek blending ratios on quality attributes of injera. For this, various ratios of teff, sorghum, and fenugreek flours were prepared using a mixture D-optimal design. The results showed that the addition of sorghum and fenugreek flours to teff flour improved the mean fiber (from 4.18 to 5.38%), fat (from 2.5 to 6.37%), protein (from 11.36 to 14.43%), and total energy (from 362.05 to 367.85 kcal) contents of the developed injera. However, the addition of these flours reduced the average mineral content [Iron (16.86 mg), Zinc (1.78 mg), and Calcium (111.39 mg)] as compared to injera made from teff flour alone [Iron:20.13 mg, Zinc: 2.36 mg & Calcium: 146.88 mg]. Injera made from composite flours was observe to have high alkaline retention capacity, lower staling rate and better sensory acceptability than injera made from teff flour alone.

Keywords: Antinutritional factors; proximate compositions; Malnutrition; Mineral contents; Staling rate

1.Introduction

In Ethiopia, teff, wheat, maize, sorghum, and barely are the major cereals that occupy almost .ate

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It also increases the amount of dietary protein and micro- macro nutrients in staple foods. As example, blending of teff, sorghum and faba bean flours significantly increase the mineral contents viz iron, zinc, and calcium, proteins, fat, carbohydrates, and ash of the blended based injera at the fermentation time of 72 hrs [7, 8]. Similarly, Godebo and coworkers reported that germinated fenugreek flour substituted injera (blending with teff flour) showed high crude protein, crude fiber and ash with an improvement of injera shelf life due to the antimicrobial property of fenugreek flour [9]. Furthermore, Abraha and Abay, studied the effect of cereal blends (teff, barley, sorghum and maize) with different ratios on the quality of injera and found that nutritionally rich injera was obtained with 50:50 blend ratio of teff + barley, teff + sorghum, teff + maize as well as with equal ratio of teff + barley + sorghum blends [3].

Although many research works have focused on injera development through the blending of teff and sorghum flours and evaluated their nutritional contents such as protein, carbohydrates, fat, fiber and ash with sensory quality, to date, no researchers were worked on teff-sorghum-fenugreek composite flours. Sorghum mix content injera recipe, on the other hand, has poor injera-making qualities, such as staling and fragile texture during storage. Fenugreek acts as a fortified component, plasticizer, providing the necessary uniformity of the dough, the elasticity of the crumb, reducing the rate of staling, and improving the nutritional value of baked products. It was reported that fenugreek has antimicrobial property, spicy flavor, good source of dietary fiber, fat, protein and minerals [10], and hence, could possibly induce some positive quality attributes to injera. Beyond to evaluating the nutritional contents plus sensory analysis of injera developed from these three blended cereals, this work also focused on conducting the anti-nutritional property, staling rate and alkaline-water retention capacity of teff-sorghum-fenugreek composite flours which were not studied by any researchers so far.

2.Materials and methods

2.1. Raw material collection, preparation and formulation

Experimental materials used for laboratory analysis consisted of sorghum (variety Melkam), fenugreek (variety Burka), and teff (variety Boni) seeds were obtained from Melkassa agricultural research center, Kulumsa agricultural research center, and Debre Zeit agricultural research center, EIAR, Ethiopia, respectively grown in the main season 2021/2022. These grain verities are reported to have high yield per unit area, have relatively good disease resistance and have been released, recently, for mass production. Because of the abundance of different varieties of sorghum, fenugreek, and teff, these study sites were chosen to obtain pure breed. The samples were manually cleaned to remove stones, dust materials, glumes, stalks and winnowing, sorted and milled. Teff and sorghum were grounded using a Perten laboratory mill (PM 120, Finland) while fenugreek was grounded using a coffee grinder mill (XFYC810, China) and sieved through a fine sieve of size 0.50 mm. Then the corresponding flours were packed separately in dry polyethylene bags and store in dry place until further analysis carried out. The laboratory activities were conducted at food science and nutrition research laboratories of Kulumsa, Melkassa and Debrezeit Agricultural research centers, EIAR head quarter quality research laboratory and Addis Ababa University in the laboratory of center for food science & nutrition, and department of applied chemistry.

After conducing preliminary study at Melkassa agricultural research center and referring reports of other researchers work [9, 11-13], the maximum and minimum values of independent variable (teff, sorghum and fenugreek) flours was set. The composite flours based on teff, sorghum and fenugreek were prepared using a formulation shown in Table 1. These 10 different formulations were obtained based on a constrained mixture D-optimal design. Each dry material was blended uniformly and packed tightly in closed clean plastic container which then kept at room temperature (25 °C). In this study injera made with 100% teff flour was used as control.

Tuble 1 . D optimit coded design for injeru preparation from terr, sorghum and rendgreek						
		Xi = Factors (Independent variables)				
Formulated samples	X1: Teff	X2: Sorghum	X3: Fenugreek			
Control	1.00	00	00			
T2	0.95	0	0.05			
Т3	0.75	0.25	0.00			
T4	0.87	0.12	0.01			
T5	0.84	0.12	0.04			
T6	0.62	0.37	0.01			
Τ7	0.73	0.24	0.03			
T8	0.62	0.34	0.04			
Т9	0.50	0.45	0.05			
T10	0.50	0.50	0.00			

Table 1. D-optimal coded design for Injera preparation from teff, sorghum and fenugreek

2.2. Dough preparation and injera baking

Injera were prepared following the traditional teff dough preparation procedure [3, 13] with minor modification. For each formulated composite flour weighed (200 g) + water (180 ml) + 16% ersho (yellow like liquid accumulated at the top of batter created during fermentation) were added and knead properly for about 5 minutes. Here, the ersho was made from the flour which used for analysis to avert contamination. The fermentation process of the resulting batter was carried out at room temperature in a closed container (plastic bowl) and proceeded in two stages: the first stage of fermentation was held for 72 hrs. After adding ersho which used as a starter culture. After the first stage of fermentation, the yellowish liquid at the surface of the dough was runoff and about 25% of the fermented dough (paste) was thinned with boiled water for 3-5 min. This formed thinning of fermented batter with water

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fermentationa (left for 30-1hr.) and baked on an electrically heated circular clay based gridle called

way that injera was baked for each fourteen formulated composite flours.

