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EFFECT OF NATURAL FERMENTATION ON THE HCl-EXTRACTABILITY OF MINERALS FROM TEF (*ERAGROSTIS TEF*)

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ABSTRACT. Tef flour was fermented at room temperature for 4 days and then baked into *injera*. *Kitta* was prepared without further fermenting the tef flour. The HCl-extractability of minerals in tef *kitta* increased marginally. Fermentation decreased phytic acid, increased inorganic phosphorus and HCl-extractability of phosphorus, iron, calcium, and zinc; the extractability increased with an increase in the period of fermentation. The level of phytic acid was found to have a significant negative correlation with HCl-extractability of minerals in *injera*. The phytate concentrations and phytate: zinc molar ratios in *kitta* were similar to that of the raw seeds, but much higher than those of *injera*. Improved extractability of minerals due to fermentation in tef is particularly important from a nutritional view point as consumption of such food may be instrumental in mitigating mineral deficiencies,and in improving the nutritional status of population consuming such food.

INTRODUCTION

In Ethiopia, the cereal grain tef (*Eragrostis tef*, (Zucc.) Trotter) is one of the major cereal crops where it is believed to have originated and accounts for about two-thirds of the daily protein content in the diet of the population [1]. Ketema has recently reviewed major features of the cereal tef [2]. More than 95% of the produced tef is utilised in the form of *injera*, a fermented pancake-like sour bread. However, a longer duration of fermentation decreases the protein quality of *injera* [3].

Besides providing protein and calories, tef is a good source of minerals particularly iron [4]. In a separate study, tef was reported to contribute 200-300 mg of iron daily to the diet [5]. According to Ebba, eating of tef overcomes the iron deficiency anaemia associated with hookworm infestation [6]. But studies have revealed that most of the iron in tef came as contamination from the soil during the threshing process under the hooves of cattle [4, 7-9]. The bioavailability of contaminant iron, however, is low and sometimes negligible [10]. In a recent study, Areda *et al.* confirmed the earlier reports of high iron content of tef due to soil and species contamination and further revealed the fact that genetic and environmental factors influence the iron content of tef [11].

Phytic acid (the hexakisphosphate ester of *myo*-inositol) is a common constituent of most cereals, legumes, some vegetables and fruits. Phosphorus in cereals occurs in the form of several classes of compounds, mostly phytate and inorganic, the remainder phospholipids, with small amounts of phosphosugars in nucleic acids [12]. Phytate phosphorus is not biologically available and is known to affect adversely bioavailability of divalent minerals [13]. Under

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physiological conditions, phytic acid is extensively ionized and is capable of interacting strongly with protein and metal ions [14]. The interaction between phytic acid and minerals gives, in some instances, complexes that precipitate in the duodenum. As it is from here that maximum absorption of divalent cations occurs, it shows that bioavailability of minerals is affected by the presence of phytate.

Fermentation is a process with a potential for improving nutritional value of cereal grains. It has been reported that a natural lactic acid fermentation improved the nutritive value of corn meal and pearl millet [15, 16]. Fermentation has also been reported to reduce significantly the phytic acid level of several plant foods including cereals such as rice [17], corn [16], millet [17] and sorghum [18], thereby converting bound forms of minerals to free forms which is responsible for increased HCl-extractability of minerals of the fermented product [19]. Previous studies have shown that fermentation significantly improved the HCl-extractability of minerals of the fermented products [16, 19]. Previous studies on utilisation of tef-based diets have focused on the micro-organisms involved in the fermentation process [20, 21], the effect of fermentation on the carbohydrates [23], and nutritional composition [10, 23]. Ramachandran and Bolodia used dialysability of iron, zinc and phosphorus against deionized water as index of the bioavailability of these minerals from tef [24]. However, the HCl-extractability of minerals in fermented tef has not been studied to any degree.

