ISSN: 2226-7522(Print) and 2305-3327 (Online)
Science, Technology and Arts Research Journal
July-Sep 2013, 2(3): 51-58
www.starjournal.org

Original Research

Effect of Grain Tef (*Eragrostis tef* (Zucc.) Trotter) Flour Substitutions with Flaxseed on Mineral Content, Antioxidant Activity, Phytic Acid Content and Microbial Quality of Injera

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Abstract

There is an increased global interest in the food industry to develop and market functional foods in which scientific investigations are limited in Ethiopia. Tef injera functional character can be further enhanced by substitution with flaxseed which is known to bear functional ingredients (α-linolenic acid an ω-3 fatty acid, secoisolariciresinol diglycoside lignans, dietary fibre and proteins). Two flaxseed forms (whole and flour) and three flaxseed substitution levels (3%, 6% and 9% flaxseed) arranged in a factorial experimental design in three replications were co-fermented to find out whole or flour and at what substitution level injera with better nutrient and functional potential can be processed. Tef injera (100%) was used as a control. Tef injera substituted with flaxseed (whole and flour) at 3%, 6% and 9% showed a significant (P<0.05) effect on minerals (except P), ferric ion reducing antioxidant power (FRAP), phytic acid and microbial quality of injera. With 9% flaxseed substitution FRAP, Zn and Ca contents percentage increase were: 102, 110 and 16; whereas phytic acid and Fe decreased by 76 and 19, respectively from the control. Between 2 to 6 injera storage days, yeast-mould (2.27 to 3.93 log cfug^{-1}) and total aerobic plate counts (ND to 3.77 log cfug^{-1}) were lowest for 9% flaxseed substitution and highest for the control injera (2.85 to 4.08 log cfug^{-1} and 3.70 to 4.30 log cfug^{-1}, respectively). Coliforms were not detected. Whole flaxseed substituted injera had high minerals, antioxidant and microbial stability than flour flaxseed substituted injera. Injera with high minerals (except iron) and antioxidant of improved microbial stability, low phytic acid contents can be processed by 9% flaxseed substitution.

Article Information

Article History:

Received : 13-08-2013

Revised : 20-09-2013

Accepted : 22-09-2013

Keywords:

Cfu

FRAP

Minerals

Phytic acid

Tef-flaxseed injera

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INTRODUCTION

Tef (*Eragrostis tef*) is a cereal crop widely cultivated in Ethiopia mainly to process its grain flour into injera (a fermented, staple food for the majority of Ethiopians) (Bultosa *et al*., 2008). In its injera making features tef grain is superior as compared to other cereal grains used (Yetneberk *et al*., 2005). Tef injera is a gluten free product and being fermented food from whole grain flour dough by lactic acid bacteria and yeast has pre- and pro-biotic potential. In many respects it favours toward complete nutrient supply with functional food character for consumers, particularly for celiac patients (Bergamo *et al*., 2011). Tef injera is known to be high in its micronutrients (Fe, Ca and Zn) partly because of agronomic practices used in tef productions in Ethiopia and reduction of mineral inhibitors like phytates on fermentation that enhances bio-availability (Umeta *et al*., 2005; Abebe

et al., 2007). Tef grain flour when incorporated in wheat bread is known to improve iron and antioxidant contents of the bread (Mohammed *et al*., 2009; Alaunyte *et al*., 2012).

Flax (*Linum usitatissimum* L.) seed is known to bear functional food properties because of its high α-linolenic acid (ω-3 fatty acid), secoisolariciresinol diglycoside lignans, dietary fibre (Tour'e and Xueming, 2010) and proteins (Rabetafika *et al*., 2011). Flaxseed is incorporated in various baked foods as whole and in crushed forms as healthy food ingredients (Hussain *et al*., 2011). Processing of injera by substitution of tef flour with flaxseed has thus a potential to improve the ω-3 fatty acid, lignans and protein contents of the injera. It may also lead to microbiologically stable of fresh keeping quality injera because of high antioxidant lignans in the flaxseed, lipid starch complex formation on baking which slow stalling of baked foods (Ingrid *et al*., 1997) and the presence of mucilage (Hussain *et al*., 2011) in the flaxseed.

There is an increased global interest in the food industry to develop and market functional foods (Van der Made *et al*., 2012) in which the scientific investigations are limited in Ethiopia. In view of this, injera processed by substitution (3%, 6% and 9%) with whole and flour flaxseed to tef grain flour showed with 9% flaxseed substitution, percentage energy, moisture, ash, crude protein, fibre and titratable acidity (TA) increase were 3.5, 27.3, 25.9, 20.4, 114.3 and 10.1, respectively (Girma *et al*., 2013). With an increase in the flaxseed substitution, most injera sensory acceptance increased, whereas injera eyes and colour were decreased and appeared superior for the control (100% tef injera). It was noted that injera structure has a potential to deliver ω-3 fatty acid, antioxidant lignans of improved protein contents by substitution with flaxseed. As part of the tef-flaxseed injera characterisation, in this paper selected mineral nutrients, antioxidant, phytic acid contents and microbial stability of injera are reported.

