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EFFECT OF FERMENTATION BY MIXED CULTURES OF LACTIC ACID BACTERIA ON THE HCl-EXTRACTABILITY OF SOME MINERALS FROM TEF (*ERAGROSTIS TEF*) *ATMIT*

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ABSTRACT: Tef *atmit* (thin gruel) was fermented by mixed cultures of lactic acid bacteria (LAB), namely, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Pediococcus pentosaceus*, at 30° C for 2 days. The fermentation brought about a significant increase in inorganic phosphorus, and HCl-extractability of phosphorus with a corresponding decline in phytic acid content of tef *atmit*. The contents of phytate to zinc molar ratio, an index of zinc bio-availability, in the fermented products decreased corresponding to a decrease in phytic acid. Fermentation also improved the HCl-extractability of iron, calcium and zinc in *atmit*. Reduction in phytic acid and phytate to zinc molar ratio and increase in inorganic phosphorus was more pronounced in *atmit* fermented with *Lactobacillus casei* + *Lactobacillus plantarum* compared to the other cultures. Unfermented tef *atmit* as a weaning food may not be necessarily beneficial for iron and zinc bio-availability.

Key words/phrases: Fermentation, HCl-extractability of minerals, lactic acid bacteria, phytate, tef *atmit*

INTRODUCTION

In Ethiopian cultures, most traditional weaning foods are non-milk family foods, based on the local staple-usually a cereal, such as tef (*Eragrostis tef*), maize, sorghum or barley. In the South-western regions, however, infant diets are prepared from a root crop enset (*Ensete ventricosum*), which is the common staple of the regions.

Infant weaning foods are usually given in the form of gruels or boiled semi-liquid preparations called *atmit*. To achieve the desired free-flowing consistencies, slurries containing about 10% flour are used in the preparation of the gruels (*atmit*).

Tef is a staple food for a large segment of population in Ethiopia where it contributes a major part of dietary nutrients (Ethiopian Nutrition Survey, 1959). The cultivation of tef as a cereal is virtually confined to Ethiopia.

A high intake of cereal grains has been associated with iron deficiency anaemia and zinc deficiency in the population of developing countries, because the availability of minerals from plant foods such as cereals is limited due to the presence of anti-nutrients (Hallberg, 1981).

In common with other cereals, tef may contain the anti-nutrient phytic acid which complexes with divalent cations rendering the minerals in tef biologically unavailable for human system.

Tef fermentation is commonly used in Ethiopia for making, in addition to beverages, a number of foods such as *injera*, and thin gruel (*atmit*). *Injera*, a flat pan-cake type sour bread, constitutes a major part of the staple diet in the highlands of Ethiopia. The use of fermented *atmit* is popular in rural areas while unfermented *atmit* is used as infant diet in urban centres. Fermented *atmit* is usually prepared for sick people and children of all ages. Because it is believed to increase milk output, *atmit*, fermented and unfermented, is traditionally given to lactating mothers. It is not uncommon to invite a visiting guest a glass of fermented *atmit* prepared from tef in Western Ethiopia.

It is a common practice to prepare fermented *atmit* from a previously fermented tef dough. Such fermented product is less susceptible to infection by food poisoning bacteria than similar unfermented foods and offers safe weaning foods for children. Tef fermentation has been shown to be efficient in inhibiting and eliminating pathogenic micro-organisms (Meaza Girma *et al.*, 1989). Traditionally, the naturally occurring micro-organisms in tef flour are utilised in these fermentations. Berhanu Abegaz Gashe (1985) isolated five species of lactic acid bacteria, namely, *Leuconostoc mesentroides*, *Pediococcus cerevisiae*,

Lactobacillus plantarum, *Lactobacillus fermentum*, *Lactobacillus brevis* and yeasts from the fermenting tef flour/water slurry.

Fermentation is known to reduce phytic acid content of several plant foods including rice, corn, millet, sorghum (Daniels and Fisher, 1975; Reddy and Salunkhe, 1980; Lopez *et al.*, 1983; Binyam Kebede and Kelbessa Urga, 1995) converting bound form of minerals to free form which is responsible for increased HCl-extractability of the minerals of the fermented products (Chompreeda and Fields, 1984). Improvements in HCl-extractability of minerals from pearl millet by fermentation with pure cultures of yeasts and lactobacilli has also been reported earlier (Khetarpaul and Chauhan, 1989). Data on HCl-extractability of minerals from fermented tef *atmit*, however, is lacking.

