Bull. Chem. Soc. Ethiop. 1997, 11(2), 101-109.
Printed in Ethiopia

ISSN 1011-3294

EFFECTS OF NATURAL AND MIXED CULTURE OF LACTOBACILLI FERMENTATION ON *IN VITRO* IRON AND ZINC BIOAVAILABILTY IN TEF (*ERAGROSTIS TEF*) *ATMIT*

Kelbessa Urga[1]*, N. Keshava [2] and H.V. Narasimha [2]

[1] Ethiopian Health and Nutrition Research Institute, P.O. Box 5654, Addis Ababa, Ethiopia
[2] Central Food and Technological Research Institute, Mysore 570013, India

(Received June 18, 1997; revised October 24, 1997)

ABSTRACT. The *in vitro* availability of iron and zinc in fermented white and brown tef *atmit* (gruel) was studied. Fermentation was carried out naturally and using combinations of single starter cultures of lactobacilli namely; *L. casei, L. fermentum, L. plantarum* and *P. pentosaceus*. Naturally fermented white and brown tef *atmit* had phytic acid levels reduced by 47.6 and 45% whereas mixed culture fermented *atmit* had reduced the phytic acid levels by 73 and 71%, respectively. Increase in zinc solubility and reduction in phytate:zinc molar ratios were significantly higher ($p < 0.05$) in mixed culture fermentation than in naturally fermented tef *atmit*. Natural fermentation increased ionizable iron by more than six-fold whereas, it increased by an average of 68.4 and 78.6%, respectively ,in white and brown tef *atmit* following mixed cultures of lactobacilli fermentation. Ionizable iron in both naturally and mixed cultures of lactobacilli fermented tef *atmit* was significantly higher ($p < 0.05$) in brown tef compared to white tef. Natural and mixed cultures of lactobacilli fermentation improved native ionizable iron and soluble zinc in tef gruels (*atmit*). Unfermented tef *atmit* as a weaning food may not be necessarily beneficial for iron and zinc bioavailability.

INTRODUCTION

Porridges whether fermented or not, have been used for a long time as weaning food in Ethiopia. Traditionally, cereals like tef, sorghum, barley, wheat, and corn or a mixture of the flours from these cereals are used to make stiff porridge (*ganfo*), thin porridge or gruel (*atmit*) and a wide range of fermented beverages including different types of local beers.

Ganfo is made from unfermented meal and is usually eaten in the morning or evening with spices-seasoned soft cheese or butter. *Ganfo* is a popular dish especially with mothers who have just given birth.

Special weaning foods are seldom available at cost affordable by most households. As a result, staple foods, cooked in water are normally fed to weaning age children in the form of gruels (*atmit*). Because it is believed to stimulate milk production, *atmit* supplemented with spiced butter or fermented milk is prescribed for lactating mothers to enhance the nutritional value.

Fermented *atmit* is commonly consumed in rural areas of the country. Fermentation is carried out for two days using a leftover (backslopping) from the previous fermentation lot as an inoculum. Fermented *atmit* is usually prepared for sick people and children of all ages. Traditionally, the naturally occurring micro-organisms in tef flour are utilized in these fermentation. Studies by Gashe [1] showed that lactic acid bacteria play a dominating role in the fermentation of tef dough during *injera* production[1]. The low pH value and presence of fermentation acids means that the fermented *atmit* is less susceptible to infection by food poisoning bacteria than similar unfermented foods and offer safer weaning foods [2-4].

The cereal grain tef (*Eragrostis tef* (Zucc.) Trotter), indigenous to Ethiopia, is one of the

major cereal crops and contributes up to two-thirds of the protein intake of the population where it is commonly consumed [5]. Two types of tef, white and brown are known. Tef is used to make a variety of food items including a sour dough pancake-type baked product called *injera*, porridge and *atmit* (thin gruel). When compared to other cereals tef is better because of minerals like iron, calcium, and zinc [6]. Most of the iron in tef is due to variety (genotype) and environment specific contamination [7-10].

