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The Effect of Blending Ratio of Tef [*Eragrostis Tef (Zucc) Trotter*], Sorghum (*Sorghum bicolor* (L.) Moench) and Faba Bean (*Vicia faba*) and Fermentation Time on Chemical Composition of Injera

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Abstract

Background: This study was conducted to determine the effect of tef [*Eragrostis tef (Zucc) Trotter*], sorghum (*Sorghum bicolor* (L.) Moench) and faba bean (*Vicia faba*) blending ratio and fermentation time on chemical composition of Injera.

Methods: The effect of two factors, blending ratio (tef, sorghum and faba bean) and fermentation time at 24 h, 48 h and 72 h were studied. The experiment was conducted using custom design.

Results: Both the linear terms and the interaction terms of tef, sorghum and faba bean had significant ($P<0.05$) effect on the proximate composition of the blend Injera. The mixture of faba bean and sorghum with tef increased the ash, carbohydrate, energy content, fiber, moisture and protein content of the blend injera. The result shows the increased protein content which less in amount in tef Injera was because of faba bean and sorghum which contain more protein as compared to tef. Significant ($P<0.05$) increased in moisture and protein content was observed on long fermentation time (72 h). However ash, fat, fiber, carbohydrate and energy were shown on short fermentation time. Thus, short fermentation time gives more caloric food than long fermentation time. On the two dimensional mixtures contour plot the optimum value (maximum amount) of protein was observed when 55% tef, 30% sorghum and 15% faba bean were blended and fermented at 72 h. on carbohydrate the maximum amount or optimum was shown when 65% tef, 30% sorghum and 5% faba bean were mixed and fermented for 24 h. Also on the energy content the optimum value or maximum amount was shown when 70% tef, 20% sorghum and 10% faba bean were mixed and fermented at 24 h.

Conclusion: The result showed that good quality Injera in nutritional composition can be obtained by the blend of 55% tef, 30% sorghum and 15% faba bean at 72 h fermentation time. The optimum value of all chemical composition was obtained when more sorghum (30%) and faba bean (15%) were mixed in to 55% tef flour and fermented at 72 h.

Keywords: Blending ratio; Fermentation time; Injera; proximate composition; Tef

Abbreviations:

AACC: American Association of Cereal Chemists; ANOVA: Analysis of Variance; AOAC: Association of Official Analytical Chemists; CHO: Carbohydrate; DMRT: Duncan's Multiple Range Test; FAO: Food and Agriculture Organization of the United Nations; FT: Fermentation Time; HARC: Holeta Agricultural Research Center; HURC: Haramaya University Research Center; MC: Moisture Content; NRC: National Research Council of USA; USA: United States of America

Introduction

Grain tef was reported to have proximate composition of 11% protein, 73% carbohydrate, 3% crude fiber, 2.5% fat and 2.8% ash [1]. It was also reported to have a relatively higher iron content than other common cereals [2] this was due to agronomic practices used in Ethiopia and fermentation during Injera making [3]. The grain is mainly used for making popular pancake-like local bread called Injera; sometimes also used as porridge and an ingredient of home-brewed alcoholic drinks.

Tef has been reported to be used in mixtures with soybean, chickpea and other grains because of its higher mineral content [2]. It is gluten-free, and is gaining popularity in the whole food and health food industry in Netherlands as an alternative grain for persons with gluten sensitivity [4].

Grain sorghum was reported to have proximate composition of 74.7% carbohydrates, 1.8% ashes, 12.3% protein, 4.2% fat and 1.7% fiber [5]. Sorghum is used as the second most preferred cereal for Injera preparation in Ethiopia [6]. This is because sorghum injera shows brittleness and dryness after storage [7]. It is also used to produce many African and Asian traditional foods like, roti, chapatti, tuwo, tortillas and porridges [8].

Faba bean was reported to have carbohydrate (50-60%), protein (25-35%) and lipid (1-2.5%) (With oleic and linoleic acid representing

75% of fats) [9]. Also Muehlbauer and Abebe [10] reported that the whole dried seeds contain (per 100 g) 344 calories, 10.1% moisture, 1.3 g fat, 59.4 g total carbohydrate, 6.8 g fibre, 3.0 g ash, 104 mg Ca, 301 mg P and 6.7 mg Fe. The bean is a common breakfast meal in the Mediterranean region, Central and East Asia, Ethiopia, America and Oceania [11]. It is used to make stew (shiro wot in the Ethiopian national language, Amharic) mixed with other pulses like pigeon pea and chickpea. It is served with Injera [12].

