Original article

Effect of natural fermentation on nutritional and antinutritional factors of tef (*Eragrostis tef*)

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Abstract: Tef flour mixed with water in a 1:1.6 (w/v) ratio was allowed to ferment at 22^0 C for 96 hrs by the action of endogenous microflora in the batter. After 96 hrs total protein content in tef dough decreased by 12% whereas the NPN, free amino acids, free amino acid nitrogen, soluble protein and fat acidity increased 7.4-, 7.0-, 6.6-, 7.7- and 10.7- fold, respectively. Fermentation also resulted in significant drop in pH and sharp rise in titratable acidity of the 96 hrs fermented dough. Iron, phosphorus and calcium decreased by 43%, 35% and 41%, respectively, in the dough fermented for 96 hrs. Phytic acid, tannins and trypsin inhibitor contents were reduced by 72%, 55% and 69%, respectively. In *ersho*, the liquid portion drained off from the fermented dough, total protein, NPN, free amino acids, iron, calcium and phosphorus increased significantly whereas total and reducing sugars decreased during the two days of fermentation. The pH of *ersho* dropped slightly but the titratable acidity increased by 35%. These results could provide useful indices for the improved evaluation of tef fermentation. [*Ethiop. J. Health Dev.* 1997;11(1):61-66]

Introduction

Tef *(Eragrostis tef)* is the second most extensively cultivated crop indigenous to Ethiopia (1). Tef is commonly used in Ethiopia in the production of beverages and a number of foods, such as *injera* (leavened pancake), *kita* (unleavened pancake), porridge and gruel (2). *Injera* is a pancake-like soft, thin leavened bread obtained by natural fermentation of cereal grains including tef. The fermentation process lasts for a total of two to four days. Sequences of events and successions of microorganisms during the fermentation of tef have been previously reported (3,4). Tef *injera* contributes a major part of the diet for the Ethiopian population with the exception of those living in areas where Enset *(Ensete ventricosum)*, maize and sorghum are the main diets. *Injera* is eaten with stew prepared from legumes or meat.

Like other cereals, tef may contain considerable amount of antinutritional factors like, phytic acid, tannins and trypsin inhibitors. Phytic acid makes iron, calcium, zinc and magnesium unavailable for use by the body and also binds with proteins (5). Tannins might also reduce protein digestibility by inhibiting the digestive enzymes (6). High levels of trypsin inhibitor activity stimulate pancreatic juice secretion and cause pancreatic hypertrophy and growth (7).

Fermentation has been reported to significantly decrease phytic acid, tannins and trypsin inhibitors (8) and improve protein digestibility *(in vitro)* and the nutritive value of cereals and legumes (9,10). The effect of natural fermentation on aninutrients in tef is, however, lacking. On the other hand, fermentation processes negatively affect the chemical composition and the overall nutritive value of a diet (9,11). A substantial loss of protein (40%) was reported during the fermentation of maize and millet to *ogi* (12). Fermentation of enset also resulted in loss of protein, carbohydrate and calcium (13,14). Natural fermentation has also been reported to decrease the total

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nitrogen content of tef dough (3). *Ersho*, the clear yellow liquid that accumulates on the surface of the fermenting tef-flour batter is discarded during *injera* preparation. The microbial flora and chemical properties of *ersho* has been reported (15). There is, however, paucity of data indicating the loss of nutrients during tef dough fermentation. The present communication deals with studies on the levels of antinutritional components and loss of nutrients during tef dough fermentation.

Methods

White variety tef *(Eragrostis tef)* was purchased from an open market in Addis Ababa, Ethiopia. The seeds were cleaned by sieving to remove foreign matter. The cleaned tef was ground through a 0.5 mm screen in Cyclotec sample mill (Tecator, Sweden). Dough was prepared by mixing the flour with water in a 1:1.6 (w/v) ratio and homogenized. The homogenous slurry was allowed to ferment at 22^0 C for 96 hrs in a glass jar covered with aluminium foil. Samples were removed at the beginning of fermentation, and thereafter at 24 hr intervals, for pH determination and chemical analysis for up to 96 hrs. *Ersho* was collected and its volume measured. To measure the pH of the dough, 10g of the fermenting mass was suspended in equal volume of deionized water and homogenized. pH of the suspension was measured using an Orion (Orion, USA) pH meter. The titratable acidity expressed as percent lactic acid was determined by titrating the decanted homogenate used for pH measurement against 0.1 NaOH to pH 8.3 end point. pH and titratable acidity of *ersho* was determined without further treatment.