In common with other cereals tef may contain considerable amount of phytic acid. Human studies indicated that phytic acid and other inositol phosphates had very strong inhibitory effect on iron and zinc absorption [11-12].

Degradation of phytic acid and other possible iron and zinc complexes by enzymes of micro-organisms should liberate iron and zinc and increase their availability. Svanberg *et al.* [13] observed that when phytic acid was completely hydrolyzed after germination and fermentation of white sorghum, the amount of bioavailable iron was significantly increased [13]. Fermentation is also known to reduce phytic acid of several plant foods including cereals [14-16].

Recent nutritional research on tef has focused on protein quality [17], microbiology [1, 18] and nutrient composition [10, 19-20] of the grain and fermented products. Few data are, however, reported on availability of minerals from tef foods [21]. The present study was designed to evaluate and compare the effects of natural and mixed cultures of lactobacilli fermentation of tef on iron and zinc bioavailability, estimated *in vitro*, and to measure the degree of phytate hydrolysis in fermented tef (*atmit*) gruels. Solubility at pH 7.5 was used as index bioavailability for zinc; both total soluble and ionizable levels were considered for iron.

EXPERIMENTAL

Preparation of samples. White and brown tef variety seed samples were procured from a local market in Bishoftu, Shoa, Ethiopia. The seed samples were transported to India and stored at 4 °C until used. Tef grains were cleaned of dust and other foreign material, and ground into flour in an electric grinder (M/S Milone, Rajkot, India) using 0.5 mm sieve.

Ersho preparation. A small portion of a left-over from the previous fermented batch was kept viable by recycling about 10% of the previous batch into the same amount of tef flour slurry (10% dm) with incubation at 30 °C until the pH reached about 3.8. Traditionally, this type fermentation is called *ersho* (starter culture). The microbiology of tef *ersho* has been reported earlier [22].

Flour fermentation. Flour and double distilled water (1:3 w/w) were mixed with *ersho* (19 parts flour-water slurry and 1 part *ersho*). The mixture was incubated at 30 °C for two days. Double distilled water was added to the fermented slurry to make a 10% dry matter concentration and then it was cooked for 20 min.

Gruel (atmit) fermentation. Flour and double distilled water were mixed to give 10% dry matter and autoclaved (15 psi for 15 min). After cooling to 45 °C, mixtures of cultures of lactobacilli were added and the *atmit* incubated at 30 °C for two days. The mixed cultures of lactobacilli include *L. casei* (Lund 2) + *P. pentsaceus* (CF 1); *L. fermentum* (MTCC 911) + *L. plantarum*

(NCIM 2083); *L. casei* (Lund 2) + *L. fermentum* (MTCC 911); *L. plantarum* (NCIM 2083)+ *L. casei* (Lund 2); *L. fermentum* (MTCC 911) + *P. pentosaceus* (CF 1) and *P. pentosaceus* (CF 1) + *L. plantarum* (NCIM 2083). Each of the micro-organisms supplied 10^5 cells/ml. The autoclaved unfermented and unfermented cooked *atmit* and tef flour served as controls. Single cultures of the lactic acid bacteria were obtained from the Department of Microbiology, CFTRI (Central Food Technological Research Institute), Mysore, India. All samples were dried for 48 h at 65°C to constant weight and ground as described earlier.

Analyses. Changes in pH during the fermentation process were monitored periodically with a pH meter. Titratable acidity was estimated according to the method of Nout *et al.* [4] and reported as % lactic acid.

For total iron and zinc estimation, the samples were wet-acid digested using nitric acid and perchloric acid mixture (HNO_3 : $HClO_4$, 5:1 v/v). The amount of iron and zinc in the digested samples were determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 3110, Norwalk, USA) according to the method of Lindsey and Norwell [23].