2.3. Proximate composition analysis

2.3.1. Moisture content

The moisture content of the samples was determined using eqn. (1) according to the Association of Official Analytical Chemistry [14] using the official method 925.10 by drying of 2.5 g of injera sample at 105 °C for 1 hour in oven (TR-TC-YHG-300-BS-11, China).

Moisture (%) =
$$\frac{W_2 - W_3}{W_2 - W_1}$$
 X100
eqn. (1)

Where W_1 : the weight of the crucible, W_2 : initial weight of sample, and W_3 : Final weight of sample after dried.

2.3.2. Total ash content

The ash content of injera which literally measures the mineral content of the injera was determined using eqn. (2) according to the Association of Official Analytical Chemistry [14] using the method 923.03 by taking about 2.5 g sample (in duplicate) after carbonization and ignition at 550 °C for 3 hours in the muffle furnace (Nobertherm, Germany).

Ash (%) =
$$\underline{W_1 - W_2}$$
 X100
W eqn. (2)

Where W1: the weight of the ash+ crucible after ashing, W2: the weight of the empty crucible, and

W: the weight of the sample.

2.3.3. Fat content

Crude fat was determined according to the Association of Official Analytical Chemistry [14] using the official method 4.5.01. About 2 g of injera sample (in duplicate) was extracted with 50 ml petroleum ether or diethyl ether for a minimum period of 4 hours in the soxhlet extractor. The solvent was then evaporated and the extracted fat was dried in the oven and cooled in a desiccator. The crude fat was determined according to eqn. (3).

Fat (%) =
$$\frac{W_2 - W_1}{S_W}$$
 X100
eqn. (3)

flask, W_2 : weight of the extraction flask plus the dried crude fat, and Sw: weight of the sample.

2.3.4. Crude fiber content

Crude fiber content of the injera samples was determined using eqn. (4) according to the method 962.09 of the Association of Official Analytical Chemistry (AOAC) [14]. The fresh injera samples of 2 g (in duplicate) (W₃) was placed into a 660 ml beaker; 200 ml of 1.25% sulfuric acid were added, and boiled gently for 30 minutes. Then, after 30 minutes heating, 20 ml of 28% KOH was added and boiled gently for further 30 minutes, with occasional stirring. The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with distilled water. The solution was poured into sintered glass crucible and filtered with the aid of vacuum pump (High performance vacuum pump, Robin Air way, SPX Corporation, Monteplier, USA). The wall of the beaker was rinsed with hot distilled water several times; washings was transferred to the crucible and filtered. The residue in the crucible was washed with hot distilled water and filtered (repeated twice). Then the residue was transferred to crucible cup and dried in oven for 2 hours at 130 °C and cooled for 30 minutes in a desiccator, and then weighed (W_1) . The crucible was cooled in desiccators and weighed (W_2) .

Crude fiber (%) =
$$\frac{W_1 - W_2}{W_3}$$
 X100

Where W_1 : weight of crucible with sample after drying; W_2 : weight of crucible with sample after ashing; W_3 : fresh sample weight.

eqn. (4)

2.3.5. Crude protein

Protein content was determined according to AOAC [14] using the official method 920.87 by the Kjeldahl method. Dried injera flour samples of 0.5 g was taken and mixed with 6 ml of acid mixture (ortho-phosphoric acid and concentrated sulfuric acid) and then 3.5 ml of 30% hydrogen peroxide was added to it step by step. Then 3 g of catalyst mixture (0.5 g of selenium metal with 100 g of potassium sulfate) was added into different test tubes, and allowed to stand for about 10 minutes. Then the digestion was allowed until a clear solution was obtained. About 25 ml of de-ionized water was added, and shaken to avoid precipitation of sulfate in the solution. To conduct the distilation process, 25 ml of boric acid, 25 ml of distilled water poured in a 250 ml conical flask. The digested solution was transferred into the sample compartment of the distiller.

Sodium hydroxide solution (35%) was added (40 ml) into the digested and diluted solution. The distillation process was continued for 9 minutes until a total volume reached between 200 ml and 250 ml. Finally, the distillate was titrated using 0.1 N hydrochloric acid until reddish color appeared. Then the crude protein was determined using eqn. (5) after calculated the Nitrogen % from the titration process.

Protein (%) = $6.25 \times \%$ Nitrogen eqn. (5)

2.3.6. Carbohydrate

The carbohydrate content of the developed injera was calculated by the method of difference [15] that is carbohydrate (%) = 100 - (Protein value + Fiber value + Moisture value + Ash value).

2.3.7. Gross food energy

The gross energy was calculated by difference method and Atwater's conversion factors [16]: Energy (kcal/g) = (% carbohydrate - % crude fiber) x 4 + (% crude fat x 9) + (% crude protein x 4)

2.4. Determination of minerals

The Iron, Zinc and Calcium contents of the samples were calculated using eqn. (6) obtained from the flame atomic absorption spectrophotometer (Model No. AAS-700, Perkin Elmer) process of each formulated injera sample employing the standard AOAC, [14] method 985.35. After obtained ash using eqn. (2), a blank solution was prepared in 50 ml volumetric flask with the same procedure for minerals reading. Then sample reading was conducted. Preparation of standard solutions: Six series of working standard metal solutions (0.0, 0.5, 1, 1.5, 2.0, and 2.5 ppm for Ca, and 0.0, 0.5, 1.0, 2.0, 3.0 and 4.0 ppm for Fe & 0 .000, 0.125, 0.250, 0.500, 0.750, and 1.000 for Zn) of the minerals were prepared by appropriate dilution of the metal stock solution (nitrate of the metal) with deionized water in 10 ml volumetric flask. Calibration curve (concentration vs. absorbance) for each element were derived using the absorbance process. Finallyt, the mineral element content was calculated as follow:

eqn. (6)

Where, W: Weight of sample on a dry matter basis, V: Volume of extract in liters, A: Concentration (mg/l) of sample solution, B: Concentration (mg/l) of blank solution and Df: Dilution factor (50 ml for Ca, Fe and Zn).