For chemical analyses, the fermented dough was dried at 60^0C in an oven. The dried, fermented dough was reground in the Cyclotec sample mill and stored at 4^0C until analyzed.

Total protein of the dough and *ersho* was calculated using the factor 6.25xN after determination of total nitrogen by micro-Kjeldahl method described in the Association of Official Analytical Chemists (AOAC) (16). Total sugar in *ersho* was estimated colorimetrically according to Dubois *et al* (17) and reducing sugars by the method of Miller (18). The difference between total and reducing sugars was taken as non-reducing sugars.

The method Vanillin-HCl described by Maxon and Rooney (19) was employed for the estimation of tannin in tef dough using catechin as a standard and expressed as catechin equivalents (mg/100 g, dry weight).

Trypsin inhibitor activity in tef dough was determined by the method of Kakade *et al* (11) using N-benzoyl-DL-arginine-p-nitroaniline (BAPNA) the trypsin substrate and expressed as TIU/g (dry, weight).

Total iron in tef dough and *ersho* was determined using bathophenanthrolein as described in AOAC (16), phosphorus by the Fiske-Subbarrow method (20) and calcium according to the AOAC (16).

Nonprotein nitrogen (NPN) in tef dough and *ersho* was determined by the procedure of Concon and Soltess (21). The ninhydrin method of Rosen (22) was used to estimate free amino acids in tef dough and *ersho* using leucine as a standard. The degree of protein hydrolysis in the fermented tef dough and the discarded liquid portion was determined according to Tangnual *et al* (23). Fat acidity was determined in 2.0 g samples extracted with 20 ml toluene-ethanol (1:1 v/v), and titrated with 0.0178N KOH. It is expressed as mg KOH/100 g sample (24).

Data analysis: Data were subjected to analysis of variance (ANOVA). Differences were considered significant at $p<0.05$.

Results

Table 1 indicates changes in protein components, fat acidity and protein solubility of fermenting tef dough. As fermentation time increased total protein of the dough decreased. Total protein content of the dough decreased by about 12% after 96 hrs of fermentation.

Nonprotein nitrogen (NPN) increased significantly ($p<0.05$) throughout the fermentation process. This fraction presumably consisted, predominantly, of the products of storage protein degradationamino acids and peptide. The increase accounted for the loss in true protein which was not estimated in this study. The proportion of NPN relative to protein gradually increased. After 96 hrs of fermentation, more than 75% of the nitrogen was in the form of protein. The total amount of amino acid nitrogen, as a measure of protein hydrolysis, increased more than 9-fold during the 96

hrs fermentation as proteins were degraded. Free amino acids and protein solubility similarly increased nearly 7- and 8 - fold, respectively, after 96 hrs of fermentation period.

Fat acidity increased significantly ($p<0.05$) during the first 48 hrs of fermentation which then remained unchanged throughout the fermentation period.

Table 1: **Changes in protein components, fat acidity and protein solubility of fermenting tef dough**

Period hr	Total protein %	Nonprotein Nitrogen %	Amino acid Nitrogen mg/g	Free amino Acids mg/g	Soluble Protein %	Fat acidity mgKOH/100g
0	23.10±0.10 [a]	0.11±0.01 [a]	5.73±0.13 [a]	75.3±1.2 [a]	0.41±0.03 [a]	0.11±0.01 [a]
24	22.92±0.05 [a]	0.22±0.03 [b]	26.24±0.11 [b]	359.3±3.6 [b]	4.33±0.12 [b]	0.91±0.07 [b]
48	22.42±0.13 [b]	0.53±0.02 [c]	27.93±0.08 [b]	393.4±4.7 [c]	5.41±0.09 [c]	1.15±0.04 [c]
72	21.59±0.07 [c]	0.68±0.03 [d]	30.17±0.11 [c]	424.3±7.3 [d]	5.93±0.11 [d]	1.16±0.07 [c]
96	20.33±0.11 [d]	0.81±0.04 [e]	37.60±0.12 [d]	527.2±8.7 [e]	6.97±0.13 [e]	1.18±0.06 [c]

Values are means ± S.D. of five determinations. Values in a column followed by different superscripts are significantly different ($p<0.05$).