In vitro method for predicting bioavailalbility of iron in the *atmit* samples was carried out as described by Rao and Prabhavathi [24]. Optimal conditions for the enzyme (pepsin) activity were standardized by using various enzyme concentrations ranging from 0.1-0.8% against known substrate. Two g of the study samples was mixed with 25 mL of 0.5% pepsin (P-7000, Sigma in 0.01 M HCl. The pH of the mixture was adjusted to 1.35 with 2 M HCl and incubated in a 100 mL conical flask at 37°C for 90 min in a an incubator shaker (Environ Shaker Model 3597-I, LabLine Instruments, Melrose Park, Ill., USA) set at 100 rpm. At the end of the incubation, contents were centrifuged at 4500 rpm for 30 min and the supernatant filtered through Whatman No. 44 filter paper. The pH of the filtrate was adjusted to 7.5 with 2 M and 0.1 M NaOH and the contents again incubated, centrifuged and filtered as described earlier. The supernatant which was filtered again was used for the determination of soluble iron and zinc as well as ionizable iron. Free form of the iron (ionizable iron) in the filtrate which reacts with α, α'-dipyridyl to yield colour, was determined by AOAC [25]. Soluble iron was estimated by the method of Tennat and Greenman [26] by digesting the filtrate with potassium permanganate followed by decolorizing with ascorbic acid and filtration. The iron in the filtrate was determined by the α, α'-dipyridyl method [25]. For estimation of zinc in the *in vitro* digestion samples, the supernatant which was used for soluble and ionizable iron determination was evaporated to dryness on a hot plate. The dry residue was wet-acid digested using perchloric acid and nitric acid mixture and zinc determined by atomic absorption spectrophotometry as described earlier. Phytic acid in the samples was estimated colorimetrically [27].

Statistical analysis. Data from the experiments were evaluated by analysis of variance, followed by the student's t test to compare bioavailability of iron and zinc and phytate contents of *atmit* fermented using starter cultures of lactobacilli to that from naturally fermented *atmit* at the $p < 0.05$ probability level [28].

RESULTS AND DISCUSSION

Table 1 indicates the pH of unfermented and fermented white and brown tef *atmit*. The pH the fermented *atmit* had decreased significantly ($p < 0.05$) following two days of natural lactic acid

fermentation. With the drop in pH, a concomitant rise in titratable acidity of the *atmit* was also observed.

Fermentation with inocula of mixed cultures of lactobacilli significantly decreased ($p < 0.05$) the pH of both white and brown tef *atmit* by an average of 50% following two days of fermentation (Table 1). This sharp drop in pH is essential to the prevention of growth of food poisoning bacteria [3-4]. Concomitantly with drop in pH was a significant rise ($p < 0.05$) in titratable acidity of the *atmit* (Table 1). *L. fermentum* + *L. plantarum* and *P. pentosaceus* + *L. fermentum* exhibited the greatest pH lowering effect and the corresponding higher titratable acidity in both tef varieties. The lower pH and higher titratable acidity of the fermented *atmit* may be attributed to the production of organic acids by the microflora [2]. Rapid drops in pH with corresponding increase in titratable acidity have been reported in lactic acid fermentation of corn [15].

Table 1 also summarizes the phytic acid (expressed as phytate phosphorus) content of fermented and unfermented tef *atmit*. Cooking of the unfermented slurries did not affect the phytate content of the tef *atmit*. However, autoclaving of the unfermented *atmit* resulted only in marginal reduction in phytic acid content. In contrast, natural lactic fermentation of tef flour slurry decreased the phytate content of the white and brown tef varieties by 47.6 and 45%, respectively, following 48 h fermentation and then cooking into *atmit*. Hydrolysis of phytate has been reported earlier in lactic fermented corn [15], sorghum [16] and *uji* [29].