2.5. Determination of anti-nutritional factors

2.5.1. Determination of Tannin content

The condensed tannin was determined by using vanillin-HCL assay methods using a UV spectrophotometer [17] as modified by [18] cereal chemistry. A gram of sample in a screw cap test tube was measured and then10 ml of 1% HCl in methanol was added to the tube containing the sample. The sample-containing tube was placed on a mechanical shaker (KS501 digital, ink laboratory CHNIK) for 24 hours at room temperature. Then, the tube was centrifuged at 1000 rpm for 5 minutes. A milliliter of supernatant was taken and mixed with 5 ml of vanillin-HCl in another test tube. Finally, the sample was allowed to wait for 20 minutes to complete the reaction, and then, the absorbance of the colored intensity of the sample UV-visible was measured using а spectrophotometer at 500 nm.

Preparation of standard solutions: A 40 mg Dcatechin standard was dissolved in 1000 ml 1% HCl solution in methanol, from which a series of standard solutions (0, 0.2, 0.4, 0.6, 0.8 and 1 ml) was taken in test tube. After 20 minutes, the absorbance of standard solutions was measured at 500 nm with a UV-VIS Spectrophotometer. The content of condensed tannins was estimated using the D-catechin calibration. Then the Tannin content evaluated as follow:

Tannin content (mg/100g) =
$$\underline{As - Ab} - \underline{int. x 10}$$

Slope x D x W eqn. (7)

Where, As: absorbance of sample solution, Ab: the blank absorbance, Int.: intercept from the absorbance equation curve, D: density of solution (0.791g/ml), and W: weight of the sample in gram, and 10 is the aliquot.

2.5.2. Determination of phytate content

Phytate was determined by a Modified colorimetric method [19]. About 0.5g of samples (in duplicates) was taken in 15 ml centrifuge tubes and 10 ml of 2.4% (0.64N) HCl was added and mix vigorously with vortexer, and shaked mechanical shaker (KS501 digital, ink laboratory CHNIK) at 300 rpm for 16 hours at room temperature. Then the samples were centrifuged at 3000 rpm for 20 minutes.

The supernatant was filtered through Whatman grade 1 filter paper into the tubes with the previously weighed NaCl and mixed thoroughly on vortex for the salt to dissolve. One ml of supernatant was taken into 25 ml volumetric flask and dilute to the level with deionized water. One milliliter (1 ml) of Wade reagent (0.03% solution of FeC1₃.6H₂O containing 0.3% sulfosalicylic acid in water) was added to 3ml of the diluted sample, and the mixture was mixed. Finally, the read absorbance of color reaction in a spectrophotometer at 500 nm wavelength was measured and distilled water was used to zero spectrophotometer.

Preparation of standard solutions: Seven series of standard solutions containing 5 40 mg/ml phytic acid in in 0.2M HCl were prepared. The concentration of standards was (0, 1, 4.5, 9, 18, 27 and 36) ppm. About 3ml of the standard was pipetted into15ml centrifuge tubes, plus on blank consisting of 3ml of distilled water. Then 1 ml of the wade reagent was added and the solution was mixed on a vortex mixer for 5sec. The mixture was centrifuged at 3000 rpm for 10 minutes and supernatant read at 500nm using UV spectrometer was read by using distilled water as a blank.

Phytate content (mg/100g) =
$$(As - Ab) - int. \times 10$$

 $Slowary Ward - eqn. (8)$

Where As: absorbance of sample solution, Ab: the blank absorbance, Int.: intercept from the absorbance equation curve, D: density of the solvent and W: weight of the sample in gram

2.6. Determination of staling rate and alkaline water retention capacity

The alkaline water retention capacity (AWRC) and staling rate of samples were determined using eqn. (9) as modified by [20]. A reagent containing 0.1N sodium bicarbonate (NaHCO₃) solution was prepared by dissolving 8.4 g sodium bicarbonate in 1 liter water. The AWRC was determined by weighing 1g (in duplicate) of samples of in 15 ml tubes (W₁), was added, then 5 ml of 0.1 N NaHCO₃ was added, mixed, and left at room temperature for 20 minutes. The slurry was centrifuged for 15 minutes at 3000 rpm, the supernatant was discarded, and the tubes were left to drip upside down for 10 minutes. After that, the dried tubes were weighed (W₂).

eqn. (9)

 $AWRC (\%) = (\underline{W_2 - W_1}) \times 100$ W_1

Where W_1 : weight of tube containing the dry sample; W_2 : weight of tube containing the dripped sample.

Then staling rate is calculated as follows:

Staling rate (%) =
$$(\underline{AWRC_0 - AWRC_n}) \ge 100$$

AWRC₀ eqn. (10)

Where: AWRC₀: AWRC at zero time, and AWRC_n: AWRC at a specific day of storage.

2.6.1. pH determination

The pH of the samples was measured using a digital pH meter (pH-013 High Accuracy Portable pH Meter). The pH meter was calibrated with standard bufering solutions at pH 4 and 7, and then each injera suspension (a well homogenized mixture of 10 g of ground injera with 100 ml distilled water) was measured [21].

2.7. Sensory attributes

In order to optimize the maximum acceptable limit of sorghum- teff- fenugreek substitution level in the injera making process, a preliminary sensory acceptability test of injera was conducted. The sensory evaluation of injera samples was carried out by 32 semi-trained panelists composed of males and females between the age of 24 and 35 who worked at Melkassa Agricultural Research Center. The samples were tested following [22] procedures for softness, stickiness, rollability, sourness, bitterness, color, odour, flavor, injera eyes, and overall sensory acceptability parameters using a seven-point hedonic scale with the crateria: 1=dislike extremely, 2=dislike moderately, 3=dislike slightly, 4=neither like nor a dislike, 5=like slightly, 6=like moderately, and 7=like extremely.