MATERIALS AND METHODS

Experimental Materials and Injera Sample Processing

Tef grain and flaxseed used are as reported in Girma *et al*. (2013). The experiment was conducted in two factorial designs of two flaxseed forms (whole and flour) and three flaxseed substitution levels (3%, 6% and 9% flaxseed) in three replications were co- fermented. Tef injera containing flaxseed was prepared by mixing tef-flaxseed: water (1kg: 2L) and about 160mL of *irsho* (starter culture saved from preliminary trail of the work) and made into dough by hand kneading, fermented and baked along with the control (100% tef injera) as it is done traditionally of the detailed processing stages described in Girma *et al*. (2013). Fresh baked injera was dried for 24h at 65^{O}C in an oven (Model: 101-1A; Tianjin Taisite Instrument Co., Ltd, Tiajin, China) and ground on a mortar and pestle to pass 750µm sieve. This sample was kept in sealed plastic bag at refrigeration temperature (5^{O}C) and was used for minerals, antioxidant and phytic acid contents analysis.

For microbiological stability analysis, fresh baked injera was received from baking griddle on traditional grass straw made plate. Immediately after cooling for a while (3 to 4 min) the baked injera was stacked in a *masob* (a traditional grass straw made storage basket) in which clean clear polyethylene plastic was placed underneath of injera. Injera samples were then wrapped with polyethylene plastic before placing the *masob* lid as is practiced traditionally. The samples were kept at room temperature (23^{O}C) for 6 days for sampling on each analysis date. Unless specifically mentioned all chemicals used were of analytical reagent grade.

Determination of Iron, Zinc & Calcium Contents

These were determined by Atomic Absorption Spectrophotometer (Model 210 VGP spectrophotometer, Buck Scientific, East Norwalk, CT, USA) after digestion of about 3.0 g dried injera using air –acetylene as a source of energy for atomisation (AACC, 2000). For iron content determination absorbance was measured at 248.3nm and iron was estimated from a standard calibration curve (3-8µg Fe/mL) prepared from analytical grade iron wire. For zinc content determination, absorbance was measured at 213.8nm and zinc level was estimated from a standard calibration curve (0.1-1.0µg Zn/mL) prepared from ZnO. For calcium content determination, absorbance was measured at 422.7nm after addition of 1% lanthanum (i.e., 1mL La solution/5mL) to sample and standard to suppress interferences. Calcium content was then estimated from standard solution (0.1-1.0µg Ca/mL) prepared from $CaCO_3$.

Determination of Phosphorus

Phosphorous was determined after digestion of 10 mg dried injera by measuring absorbance of blue colour of phosphomolybdate at 822nm with UV-Vis Spectrophotometer (Model 6505, Genway, U.K) (Morrison, 1964). Phosphorus level was estimated from a series of standard (0.2-1.2 µg P/mL) calibration curve prepared from K_2HPO_4.

Determination of Phytic Acid

Phytic acid was determined after 0.25 g of flour sample was extracted with 12.5 mL of 3% TCA,

precipitation of phytate as ferric phytate with addition of 4 mL of $FeCl_3$ (2mg/mL) (Poiana *et al*., 2009) followed by phytate phosphorus (Ph-P) analysis (Morrison, 1964) using conversion factor i.e., phytate = P × 3.55 (Poiana *et al*., 2009).

Antioxidant Activity by FRAP Method

Antioxidant activity was determined by the ferric ion reducing antioxidant power (FRAP) method (Ciou *et al*., 2008). About 0.5 g of milled injera samples were extracted with 80% methanol (1mL) by vortex mixing (REAX top, Germany) for 2 h. To the sample 2.5mL of phosphate buffer (200 mM; pH 6.6) and 2.5mL of potassium ferricyanide (1%) were mixed and incubated at 50^{O}C for 20 min in water bath (GLS 400 water bath, England) and mixed with 2.5mL of 10% trichloroacetic acid (TCA), centrifuged (Centrurion Scientific Model 1020 DE, United Kingdom) at 3000 rpm for 10 min. Supernatant (2.5mL) was mixed with 2.5mL of distilled water and 0.5mL of $FeCl_3$ (0.1%) and absorbance was measured at 700nm using UV-VIS spectro-photometer (Model 6505, Genway, U.K). An increase in absorbance of reaction mixture indicates an increase of reducing power. Ascorbic acid (0.1-1.0µg/mL) was used as standard from which FRAP value was expressed as ascorbic acid equivalent (AAE) in µmol per g of sample.

Microbiological Analysis of Injera

Yeasts-Moulds, Total Aerobic Plates and Coli Forms Counts: These were determined by pour plate technique after suspending injera samples (10g) in 90mL of buffered peptone water for each analysis. Serial dilutions of the suspension were prepared in buffered peptone water and each dilution (1mL) was transferred into a duplicate sterile petri plates. For yeasts-moulds counts Potato Dexatrose Agar (PDA) was aseptically poured into the plates and after incubation aerobically at 25°C for 3 to 5 days yeast-moulds were enumerated on plates bearing 30 to 300 colonies as colony forming units/g injera (cfu/g) according to ISO 7954:E (1987). For total aerobic plate count the Plate Count Agar (PCA) was used and after the plates were incubated aerobically at 30°C for 72 h the total aerobic bacteria were enumerated on plates bearing 20 to 200 colonies using colony counter as cfu/g (ISO 4833: (1991). For coli forms count Violet Red Bile Lactose Agar (VRBLA, Merck) was used and after the plates were incubated aerobically at 37°C for 24 h examined for typical purplish red colonies signifying coli form as cfu/g (ISO 4832:1991).