The objective of this research was to investigate the effects of natural lactic acid fermentation of tef flour on HCl-extractability of iron, calcium, zinc and phosphorus in 0.03 N HCl (within the concentration of HCl found in the stomach of adults).

EXPERIMENTAL

Materials. Tef, white and brown varieties, were procured from the local market in Bishoftu, Eastern Shoa, Ethiopia. The grains were cleaned to remove dust and other foreign matter, and were ground in an electric grinder (M/s Milone, Rajkot, India) using 0.5 mm sieve size. Double distilled water was used throughout.

Preparation of ersho. To prepare fresh *ersho* (starter), 300 g flour and 1000 mL double distilled water were mixed into a slurry and the slurry was allowed to ferment for 3-4 days at room temperature. The yellowish liquid on top of batter was discarded and the batter used as *ersho*.

Batter preparation for injera. Tef flour (1000 g), water (1600 mL) and *ersho* (260 g) were mixed. The mixture was well stirred and left for 4 days to ferment and sour. Fermentation was considered sufficient, when the yellowish liquid (*ersho*) settled over the dough. About 250 g of the total dough was removed, mixed with 500 mL boiling water and cooked for 15-20 min to gelatinize the starch. This cooked portion, known as *absit*, was cooled to 50-60°C and then returned to the main fermented batch and mixed thoroughly. The batter is then allowed to ferment for a further of 2-3 h. Finally, portions of the batter were poured on to a pre-heated *Chapatti*-making pan.

Preparation of unleavened bread (kitta). Flour and water were mixed in a 1:1 (w/v) ratio and kneaded until a consistent dough is formed. The dough was immediately baked on a pre-heated plate on both sides. The baking time depends on the thickness of the bread to be baked. The fermented as well as unfermented samples were oven-dried at 65° for 48 h to a constant weight then finely ground with the same electric grinder and sieve size.

Phytate and inorganic phosphorus. The samples were extracted in 0.3 N HCl with continuous shaking (100 rpm) for 3 h in a mechanical shaker at room temperature. Phytic acid in the extract was estimated colorimetrically [25]. Phytate phosphorus was calculated by using the following formula [14]:

$$\text{Phytate phosphorus (mg)} = \frac{\text{(phytate content)}}{100} \times 28.18 \qquad (1)$$

Inorganic phosphorus in the sample was extracted in water by shaking (100 rpm) at room temperature for 3 h. Inorganic phosphorus in the extract was determined colorimetrically [26].

Mineral analysis. The samples were acid-digested using a nitric acid-perchloric acid mixture (HNO_3:$HClO_4$, 5:1 (v/v)). The amounts of iron and zinc in the digested samples were determined by atomic absorption spectrometry (Perkin-Elmer, Model 3110, Norwalk, CT, USA), according to the method of Lindsey and Norwell [27]. Phosphorus in the digested samples was estimated colorimetrically [26], whereas calcium was determined by the titration method [28].

HCl-extractablity of minerals. The minerals in the fermented and unfermented samples were extracted with 0.03 N HCl by shaking (Environ Shaker, Model 3597-I, LabLine Instruments, Melrose Park, Ill., USA) the contents (100 rpm) at 37° for 3 h. The clear extract obtained after filtration with filter paper (Whatman No. 42) was oven-dried at 100° and wet-digested with acid mixture. The amounts of extractable phosphorus, iron, calcium, and zinc in the digested samples were determined by the methods described earlier.

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0.03 N HCl}}{\text{Total mineral}} \times 100 \qquad (2)$$

Statistical analysis. Triplicates analyses were made on each sample for minerals, titratable acidity, pH, phytic acid and HCl-extractability of the minerals reported. The data were subjected to analysis of variance to estimate the level of significance and correlation coefficients according to standard methods [29].

RESULTS AND DISCUSSION

Phytate in brown tef constituted more than 55% of total phosphorus; this value was 58% for white tef. Brown tef seeds also contained 12% higher total phosphorus compared to white tef seeds. The inorganic phosphorus content of brown tef seeds was 37% higher than that of white seeds. However, iron, calcium, zinc, and phytate concentrations as well as phytate:zinc molar ratios were similar in both tef varieties (Table 1).