2.8. Statistical data analysis

Quantitative data analysis was carried out using

statistical software package, version 23.0 and design expert software version 13.0.5.0. A duplicate data obtained was subjected to a design expert one-way statistical analysis of variance (ANOVA) to test the statistical difference among the baked injera and the significance level was set at p < 0.05.

3. Results and discussion

3.1. Proximate composition of the developed injera

Moisture, total ash, crude fat, crude fiber, crude protein, carbohydrate content and energy contents of all formulated injera are presented in Table 2. According to the result, the highest moisture content (69.92%) was observed on injera developed from teff (50%), Sorghum (45%) and Fenugreek (5%) composite flours while the lowest one (60.04%) was seen on injera developed from Teff flour (100%) [control]. This explicitly showed that addition of sorghum and fenugreek flours have tendency to increase the moisture content of injera which then enhanced its softness. On average wise, the mean moisture content of the developed injera was found to be 63.73%.

 Table 2. Proximate composition of Injera based on composite flour formulations (%)

Samples	Moisture	Ash	Fat	Fiber	Protein	. CHO	Energy (kcal)
Control	60.04 ± 0.17^{h}	3.14±0. 01 ^b	2.50 ± 0.00^{h}	4.18 ± 0.00^{f}	11.36±0.01 ^g	77.71±0.01ª	362.05 ± 0.00^{f}
T_2	66.44±0.11 ^{cd}	3.06±0.01°	10.34±0.00 ^b	4.30±0.00 ^{ef}	14.73±0.04°	62.72±0.00 ⁱ	385.62±0.49 ^a
T ₃	62.19 ± 0.12^{f}	2.92±0.01 ^e	2.58 ± 0.00^{h}	5.38±0.03 ^{bcd}	11.71±0.01 ^f	76.91±0.00 ^b	356.14±01. ^g
T_4	61.75±0.35 ^{fg}	3.02±0.01 ^{cd}	3.24±0.03 ^g	4.64±0.01 ^{cdef}	12.67±0.01e	74.84±0.05 ^d	360.64 ± 0.22^{f}
T ₅	65.99±0.01 ^d	2.97±0.01 ^{de}	8.66 ± 0.00^{d}	4.71±0.07 ^{cdef}	14.71±0.01°	65.01±0.01 ^g	378.00±0.03 ^{cd}
T_6	63.79±0.01e	2.78 ± 0.01^{f}	3.52 ± 0.00^{f}	5.54±1.41 ^{def}	14.04±0.003 ^d	73.07±001e	357.96 ± 5.71^{f}
T ₇	56.16 ± 0.68^{i}	3.91±0. 01 ^a	7.00±0.03 ^e	5.16±0.03 ^{bcde}	14.80±0.42°	66.29±0.30 ^f	366.73±2.02 ^e
T8	67.79±0.34 ^b	2.76±0.01 ^f	8.91±0.00°	5.50±0.00 ^{bc}	15.91±0.01 ^b	63.56±0.06 ^h	376.06±0.18 ^d
T9	69.92±0.03 ^a	2.64±0.01g	10.84±0.00 ^a	6.00±0.12 ^b	17.20±0.00 ^a	59.54±0.01 ^k	380.79±0.45 ^{bc}
T10	63.21±0.30 ^e	2.68±0.01 ^g	2.26 ± 0.00^{i}	6.99±0.04 ^a	14.08±0.01 ^d	74.99±0.03 ^d	348.68±0.15 ^h
Mean	63.73	2.99	5.99	5.24	14.12	69.46	367.28

N.B.: Values are mean \pm standard deviation in duplicate run. Values followed by different letters within a column indicate significant difference (p < 0.05); CHO: Carbohydrate content

This value is in line with previous research findings [23, 24] which reported that injera made from various cereals has a moisture content of 59.34-66.97%. On the other hand, the ash content which is the measure of mineral was higher (3.91%) in injera developed based on teff (73%), sorghum (24%), and fenugreek (3%) composite flours and the lost ash content (2.64%) was found in injera made based on teff (50%), sorghum (45%) and fenugreek (5%) composite flours as observed on Table 2. Here as observed from the Table, injera developed from teff only showed relatively has higher ash content than injera developed from the composite flours except for sample T7. The ash content was somewhat influenced by the ratio of teff, sorghum, fenugreek which was supported by previous reports [25, 26] where the moisture content was a little be raised up to 3.38% when the amount of fenugreek higher whereas the amount changed to 2.29% when the amount of sorghum fluctuates. Showing that finding an optimum combination of these cereals is crucial to obtain a reasonably palatable injera. Furthermore, the fat and protein content of the developed injera were evaluated and found that the maximum content [fat (10.84%), protein (17.20%)] was obtained at the combinations of teff (50%), sorghum (45%) and fenugreek (5%) composite flours, while the lowest fat content was observed in samples (control), T3, and T10 combinations which showed that a significant difference among each sample at P<0.05 was seen. Similarly, the lowest amount of protein content was observed in samples (control) and T3 [teff:75%, sorghum:25% and fenugreek:0%] and a significant difference was observed among the samples at P<0.05. Interestingly, addition of sorghum and fenugreek flours significantly improved the fat and protein content of injera regardless of the ratio of these two cereals flour added to the teff flour as clearly observed in Table 2. This results exactly agreed with pervious report [27] which claimed that injera containing fenugreek flour resulted in a higher crude fat and protein content than injera teff flour based alone. Moreover, the fiber content of the developed injera was highly influenced by the addition of sorghum and fenugreek flours as observed in the Table. Consequently, all injera based on the composite [teff, flours sorghum, fenugreek] possessed relatively higher amount of fiber content than teff flour only. The highest fiber content was observed on injera developed from 50% (teff) and 50% (sorghum) blended flours. This showed that the fiber content of these sample injera was drastically

influenced by sorghum instead of fenugreek flour due to the fibrous nature of sorghum flour [26]. The carbohydrate (CHO)content of the developed injera based on composite flours had relatively lower than teff flour alone injera (77.71%).