Data Analysis

At least triplicate data were analysed using two factors analysis of variance (ANOVA) using SAS (Version, 9.1; SAS Institute, Cary, NC, USA). DMRT was used for mean separation at P<0.05.

RESULTS AND DISCUSSION

Grain Tef and Flaxseed Minerals, Phytic Acid Content and Frap Antioxidant Activity

The mineral and anti-nutrient contents for injera processed are contributed from the tef and flaxseed grains. Accordingly, iron, zinc, calcium, phosphorus, phytic acid and FRAP antioxidant contents of grain tef and flaxseed samples were analyzed. Grain tef has high iron content (36.2mg$100g^{-1}$) than flaxseed (8.1mg$100g^{-1}$) because grain tef is known for its high (37.7mg$100g^{-1}$) iron contents (Abebe *et al*., 2007). Grain tef has low zinc (1.50mg$100g^{-1}$), calcium (66.6mg$100g^{-1}$) and phosphorus (157.6mg$100g^{-1}$) contents than flaxseed (4.3mg $100g^{-1}$, 188.5mg $100g^{-1}$ and 267.7mg $100g^{-1}$, respectively). Phytic acids in grain tef and flaxseed grains were 7.3mgg^{-1} and 3.3mgg^{-1}, respectively. The tef grain phytic acid content was somewhat lower than 8.4mgg^{-1} (Abebe *et al*., 2007) probably because of grain variety and analysis method differences. The phytic acid content of flaxseed was similar (3.2 mgg^{-1}) as reported by Rajesha *et al*. (2011). The FRAP antioxidant contents of flaxseed and tef flours were 238.8µmolg^{-1} and 97.2µmolg^{-1}, respectively. The analysis shows that into the injera processed, potentially high Zn, Ca, P and FRAP antioxidant will be contributed by flaxseed. High iron content will be contributed by the grain tef.

Effects of Fermentation & Flaxseed Substitution on Mineral, Phytic Acid content and FRAP Antioxidant Activity of Injera

Except for the phosphorus content, flaxseed forms had a significant effect (P<0.05) on iron, zinc, calcium, phytic acid and FRAP antioxidant contents of the injera (Table 1). Whole flaxseed substituted injera were found to have high iron, zinc, calcium, phytic acid and FRAP antioxidant contents than flour flaxseed substituted injera. This is probably because of some mineral, phytic acid and phenolic compounds loss along with fibre on milling, by leaching and fermentation degree variations between the use of whole and flour flaxseed in the course of injera processing.

The iron content of injera processed form 3% flaxseed is significantly (P<0.05) higher (33.77mg$100g^{-1}$) than the injera processed from 9% (29.52mg$100g^{-1}$) flaxseed substituted injera because grain tef has high iron contents than flaxseed. The highest iron (36.32mg$100g^{-1}$) content was for the control (100% grain tef) injera sample. This value is in the range (30 to 39mg$100g^{-1}$) reported by Umeta *et al*. (2005). The study showed flaxseed substitution has a negative impact on the iron content of injera (i.e., with 9% flaxseed substitution, 19% iron content reduction from the control injera was observed). The zinc content of

Table 1: Main effects of flaxseed forms and flaxseed substitution levels on mineral, phytic acid conent and FRAP Antioxidant activity of injera.

Factors	Fe ($mg100g^{-1}$)	Zn ($mg100g^{-1}$)	Ca ($mg100g^{-1}$)	P ($mg100g^{-1}$)	PhA (mgg^{-1})	FRAP ($\mu molg^{-1}$)
Effect of flaxseed forms						
F	31.38 ± 2.16^{c}	2.61 ± 0.16^{b}	67.80 ± 2.48^{b}	170.29 ± 2.79^{a}	0.57 ± 0.05^{c}	55.19 ± 9.10^{b}
W	32.28 ± 1.99^{b}	2.65 ± 0.19^{a}	68.42 ± 2.68^{a}	171.77 ± 3.83^{a}	0.59 ± 0.05^{b}	57.79 ± 10.32^{a}
Cont.	36.32 ± 1.22^{a}	1.35 ± 0.02^{c}	61.33 ± 1.37^{c}	159.62 ± 3.60^{b}	0.93 ± 0.02^{a}	34.19 ± 0.86^{c}
Effect of flaxseed substitution levels						
3%	33.77 ± 0.87^{b}	2.45 ± 0.05^{c}	65.24 ± 1.28^{c}	169.84 ± 2.59^{a}	0.64 ± 0.02^{b}	46.38 ± 1.15^{c}
6%	32.19 ± 0.91^{c}	2.61 ± 0.08^{b}	68.18 ± 0.70^{b}	170.20 ± 1.80^{a}	0.57 ± 0.02^{c}	54.09 ± 1.11^{b}
9%	29.52 ± 1.55^{d}	2.83 ± 0.07^{a}	70.91 ± 1.08^{a}	173.05 ± 4.46^{a}	0.53 ± 0.02^{d}	69.01 ± 2.59^{a}
Cont.	36.32 ± 1.22^{a}	1.35 ± 0.02^{d}	61.33 ± 1.37^{d}	159.62 ± 3.60^{b}	0.93 ± 0.02^{a}	34.19 ± 0.86^{d}
Mean	32.17	2.53	67.59	170.15	0.61	54.78
CV (%)	1.43	1.93	1.36	2.01	1.62	1.07