The freshly prepared tef dough started to ferment soon after the addition of water and the pH of fermenting tef dough decreased significantly ($p<0.05$) from about 6.1 to 3.8 during the first 72 hrs. Thereafter, the pH of the fermented tef dough remained at similar low levels. Concomitantly with the drop in pH was a sharp rise in titratable acidity of the medium. The highest titratable acidity attained in tef dough fermented at room temperature was 1.26%.

A significant difference was observed in the nonfermented and fermented tef dough samples in their phytic acid contents (Table 2). The amount of phytic acid decreased by 72% by the 96 hr of fermentation. Similarly, tannin and trypsin inhibitor activity contents decreased significantly ($p<0.05$) by 55% and 68.9%, respectively, after 96 hrs of fermentation.

The changes in minerals content is shown in Table 2. All the minerals studied, (calcium, iron and phosphorus), significantly ($p<0.05$) decreased throughout the fermentation period.
The highest decrease was observed in iron (43%) after 96 hrs of fermentation. Table 3 indicates changes in protein components and minerals in *ersho*. The total protein, NPN, amino acid nitrogen and free amino acids content increased significantly ($p<0.05$) with the fermentation time. The content of calcium, phosphorus and iron in *ersho* also increased with the fermentation time. However, the content of phosphorus and iron did not show any noticeable change after 72 hrs of fermentation. As fermentation progressed, total sugar and non-reducing sugars decreased (Table 4). The decrease in the pH of *ersho* with fermentation time was not so rapid as they were in the dough. The pH of *ersho* dropped from 3.93 during 48 hrs of fermentation to 3.77 after 96 hrs of fermentation. *Ersho* had a slightly lower pH than that of the tef dough. The fall in pH resulted in increased titratable acidity of *ersho* which rose from 1.83% at 48 hrs fermentation to 2.47% after 96 hrs of fermentation. The volume of *ersho* discarded from the fermenting tef dough significantly ($p<0.05$) increased with the increases in fermentation time (Table 4).

Table 2: **Changes in antinutritional factors, minerals, pH and titratable acidity of fermenting tef dough**

Time hr	TIA TIU/g	Tannin mg/100g	Phytate mg/100g	Ca mg/100g	P mg/100g	Iron mg/100g	pH mg/100g	TA %
0	5584±67^{a}	881±9^{a}	707±8^{a}	231.5±1.8^{a}	18.4±0.1^{a}	6.1±0.2^{a}	0.14±0.01^{a}	
24	4353±72^{b}	615±4^{b}	510±9^{b}	232.1±2.3^{a}	18.3±0.2^{a}	4.3±0.3^{b}	0.81±0.02^{b}	
48	2761±98^{c}	530±9^{c}	310±5^{c}	214.0±3.7^{b}	15.7±0.1^{b}	4.0±0.1^{b}	0.84±0.03^{b}	
72	2651±83^{d}	471±3^{d}	245±9^{d}	175.6±2.8^{c}	12.5±0.2^{c}	3.8±0.1^{b}	1.05±0.01^{c}	
96	1766±54^{e}	393±8^{e}	198±9^{e}	137.2±1.6^{d}	10.5±0.1^{d}	3.8±0.2^{b}	1.26±0.03 ^{d}	

Values are means ± S.D. of five determination. Values in each column followed by different superscripts are significantly different ($p<0.05$).
TIA -trypsin inhibitor activity; TA-titratable acidity.

Changes in the titratable acidity of the dough were significantly correlated with NPN, free amino acids and amino acid nitrogen ($p<0.05$).

Discussion

Fermentation of tef dough was spontaneous just as in other traditional cereal dough fermentations during which various biochemical changes take place. The rise in the levels of NPN, free amino acids, soluble
proteins and amino nitrogen may be due to hydrolytic action of proteolytic enzymes on dough proteins during tef dough fermentation. The formation of more simple and soluble products after

fermentation may enhance available amino acids content, protein digestibility and the nutritional value of tef.

Previous studies also reported that natural fermentation increased the proteolytic activity and the contents of peptides and amino acids resulting in increased protein digestibility and nutritional values of different foods (9, 10, 25). The increase in fat acidity might be due to increased activity of lipolytic enzymes which produced more free fatty acids which impart their flavour to the final product. A similar observation was reported by Kazanas and Fields (9).

There was a 12% decrease in total protein in fermenting tef dough. The decrease can be attributed to leaching out of the protein into *ersho* which was discarded after 96 hrs of fermentation. Similarly, Gashe et al (3) observed 4 - 13% decrease in total nitrogen content during tef dough fermentation.