To get sufficient nutrition, cereals have been found to have nutrient potentials that could complement one another if properly processed and blended with legumes [13]. Fermentation also has contribution to improve the nutritional values of those cereal products. Fermented foods can have the added benefits of enhancing flavour, increased digestibility and improving nutritional value [14]. This is due to growth and action of the bacteria during fermentation [15].

Therefore, the objective of this study is to investigate the effect of faba bean, sorghum and tef grains blending ratio and fermentation time on nutritional composition Injera.

Materials and Methods

Experimental site

The research was conducted at Food Science and Postharvest Technology, and Animal Nutrition Laboratory, Haramaya University, Ethiopia.

Sample collection

The experimental materials such as white tef, faba bean and sorghum were collected from Debre Zeyit Agricultural Research Center (DZARC); Holeta Agricultural Research Center (HARC) and Haramaya University Research Center (HURC) respectively.

Experimental design

Mixture design was used in this study to determine the ratio of the blends cereals (tef and sorghum) with legume faba bean. In this study the effect of three mixtures of products namely tef (T), sorghum (S) and faba bean (F) flour and three fermentation time at 24 h, 48 h and 72 h were studied to determine appropriate formulation. The proportion of tef from 55-70%, sorghum from 20-30% and faba bean from 5-15% were used. The constrained proportion was planned after doing preliminary test. Thus to compare the blend Injera, 100% tef flour was used [12].

Sample preparation

The tef, sorghum, faba bean grains were manually cleaned. Then tef grain was milled by disk attrition mill to whole flour to the fineness level traditionally used for Injera processing at Haramaya University public grain milling house. The sorghum grain was milled after debraning using mortar and pestle. Faba bean was milled after seed coat was removed on a disc attrition mill. The flour was kept in air tight sealed plastic bucket at room temperature [16] for the duration of the analysis. The blend mixture was prepared and Injera was processed.

Dough processing and fermentation

Injera containing tef, sorghum and faba bean was prepared using standard recipes. The process involved dough processing and fermentation and then baking of the batter (thin fermented dough). Prior to fermentation 1 Kg of the blend flour (tef, sorghum and faba bean flour) was mixed with about 2 L water and about 80 mL of ersho (starter saved from preliminary fermentation) and then the dough was kneaded by hand for each treatment/blend. The dough was kept by covering the lid/bowl at room temperature at Food Science and Postharvest Technology (FSTP) Laboratory.

Fermentation

On the fermentation during 24 h notably vigorous gas evolution and maximum dough expansion were observed. During 48 h and 72 h fermentation time an acidic yellowish liquid on dough surface were observed. The layer of the liquid was then removed. For Injera baking from dough fermented for 24 h, 48 h and 72 h, about 10% of fermenting dough was withdrawn for absit preparation. 10% of the fermented dough of the blend flour (for each fermentation time: 24 h, 48 h and 72 h) was taken and boiled at 100°C and then after the absit was cooled at 46 °C mixed back to the fermented dough. After the 2nd fermentation (2 h to 3 h) Injera was baked.

Baking

Injera was baked after fermentation by diluting the batter slightly with water to uniform consistency. Then it was poured using circular motion from the outer perimeter towards the center, onto a hot-round smooth baking griddle called metad. The metad was then covered with a metad lid called akambalo to prevent steam from escaping. Prior to pouring the batter, the metad surface was swapped down by the rapeseed flour using a piece of cloth. This prevents the Injera from sticking to the metad surface. Finally Injera was baked for about 3 mints.

Proximate composition of raw materials and dried Injera products

Moisture, ash, crude protein, crude fat, crude fiber and carbohydrate contents of both the raw materials (tef, sorghum and faba bean flour) and the blend Injera was analyzed with standard methods. Injera was dried for 8-12 h at 70°C in an oven (Electric Heated Blast Dry Box 101-1a, Tianjin city). Then it was ground with mortar and pestle to fine level to pass sieve 750 µm. The dried and milled Injera products were kept in air tight sealed plastic bucket at room temperature [16] for the duration of the analysis.