Figure 1. depicts comparison of the current research findings against literature data (data was Calculated from literature papers: green color [8, 24])





The lowest CHO content of composite flours based injera was observed in sample T9 (59.54%). In contrast to CHO content of injera based on various cereals flour reported by [4, 23] which was in the range of (73.89-86.8%). Such kinds of variations potentially was observed due to the short or long fermentation time of the dough [8]. Similarly, the energy content of the developed injera based on the composite flours were calculated and found that a 385.62% (control) recorded as the highest energy lot compared to injera on other blends and teff flour based alone. This result was a better track record compared to energy for injera made from [teff, sorghum, faba bean] reported by [8, 24] which was in the range of 60.74 to 75.62%.

To further assess the impacts of sorghum and fenugreek addition to teff flour on the quality and nutritional change of injera, the current research findings were thoroughly compared with other researchers results. Figure 1 showed how average nutritional value of Injera developed from the composite flours enhanced compared to sole teff injera and compared this output with literature data.

The fiber, fat and protein contents of injera developed from the addition of sorghum and fenugreek to teff flour were positively enhanced compared to Injera based on teff alone. Similarly, except the protein content, the ash, fiber, and fat contents of injera based on sorghum and fenugreek added teff flour and teff flour alone were significantly higher compared to injera based of sorghum and faba bean added teff flour as shown in Figure 1. In the meantime, the moisture and energy content of injera developed from the blends [teff, sorghum, fenugreek] were shown a positive change compared to injera based on teff alone (including the literature data) as observed in Figure 2. However, the CHO content of injera developed from sorghum and fenugreek flour added to teff flour as well as injera developed from the blend of teff, sorghum and faba bean was a little be lowered. This was probably associated with the effects of interactions of fiber, protein and fat which potentially promote a stable CHO monomers [28, 29]. This of course important for health due to lowering and stabilizing glucose level during metabolic process.

3.2. Mineral profiles, concentrated tannin and phytic acid of the developed injera

The mineral contents [Fe, Zn, Ca] of injera developed from the sorghum and fenugreek added teff flours was not improved compared to teff flour alone based injera as shown in the Table 3. However, improvements on Iron (21.21 mg) and Calcium (151.13 mg) contents were observed on sample T2 [95% teff flour: 5% fenugreek flour]. According to the report of [30, 31] teff flour has 6.3 mg/25.13 mg Fe and 104 mg Ca whereas [7] reported that injera developed on sole teff flour based was 19.18 mg Fe, 14.66 Zn and 136 mg Ca on a 72 hrs. Fermentation time. Interestingly, injera baked involving sorghum flour was potentially decreased Fe, Zn, and Ca contents; in contrast, a 5% fenugreek flour addition showed a significant change at P<0.05 which probably imparts the flour due to having higher Fe (33.5) than sorghum (4.1 mg) [31, 32].

Samples	Iron [Fe]	Zinc [Zn]	Calcium [Ca]	Condensed Tannin	Phytic Acid
Control	20.13±0.014 ^a	2.36±0.014 ^b	146.88+0.000 ^c	0.89±0.565ª	132.93±0.014 ^b
T ₂	21.21±0.028 ^d	2.31±0.014 ^e	151.13 ± 0.028^{f}	4.11±0.030°	174.84 ± 0.042^{d}
T ₃	16.48±0.000g	1.86±0.014°	111.37 ± 0.000^{f}	0.67 ± 0.000^{a}	102.64±0.000 ⁱ
T_4	18.59±0.000 ^d	2.13±0.028 ^b	130.65±0.014 ^d	1.23±0.001e	126.36 ± 0.028^{f}
T5	19.24±0.014 ^c	2.10±0.014 ^b	133.16±0.028°	2.76±0.001 ^b	152.03±0.000 ^b
T6	14.94 ± 0.000^{h}	1.59 ± 0.000^{d}	95.13±0.014 ^h	1.01 ± 0.000^{ef}	96.82±0.000 ^k
T ₇	17.47±0.028e	1.89±0.000°	116.74±0.028e	2.02±0.001 ^d	130.59±0.000e
T ₈	16.34±0.014 ^g	1.63 ± 0.000^{d}	101.93±0.000 ^g	2.33±0.001°	126.14±0.028 ^g
T ₉	14.63 ± 0.014^{i}	1.33±0.028 ^e	87.11 ± 0.000^{i}	2.71±0.000b	121.56±0.014 ^h
T ₁₀	12.84±0.014 ^j	1.19 ± 0.000^{f}	75.28±0.000 ^j	0.45 ± 0.001^{g}	72.83 ± 0.028^{1}
Mean	16.86	1.78	111.39	1.92	122.65

Table 3. Calculated mineral profiles, Tannin and Phytic Acid of the developed Injera [mg/100g]

NB.: Values are mean \pm standard deviation in duplicate run. Values followed by different letters within a column and row indicate significant difference at p < 0.05