This paper reports the effect of fermentation by mixed pure cultures of LAB on HCl-extractability of minerals from tef *atmit* in 0.03N HCl (the concentration of the acid content in the human stomach).

MATERIALS AND METHODS

Preparation of samples

White 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.

Gruel (atmit) *fermentation*

Pure cultures of the lactic acid bacteria were obtained from the Department of Microbiology, CFTRI (Central Food Technological Research Institute), Mysore, India. The LAB were propagated and grown in reconstituted MRS broth [de Mann, Rogosa and Sharpe (Oxoid)] incubated at 30° C with shaking for 24 hr. This was followed by three successive sub-culturing in MRS broth at 30° C at 24 hr intervals until a final concentration of 10^8 or 10^9 cells $(ml)^{-1}$ was obtained. The cells were harvested by centrifugation at 5000 X g for 10 min, washed

twice and resuspended in sterile distilled water. These suspensions were used to inoculate (10% v/v) the porridges.

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 pure cultures of lactobacilli were added to the *atmit* to give a final inoculum level of 10^5 cells $(ml)^{-1}$ and incubated at 30° C for two days. The pure culture combinations of lactobacilli include *Lactobacillus casei* (Lund 2)+ *Pediococcus pentosaceus* (CF 1), *Lactobacillus fermentum* (MTCC 911)+ *Lactobacillus plantarum* (NCIM 2083), *Lactobacillus casei* (Lund 2)+ *Lactobacillus fermentum* (MTCC 911), *Lactobacillus plantarum* (NCIM 2083)+ *Lactobacillus casei* (Lund 2), *Lactobacillus fermentum* (MTCC 911)+ *Pediococcus pentosaceus* (CF 1), *Pediococcus pentosaceus* (CF 1)+ *Lactobacillus plantarum* (NCIM 2083). The autoclaved unfermented and unfermented cooked *atmit* and tef flour served as controls. All samples were dried for 48 hr at 65° C to constant weight and ground as described earlier.

pH and titratable acidity

During the period of fermentation, the changes in pH were monitored periodically with a pH meter. Titratable acidity was estimated according to the method of Nout *et al.* (1989) and reported as % (w/w) lactic acid.

Phytate and inorganic phosphorus

The samples were extracted in 0.3 N HCl with continuous shaking for 3 hr in a mechanical shaker at room temperature. Phytic acid in the extract was estimated colorimetrically (Haug and Lantzsch, 1983).

Phytate phosphorus was derived by using the following formula (Reddy *et al.*, 1982):

$$Phytate\ phosphorus\,(mg) = \frac{A x 28.18}{100},$$

where A is phytate content.

Inorganic phosphorus in the sample was extracted in double distilled water by shaking at room temperature for 3 hr. Inorganic phosphorus in the extract was determined colorimetrically (Fiske and Subarrow, 1925).

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 spectrophotometry (Perkin-Elmer, Model 3110, Norwalk, CT, USA) according to the method of Lindsey and Norwell (1969). Phosphorus in the digested samples was estimated colorimetrically (Fiske and Subarrow, 1925), whereas calcium was determined by the titration method (AOAC, 1995).

HCl-extractability 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 at 37°C for 3 hr. The clear extract obtained after filtration with Whatman #42 filter paper was oven-dried at 100°C and wet-digested with diacid mixture. The amounts of extractable phosphorus, iron, calcium and zinc in the digested samples were determined by the methods described earlier.

$$Mineral\ extractability\,(\%) = \frac{Miniral\ extractable\ in\ 0.03N\ HCl}{Total\ mineral} x\,100.$$

Statistical analysis

The data were subjected to analysis of variance in a completely randomised design (Panse and Sukhatme, 1961).

RESULTS AND DISCUSSION

The unfermented tef flour contained phytate phosphorus constituting 57.7% of total phosphorus (Table 1). Cooking into gruel (*atmit*) of the unfermented flour/water slurry had no effect on the contents of phytic acid while autoclaving

the flour/water slurry reduced the phytic acid content only marginally (9%). Similarly, the phytate:zinc molar ratio contents in the unfermented *atmit* and tef flour were not affected. No apparent changes in the concentrations of the minerals (iron, calcium, zinc and phosphorus) were observed during the preparation of tef flour/water slurry into fermented and unfermented *atmit* (Table 1).