Statistical analysis

At least a triplicate data were analyzed and modelled using the statistical software JMP™ 8, 2008 (by SAS Institute Inc., Cary, NC, USA). Mixture response surface methodology was applied to the experimental data using JMP version 8. A polynomial equation was fitted to the data to obtain a regression equation. Statistical significance terms in the models were identified. Summary of fit, ANOVA, lack of fit and parameter estimates were generated by the JMP 8. Data was also analyzed by ANOVA and mean comparison was done using Duncan's Multiple Range Test (DMRT) by SAS 9.1.3. Significance was judged if the probability level of the F-statistic calculated from the data was less than 0.05. The model adequacy was checked by R2, R2 adj and lack of fit test. The tertiary contour plots were drawn to develop the optimum blending ratio for the mixture of tef, sorghum and faba bean that are used as an ingredient for Injera making

Results and Discussions

Proximate compositions of the Injera from the blended products

Moisture

The moisture content of the blended Injera had ranged from 5.55-8.25% (Table 1). The highest moisture content was (8.25%) obtained from the blend 55% tef, 30% sorghum and 15% faba bean fermented for 72 h while the lowest moisture content (5.55%) was obtained from the blend 65% tef, 30% sorghum and 5% faba bean fermented for 24 h. This may be due to higher water absorption capacity of faba bean flour. This work is similar to who found that water absorption of the bread was increased as the amount of decorticated cracked faba bean flour increased [17-19]. The moisture content of the blend Injera was also significantly ($P<0.05$) influenced by the linear term of fermentation time. From the blend Injera when fermentation time increased the moisture content was significantly increased [20]. Reported that moisture content of the product increased when fermentation time increased. The following model was developed to predict the moisture content.