Means ± standard deviation with different letters after data within a column represents differences at 95% probability levels, CV= coefficient of variance, Cont. = control (100% pure tef *injera*) sample; Where: Fe= iron, Zn= zinc, Ca= calcium and P= phosphorus, PhA = Phytic acid, FRAP= ferric-ion reducing antioxidant; F and W= flour and whole flaxseed, respectively, Number of observation = total of 21 *injera* samples (6 x 3 + 1 x 3 control).

injera sample showed a significant difference (P<0.05) among the flaxseed substitution levels. The highest ($2.83mg100g^{-1}$) zinc content was for injera containing 9% flaxseed. Lowest was for the control ($1.35mg100g^{-1}$) injera, which is in the range of 1.0 to 1.4 $mg100g^{-1}$ zinc content reported (Umeta *et al*., 2005) for 100% grain tef injera. With 9% flaxseed substitution, the zinc content was increased by 110% from the control injera. Similar to this, a significant increase in the mineral contents (Fe, Zn and Mn) in wheat bread was reported (Gambuś *et al*. 2004) with substitution by full fat flaxseed flour except for the Fe content in which in the flaxseed substituted injera is decreasing.

With an increase in the flaxseed substitution level to 9%, the injera calcium content increased because of high calcium content in the flaxseed. According to Gambuś *et al*. (2004), with 13% replacement in wheat bread flour resulted in a 5 fold increase of micro and macro- minerals contents in bread. Significant difference (P<0.05) in the phosphorus content was observed only between 100% grain tef injera and flaxseed substituted injera even though phosphorus contents are high in the flaxseed. Such leveling off in the flaxseed substituted injera is most probably related to phosphorus containing compounds like phytic acid losses during yellowish liquid discarding in the 1st phase of fermentation for injera processing.

The phytic acid in whole grain flaxseed substituted tef injera was higher than flour flaxseed substituted tef injera which most probably related to the phytic acid loss in the flour substituted during milling, flour dough processing by leaching and fermentation degree differences toward phytic acid destruction (in whole flaxseed less available). Flaxseed substitution levels had also significant effect (P<0.05) and high phytic acid content was observed in 3% flaxseed substituted tef injera and lowest was for 9% flaxseed containing injera. The injera fermentation process has reduced phytic acid contents in the injera significantly by 87% in the control injera. In the 3% and 9% substituted injera, the phytic acid content of injera was reduced by 91% to 92%, respectively. Such phytic acid destruction is nutritionally important since it makes the minerals (Fe, Zn and Ca) bioavailable. Previous studies (Umeta *et al*., 2005; Abebe *et al*., 2007) on 100% grain tef injera also indicated a destruction of 91% to 93% phytic acid.

Whole flaxseed substituted injera had higher FRAP antioxidant content than flour flaxseed substituted injera. Processing by fermentation to injera has reduced FRAP antioxidant potential by somewhat 65% in the control injera. For 9% and 3% flax seed substituted injera, this reduction was 37% and 54%, respectively. This is probably because of some grain bran portions (like pericarp) losses in the course of milling (the case of flour substituted injera), leaching and fermentation process for injera which are known to be rich in the antioxidant compounds (Verma *et al*., 2008). However, substitution with flaxseed has improved the FRAP antioxidant potential of injera. Among the substitution levels, the FRAP antioxidant content of 9% flaxseed injera was the highest and the lowest was for 3% flaxseed containing injera. This is at large due to the high lignan contents of flaxseed that acts as an antioxidant. The total antioxidant activities of the flaxseed lignan extract and L-ascorbic acid (as a standard) were reported to be 2.10 and 7.35µmol Fe^{2+}/mg dry weight, respectively (Barbary *et al*., 2010).