On the other hand, the presence or absence of concentrated tannin in teff flour depends on the type of grain teff. According to the report of [13] concentrated tanning was not detected or insignificant [0.012 mg] in the white teff flour which was supported by the finding of [7] that reported the concentrated tannin in white flour was

about 0.008 mg which is insignificant. Nevertheless, in this study the type of teff sample used was red teff flour and found that the concentrated tannin content was about 0.89 mg as observed in Table 2. This value is higher than the value reported on redteff flour concentrated tannin [0.111 mg] by [33]; this variation may be raised due to method of calculation employed and environment conditions different where the samples obtained. The tannin amount found in almost all Injera developed from sorghum and fenugreek added teff flour was significant at P < 0.05 among the developed injera. The amount ranged from 0.45 to 4.11 mg where the highest tannin amount found in the blending of Teff flour (95%) and Fenugreek flour (5%) while the lowest was found in teff (50%) and Sorghum (50%). It was clearly observed that the tannin amount entirely depended on the amount of fenugreek flour added to teff flour because of tannin concentration in fenugreek flour is higher [9.88 mg] than teff as reported by [13]. In supporting of this finding, injera developed from a 4% fenugreek flour addition to teff flour was increased the tannin amount from 0.012 to 3.86 mg and even increased to 7.41 mg as addition of fenugreek flour raised to 16% [13]. Similarly, the phytic acid content of the developed injera was calculated and found that the phytic amount ranged 72.83 to 174.84 mg. The highest phytic acid amount was found in Injera developed from the blend of teff flour (95%) and fenugreek flour (5%) while the lowest amount recorded in injera developed from the blend of teff (50%) and sorghum (50%) which are significant at P< 0.05 among the developed injera. The phytic acid amount in injera made from the sole red-teff flour was 132.93 mg which is higher than 30 mg that reported by [33] and much higher than white teff injera (1mg) as reported by [13] but significantly smaller than value (234.46 mg) reported by [7]. In any cases, both condensed tannin and phytic acid amount significantly increased as the addition ratio of fenugreek flour increased. In contrast, addition of sorghum in teff flour for injera baking, absolutely decreased both condensed tannin and phytic acid concentrations. That is why the minerals content of the developed injera based on teff, sorghum and fenugreek flours were diminished in almost all baked injera, Table 3. Consequently, tannin and phytic acid able to bind to minerals such as iron, calcium and zink and make them unavailable for bio-absorption during metabolic process of energy harvesting [34].

3.3. Alkaline water retention capacity and staling rate of the formulated injera

The alkaline water retention capacity (AWRC) and staling rate (SR) of the developed injera based on the composite flours have been summarized on Table 3.

The alkaline water retention capacity (AWRC) which measures the amount of alkaline water held by the baked products [35] and the staling rate (SR) that determines the products

softening and loss of the characteristics fresh flavor [36] are crucial quality parameters. In the current study, the highest AWRC of the developed Injera was 84.01% in the blend of teff flour (50%), sorghum flour (45%), and fenugreek flour (5%) while the lowest AWRC was 49.42% in the blend of teff flour (50%) and sorghum flour (50%) at initial time (0 hr.) as observed on Table 4. However, injera developed from sole teff flour (100%) has the lowest AWRC (52.09%) even compared with the mean value (65.29%) of composite flours based injera. Implied that addition of sorghum flour and fenugreek flour to teff flour significantly enhanced the AWRC of the developed injera and a significance difference in AWRC was seen among the baked injera at P<0.05. In general, as a storage time of the baked injera goes from 0hr (84.01%), 24hr (76.49%), 48hr (76.01%), to 72 hr (73.66%)., the AWRC of injera somewhat decreased but not significance at P<0.05. This AWRC highlights the hydration capacity of injera could be improved by adding a proper ratio of sorghum and fenugreek flours to teff flour because of hydration capacity is correlated with AWRC as [37] reported in the study of relationship between AWRC and some quality parameters of chickpea. In addition, the fibrous nature as well as the interaction between fiber and starch of the developed injera promote AWRC property by strengthening water binding capacity which prevents water loss during storage [38].

Similarly, the staling rate of the formulated injera was evaluated and found that a significance change was not observed among the formulated injera and the sole teff flour based injera (within the column) at P<0.05 after a 24-hr. storage. Nevertheless, a staling rate of the formulated injera was significantly changed among the developed injera after 48-hr and 72-hrs. storage at P<0.05. Even a significance change has been observed within the group (within the row) of the developed injera and the sole teff flour based injera through 24, 48 and 72-hrs. storage at P<0.05. The softness and freshness of injera based on sole teff flour alone was much compromised due to the staling rate increased from 23.54 % at 24-hr to 40.35% at 72-hr. Injera developed by adding sorghum and fenugreek flours to teff flour was compromised its softness and freshness in a delayed manner except injera from

samples T3 (from 23.55 % to 41.92%), T4 (from 21.33% to 40.87%), and T6 (from 23.725 to 41.37%) after 24 and 72 -hrs.as observed on Table 4. However, a higher compromise in softness and freshness of Injera developed from the composite flours were observed in the blend of teff flour (50%) and sorghum flour (50%) or sample T10 due to the staling rate drastically increased from 33.95% to 52.31% through 24 to 72-hrs. of storage. Interestingly, the staling rate was low in injera developed from the blend of teff flour (95%) and fenugreek flour (5%)-T2, and the blend of teff flour (50%), sorghum flour (45%), and fenugreek flour

(5%)-T9 with the value of 17.93% and 12.32% after 72-hrs. storage, respectively. This is because injera developed from the formulated sample T2 and sample T9 has higher fat and protein contents [Table 2] which was agreed with the report of [39] that claimed fat and protein contributed to a reduction in the staling rate. Consequently, addition a proper ratio of sorghum and fenugreek flours to teff flour for injera baking potentially favor injera to have more AWRC which promoted injera to pursue good hydration property whereas it prevented injera from crust hardening, loss of softness and freshness by reducing the staling rate.