Run	T (%)	S (%)	FB (%)	FT (h)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	CHO (%)	Energy kcal/100 g
1	55	30	15	24	7.75 ± 0.01^{d}	2.85 ± 0.01^{a}	17.53 ± 0.02^{c}	2.26 ± 0.03^{h}	2.79 ± 0.04^{a}	74.92 ± 0.05^{b}	366.43 ± 0.31^{e}
2	70	20	10	24	5.89 ± 0.01^{l}	2.24 ± 0.02^{e}	16.67 ± 0.02^{h}	2.89 ± 0.04^{a}	2.65 ± 0.04^{d}	75.66 ± 0.01^{a}	376.33 ± 0.38^{a}
3	65	20	15	24	7.71 ± 0.03^{e}	2.85 ± 0.04^{a}	17.33 ± 0.03^{f}	2.37 ± 0.03^{f}	2.76 ± 0.04^{a}	74.68 ± 0.03^{d}	375.57 ± 0.29^{b}
4	65	30	5	24	5.55 ± 0.01^{m}	1.23 ± 0.01^{f}	14.46 ± 0.01^{i}	2.83 ± 0.03^{b}	1.31 ± 0.01^{j}	67.82 ± 0.04^{j}	352.74 ± 0.16^{i}
5	70	20	10	48	6.14 ± 0.02^{j}	2.24 ± 0.01^{e}	16.75 ± 0.03^{g}	2.83 ± 0.02^{b}	2.63 ± 0.03^{e}	70.43 ± 0.04^{h}	365.19 ± 0.38^{f}
6	65	30	5	48	6.11 ± 0.05^{k}	1.18 ± 0.04^{g}	14.47 ± 0.02^{i}	2.76 ± 0.04^{c}	1.27 ± 0.04^{k}	67.21 ± 0.09^{k}	351.56 ± 0.37^{j}
7	65	20	15	48	8.12 ± 0.02^{b}	2.79 ± 0.03^{c}	17.39 ± 0.01^{e}	2.32 ± 0.01^{d}	2.71 ± 0.02^{b}	74.67 ± 0.05^{d}	362.57 ± 0.48^{g}
8	55	30	15	48	7.89 ± 0.04^{c}	2.81 ± 0.02^{b}	17.63 ± 0.03^{b}	2.18 ± 0.04^{i}	2.71 ± 0.03^{b}	74.78 ± 0.13^{c}	372.26 ± 0.22^{c}
9	65	20	15	72	8.21 ± 0.02^{a}	2.75 ± 0.01^{d}	17.45 ± 0.04^{d}	2.27 ± 0.03^{h}	2.68 ± 0.03^{c}	74.64 ± 0.03^{e}	361.51 ± 0.02^{h}
10	55	30	15	72	8.25 ± 0.02^{a}	2.75 ± 0.02^{d}	17.69 ± 0.03^{a}	2.12 ± 0.02^{k}	2.68 ± 0.05^{c}	74.54 ± 0.04^{f}	371.00 ± 0.19^{d}
11	65	30	5	72	6.25 ± 0.04^{i}	1.14 ± 0.01^{h}	14.47 ± 0.01^{i}	2.71 ± 0.01^{d}	1.25 ± 0.05^{l}	66.91 ± 0.02^{l}	350.91 ± 0.15^{k}
12	70	25	5	72	6.58 ± 0.02^{g}	1.13 ± 0.03^{h}	14.47 ± 0.01^{i}	2.76 ± 0.03^{c}	1.23 ± 0.01^{m}	66.81 ± 0.03^{m}	349.60 ± 0.14^{l}
Cont 1	100	0	0	24	6.54 ± 0.03^{h}	1.08 ± 0.04^{i}	11.08 ± 0.02^{l}	2.53 ± 0.05^{e}	1.45 ± 0.03^{g}	69.60 ± 0.08^{i}	348.07 ± 0.21^{m}
Cont 2	100	0	0	48	6.66 ± 0.03^{f}	1.04 ± 0.09^{j}	12.52 ± 0.02^{k}	2.26 ± 0.01^{g}	1.43 ± 0.01^{h}	63.09 ± 0.03^{n}	343.78 ± 0.18^{n}
Cont 3	100	0	0	72	7.73 ± 0.02^{d}	0.89 ± 0.01^{k}	12.66 ± 0.01^{j}	2.15 ± 0.01^{j}	1.35 ± 0.03^{i}	60.74 ± 0.04^{o}	337.37 ± 0.17^{o}
Mean					7.49 ± 1.81	2.32 ± 0.19	16.12 ± 0.94	2.51 ± 0.42	1.47 ± 0.17	71.78 ± 1.63	362.44 ± 7.66
Range					5.55-8.25	0.89-2.85	11.08-17.69	2.12-2.89	1.23-2.79	60.74-75.66	337.37-376.33

Values are in Mean of a triplicate data ± STDEV. Means (n) within a column with the same letter are not significantly different ($p>0.05$). T: tef; S: Sorghum; FB: Faba Bean; FT: Fermentation Time; CHO: Carbohydrate

Table 1: Proximate composition of the blend Injera.

MC=-0.139T-1.298S+1.648Fb+0.0259(T*S)-0.0164(T*FB) +0.0134FT[x] (13)

Where MC: Moisture content (%) predicted, T is the proportion of tef in percent (%), S is the proportion of sorghum in percent (%), FB is the proportion of Faba bean in percent (%) and FT[x] is fermentation time (x=24 h, 48 h and 72 h).

The predicted formula has the coefficients of the effects, which is estimates of the parameter. By substituting the value of the proportion of tef, sorghum, faba bean and fermentation time to the predicted formula, the predicted moisture content would be estimated. For example, if the proportion of tef (55%), sorghum (30%), faba bean (15%) and fermentation time (72 h) inserted to predicted formula on equation 13, the predicted moisture content (8.28%) with a residual value of -0.0277. However, the predicted value is different based on the substitution of the components proportion and fermentation time.

Ash

The ash content of the blend Injera was in the range of 0.89-2.85% (Table 1). The highest ash content was 2.85% obtained from the blend 55% tef, 30% sorghum and 15% faba bean fermented for 24 h. The least (1.13%) was obtained by blending 70% tef, 25% sorghum and 5% faba bean fermented for 72 h. The ash content of the control samples were 1.08%, 1.04% and 0.89% for 24 h, 48 h, and 72 h fermentation time, which were significantly ($P<0.05$) increased on blending with faba bean and sorghum flour since faba bean and sorghum flour had high ash contents 3.03% and 1.64%. Increased fermentation time somewhat showed decreased in the ash content of the blend Injera in most treatments. This finding was similar with they reported that there is gradual decrease in the ash contents with the fermentation days [21].