Interaction Effect of Flaxseed Forms by Substitution Levels On Mineral, Phytic Acid Content and FRAP Antioxidant Activity of Injera

The flaxseed form and blending ratio interactions have a significant effect (P<0.05) on iron, zinc, calcium, phytic acid and FRAP antioxidant contents of the injera (Table 2). Iron content was highest for 3% whole flaxseed containing injera and lowest was for 9% whole and flour flaxseed substituted injera. As flaxseed substitution increased, the iron content decreased because 100% grain tef injera is known to have high iron contents (Umeta *et al*., 2005; Abebe *et al*., 2007; Alaunyte *et al*., 2012). Unlike the iron content, the interaction of flaxseed forms and substitution levels showed an increase in the zinc and calcium contents of the injera because zinc and calcium contents were high in the flaxseed. The control injera has the lowest zinc and calcium contents. In the phosphorus content, significant difference was only observed between the 9% whole flaxseed and flour (3% and 6%) substituted injera and the lowest phosphorus content was for the control injera. Among the flaxseed substitution levels, highest phytic acid content was obtained in 3% whole and flour flaxseed interaction and the lowest was obtained in 9% flour flaxseed substituted injera. Substitution has reduced the phytic acid content as compared to the control injera. The FRAP antioxidant content among the flaxseed substitution levels was highest in the interaction of whole flaxseed substituted injera at 9% and the lowest was for flour flaxseed substituted at 3%. Flaxseed substitution (from 3% to 9%) has increased by 1.3 up to 2.0 folds the FRAP antioxidant contents as compared to the control injera because of high antioxidant contents in the flaxseed (Tour'e & Xueming, 2010). The interaction, shows the flaxseed substitution levels has a dominant effect except in the FRAP antioxidant contents where flaxseed forms also appeared to have strong effect.

Table 2: Interaction effects between flaxseed forms and flaxseed substitution levels on mineral, phytic acid and FRAP contents of injera.

Treatment	Fe ($mg100g^{-1}$)	Zn ($mg100g^{-1}$)	Ca ($mg100g^{-1}$)	P ($mg100g^{-1}$)	PhA (mgg^{-1})	FRAP ($\mu molg^{-1}$)
F*3%	33.34 ± 0.87^{bc}	2.45 ± 0.06^{c}	64.96 ± 1.63^{d}	169.12 ± 2.66^{b}	0.64 ± 0.01^{b}	45.55 ± 0.93^{f}
F*6%	31.71 ± 0.85^{d}	2.60 ± 0.10^{b}	68.19 ± 0.93^{c}	170.07 ± 2.23^{b}	0.57 ± 0.01^{c}	53.21 ± 0.84^{d}
F*9%	29.09 ± 1.85^{e}	2.79 ± 0.03^{a}	70.26 ± 0.53^{b}	171.67 ± 3.25^{ab}	0.52 ± 0.01^{e}	66.80 ± 1.40^{b}
W*3%	34.20 ± 0.67^{b}	2.45 ± 0.02^{c}	65.51 ± 0.89^{d}	170.56 ± 2.53^{ab}	0.65 ± 0.02^{b}	47.21 ± 0.64^{e}
W*6%	32.68 ± 0.73^{cd}	2.62 ± 0.07^{b}	68.17 ± 0.46^{c}	170.32 ± 1.46^{b}	0.58 ± 0.02^{c}	54.96 ± 0.45^{c}
W*9%	29.96 ± 1.19^{e}	2.87 ± 0.08^{a}	71.57 ± 1.12^{a}	174.44 ± 5.35^{a}	0.54 ± 0.01^{d}	71.21 ± 1.06^{a}
Cont.	36.32 ± 1.22^{a}	1.35 ± 0.02^{d}	61.33 ± 1.37^{e}	159.62 ± 3.60^{c}	0.93 ± 0.02^{a}	34.19 ± 0.86^{g}
Mean	32.17	2.53	67.59	170.15	0.61	54.78
CV (%)	3.45	2.53	1.53	1.87	2.63	2.21

Means ± standard deviation with different letters after data within a column represents differences at 95% probability levels CV= coefficient of variance, F*3%, F*6% and F*9% = *injera* baked from flour flaxseed with 3%, 6% and 9% substitution levels, respectively; W*3%, W*6% and W*9% = *injera* baked from whole flaxseed with 3%, 6% and 9% substitution levels, respectively. PhA = Phytic acid, FRAP= ferric-ion reducing antioxidant power; Cont.= control sample (100% tef *injera*), Number of observation = total of 21 *injera* samples (6 x 3 + 1 x 3 control).

The Effects of Flaxseed Forms and Flaxseed Substitution Levels on Microbial Count of Flaxseed Substituted Tef Injera Product

Yeast-mould and total aerobic plate counts were significantly different (P<0.05) between flaxseed application forms for all injera storage days evaluated (Table 3). Flour substituted injera were appeared to have high yeast-mould and total aerobic plate counts than whole flaxseed substituted injera. This could be in part because in the flour fermented dough, nutrients such as proteins and vitamins from grains on grinding might be easily available for microorganisms leading to higher counts (Blandino *et al*., 2003).

Between 2 and 6 storage days for flour flaxseed substituted injera, the yeast-mould counts were increased from 2.51 $logcfug^{-1}$ to 4.02 $logcfug^{-1}$ and in the whole flaxseed substituted injera from 2.45 $logcfug^{-1}$ to 3.99 $logcfug^{-1}$. Total aerobic plate counts were increased for flour substituted injera from 2.41 $logcfug^{-1}$ to 3.95 $logcfug^{-1}$ and in the whole flaxseed substituted injera from 2.39 $logcfug^{-1}$ to 3.85 $logcfug^{-1}$. The control injera had high yeast-mould and total aerobic plate counts than the flaxseed substituted injera samples because in the flaxseed substituted injera there is an anti-microbial effect from flaxseed constituents like the phenolic compounds of antioxidant character (Tour'e & Xueming, 2010).