Baking of *kitta* had little or no effect on the concentration of phytic acid as compared with raw ingredients (Table 1). The concentration of phytic acid in the prepared *kitta*, therefore, remained high. The phytate : zinc molar ratios also remained unaffected in *kitta* compared to the raw ingredients (Table 1). The phytate : zinc molar ratio has been suggested to be an important determinant of zinc bioavailability in human diets. Oberleas and Harland showed that foods with a molar ratio of phytate : zinc, less than 10 showed adequate availability of zinc and problems were encountered if this value was greater than 20 [30]. In studies in rats, high molar ratios have been shown to reduce the availability of zinc significantly. Morris and Ellis also reported that breakfast cereals with phytate : zinc ratios above 15 depressed growth in rats [31].

In the present study, all the samples of *kitta* had values greater than 28, indicating that zinc

Table 1. Composition of *tef* products (mg /100 g) and phytate : zinc mole ratio (Pp = phytate phosphorus; Pa:Zn = phytate:zinc mole ratio; P_t = total phosphorus; Pi = inorganic phosphorus).

Comp.	Seeds		Prepared food			
	White tef	Brown tef	White tef *Kitta*	B. tef *Kitta*	White tef *Injea*	Brown tef *Injea*
Fe	9.31±2.97	9.15±3.17	9.15±2.23	9.34±3.13	9.19±2.93	9.36±3.47
Ca	197.32±4.13	205.61±7.32	201.09±9.33	206.18±2.76	202.43±5.23	206.89±7.69
Zn	2.3±0.27	2.33±0.71	2.31±0.63	2.33±0.61	2.31±0.81	2.34±0.67
Pp	188.21±2.46	197.26±2.51	187.38±2.21	197.09±1.92	38.43±1.32	54.31±1.82
Pt	324.63±4.17	361.66±4.57	362.11±4.87	361.61±4.36	362.83±5.07	361.88±4.73
Pi	58.83±1.87	92.33±1.35	58.13±2.21	92.30±1.43	277.21±4.13	290.32±3.71
Pa:Zn	28.64±4.17	29.63±2.51	28.25±2.23	29.58±1.13	6.19±0.41	8.30±0.21

* Values are means ± S.D. of three tests and are expressed on dry weight basis.

availability from these diets would be low. Unleavened breads with a phytate:zinc molar ratio of greater than 20 comprise over 90% of the diet in the Middle East, where clinical zinc deficiency was first described [30].

Natural fermentation of tef at room temperature has resulted in various biochemical changes (Table 2). After 96 h fermentation of tef flour, the pH decreased with a corresponding increase in titratable acidity. Values for titratable acidity (expressed as percent lactic acid) and pH were not, however, significantly different among both varieties of tef. At the end of the 4th day, the pH of dough was 3.83 and 3.85 and the titratable acidity was about 1.31 and 1.17 % in white and brown tef varieties, respectively, indicating that normal lactic acid fermentation occurred. A significant ($p < 0.05$) negative correlation ($r = -0.9820$, $r = -0.9973$) has been noted between pH and titratable acidity in white and brown tef varieties. A rapid drop in pH with a corresponding increase in titratable acidity has been observed in lactic acid fermentation of corn meal [15].

Injera, prepared from both white and brown tef varieties, had higher pH compared with pH of the doughs. The increase in pH may be attributed to the evaporation of low boiling volatile short chain fatty acids during the baking process.