 Table 4. Effect of ingredients formulation on alkaline water retention capacity and staling rate of the developed Injera at different times

The Jack Transformed Action of the Contract of							
Samples	Alka	line Water Ret	ention Capacit	Staling Rate [Hr.]			
-	0	24	48	72	24	48	72
Control	52.09±0.21 ^b	39.83±0.13 ^b	38.93±0.31 ^b	31.07±0.12 ^b	23.54±0.15 ^b	25.26±0.25 ^j	40.35±0.18e
T_2	78.68±0.21 ⁱ	71.11±0.23 ^h	68.25±0.11 ⁱ	64.57±0.12 ^f	9.62±0.22 ^b	13.26±0.12 ^d	17.93±0.21 ^h
T ₃	53.75±0.14 ^h	41.09±0.09 ^g	40.65±0.21 ^h	31.22±0.16 ^f	23.55±0.12 ^b	24.37±0.18e	41.92±0.30°
T_4	58.28±0.06 ^g	41.09±0.15 ^g	43.22±0.23 ^f	34.46±0.21 ^{ef}	21.33±0.11 ^b	25.84±0.15°	40.87±0.13 ^e
T ₅	67.08±0.44 ^d	53.44±0.23 ^d	52.61±0.41 ^d	45.7±0.28 ^{cd}	20.33±0.32 ^b	21.57±0.39 ⁱ	31.87±0.36 ^g
T ₆	60.87 ± 0.16^{f}	46.43±0.24 ^f	46.28±0.51 ^f	35.69±0.41 ^{ef}	23.72±0.21 ^b	23.97±0.32 ^f	41.37±0.27°
T ₇	62.62±0.33 ^e	48.43±0.29 ^e	47.99±0.35 ^e	39.41±0.1 ^{de}	22.66±0.31b	23.36±0.34 ^g	37.06 ± 0.25^{f}
T_8	72.93±0.31°	55.88±0.24 ^c	56.93±0.34°	49.86±0.18°	23.39±0.22 ^b	21.94±0.33 ^h	31.63±0.24 ^g
T 9	84.01±0.42 ^a	76.49±0.36 ^a	76.01±0.28 ^a	73.66±0.27 ^a	8.95±0.38 ^b	9.52 ± 0.35^{1}	12.32±0.34 ^g
T ₁₀	49.42±0.16 ^j	32.92 ± 0.08^{j}	32.64 ± 0.15^{j}	23.57±0.09 ^{gh}	33.39±0.12 ^b	33.95±0.17 ^b	52.31±0.13 ^a
Mean	65.29	51.88	51.62	44.24	20.77	21.98	34.14

NB.: Values are mean \pm standard deviation in duplicate run. Values followed by different letters within a column and row indicate significant difference at p < 0.05

3.4. Effect of blending ratios on the sensory quality of formulated injera

The color of the baked Injera from the composite flours was ranged from 5 to 6.14 while injera baked from sole teff flour was 5.84 as observed on Table 5 (A). The highest preferred color of injera was scored for T8 (6.0), T9 and T10 (6.14). Across the experimental samples, the panelists were chosen considerably various formulated Injera at P< 0.05. In terms of color attractiveness, injera baked on the blend of teff flour (50%) and sorghum flour (50%) was placed top in the color approval process. As observed on Figure 3 and Table 5 (A), the color intensity of the baked injera was decreased as the ratio of fenugreek flour substitution increased. This color variations of the baked injera with the addition of fenugreek seed flour was consistent with the report of [27]. The overall mean color intensity of the formulated injera was recorded as 6.25. Similarly, the eye distribution [small pore] on all formulated injera was ranged from 5.57 to 6.43 whereas the sole teff flour Injera was 6.14. This small pore or the eye distribution on injeras is indicated the perfect fermentation of the dough which as a result much of carbon dioxide was purged. This determined eye distribution on injera and became attractive for consumption. In this regard, the perfect eye distribution was observed on injera developed from the formulated samples T7 (6.43), T8 (6.43), and T9 (6.29) while the lowered one was T4 (5.57) as chosen by the panelists. These values are almost relatively higher than with their mean value. According to the findings, the eye distribution on the some formulated injera and sole teff flour injera have shown almost similar scored on hedonic rating system. Apparently, the inclusion of fenugreek flour reduced the injera's eye appearance when compared to injera prepared form sole teff flour as pinpointed by the panelists due to the antimicrobial nature of fenugreek seed [40] which potentially affect fermentation process and so does on the release of CO₂.

The ANOVA odor analysis of all formulated injera have a significance impact on the formulation samples at P < 0.05. Injera baked based on samples T7 and T8 where their blends were 73 % teff flour, 24 % sorghum flour, and 3 % fenugreek flour, and 62 % teff flour, 34 % sorghum flour, and 4 % fenugreek flour were preferred by the panelists.



Figure 3. Injera made from the formulated ratios of teff, sorghum and fenugreek flours

Furthermore, the formulated injera's softness and its interaction impact were not substantially different at P < 0.05. According to the panelists, the baked injera rollability was ranged from 5.93-6.43. Injera baked from sample T5 (84% teff, 12% sorghum, and 4% fenugreek) have the lowest softness while the best was obtained at the blend of 75% teff and 25 % sorghum. It was found that injera based on sole teff flour was relatively softer (6.19) than the mean value (5.96) of the formulated injera which agreed with the report of [12]. The stickiness property of the sole teff flour Injera (5.88) and the mean value (5.75) of the formulated Injera was found to be similar and insignificance at P < 0.05. The rollability behavior of the developed injera was almost shown similar rollability except injera incorporated 5% fenugreek flour (5.93) but the change was insignificance at P<0.05.