With an increase in the flaxseed substitution levels from 3% to 9%, yeast-mould and total aerobic plate counts showed a significant (P<0.05) decreasing trend for 2 to 6 day evaluated. The highest yeast-mould and total aerobic plate counts

Table 3: Effect of flaxseed form and flaxseed substitution levels on microbial count of fresh injera product.

	Yeast–mould count (log cfug^{-1})			Total aerobic plate count (log cfug^{-1})		
	Days of storage			Days of storage		
Treatment	**2**	**4**	**6**	**2**	**4**	**6**
Effect of flaxseed forms						
F	2.51 ± 0.17^{b}	2.92 ± 0.09^{b}	4.02 ± 0.05^{b}	2.41 ± 1.76^{b}	3.76 ± 0.15^{b}	3.95 ± 0.16^{b}
W	2.45 ± 0.18^{c}	2.88 ± 0.11^{c}	3.99 ± 0.07^{c}	2.39 ± 1.74^{c}	3.71 ± 0.15^{c}	3.85 ± 0.09^{c}
Cont.	2.85 ± 0.00^{a}	3.06 ± 0.00^{a}	4.08 ± 0.00^{a}	3.70 ± 0.00^{a}	4.07 ± 0.03^{a}	4.30 ± 0.00^{a}
Effect of flaxseed substitution levels						
3%	2.67 ± 0.07^{b}	3.01 ± 0.02^{b}	4.06 ± 0.01^{b}	3.67 ± 0.03^{b}	3.90 ± 0.05^{b}	4.01 ± 0.11^{b}
6%	2.50 ± 0.05^{c}	2.91 ± 0.03^{c}	4.02 ± 0.01^{c}	3.54 ± 0.04^{c}	3.74 ± 0.04^{c}	3.92 ± 0.12^{c}
9%	2.27 ± 0.07^{d}	2.78 ± 0.06^{d}	3.93 ± 0.04^{d}	ND^{d}	3.56 ± 0.07^{d}	3.77 ± 0.04^{d}
Cont.	2.85 ± 0.00^{a}	3.06 ± 0.00^{a}	4.08 ± 0.00^{a}	3.70 ± 0.00^{a}	4.07 ± 0.03^{a}	4.30 ± 0.00^{a}
Mean	2.51	2.91	4.01	2.5	3.78	3.93
CV (%)	0.14	0.06	0.03	0.12	0.22	0.08

Means ± standard deviation with different letters after data within a column represents differences at 95% probability levels; cfug^{-1} is colony forming units/g fresh *injera*; ND = not detected, CV= coefficient of variance, F and W= flour and whole flaxseed substitution, respectively; 3%, 6% and 9% = flaxseed substitution levels; Cont.= control (100% tef *injera*), Number of observation = total of 21 *injera* samples (6 x 3 + 1 x 3 control).

were for control injera sample. Between 2 and 6 storage days for 3% flaxseed substituted injera the yeast-mould counts were increased from 2.67 logcfug^{-1} to 4.06 logcfug^{-1} and for 9% flaxseed substituted injera from 2.27 logcfug^{-1} to 3.93 logcfug^{-1}. Whereas, the total aerobic plate counts increased from 3.67 logcfug^{-1} to 4.01 logcfug^{-1} for 3% and from not detectable level to 3.77 logcfug^{-1}. In the control injera sample for 2 to 6 storage days, yeast-mould and total aerobic plate counts were varied from (2.85 logcfug^{-1} to 4.08 logcfug^{-1}) and (3.70 logcfug^{-1} to 4.30 logcfug^{-1}), respectively. Such difference in the microbial counts between control injera and flaxseed substituted injera is due to flaxseed antimicrobial activity (Shin *et al*., 2007; Xu *et al*., 2008; Barbary *et al.,* 2010). The increase in acidity because of the presence of more quantity of fermentable carbohydrates contributed by flaxseed is also additional factor in the reduction of microbial load (Blandino *et al*., 2003). Coli forms were not detected in all injera samples. This is due to a low pH of injera (4.37 to 4.49) (Girma *et al*., 2013), bacteriocin produced by dominating lactic acid bacteria (LAB) (Blandino *et al*., 2003) and the injera steam baking action. The pH range of 3.6 to 4.1 is known favorable for eliminating coli forms in fermented foods (Blandino *et al*., 2003). Lignans from flaxseed is also effective antibacterial (Barbary *et al.,* 2010*)*.

Interaction Effects of Flaxseed Forms With Flaxseed Substitution Levels on Microbial Count of Flaxseed Substituted Tef Injera Product

The interaction between flaxseed forms and substitution levels was significant on the injera yeast-mould and total aerobic plate counts evaluated for all storage days (Table 4).

Table 4: Interaction effect of flaxseed forms by flaxseed substitution levels on microbial count of fresh injera.