Similarly, mixed culture fermented tef *atmit* had lower phytate content compared to the autoclaved unfermented white and brown tef *atmit* (Table 1). Among the mixed cultures, *L. fermentum* + *L. casei* had the most pronounced phytate lowering effect in both white and brown tef *atmit*. On average, the phytate content of mixed culture lactobacilli fermented tef *atmit* (white and brown varieties) decreased by about 73 and 71%, respectively. Fermentation by mixed cultures of lactobacilli thus significantly decreased ($p < 0.05$) the phytate content of tef *atmit* compared to the *atmit* prepared from naturally fermented tef flour slurries. However, phytic acid concentration in both tef variety *atmit* was not completely degraded by natural lactic or mixed culture lactobacilli fermentation.

Microbial phytase, as reported in several micro-organisms [15, 30], may hydrolyze phytate during fermentation and account for the reduction in phytate content in the fermented *atmit*. Acidic pH of the fermented *atmit* may also provide favorable conditions for phytase activity. Phytate reduction by pure cultures of yeasts and lactobacilli has similarly been reported earlier in *rabadi*, a fermented pearl millet food product [31].

Table 2 and 3 summarize soluble zinc and phytate:zinc molar ratios of fermented and unfermented *atmit*. The solubility of zinc in the unfermented cooked and autoclaved unfermented *atmit* with a phytic acid content 981 and 934 μmol was about 8 and 9% and 9 and 10%, respectively (Tables 2 and 3). These values can be compared to whole grain cereal-based meals where as low as 5% absorption has been observed from meals high in phytate content [12, 14].

The phytate:zinc molar ratios were more than 28 in flour, unfermented cooked and autoclaved unfermented *atmit* of white tef, whereas this value was more than 29 in brown tef flour and unfermented *atmit* (Table 3) which still showed low zinc solubility. Such habitually low soluble zinc and high molar ratios in the unfermented *atmit* may jeopardize zinc status of the infants if consumed as the sole weaning food. This would also be consistent with earlier findings of low zinc status in populations consuming larger quantities of unleavened bread [32].

Table 1. pH, titratable acidity (%), and phytic acid (μmol) contents of the control and fermented tef *atmit*[*]

Food	Ino-culum	White tef			Brown tef		
		pH	TA	Phytate P	pH	TA	Phytate P
Flour	-	-	-	986.57 ±3.21[a]	-	-	1042.56 ±3.54[a]
Control [1]	-	6.56 ±0.10[a]	0.31 ±0.02 [a]	981.43 ±1.75[a]	6.37 ±0.07 [a]	0.26 ±0.01 [a]	1034.12 ±3.51 [a]
Atmit	Natural	4.23 ±0.08[b]	1.23 ±0.09 [b]	514.13 ±2.12[b]	4.40 ±0.02 [b]	1.01 ±0.09 [b]	570.27 ±4.84 [b]
Control [2]	-	6.66 ±0.07[a]	0.31 ±0.06 [a]	934.83 ±2.74[a]	6.64 ±0.07 [a]	0.27 ±0.03 [a]	962.88 ±1.37 [a]
Atmit [3]	Lc+Pp	3.28 ±0.05[c]	1.25 ±0.08[c]	259.29 ±3.36[c]	3.35 ±0.04[c]	1.24 ±0.04[c]	308.94 ±4.31[e]
	Lf+Lp	3.26 ±0.03[c]	1.27 ±0.06[c]	294.10 ±1.01[d]	3.20 ±0.05[c]	1.29 ±0.07[c]	230.88 ±2.07[d]
	Lc+Lf	3.35 ±0.06[c]	1.24 ±0.05[c]	240.16 ±3.12[c]	3.25 ±0.03[c]	1.26 ±0.04[c]	258.71 ±2.91[e]
	Lp+Lc	3.35 ±0.04[c]	1.26 ±0.06[c]	202.99 ±2.43[e]	3.41 ±0.04[c]	1.23 ±0.06[c]	282.09 ±4.43[f]
	Lf+Pp	3.27 ±0.05[c]	1.28 ±0.09[c]	255.04 ±2.31[c]	3.35 ±0.03[c]	1.25 ±0.07[c]	282.88 ±2.51[f]
	Pp+Lp	3.42 ±0.03[c]	1.24 ±0.03[c]	240.72 ±2.23[c]	3.43 ±0.04[c]	1.21 ±0.05[c]	294.10 ±5.23[f]