Table 5 (A). Sensory acceptability test result of the mean formulated fresh injera using 7- point nedolic sc

Samples	Color	Eye distribution	Odor	Softness	Stickiness	Rollability
Control	5.84±0.04 ^b	6.14±0.17 ^{ab}	5.71±0.01 ^{bc}	6.19±0.26 ^{abc}	5.88±0.0 ^{ab}	6.24±0.33 ^{ab}
T2	5.00±0.28 ^{cd}	5.86±0.09 ^{bc}	5.71±0.85 ^{bc}	6.14±0.11 ^{abc}	5.64±0.24 ^{abc}	5.93±0.04 ^b
T3	5.86±0.03 ^b	5.86±0.03 ^{bc}	5.57±0.14°	6.34±0.11 ^{abc}	5.86±0.07 ^{abc}	6.29±0.11 ^{ab}
T4	5.00±0.00 ^{cd}	5.64±0.13°	5.57±0.14°	5.93±0.03abc	5.86±0.16 ^{abc}	6.29±0.07 ^{ab}
T5	5.29±0.13°	6.29±0.10 ^a	6.29±0.13 ^{ab}	5.14±0.17 ^d	5.14±0.18°	6.00±0.28 ^b
T6	5.14±0.04 ^{cd}	5.57±0.06°	5.86±0.09 ^{bc}	6.14±0.2 ^{abc}	5.71±0.98 ^{abc}	6.00±0.14 ^{ab}
T7	5.86±0.09 ^b	6.43±0.11 ^a	6.57±0.03 ^a	5.86±0.06 ^{bc}	6.00±0.14 ^{ab}	6.43±0.03 ^{ab}
T8	6.00±0.14 ^{ab}	6.43±0.03 ^a	6.57±0.14 ^a	6.43±0.04 ^{ab}	6.29±0.0 ^{ab}	6.29±0.16 ^{ab}
T9	6.14 ±0.1 ^{ab}	6.29±0.13 ^a	5.71±0.16 ^{bc}	5.86±0.1 ^{bc}	6.29±0.28 ^{ab}	6.43±0.04 ^{ab}
T10	6.14±0.09 ^{ab}	5.81±0.0 ^{bc}	6.10±0.0 ^{abc}	5.79±0.14 ^{bc}	6.00±0.28 ^{ab}	6.27±0.1 ^{ab}
Mean	6.25	6.02	5.99	5.96	5.75	6.21

Note: Values are mean \pm standard deviation in duplicate run. Values followed by different letters within a column indicate significant difference (p < 0.05).

Table 5 (B). Sensory acceptability test result of the mean formulated fresh in	jera using 7	- point hedonic scale
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Samples	Flavor	Sourness	Bitterness	pН	Over all acceptability
Control	5.71±0.028 ^{def}	6.37±0.099ª	5.86±0.085 ^{ab}	3.82±0.001 ^{cd}	5.99±0. 047 ^{ab}
T_2	6.64±0.184 ^a	5.57±0.156 ^{bc}	6.29±0.41 ^a	3.83±0.014 ^{cd}	5.86±0. 219 ^{ab}
T ₃	5.81±0.127 ^{cde}	5.71±0.127 ^{bc}	5.87±0.028 ^{ab}	3.98±0.014 ^b	5.91±0. 083 ^{ab}
T_4	5.43 ± 0.028^{f}	5.57±0.141 ^{bc}	5.71±0.268 ^{ab}	4.08±0.014 ^a	5.67±0.024 ^{ab}
T ₅	6.07±0.099 ^{bc}	5.64±0.566 ^{bc}	5.29±0.569 ^b	4.11±0.000 ^a	5.68±0. 778 ^{ab}
T ₆	$5.57 \pm 0.0.014^{ef}$	5.57±0.156 ^{bc}	5.93±0.042 ^{ab}	3.98±0.003 ^b	5.72±0. 016 ^{ab}
T ₇	6.71±0.042 ^a	6.00±0.141 ^{ab}	6.00 ± 0.848^{ab}	3.86±0.014°	6.21±0. 125 ^a
T ₈	6.14±0.084 ^{bc}	6.00±0.424 ^{ab}	5.86±0.071 ^{ab}	3.67±0.000e	6.22±0. 089 ^a
T ₉	5.86±0.141 ^{cde}	5.00±0.141 ^d	5.43±0.042 ^b	3.75±0.000 ^{de}	5.89±0. 026 ^{ab}
T ₁₀	5.91±0.000 ^{cde}	5.40±0.283 ^{cd}	5.24±0.283 ^b	3.80±0.014 ^{cd}	5.85±0. 447 ^{ab}
Mean	6.02	5.61	5.74	3.89	5.89

Note: Values are mean \pm standard deviation in duplicate run. Values followed by different letters within a column indicate significant difference (p < 0.05).

Regardless of the ratio [teff, sorghum, fenugreek], the flavor property of almost all injera formulated from the composite flours have higher flavor than the Injera based on sole teff aflour alone. Causing the ANOVA analysis on the interaction between flavor acceptance and injera mixing ratio was substantially different at P < 0.05. This showed that blending could improve not only the nutritional content but also the flavor at a complete fermentation time. In contrast, the sourness property of the control based injera has shown higher sourness (6.37) taste compared to the composite flour based injera (higher value: 6.0) even with their mean value (5.61) as shown on Table 5 (B). This explained that sole teff flour dough was fermented quickly before 72-hrs. and further fermentation has encouraged the production of more weak acids such as lactic acid which made the injera sourer. Whereas in the composite flours, due to the antimicrobial and antinutritive nature of fenugreek seed flour it could possible slow the dough fermentation process and hence the chance of getting more lactic in the formulated injera was lower [41, 42]. Besides, such property could lead injera to have additional taste such as bitterness and hence the acceptance of the taste of the formulated injera was reduced as the addition ratio of fenugreek substitution increased.

Similarly, the staling rate of the developed injera based on the composite flours showed lower rate compared to the teff flour alone injera across the storage time. Furthermore, the sensory analysis showed that injera developed from the composite flours have a better color, eye distribution, odor, flavor and overall acceptability than sole teff flour injera as indicated by the panelists.

In general, regardless of the blending ratios of teff, sorghum and fenugreek flours, a drastic improvement in nutrition content and overall acceptability of injera was observed. Nevertheless, the best combinations from the ten formulated samples were injera baked from the blend of teff flour (50%), sorghum flour (45%), and fenugreek flour (5%) -T9 from the standpoint of nutrition, hydration, softness, freshness and overall sensory acceptability.

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Compliance with Ethics Requirement: Authors declare

Authors declare that they have no conflict of interest.

Compliance with Ethics Requirements. Authors

requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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