	Yeast-mould count (log cfug^{-1})			Total aerobic plate count (log cfug^{-1})		
	Days of storage			Days of storage		
Treatment	**2**	**4**	**6**	**2**	**4**	**6**
F*3	2.70 ± 0.07^{b}	3.02 ± 0.02^{ab}	4.07 ± 0.00^{ab}	3.68 ± 0.02^{b}	3.92 ± 0.04^{b}	4.07 ± 0.13^{b}
F*6	2.52 ± 0.03^{c}	2.93 ± 0.02^{c}	4.03 ± 0.01^{c}	3.56 ± 0.03^{c}	3.76 ± 0.04^{c}	3.99 ± 0.15^{bc}
F*9	2.31 ± 0.06^{d}	2.81 ± 0.04^{e}	3.96 ± 0.02^{e}	ND^{e}	3.59 ± 0.07^{d}	3.79 ± 0.03^{e}
W*3	2.64 ± 0.05^{b}	3.00 ± 0.02^{b}	4.05 ± 0.01^{bc}	3.65 ± 0.03^{b}	3.87 ± 0.06^{b}	3.95 ± 0.03^{cd}
W*6	2.48 ± 0.05^{c}	2.89 ± 0.03^{d}	4.01 ± 0.01^{d}	3.52 ± 0.04^{d}	3.71 ± 0.03^{c}	3.86 ± 0.02^{de}
W*9	2.23 ± 0.05^{e}	2.75 ± 0.06^{f}	3.90 ± 0.04^{f}	ND^{e}	3.53 ± 0.05^{d}	3.75 ± 0.04^{e}
Cont.	2.85 ± 0.00^{a}	3.06 ± 0.00^{a}	4.08 ± 0.00^{a}	3.70 ± 0.00^{a}	4.07 ± 0.03^{a}	4.30 ± 0.00^{a}
Mean	2.51	2.91	4.01	2.5	3.78	3.93
CV (%)	2.1	1.12	0.46	0.95	1.32	2.12

Means ± standard deviation with different letters after data within a column represents differences at 95% probability levels; cfug^{-1} is colony forming units/g fresh *injera*; ND = not detected, CV= coefficient of variance, F*3%, F*6% and F*9% = *injera* baked from flour flaxseed with 3%, 6% and 9% substitution levels, respectively; W*3%, W*6% and W*9% = *injera* baked from whole flaxseed with 3%, 6% and 9% substitution levels, respectively, Number of observation = total of 21 *injera* samples (6 x 3 + 1 x 3 control).

On the 2 days of storage, among the flaxseed substitution yeast-mould counts was lowest for whole grain substituted at 9% and highest for flour and whole flaxseed substituted at 3%. The total aerobic plate counts on the 2 day stored injera was not detected at 9% substitution (both whole and flour) and highest was for flour and whole flaxseed substituted at 3%. The control injera has the highest yeast-mould and total aerobic plate counts at all respective storage days in the interaction. The result shows flaxseed substitution levels has a dominant role in reducing the microbial counts. The whole flaxseed substitution showed also somewhat lower microbial counts than flour flaxseed substituted injera.

CONCLUSIONS

This study shows that flaxseed forms (grain or flour) and flaxseed substitution levels had significantly influenced mineral, phytic acid, FRAP antioxidant contents of flaxseed substituted tef injera product. Whole flaxseed containing injera sample showed significantly increased mineral content and lower microbial load as compared to flour flaxseed containing injera. With the flaxseed substitution, the iron content was found to be negatively influenced because of 100% grain tef injera is known for its high iron contents. With increasing the flaxseed proportion in the injera product, the microbial count was decreased and this is contributed by the antimicrobial activity of flaxseed and the acidity of fermented injera. In all fresh injera samples during the 6 days storage, coli form counts were not detected. The injera made with 9% flaxseed flour and whole as partial replacement of tef flour had showed improved antioxidant potential and better microbial shelf life stability.

ACKNOWLEDGEMENTS

The authors thank NARF Functional Food Development Project (NARF/CR/01/004/09) at FSPT department, Haramaya University for financial support, DZARC for grain tef and KARC for flaxseed offers. Centre of Research on Grain Quality, Processing and Technology Transfer (CRGQPTT) at FSPT department is acknowledged for supporting with facilities.

REFERENCES

AACC. (2000). Approved Methods of the American Association of Cereal Chemists, 10th edn. American Association of Cereal Chemists, St Paul: MN., USA.

Abebe, Y., Bogale, A., Hambidge, K.M., Stoecker, B.J., Bailey, K. & Gibson, R.S. (2007). Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability. *Journal of Food Composition and Analysis* 20: 161-168.

Alaunyte, I., Stojceska, V., Plunkett, A., Ainsworth, P. & Derbyshire, E. (2012). Improving the quality of nutrient-rich teff (*Eragrostis tef*) breads by combination of enzymes in straight dough and sourdough bread making. *Journal of Cereal Science* 55: 22-30.

Barbary, O.M., El-Sohaimy, S.A., El-Saadani, M.A. & Zeitoun, A.M.A. (2010). Antioxidant, antimicrobial and anti-HCV activities of lignan extracted from flaxseed. *Research Journal of Agriculture and Biological Sciences* 6(3): 247-256.