All the linear terms of fermentation time showed significant ($P<0.05$) effect on the ash content of the blend Injera. An increased in fermentation time had a significant reduction effect in all treatment. This is probably due to contribution of microorganisms during longer fermentation time. The previous study has reported a significant decrease of ash content after four days of fermentation. The same trend was observed by Gourdouvelis et al. [22] on the effect of fermentation on the nutrient status of locust bean where a decrease of about 30% in ash content was recorded after fermentation. The following model was developed to predict the ash content (Eq. 14).

A=0.0529T+0.3201S-0.133FB-0.0062(T*S) +0.00322(T*FB)-0.00186FT[x] (14)

Where: predicted ash (%), T: The proportion of raw tef flour in percent (%), S: The proportion of raw sorghum flour in percent (%), FB: The proportion of raw faba bean in percent, and FT[x]: Batter fermentation time (x=24 h, 48 h and 72 h).

The coefficients of all effects as shown in the predicted equation (14) are the estimates of the effects, which help to test the fit model and the significance of the effects on the predicted ash content. By using the predicted formula, more predicted ash content (2.84%) with highly reduced residual value of 0.00205 was obtained on the blend Injera made from 55% tef, 30% sorghum and 15% faba bean fermented for 24 h. However, this value is reduced to 2.80% with increased residual value of 0.00667.

Crude protein

The protein content of the blend Injera was significantly ($P<0.05$) influenced by blending ratio as shown (Table 1). This might be attributed due to the addition of different proportion of blending components (tef, sorghum and faba bean). The protein content of the blend Injera was ranged from 11.08% to 17.69%. Blending of 30% sorghum and 15% faba bean flour with 55% tef flour has given significantly higher amount of crude protein (17.69%) than 65% or 70% tef flour. This means, blending of 30% sorghum and 5% faba bean flour to 65% tef flour was likely to give the lowest protein content of 11.08%. This might be due to the presence of more protein in sorghum and faba bean flour compared to tef flour. Due to this, the entire blend Injera had more protein content than the control Injera. And increasing the faba bean and sorghum flour proportion in the blend had significantly ($P<0.05$) increased the protein content of the control Injera. This is similar to finding who reported that when decorticated cracked faba bean flour fortification was increased from 0 to 20%, there was an increase of 36% in protein content [19].

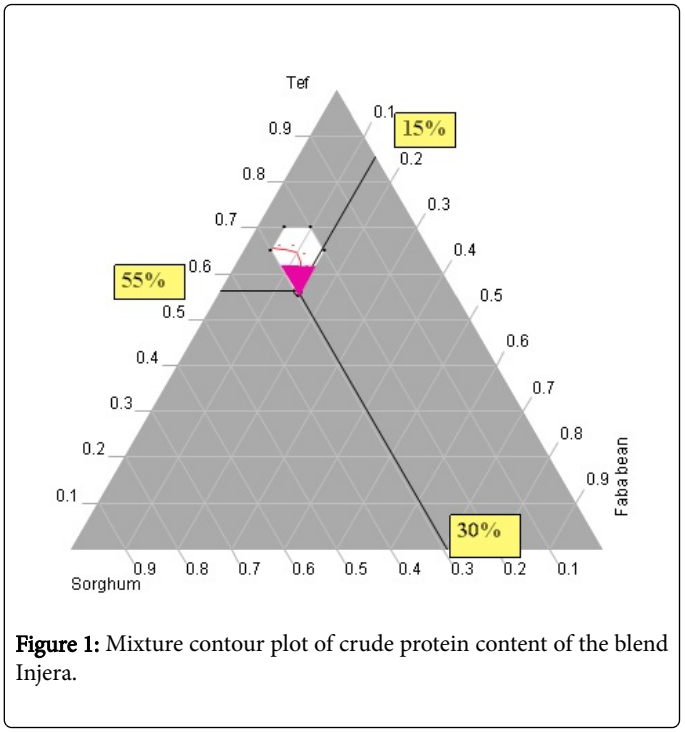

Figure 1: Mixture contour plot of crude protein content of the blend Injera.