Table 1. Composition of tef products [mg(100g)$^{-1}$]*.

Composition	Products			
	Flour	Atmit[1]	Atmit[2]	Atmit[3]
pH	6.62±0.13	6.56±0.10	6.66±0.07	3.32±0.04
Iron	9.31±0.19	9.32±0.26	9.32±0.02	9.33±0.27
Calcium	197.09±7.65	197.32±4.13	198.03±5.12	203.13±7.58
Zinc	2.30±0.02	2.26±0.04	2.30±0.09	2.31±0.13
Total phosphorus	324.63±4.17	320.93±5.23	323.13±8.21	326.28±7.18
Inorganic phosphorus	58.83±1.87	72.38±1.27	80.16±4.14	229.89±3.17
Phytate phosphorus	188.11±2.25	187.21±1.75	178.32±1.27	47.36±2.69
Phytate:zinc molar ratio	28.66±2.13	28.42±1.98	26.93±1.17	7.16±0.25

*, Mean values ± standard deviation of three tests are expressed on dry basis;
[1], Tef *Atmit* cooked without fermentation; [2], Autoclaved unfermented tef *Atmit*;
[3], Average values of *atmit* fermented by: Lc (*Lactobacillus casei*); Pp (*Pediococcus pentosaceus*); Lf (*Lactobacillus fermentum*); and Lp (*Lactobacillus plantarum*).

Fermentation with inocula of mixed cultures of LAB significantly ($p<0.05$) decreased the pH of the *atmit* to an average value of 3.2, following two days of fermentation with a corresponding increases in titratable acidity (expressed as % lactic acid) (Table 2). In mixed pure culture fermentation by LAB, *L. fermentum* + *L. plantarum*, *L. fermentum* + *P. pentosaceus*, *L. casei* + *P. pentosaceus* exhibited the greatest pH lowering effect and the corresponding high titratable acidity in the *atmit*. The low pH and high titratable acidity in the fermented *atmit* may be due to the production of organic acids by the microflora. Rapid drop in pH with a corresponding increase in titratable acidity

has been reported in lactic fermentation of various foods including corn and sorghum (Lopez *et al.*, 1983; Nanson and Fields, 1984).

Table 2. Fermentation characteristics of tef *atmit* fermented by mixed cultures of lactobacilli*.

Inoculum	pH	TA %	Pp mg(100g)$^{-1}$	Pi mg(100g)$^{-1}$	Pa/Zn
Atmit[2]	6.66±0,07[a]	0.21±0.04[a]	178.32±1.75[a]	80.1±4.14[a]	26.93±1.17[a]
L. casei + *P. pentosaceus*	3.28±0.05[b]	1.25±0.08[b]	49.46±1.38[b]	227.9±2.83[b]	7.47±0.12[b]
L. fermentum + *L. plantarum*	3.26±0.03[b]	1.27±0.06[b]	56.10±6.01[c]	223.1±3.21[b]	8.48±0.17[c]
L. casei + *L. fermentum*	3.35±0.06[b]	1.24±0.05[b]	45.81±2.12[d]	230.6±3.81[b]	6.90±0.14[b]
L. plantarum + *L. casei*	3.35±0.04[b]	1.26±0.06[b]	38.72±1.24[e]	235.2±1.95[c]	5.84±0.27[d]
L. fermentum + *P. pentosaceus*	3.27±0.05[b]	1.28±0.04[b]	48.65±2.31[b]	232.2±3.17[c]	7.37±0.32[b]
P. pentosaceus + *L. plantarum*	3.42±0.03[b]	1.24±0.04[b]	45.41±3.12[d]	230.2±4.01[c]	6.90±0.46[b]

*, Mean values ± standard deviation. TA, titratable acidity; Pp, phytate phosphorus; Pi, inorganic phosphorus; Pa/Zn, phytate:zinc molar ratio; [2], Autoclaved unfermented tef *atmit*. Means in a column with different superscript letters are significantly different ($p < 0.05$).