*Means in a column with different superscript letters are significantly different ($p < 0.05$). [1] Tef gruel cooked without fermentation; [2]autoclaved unfermented tef gruel; [3]Lc = *Lactobacillus casei*; Pp =*Pediococcus pentosaceus*; Lf=*Lactobacillus fermentum*; Lp = *Lactobacillus plantarum*.

However, when the phytate content was reduced by natural lactic acid fermentation to approximately 514 and 570 μmol, solubility of zinc in white tef (Table 2) and brown (Table 3) tef *atmit* increased by 55 and 50%, respectively. The phytate: zinc molar ratio has, in contrast, decreased by 51% and 48%, respectively, in white tef (Table 2) and brown tef (Table 3) *atmit* following 48 h fermentation compared with the unfermented *atmit*.

Mixed culture fermentation by lactobacilli also significantly increased ($p < 0.05$) zinc solubility and reduced the phytate:zinc molar ratios in fermented white and brown tef *atmit*. The phytate : zinc molar ratios decreased by an average value of 71% in white tef *atmit* (Table 2) and 73% in brown tef *atmit* (Table 3) compared with the controls. This is in agreement with results from earlier studies of cereal-based meals which indicated that a molar ratio of phytic acid to zinc of less than 10 is desirable and low phytate to zinc molar ratio in various foods is associated with

Table 2. Soluble Zn (%), ionizable Fe, soluble Fe, predictable Fe and phytate:zinc molar ratio of the control and fermented white tef *atmit*[*].

Diet	Inoculum	Soluble Zn at pH 7.5	Ionizable Fe, %	Soluble Fe, %	Predicted Fe**, %	Pa/Zn
Flour	-	8.13±0.19 [a]	9.56±0.15[a]	10.22±0.20[a]	4.98[a]	28.66±2.13[a]
Control [1]	-	9.41±0.05 [a]	8.52±0.83[b]	13.52±0.56[b]	4.49[a]	28.43±1.98[a]
Atmit	Natural	54.78±0.18 [b]	50.68±0.31[c]	52.27±0.88[c]	24.34[b]	13.89±0.69[b]
Control [2]	-	13.10±0.16[c]	11.08±1.08[a]	14.36±1.26[b]	5.70[c]	26.93±1.17[a]
Atmit [3]	Lc+Pp	74.72±0.17[d]	61.22±0.50[d]	66.46±0.83[d]	29.29[d]	7.47±0.12[c]
	Lf+Lp	53.70±0.20[b]	70.68±1.59[e]	73.69±1.58[e]	33.76[e]	8.48±0.17[c]
	Lc+Lf	58.10±0.22[e]	63.44±1.34[d]	69.91±0.84[d]	32.49[e]	6.90±0.14[d]
	Lp+Lc	65.15±0.31[f]	61.90±0.45[d]	69.26±1.21[d]	29.56[d]	5.84±0.27[e]
	Lf+Pp	72.05±0.41[d]	87.79±2.65[e]	89.37±1.63[f]	41.75[f]	7.37±0.32[c]
	Pp+Lp	59.13±0.36[e]	64.96±0.82[d]	75.28±0.81[e]	31.30[d]	6.90±0.46[e]

[*] Mean ± standard deviation of three tests and are expressed on dry basis. [**] Based on prediction equation [24] $y = 0.4827 + 0.4707x$, where x = % ionizable iron at pH 7.5. Pa/Zn = phytate: zinc molar ratio. Means in a column with different superscript letters are significantly different ($p < 0.05$). [1,2,3]defined in Table 1.

increased zinc absorption in rats [33-34]. Similarly, Navert *et al.* [14] reported that zinc absorption from a high phytate bread can be increased by increasing the fermentation time and thereby decreasing the phytate content and phytate : zinc molar ratios [14]. Stuart *et al.* [35] also observed that rats were able to absorb significantly higher amount of zinc from the fermented sorghum food than from the unfermented sorghum or maize gruels presumably due to the lower phytate : zinc molar ratio in the product [35].