As a result of natural fermentation, a significant reduction in phytic acid (expressed as phytate phosphorus) content was noticed in both tef varieties (Table 2). Phytate phosphorus decreased by 76.5% and 65.4%, respectively in white and brown tef varieties following 96 h fermentation. These results may be compared with reductions of phytic acid content reported in corn meal [15] and millet flour [16]. Baking the fermented doughs into *injera* further decreased (79.6 and 72.5% in white and brown tef, respectively) the residual phytate to lower levels. The residual phytate content was noticeably higher in brown tef *injera* than in white tef *injera* (Table 2). Natural fermentation, however, did not eliminate completely the content of phytic acid in tef flour.

Similarly, the inorganic phosphorus increased by 4.6 and 3.0-fold, respectively, in white and brown tef flour varieties following 4 days of fermentation. *Injera* baking also further increased the amount of inorganic phosphorus. The increase in inorganic phosphorus corresponds to the gradual decrease in phytate phosphorus. The results suggest that the increase in inorganic

Table 2. Fermentation characteristics of naturally fermented tef dough and *injera*.*

White tef	Fermentation time, h					
	0	24	48	72	96	*Injea*
pH	6.56±0.09^a	5.03 ± 0.01^b	4.23±0.09^c	3.94±0.03^d	3.83±0.04^e	4.10±0.02^f
TA %	0.26±0.01^a	0.53 ± 0.01^b	1.23±0.08^c	1.27±0.04^d	1.31±0.05^e	1.29±0.03^d
Pp mg/100g	188.21±2.46^a	104.12±3.17^b	98.07±5.21^b	60.32±1.62^c	44.26±2.12^d	38.43±1.32^d
Pi mg/100g	59.10±2.63^a	103.87±7.02^b	185.77±9.31^c	262.68±7.14^d	270.17±5.62^e	277.72±4.55^f
Brown tef						
pH	6.37±0.11^a	5.61±0.06^b	4.40±0.12^c	4.11±0.06^d	3.85±0.05^e	4.31±0.12^d
TA %	0.21±0.02^a	0.47±0.05^b	1.01±0.03^c	1.15±0.04^d	1.17±0.02^d	1.19±0.06^d
Pp mg/100g	197.26±1.27^a	130.31±5.13^b	104.21±4.13^c	86.63±2.71^d	68.22±2.17^e	54.31± 1.82^f
Pi mg/100g	93.77±3.62^a	154.33±5.69^b	223.07±3.41^c	257.16±6.62^d	277.21±4.13^e	290.13±3.71^f

* Values are means ± S.D., n = 3. , TA = titratable acidity; Pp = phytate phosphorus; Pi = inorganic phosphorus; any two means in columns bearing different letter superscripts differ significantly ($p < 0.05$).

phosphorus in the fermenting mixtures may have been due to the enzyme phytase. Phytase, a normal constituent of cereals and legumes and which may also be produced by the fermenting microflora [15] hydrolyse the phytate phosphorus in fermenting tef flour and release inorganic phosphorus from phytic acid. Correlation coefficients showed significant ($p < 0.05$) negative correlation ($r = -0.9784$ and $r = -0.9492$) between phytate phosphorus and inorganic phosphorus in both white and brown tef varieties, respectively. A decrease in phytic acid and simultaneous increase in inorganic phosphorus has been reported during natural fermentation of pearl millet [16].

The phytate : zinc molar ratios for fermenting white tef is shown in Table 3. Fermentation had significantly reduced phytate : zinc molar ratios; the longer the period of fermentation, the greater the extent of decrease. Compared to the raw seeds, phytate : zinc molar ratio was reduced by 77% in white tef (Table 3) and by 71% in brown tef (Table 4) following 4 days of fermentation. Further, a significant ($p < 0.05$) negative correlation was found between the phytate phosphorus and phytate : zinc molar ratio ($r = -0.9470$ and $r = -0.9971$) in both white and brown tef varieties. Thus, *injera* has considerably low phytate : zinc molar ratio, and, using this as an indicator, zinc bioavailability in *injera* might be high.