Fermentation of the autoclaved *atmit* resulted in a significant decline in phytic acid (expressed as phytate phosphorus) presumably due to its hydrolysis which was demonstrated by more than two-fold increase in inorganic phosphorus content following two days of fermentation (Table 2). Among the mixed culture fermentations, *L. plantarum* + *L. fermentum* lowered the phytate phosphorus to a marked extent followed by *L. plantarum* + *P. pentosaceus* and *L. casei* + *L. fermentum*. Therefore, tef *atmit* fermented with *L. plantarum* + *L. casei* had the lowest amount of phytate phosphorus and highest amount of inorganic

phosphorus. Complete removal of phytic acid from the fermented *atmit*, however, was not observed.

The marked phytase activity as found in various microorganisms may hydrolyse phytic acid during mixed culture fermentation of tef *atmit* which may account for reduction in phytate phosphorus content in the fermented products (Daniels and Fisher, 1975; Lopez *et al.*, 1983). Acidic pH of the fermented product may also provide favourable conditions for microbial phytase activity. Reduction in phytic acid has similarly been reported earlier during single and mixed culture fermentation of pearl millet flour using lactobacilli and yeasts (Khetarpaul and Chauhan, 1989a; 1991).

Poor bio-availability of zinc is thought to be due to the high content of phytate in plant food products (Davis and Olpin, 1979). One way to reduce phytic acid content was through fermentation as demonstrated in this study. The molar ratio, phytate:zinc, is used as an important determinant of zinc bio-availabilty from cereal-based diets to humans (Navert *et al.*, 1985). Results recorded to date suggest that daily phytate to zinc molar ratio > 15 may be associated with increased zinc deficiency (Fordyce *et al.*, 1987).

The mean molar ratios of phytate to zinc in the unfermented *atmit* were high and above the values associated with clinical and/or biochemical evidence of zinc deficiency (Table 1). Such habitually high phytate and phytate to zinc molar ratios may jeopardise the zinc status of the infants if such *atmit* is used as a sole weaning food since infants are at an even greater risk of sub-optimal zinc status than adults because of their high zinc requirements for growth.

Using mixed cultures of LAB, fermentation has considerably decreased the ratio by an average of 71% in the fermented *atmit* (Table 3). *L. plantarum* + *L. casei*-fermented tef *atmit* had the lowest phytate:zinc molar ratio. The bioavailability of zinc from mixed cultures of LAB-fermented tef *atmit* thus seems to be high since these values are lower than the limits established by human and animal studies (Morris *et al.*, 1982). The substantial decrease in phytate:zinc molar ratio obtained by mixed pure cultures of LAB fermentation of tef *atmit* may be of practical importance to cereal-based infant diets with a low animal protein content.

Table 3. HCl-extractability (%) of minerals in tef products*.

Product	Inoculum	Fe	Ca	Zn	P
Flour	-	43.31 ± 1.97^{a}	32.21 ± 0.56^{a}	38.47 ± 0.41^{a}	42.52 ± 1.05^{a}
Atmit[1]	-	48.17 ± 1.41^{b}	35.51 ± 0.63^{a}	41.26 ± 0.61^{b}	43.08 ± 2.03^{a}
Atmit[2]	-	52.58 ± 2.17^{c}	38.14 ± 0.71^{c}	45.12 ± 0.23^{c}	45.62 ± 1.93^{a}
Atmit[3]	Lc+Pp	66.73 ± 0.86^{d}	59.26 ± 1.12^{d}	92.42 ± 0.56^{d}	67.99 ± 1.11^{b}
	Lf+Lp	69.06 ± 0.18^{d}	$60.07 \pm 2.18_{d}$	90.51 ± 0.88^{d}	63.12 ± 0.32^{c}
	Lc+Lf	72.17 ± 1.63^{e}	$58.48 \pm 1.36_{d}$	88.41 ± 0.82^{d}	68.32 ± 0.62^{b}
	Lp+Lc	62.40 ± 0.42^{f}	61.54 ± 0.97^{d}	95.86 ± 0.59^{e}	88.25 ± 0.33^{e}
	Lf+Pp	78.89 ± 0.36^{g}	62.23 ± 1.35^{d}	93.25 ± 0.86^{e}	89.88 ± 0.30^{e}
	Pp+Lp	68.45 ± 0.48^{d}	63.72 ± 1.24^{d}	88.85 ± 0.78^{d}	84.51 ± 0.56^{f}

*, Mean values ± standard deviation. [1], Tef *atmit* cooked without fermentation; [2], Autoclaved unfermented tef *atmit*; [3], Lc, *Lactobacillus casei*; Pp, *Pediococcus pentosaceus*; Lf, *Lactobacillus fermentum*; Lp, *Lactobacillus plantarum*. Means in a column with different superscript letters are significantly different ($p < 0.05$).