Ionizable iron in *in vitro* digestion is indicative of iron bioavailability and correlates well with iron absorption measurements in human subjects [24]. The ionizable iron in unfermented white and brown tef *atmit* was not different from that found in flours (Table 2). The lower ionizable iron in unfermented cooked *atmit* in this study may have been due to the binding of iron in phytate as reported by Hallberg *et al.* [11].

Natural lactic acid fermentation of tef flour *atmit* resulted in a significant increase ($p < 0.05$) in ionizable iron. Ionizable iron and soluble iron in fermented white and brown tef *atmit* increased about six-fold (Tables 2 and 3). Similarly, soluble iron in naturally fermented *atmit* increased by about four-fold in both white and brown tef *atmit*. However, the soluble iron level in both varieties of tef differed significantly ($p < 0.05$) with white tef indicating higher levels of soluble iron (Table 2) compared with brown tef (Table 3).

Natural fermentation also resulted in increases in predicted available iron in fermented *atmit*.

Table 3. Soluble Zn (%), ionizable Fe, soluble Fe, predictable Fe and phytate : zinc molar ratios of the control and fermented brown tef *atmit*[*]

Diet	Ino-culum	Soluble Zn at pH 7.5	Ionizable Fe, %	Soluble Fe, %	Predictable F**, %	Pa/Zn
Flour	-	7.76±0.07[a]	8.00±0.13[a]	6.74±0.31[a]	4.25[a]	29.69±3.17[a]
Control [1]	-	8.03±0.13[a]	9.36±0.85[b]	12.67±0.33[b]	4.89[a]	29.63±4.11[a]
Atmit	Natural	50.37±0.39[b]	58.79±0.67[b]	48.79±0.13[c]	28.16[b]	15.28±1.47[b]
Control [2]	-	9.16±0.12[a]	10.19±0.58[a]	19.93±0.84[d]	5.28[a]	27.74±1.81[a]
Atmit [3]	Lc+Pp	59.91±0.23[c]	79.02±1.35[c]	85.52±0.84[e]	37.64[c]	8.91±0.63[c]
	Lf+Lp	57.97±0.26[c]	72.50±0.86[d]	81.88±0.57[e]	34.57[c]	6.65±0.21[d]
	Lc+Lf	67.47±0.41[d]	89.59±1.46[e]	95.35±1.59[f]	42.61[d]	7.46±0.33[e]
	Lp+Lc	77.30±0.34[e]	62.44±0.81[f]	67.96±0.85[g]	29.86[b]	8.10±0.18[f]
	Lf+Pp	56.27±0.11[c]	87.84±1.67[e]	96.75±2.07[h]	41.81[d]	8.14±0.22[f]
	Pp+Lp	62.20±0.15[d]	80.53±1.43[e]	81.44±1.38[e]	38.33[c]	8.48±0.63[f]

*Mean ± standard deviation of three tests and are expressed on dry basis. ** Based on prediction equation [24] y = 0.4827 + 0.4707x, where x = % ionizable iron at pH 7.5. Pa/Zn = phytate : zinc molar ratio. Means in a column with different superscript letters are significantly different ($p < 0.05$). [1,2,3] defined in Table 1.

Predicted available iron in white and brown tef *atmit* increased more than five-fold compared to the unfermented cooked *atmit*.