Extractability of minerals in 0.03 N HCl (within the concentrations of HCl found in stomachs of adults) at 37° has been reported as an index of bioavailability of the minerals [20, 32]. Table 3 indicates the HCl-extractability of iron, calcium, zinc and phosphorus in white tef flour, *kitta* and *injera*. The HCl-extractability of iron, calcium, zinc, and phosphorus in white tef (Table 3) control sample was slightly higher than that of the brown tef (Table 4) control sample. As a result of *kitta* preparation, the HCl-extractability of iron improved marginally as compared to the

Table 3. Effect of natural fermentation on HCl-extractability (%) of minerals and phytate : zinc molar ratio in white tef dough (with time, h) and *injera**.

	Product						
	Kitta	Control	Dough				*Injera*
h →	-	0	24	48	72	96	-
Fe	47.21 ± 2.09[a]	43.31 ± 1.97[b]	53.71 ± 1.38[c]	58.61 ± 1.31[d]	61.29 ± 0.73[e]	70.67 ± 0.73[f]	72.35 ± 0.65[f]
Ca	40.29 ± 1.03[a]	32.21 ± 0.56[b]	41.33 ± 2.19[a]	50.15 ± 1.02[c]	54.27 ± 3.25[d]	59.41 ± 2.38[e]	61.61 ± 3.14[e]
Zn	46.80 ± 1.26[a]	38.47 ± 0.41[b]	44.53 ± 0.56[a]	59.52 ± 0.85[c]	69.59 ± 1.30[d]	72.98 ± 0.67[d]	81.13 ± 2.63[e]
P	42.52 ± 2.25[a]	42.52 ± 1.05[a]	53.58 ± 1.47[b]	59.58 ± 2.39[c]	69.59 ± 1.30[d]	72.51 ± 0.93[e]	79.24 ± 1.72[f]
Pa/Zn	28.01 ± 1.02[a]	28.06 ± 1.41[a]	16.86 ± 2.14[b]	13.89 ± 0.69[c]	7.01 ± 0.71[d]	6.39 ± 0.88[e]	6.19 ± 0.41[e]

* Values are means ± S.D., n =3; Pa/Zn = Phytate : zinc molar ratio;. letters in superscripts differ within columns values differ significantly ($p < 0.05$) from each other.

Table 4. Effect of natural fermentation on HCl-extractability (%) of minerals and phytate : zinc molar ratio in brown tef dough (with time, h) and *injera**.

	Product						
	Kitta	Control	Dough				*Injera*
h →	-	0	24	48	72	96	-
Fe	44.10 ±0.34[a]	42.00 ± 0.38[a]	49.33 ±0.28[b]	57.04 ±1.01[c]	62.51 ± 0.58[d]	66.95 ± 0.27[e]	71.38 ± 0.67[f]
Ca	39.63 ± 1.13[a]	31.04 ± 0.72[b]	42.29 ±1.67[a]	49.35 ±2.43[c]	55.47 ± 1.87[d]	60.12 ± 3.43[e]	62.09 ± 4.17[e]
Zn	42.12 ± 1.59[a]	36.84 ± 0.25[b]	43.54 ± 0.58[a]	62.82 ± 0.75[c]	69.21 ± 0.76[d]	74.07 ± 0.25[e]	87.63 ± 0.24[f]
P	40.58 ± 0.48[a]	40.08 ± 1.12a	50.84 ± 1.34[b]	56.35 ± 1.23[c]	60.16 ± 1.45[d]	62.53 ± 0.75[d]	66.00 ± 1.99[e]
Pa/Zn	29.13 ± 1.05[a]	29.44 ± 1.25[a]	19.44 ± 1.54[b]	15.28 ±1.47[c]	8.33 ± 1.12[d]	8.33 ± 0.67[e]	8.30 ± 0.21[e]

*Values are means ± S.D., n = 3; Pa/Zn = phytate : zinc molar ratio; superscript letters differ within columns values significantly differ ($p < 0.05$) from each other.

extractability of calcium and zinc. The HCl-extractability of iron, calcium, and zinc in white tef *kitta* increased by about 8, 20 and 18%, respectively, compared with the control. Lower percentages of iron and zinc were extracted in brown tef *kitta* (Table 4) as compared with white tef *kitta*. *Kitta* preparation from both white and brown tef had no effect on the HCl-extractability

of phosphorus. The marginal increase in the extractability of minerals particularly iron may be attributed to the non effectiveness of heat treatment on mineral-phytate chelates in unfermented tef grains during the preparation of *kitta*.