Fermentation time significantly ($P<0.05$) affected the protein content of the blend Injera. The highest value (17.69%) of protein was found at 72 h fermentation time while the lowest value (11.08%) of protein was found at 24 h. Significant differences existed among all the blend Injera samples including the control (100% tef Injera) ($P<0.05$). The result obtained is in full agreement with [20] who reported that the protein content of fermented Dabar and Tabat sorghum cultivar was slightly changed when the fermentation time increased. Thus, longer fermentation time has contribution in the enhancement of protein content in the blend Injera. This might be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles.

The following model (Eq. 14) was developed to predict the crude protein content of the blend.

$$P=0.314T+1.323S-0.606Fb-0.024(S*T)+0.0105(Fb*T)+0.00212FT[x] \quad (14)$$

Where: P is predicted crude protein (%), T: The proportion of raw tef flour (%), S: The proportion of raw sorghum flour (%), and FB: The proportion of raw faba bean (%), and FT[x]: The batter fermentation time (x=24 h, 48 h and 72 h).

The addition of more faba bean and sorghum flour to tef flour helps to approximate the predicted value of protein (17.66%) of the blend to the actual value (17.69%) by reducing its residual value. This means the predicted value lied on the fit line when the residual values decrease. On the other way, the addition of less faba bean or sorghum flour in to tef flour increases the residual value, and which indicates that the predicted value would be far from the actual value.

The shaded region shown in Figure 1 was the optimum region for protein. The product was selected to be optimized for the blend 55% tef, 30% sorghum and 15% faba bean because high protein content (16.0–17.69%) was obtained. Increasing the proportion of faba bean and sorghum flour to tef flour had increased the crude protein contents of the product. This shows that supplementation of faba bean and sorghum proteins to the control tef Injera can enrich the protein content to overcome protein energy malnutrition problem.

Crude fat

The crude fat content of the blend Injera had ranged from 2.12-2.89% (Table 1). The highest crude fat content (2.89%) was obtained by blending of 30% sorghum and 10%faba bean in to 70% tef flour and fermented for 72 h while the lowest value (2.12%) was obtained by blending 30%sorghum and 15% faba bean in to 55% tef and fermented for 24 h. Blending ratio had significant ($P<0.05$) effect on the crude fat content of the blend Injera. The addition of the more faba bean and sorghum flour in to less amount of tef flour in this experiment had reduced the fat content of the blend Injera. However, blending slightly higher amount of faba bean and sorghum flour with more tef flour (about 70%) showed increased fat content. This might be due to less crude fat content in faba bean and sorghum flour compared with tef flour. Faba bean flour with a less fat content (2.13%) was reported by Sarah et al. in contrast to sorghum and tef flour.

Significant difference on fermentation time shows that all fermentation time had significantly ($P<0.05$) decreased the crude fat content of the blend Injera. With longer fermentation time the fat contents of the blend were found to be lower. This decrease in fat contents might be attributed to the increased activities of the lipolytic enzymes during fermentation which hydrolyses fat components into fatty acid and glycerol. Similarly Abebe et al. [23] reported that crude protein and ether extract values had increased with increasing their fermentation period. The following model (Eq. 15) was developed to predict crude fat contents.

$$CF=0.0719T+0.3195S-0.332Fb+0.006(T*S)-0.00328(T*FB)+0.0025FT[x] \quad (15)$$

Where: F is the crude fat predicted (%), T is the proportion of raw tef flour (%), S is the proportion of raw sorghum flour (%), and FB is the proportion of raw faba bean flour (%), and FT[x] is batter fermentation time at (x=24 h, 48 h and 72 h).

The addition of 10% faba bean and 20% sorghum and 70% tef proportion in to the formula had given the predicted value 2.93% equal to the actual data (2.89%) with small residual value (-0.007).