The increase in ionizable and soluble iron in fermented *atmit* may be attributed to the reduction in phytic acid content as demonstrated earlier. *In vitro* experiments have shown a strong negative influence of phytic acid on the bioavailability of iron and zinc. Indumadhavi and Agte [36] also reported 30, 58 and 31% increase in ionizable iron in fermented rice, sorghum and Bengal gram, respectively [36]. Svanberg *et al.*[13] similarly observed that addition of the enzyme phytase to a naturally fermenting gruel increased the concentration of available iron by 50% [13]. Ramachandran and Bolodia [21] also observed similar trends in fermented tef. In a human study, iron absorption from maize or sorghum fermented beverage was ten times greater than that from a cooked gruel made from the same ingredients [37].

The content of ionizable iron in all the *atmit* fermented by mixed cultures of lactobacilli increased significantly ($p < 0.05$) compared with the autoclaved unfermented *atmit*. Highest ionizable iron values were observed in mixed culture fermentation of *L. fermentum* + *P. pentosaceus* and *L. fermentum* + *L. plantarum* in white tef *atmit* (Table 2); *L. casei* + *L. fermentum*, *L. plantarum* + *P. pentosaceus*, and *L. casei* + *P. pentosaceus* in brown tef *atmit* (Table 3). Percent ionizable iron values increased on average by more than six-fold and seven-fold in white tef and brown tef *atmit*, respectively, after mixed culture of lactobacilli fermentation which are comparable to values obtained by natural lactic fermentation of both tef varieties.

However, average value of percent ionizable iron in brown tef *atmit* was much higher compared with white tef *atmit*.

The levels of soluble iron in the *atmit* similarly varied depending on the mixed culture fermentation and tef variety. *L. fermentum* + *P. pentosaceus*, *L. plantarum* + *P. pentosaceus* and *L. fermentum* + *L. plantarum* combinations exhibited the highest values for soluble iron in white tef *atmit* (Table 2). Mixed culture combinations with higher ionizable values similarly resulted in brown tef *atmit* (Table 3). Predicted available iron varied in a trend similar to the ionizable and soluble iron values in both tef variety *atmit*.

Since phytate has been implicated as an interfering factor in the availability of divalent metal ions, the results of the present study suggest that decreases in phytic acid contents on fermentation of tef *atmit* had beneficial effect on iron and zinc availability as demonstrated in this and earlier studies [13-14, 34-35].

In vitro methods appear to be appropriate for making a relative comparison of the effect of food processing such as fermentation, since they show the same effect of enhancers and inhibitors as *in vivo* studies. The findings in the present study indicate the usefulness of both natural and mixed cultures of lactobacilli fermentation as a process for improving the native ionizability of iron and solubility of zinc. In contrast, unfermented tef *atmit* as weaning food may not be necessarily beneficial for iron and zinc bioavailabilty. In Ethiopia where food fortification is not a possible option, these types of fermented foods may have a potential to significantly improve the iron and zinc status of a population.

ACKNOWLEDGEMENTS

This study was financially supported by the United Nations University, Tokyo, Japan and Central Food Technological Research Institute, Mysore, India. The excellent technical assistance of B.V. Sasikala is gratefully acknowledged.

REFERENCES

1. Gashe, B.A. *J.Food Sci.* **1985**, 50, 800.
2. Umeta, M.; Faulks, R.M. *J. Cereal Sci.* **1989**, 9, 91.
3. Girma, M.; Gashe, B. A.; Lakew, B. *MICERN J. Appl. Microbiol. Biotechnol.* **1989**, 5, 61.
4. Nout, M.J.R.; Rombouts, F.M.; Huvelaar, A. *Intl. J. Food Microbiol.* **1989**, 8, 351.
5. Interdepartmental Committee for Nutrition and National Defence, *Ethiopian Nutrition Survey*, Addis Ababa, Ethiopia, 1959.
6. Mengesha, M.H. *Econ. Bot.* **1966**, 20, 268.
7. Hofvander, Y. *Acta Ped. Scand, Suppl.* **1968**, 494, 1.
8. Bisrat, A.; Admasu, A.; Ogbai, M. *Ethiop. Med. J.* **1980**. 18, 45.
9. Agren, G. *Acta Soc. Med. Upsal.* **1970**, 20, 268.
10. Areda, A. *SINET: Ethiop. J. Sci.* **1995**, 18, 221.
11. Hallberg, L.; Rosander, L.; Skanberg, A.B. *Am. J. Clin. Nutr.* **1987**, 45, 988.
12. Sandstrom, B.; Almagren, A.; Kivisto, B.; Cederblad, A. *J. Nutr.* **1987**, 117, 1898.
13. Svanberg, U.; Lorri, W.A.; Sandberg, A.S. *J. Food Sci.* **1993**, 58, 408.