Table 3: **Changes in protein components and minerals in *ersho* (mg/ml)**

Time hr	Protein	Non protein nitrogen	amino acid nitrogen	Free amino acids	Ca	P	Iron
48	40.8±0.1[a]	6.2±0.1 [a]	1.66±0.12 [a]	22.36±0.09 [a]	1.22±0.03 [a]	2.34±0.03 [a]	0.17±0.01 [a]
72	82.4±0.1[b]	8.4±0.3 [b]	1.96±0.13 [b]	26.17±0.10 [b]	3.44±0.01 [b]	4.00±0.04 [a]	0.34±0.03 [b]
96	120.6±0.3[c]	14.3±0.2 [c]	2.15±0.10 [c]	29.17±0.07 [c]	4.17±0.02 [c]	3.89±0.01 [a]	0..34±0.02[b]

Values are means ± S.D. of five determinations. Values in each column followed by different letters are significantly different ($p<0.05$).

pH and titratable acidity data on fermented tef dough are typical of cereals and legumes undergoing a natural lactic acid fermentation. A rapid drop in pH with a corresponding increase in titratable acidity has been reported in natural fermentation of various food grains (9, 10, 26).

Natural lactic fermentation of tef dough has resulted in a marked decrease in the content of antinutritional factors. The reduction of phytate content of fermented tef dough may be compared with values reported in fermented corn meal (26). Cereal-based diets are usually low in mineral content. The reduction of phytate content during fermentation would make cereal-based diets a good source of iron,

Table 4. Changes in sugars, pH, titratable acidity and liquid volume in *ersho*

Time hr	Total sugar mg/ml	Redacting sugar mg/ml	Non reducing sugar mg/ml	pH	Titratable acidity%	Liquid volume ml
48	9.05±0.13 [a]	0.41±0.01 [a]	8.74±0.10 [a]	3.93±0.13 [a]	1.83±0.07 [a]	5.32±0.13 [a]
72	7.47±0.11 [b]	.37±0.01 [b]	7.10±0.11 [b]	3.83±0.11 [a]	2.13±0.06 [b]	17.33±0.14 [b]
96	5.71±0.12 [c]	.32±0.02 [c]	5.30±0.14 [c]	3.77±0.12 [a]	2.47±0.11 [c]	23.42±0.13 [c]

Values are means ± S.D. of five determinations. Values in column followed by different letters are significantly different ($p<0/05$).

zinc and calcium since phytate is known to interfere with the absorption of these minerals.

The diminishing effect on polyphenols of tef dough may be due to the activity of the enzyme polyphenoloxidase elaborated during fermentation which has been reported in *rabadi* fermentation (27). Of the three antinutritional factors, phytic acid appeared to be more susceptible to degradation by fermentation. The extent of reduction, however, in the antinutritional factors appeared to be a function of fermentation time. Decrease in the content of these antinutrients during fermentation may improve the digestibility of proteins, and carbohydrates and enhance the availability of minerals. Traditional fermentation of tef flour, thus, seems to have certain advantages.

Ersho contains protein, amino acids, sugars and minerals. The general decrease in sugar levels in *ersho* with increased fermentation time is possibly due to the metabolism of sugars to glycolytic end products such as lactic and acetic acids. Umeta *et al* (28) also reported decreases in starch and sugar contents attributed to the activity of flour amylases during tef dough fermentation. The higher titratable acidity values observed for *ersho* compared to the dough can be attributed to the high buffering capacity of the medium due to the high content of soluble proteins and amino acids. About 41% of calcium, 43% iron and 35% phosphorus in the dough were leached into *ersho* after 96 hrs of fermentation. The concentration of iron in the dough was reduced to 10.5 mg/100 g due to the leaching out of about 8 mg/100 g iron into *ersho*. Consequently, the level of protein, minerals and amino acids in the dough was low. Similarly, minerals and proteins were leached into the steep water during processing of *ogi* (12).

Traditionally, a small portion of *ersho*, which contains a complex group of microorganisms (15), is used as a starter to initiate new fermentation. The use of ersho as inoculum may accelerate the fermentation process and is a potential method of improving the availability of nutrients in tef. New approaches, however, need to be sought to fully utilize *ersho*.

This study indicated that certain biochemical changes occur during traditional fermentation of tef flour. Knowledge gained about these changes during the fermentation process is necessary when commercial production of a fermented tef product with constant characteristics and anticipated quality is being considered.

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