Crude fibre

The crude fiber content of the blend Injera was significantly ($P<0.05$) affected by the addition of faba bean and sorghum flour (Table 1). The crude fiber content of the blend product Injera had ranged from 1.23 to 2.79%. The highest value (2.79%) was obtained when 55% tef flour, 30% sorghum and 15% faba bean flour were blended and fermented for 24 h while the lowest value (1.23%) was obtained at 72 h fermentation time where 70% tef flour, 25% sorghum and 5% faba bean were blended. The result revealed that blending ratio had significant ($P<0.05$) effect on the fiber content of blend Injera. Supplementation of the control tef Injera with faba bean and sorghum fiber was significantly ($P<0.05$) increased the fiber content of the blend Injera. This increase in finer content could be attributed to the presence of more fiber content in faba bean and sorghum flour [18]. Reported that faba bean fiber content ranged from 5.0 to 8.5%.

The result also revealed that long fermentation time reduced the fiber content of the blend Injera. The expected decrease in fiber content during fermentation could be attributed to the partial solubilization of cellulose and hemi cellulosic type of material by microbial enzymes [24].

$$CF=-0.1346T+0.809S+0.583Fb-0.016(T*S)+0.0076(T*FB)+0.0019FT[x] \quad (16)$$

Where: CF is crude fiber (%) predicted, T is the proportion raw tef flour, S is the proportion of raw sorghum flour, and FB is the proportion of raw faba bean, and FT[x] is batter fermentation time at (x=24 h, 48 h and 72 h)

The coefficients are estimates of the effects. The formula is useful to predict the response fiber content by substituting the proportion of the components (tef, sorghum and faba bean) and the factor fermentation time. In this finding, the result indicates that addition of more faba bean (15%) and sorghum (30%) flour but less tef (55%) and lower fermentation time (24 h) was found to predict the value (2.76%) which is near to the actual value (2.79%).

Carbohydrate

Blending ratio had a significant effect on the carbohydrate content of the blend Injera ($P<0.05$) (Table 1). The carbohydrate content of the blend Injera was ranged from 60.74 to 75.62%. The highest value of carbohydrate was obtained when 70% tef, 20% sorghum and 10% faba bean were blended and fermented for 24 h while the lowest value (66.81%) of carbohydrate was obtained when 70% tef, 25% sorghum and 5% faba bean were blended and fermented at 72 h. The result revealed that blending of more sorghum and slightly more faba bean in to more tef flour was found to increase the carbohydrate content of the blend Injera. This might be due to that the carbohydrate content (53.06%) of the raw faba bean flour was lower than 72% tef flour sample. Hence, because of blending the carbohydrate content of the control tef Injera was lower than the blend Injera. Significance difference was existed among the fermentation time as shown in the result. The blend Injera fermented for 72 h had a significantly ($P<0.05$) small amount of carbohydrate content (66.81%) than that of 24 h. Similar result also obtained in the control (100% tef) Injera. This decrease in total carbohydrate content might be due to, particularly starch and soluble sugars are principal substances for fermenting microorganisms; therefore degradation and a subsequent decrease in starch content are expected to occur. The following model (Eq. 17) was developed to predict the carbohydrate content.

$$CHO=0.515T-0.096S+2.089Fb +0.015(T*S)-0.0071(Fb*T)-0.016FT[x] \quad (17)$$

Where: CHO is predicted carbohydrate (%), T is the proportion of raw tef flour (%), S is the proportion of raw sorghum flour (%), and FB is the proportion of raw faba bean flour (%), and FT[x] is batter fermentation time at (x=24 h, 48 h and 72 h)

This predicted equation help us to find the predicted value of the carbohydrate. The coefficients are the estimates of the parameters. The predicted value was found by substituting the proportion of the parameters (tef, sorghum and faba bean) and the factor fermentation times (24, 48 and 72). For instance, predicted carbohydrate content (75.356%) was obtained by blending of 20% sorghum and 10% faba bean in to 70% tef flour.