14. Navert, B.; Sandstorm, B.; Cederblad, A. *Br. J. Nutr.* **1985**, 53, 47.
15. Lopez, Y.; Gordon, D.T. ; Fileds, M.L. *J. Food Sci.* **1983**, 48, 953.
16. Kebede, B.; Urga, K. *SINET: Ethiop. J. Sci.* **1995**, 18, 207.
17. Almgard, G. *Lanthbrukshogsk. Ann.* **1963**, 29, 215.
18. Gifawesen, C.; Bisrat, A. *SINET: Ethiop. J. Sci.* **1982**, 5, 21.
19. Areda, A.; Ketema, S.; Ingram, H.; Davis, R.H.D. *SINET: Ethiop. J. Sci.* **1993**, 16, 5.
20. Umeta, M.; Faulks, R.M. *Food Chem.* **1988**, 27, 181.
21. Ramachandran, K.; Bolodia, G. *Ethiop. Med. J.* **1984**, 22, 45.
22. Ashenafi, M. *World J. Microbiol. Biotechnol.* **1994**, 10, 69.
23. Lindsey, W.L.; Norwell, M.A. *Agron. Abstr.* **1969**, 61, 84.
24. Narasinga Rao, B.S.; Prabhavathi, I. *Am. J. Clin. Nutr.* **1978**, 31,169.
25. AOAC, *Official Methods of Analysis*, Washington, D.C., 1995
26. Tennat, G.B.; Greenman, D. A. *J. Clin. Pathol.* **1969**, 22, 201.
27. Haug, W.; Lantzsch, H.J. *J. Sci. Food Agric.* **1983**, 34, 1423.
28. Snedcor, G.W.; Cochran, W.G. *Statistical Methods*, 8th edn., Iowa State University Press: Ames; 1989.
29. Mbugua, S.K.; Ledford, R.A.; Steinkraus, K.H. *Food Chem. Microbiol. Technol.* **1983**, 8, 90.
30. Daniels, D.G.H.; Fisher, N. *Br. J. Nutr.* **1975**, 46, 1.
31. Khetarpaul, N.; Chauhan, B.M. *J. Food Sci.* **1989**, 54, 780.
32. Ter-Sarkissian, N.; Azar, M.; Ghavifker, H.; Ferguson, T.; Hedayat, H. *J. Am. Diet. Assn.* **1974**, 65, 651.
33. Sandstrom, B.; Kvisto, B.; Cederblad, A. *J. Nutr.* **1987**, 117, 321.
34. Lo, G.S.; Settle, S.L.; Steinke, F.H.; Hopkins, D.T. *J. Nutr.* **1981**, 111, 2223.
35. Stuart, M.A.; Johnson, P.E.; Hamaker, B.; Kirleis, A. *J. Cereal Sci.* **1987**, 6, 81.
36. Indumadhavi, M.; Agte, V. *Intl. J. Food Sci. Technol.* **1992**, 27, 221.
37. Derman, D.P.; Bothwell, T.H.; Torrance, J.D.; Bezwoda, W.R.; MacPhail, A.P.; Kew, M.C.; Sayers, M.H.; Disler, P.B.; Charlton, R.W. *Br. J. Nutr.* **1980**, 43, 271.