Natural fermentation did not basically alter the concentrations of total phosphorus, iron, calcium, and zinc in the fermenting mixtures and *injera*, but it instead increased HCl-extractability of these minerals. HCl-extractability of phosphorus improved significantly ($p < 0.05$) following natural fermentation. Longer fermentation periods resulted in higher phosphorus extractability. Fermentation released 1.9 times more extractable phosphorus after 4 days of fermentation and *injera* making than contained in the unfermented white tef samples (Table 3). *Injera* prepared from brown tef (Table 4) had about 13% lower extractable phosphorus compared to white tef *injera*. Cleavage of inorganic phosphorus by phytase from phytic acid may explain the improved HCl-extractability of phosphorus in the fermented tef varieties. On the other hand, a significant ($p < 0.05$) negative correlation ($r = -0.9975$ and $r = -0.9760$) between levels of phytate phosphorus and HCl-extractability of phosphorus was observed in white and brown tef varieties. Similar effects on phosphorus extractability have been reported with corn meal [19], tef [24] and with *rabadi* [17], a fermented pearl millet product.

The process of fermentation also enhanced HCl-extractability of iron, calcium, and zinc in tef flour, an index of their bioavailability to the human system. Extractability of iron increased in both white tef (Table 3) and brown tef (Table 4) by 1.7-fold following 4 days of fermentation and then baking into *injera*. Similarly, there was more than 2-fold increase in zinc extractability from *injera* baked from both tef varieties following 4 days of fermentation with higher values observed in brown tef (Table 4). Calcium extractability increased by two-fold in both tef varieties.

As result of hydrolytic diminution of phytate phosphorus (phytic acid) during fermentation, the divalent cations (Fe^{2+}, Ca^{2+} and Zn^{2+}) may be released in free forms; thereby increasing the extractability of iron, calcium, and zinc in the fermented product. Existence of negative significant ($p < 0.05$) correlation between the concentration of phytic acid and HCl-extractability of iron ($r = -0.9741$ and $r = -0.9772$), calcium ($r = -0.9525$ and $r = -0.9911$), and zinc ($r = -0.9434$ and $r = -0.9336$) in white and brown tef further strengthens this argument. Natural fermentation of pearl millet and wheat has been reported to significantly increase the HCl-extractability of minerals with concomitant decrease in phytic acid [16, 19]. Ramachandran and Bolodia similarly observed significant increases in dialysability of iron and zinc during natural fermentation of tef [24]. Although the HCl-extractability of minerals is higher in white tef than in brown tef, differences attributable to variety were not significant.

Natural fermentation of tef grain until the pH drops to about 3.84 and titratable acidity increases to 1.24 appears to be most essential in reducing the phytate phosphorus concentration and phytate : zinc molar ratios as well as improving the extractability of iron, calcium, zinc and phosphorus. Tef fermentation is, therefore, an effective method of improving the HCl-extractability and possibly the bioavailabilty of minerals, thus improving the nutritional quality of tef flour.

In conclusion, tef fermentation is a potential method of improving the HCl-extractability as indicator to bioavailability of essential minerals from cereals like tef. Consumption of such naturally fermented foods may be helpful in ameliorating the nutritional status of the people where tef is widely grown and commonly consumed.

The tef samples used in the present study were obtained from the local market where market samples are usually mixtures of strains and generally lumped according to colour. Such studies are further warranted on known varieties (genotypes) of tef grain harvested under controlled environment.

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