Energy

Blending ratio had a significant ($P<0.05$) effect on the energy content of the blend Injera. The energy content of the blend Injera was ranged from 337.37 to 376.43 kcal/100 g (Table 1). The highest value (376.33 kcal/100 g) was observed when 70% tef, 20% sorghum and 10% faba bean were blended and fermented at 24 h while the lowest value (349.60 kcal/100 g) was observed when 70% tef, 25% sorghum and 5% faba bean flour were blended and fermented at 72 h. The results have shown that the addition of more proportion of sorghum and faba bean flour in to tef flour increased the energy content of the blend Injera since faba bean had more protein content, and tef and sorghum also had more fat content. The result is similar to who reported that fat on its own contains about twice the food energy values of protein and carbohydrate [17]. Significant deference was also observed between the blend Injera and the control Injera. Thus all the energy contents in the blend Injera appeared greater than the energy content of the control sample.

The results also revealed that all fermentation time were found to be significant ($P<0.05$) on the blend Injera. The highest value (376.43 kcal/100 g) was shown at 24 h fermentation time while the lowest value (337.37 kcal/100 g) was found at 72 h fermentation time. This could be due to the decreased fat content because of increased activity of lipolytic enzymes during fermentation which hydrolysis fat components in to fatty acid and glycerol and decreased carbohydrate content because of the degradation and a subsequent decrease in starch content during fermentation by fermenting microorganisms. However, the results of this study regarding the effect of fermentation on energy content are contradictory with the report of Abebe et al. [23] who reported that both protein and ether extract increased when fermentation time was increased and this probably boost the energy content of the blend Injera. The following model (Eq. 18) was developed to predict the energy content.

$$E=5.014T+11.0953Fb-0.159(T*S)-0.012(T*Fb)-0.140FT[x] \quad (18)$$

Where:

E is predicted energy (kcal/100 g), T: The proportion of raw tef flour, S: The proportion of raw sorghum flour, and FB: The proportion of raw faba bean, and FT[x]: Batter fermentation time at (x=24 h, 48 h and 72 h).

The predicted value of the blend Injera made by taking the proportion 70% tef, 20% sorghum and 10% faba bean was obtained from the formula by substituting the proportion and fermentation time. The result shows that the actual energy content obtained from the experiment was approximately equal to the predicted value with a small residual value. This indicates that the data was good enough to describe the model.

Conclusion

The experiment was comprised of three blending components (tef, sorghum and faba bean) at different proportions: 55-70% tef, 20-30% sorghum and 5-15% faba bean and three fermentation time (24 h, 48 h and 72 h) in triplicate. A total of 15 (12+3 control sample) Injera samples were characterized.

Both the linear terms and the interaction terms of tef, sorghum and faba bean had significant ($P<0.05$) effect on the proximate composition of the blend Injera. The mixture of faba bean and sorghum with tef increased the ash, carbohydrate, energy content, fiber, moisture and protein content of the blend Injera. The result shows the increased protein content which less in amount in tef Injera was because of faba bean and sorghum which contain more protein as compared to tef.

Significant ($P<0.05$) increased in moisture and protein content was observed on long fermentation time (72 h). However ash, fat, fiber, carbohydrate and energy were shown on short fermentation time. Thus, short fermentation time gives more caloric food than long fermentation time. On the two dimensional mixtures contour plot the optimum value (maximum amount) of protein was observed when 55% tef, 30% sorghum and 15% faba bean were blended and fermented at 72 h. on carbohydrate the maximum amount or optimum was shown when 65% tef, 30% sorghum and 5% faba bean were mixed and fermented for 24 h. Also on the energy content the optimum value or maximum amount was shown when 70% tef, 20% sorghum and 10% faba bean were mixed and fermented at 24 h.

Normally, high moisture, protein was obtained from the blend Injera made by blending 55% tef, 30% sorghum and 15% faba bean and fermented for 72 h. High fat, carbohydrate and energy content were obtained on the blend Injera made by blending 70% tef, 20% sorghum and 10% faba bean and fermented for 24 h. Generally, the addition of more sorghum and faba bean with long fermentation time is important as compared to the control.

Conflict of Interest

All the authors of this manuscript are instructors of jig-jiga university by receiving a monthly salary of $120 USD, we all have no additional income from anybody or organization else. And we all the authors of the manuscript do not have any share with any organization. I am asking for the patent right for this manuscript that this paper is not anybody else work, it is the work done by Yimer Mihretie, and Geremew Bultosa.

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