The extractability of minerals in 0.03 N HCl (the concentration of the acid in the stomach of an adult) is an indicator of the bioavailability of the minerals to the human system (Chompreeda and Fields, 1984). Table 3 shows the HCl-extractability of minerals in tef *atmit* which for iron, calcium, zinc and phosphorus in tef flour was about 43, 32, 38 and 43 percent, respectively. Cooking the flour/water slurry into *atmit* or autoclaving did improve the HCl-extractability of minerals only marginally. This marginal increase in the extractability of minerals from the unfermented atmit may be attributed to the non-effectiveness of heat treatment on mineral-phytate chelates during *atmit* preparation.

Fermentation of autoclaved tef *atmit* with mixed cultures of LAB resulted in a considerable increase in HCl-extractability of iron, calcium, zinc and phosphorus. HCl-extractability of phosphorus increased by an average of 53% compared to the unfermented autoclaved tef *atmit*. *L. fermentum* + *L. plantarum* exhibited lower extractability enhancing effect whereas *L. fermentum* + *P. pentosaceus* and *L. plantarum* + *L. casei* had the highest enhancing effect. Thus, the extractability of phosphorus was the lowest when *L. fermentum* + *L. plantarum* mixed culture was used for fermentation of the *atmit*. The

improvement in phosphorus extractability corresponded with a proportional increase in the inorganic phosphorus and reduction in the phytic acid content. Thus, the higher the inorganic phosphorus, the greater was the extractable phosphorus in fermented *atmit*. This showed that hydrolytic reduction of phytic acid during atmit fermentation may be contributing towards the extractable phosphorus. The reduction in phytic acid during *atmit* fermentation may be due to the hydrolysis of phytic acid by phytase elaborated by the fermentative microflora (Daniels and Fisher, 1975; Lopez *et al.*, 1983). Cleavage of phosphorus from phytic acid may explain the improved HCl-extractability of phosphorus in the fermented *atmit*.

Fermentation by pure cultures of yeast and lactobacilli has been reported earlier to increase the HCl-extractability of phosphorus with corresponding decrease in phytic acid content of pearl millet flour (Khetarpaul and Chauhan, 1989; 1991).

Mixed cultures of LAB, used in the present study improved the HCl-extractability of iron, calcium and zinc, an index of their bioavailability to the human system. The HCl-extractability of iron in the fermented *atmit* increased by an average of 1.3-fold compared to the autoclaved unfermented atmit (Table 3). Maximum iron extractability was noted when the *atmit* was fermented with a mixture of *L. plantarum* + *P. pentosaceus*. *Atmit* fermented with mixed cultures of *L. plantarum* + *L. casei* exhibited the lowest iron extractability.

Of the mixed cultures of LAB fermentation, *L. plantarum* + *P. pentosaceus* fermentation brought about the highest extractability of calcium in the *atmit* (Table 3). In contrast, fermentation by *L. plantarum* + *L. casei* and *L. fermentum* + *P. pentosaceus* brought about the maximum zinc extractability (96%) compared to the other mixed cultures of lactobacilli.

Higher extractability of iron, calcium, zinc and phosphorus from the fermented *atmit* may be ascribed to the decreased phytic acid content of *atmit* due to hydrolysis. As a result of such hydrolysis, possibly by the phytase of the fermentative microflora, inorganic phosphorus is liberated from phytic acid and the chelated divalent cations (Fe^{+2}, Ca^{+2}, Zn^{+2}) may be released in free form, thereby increasing the extractability of the minerals in the fermented *atmit*. Improvement in the HCl-extractability of minerals through sequential culture fermentation of pearl millet by yeast and lactobacilli was reported previously (Khetarpaul and Chauhan, 1991).

In conclusion, lactic fermentation of tef *atmit* by mixed cultures of LAB is a potential method for improving the HCl-extractability of iron, calcium, zinc and phosphorus, an indicator of bioavailability of these essential minerals from cereals like tef. Therefore, consumption of fermented *atmit* as a weaning food may help ameliorate the prevalent mineral deficiencies (particularly iron and zinc) caused by their limited bio-availabilty and may further lead to a better mineral status of the vegetarian population where animal products are not affordable.

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