Blandino, A., Al-Aseeri, M.E., Pandiella, S.S. & Cantero, D., Webb, C. (2003). Review: Cereal-based fermented foods and beverages. *Food Research International* 36: 527–543.

Bergamo, P., Maurano, F., Mazzarella, G., Iaquinto, G., Vocca, I., Rivelli, A.R., Enrica Falco, D., Gianfrani, C. & Rossi, M. (2011). Immunological evaluation of the alcohol-soluble protein fraction from gluten-free grains in relation to celiac disease. *Molecular Nutrition and Food Research* 55: 1266-1270.

Bultosa, G., Hamaker, B. R. & BeMiller, J. N. (2008). An SEC-MALLS study of molecular features of water-soluble amylopectin and amylose of tef [*Eragrostis tef* (Zucc.) Trotter] starches. *Starch/Stärke* 60: 8-22.

Ciou, J.Y., Wang, C.C.R., Chen, J. & Chiang, P.Y. (2008). Total phenolics content and antioxidant activity of extracts from dried water caltrop (*Trapa Taiwanensis* Nakai) hulls. *Journal of Food and Drug Analysis* 16 (2): 41-47.

Gambuś, H., Mikulec, A., Gambuś, F. & Pisulewski, P. (2004). Perespectives of linseed utilisation in baking. *Polish Journal of Food and Sciences* 13(1): 21-27.

Girma, T., Bultosa, G. and Bussa, N. (2013). Effect of grain tef [*Eragrostis tef* (Zucc.) Trotter] flour substitution with flaxseed on quality and functionality of *injera*. *International Journal of Food Science and Technology* 48: 350-356.

Hussain, S., Anjum, F.M. & Alamri, M.S. (2011). Fortification of pan bread with healthy flaxseed. *Australian Journal of Basic and Applied Sciences* 5: 978-983.

Ingrid, A., Appelqvist, M. & Martine, R., Debet, M. (1997). Starch-biopolymer interactions—a review. *Food Reviews International* 13 (2): 163-224.

ISO 4833: (1991) Microbiology-general guidance for the enumeration of micro-organisms, colony count technique at 30^{O}C. International Organisation for Standardisation, Geneva.

ISO 4832. (1991). Microbiology-general guidance for the enumeration of coliforms, colony count technique. International Organisation for Standardisation, Geneva.

ISO 7954 E. (1987). Microbiology-general guidance for the enumeration of yeasts and moulds, colony count technique at 25^{O}C. International Organisation for Standardisation, Geneva.

Mohammed, M.I.O., Mustafa, A.I. & Osman, G.A.M. (2009). Evaluation of wheat breads supplemented with teff (*Eragrostis tef* (Zucc.) Trotter) grain flour. *Australian Journal of Crop Science* 3: 207-212.

Morrison, W.R. (1964). A fast, simple and reliable method for the micro determination of phosphorus in biological materials. *Analytical Biochemistry* 7: 218–224.

Poiana, M.A., Alexa, E. & Bragea, M. (2009). Studies concerning the phosphorus bioavailability improvement of some cereals used in nourishment. *Roumanian Biotechnological Letters* 14(3): 4467-4473.

Rabetafika, H.N., Remoortel, V.V., Danthine, S., Paquot, M. & Blecker, C. (2011). Flaxseed proteins: food uses and health benefits: review article. *International Journal of Food Science and Technology* 46: 221–228.

Rajesha, J., Rao, A.R., Madhusudhan, B., Ravishankar, G.A. & Karunakumar, M. (2011). Hematological and histopathological studies of endosperm-rich fraction of flaxseed in chicks. *International Journal of Pharmaceutical Science and Research* 2(6):1455-1459.

Shin, S.Y., Bajpai, V.K., Kim, H.R. & Kang, S.C. (2007). Antibacterial activity of bioconverted eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) against foodborne pathogenic bacteria. *International Journal of Food Microbiology* 113: 233–236.

Tour'e, A. & Xueming, X. (2010). Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components and health benefits. *Comprehensive Reviews in Food Science and Food Safety* 9: 261-269.

Umeta, M., West, C.E & Fufa, H. (2005) Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *Journal of Food Composition and Analysis* 18: 803-817.

Verma, B., Hucl, P. & Chibbar, R. N. (2008). Phenolic content and antioxidant properties of bran in 51 wheat cultivars. *Cereal Chemistry* 85 (4): 544-549.

Van der Made, S. M., Baumgartner, S., González-Muniesa, P., Zulet, M. Á. & Martínez, J.A. (2012). The use of functional foods in the metabolic syndrome. *Current Nutrition and Food Science* 2012 (8): 25-44.

Xu, Y., Hall III, C., Wolf-Hall, C. & Manthey, F. (2008). Fungistatic activity of flaxseed in potato dextrose agar and a fresh noodle system. *International Journal of Foo Microbiology* 121: 262–267.

Yetneberk, S., Rooney, L.W. & Taylor, J.R.N. (2005). Improving the quality of sorghum *injera* by decortication and compositing with tef. *Journal of the Science of Food and Agriculture*, 85:1252–1258.