Antinutrient Reduction and Enhancement in Protein, Starch, and Mineral Availability in Fermented Flour of Finger Millet (*Eleusine coracana*)

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Finger millet, a highly nutritious millet consumed by populations in South India, is a rich source of minerals and dietary fiber in addition to primary nutrients. However, it also has nutrient binding components such as phytate, phenols, tannins, and trypsin inhibitors. Fermentation of finger millet flour using endogenous grain microflora showed a significant reduction of these components (phytate by 20%, phenols by 20%, tannins by 52%, and trypsin inhibitor activity by 32%) at the end of 24 h. There was a simultaneous increase in HCl mineral extractability (Ca, 20%; P, 26%; Fe, 27%; Zn, 26%; Cu, 78%; Mn, 10%), soluble protein, *in vitro* protein digestibility (23%), and starch digestibility.

Keywords: *Finger millet; Eleucine coracana; fermentation; phytate; phenols; tannins; trypsin inhibitors; minerals; in vitro digestibility*

INTRODUCTION

Finger millet, cultivated and consumed widely in India, has a high nutrient potential, being comparable to other major cereals in its primary nutrient content (Malleshi and Hadimani, 1993). Being consumed whole, it is superior in mineral and vitamin content compared to refined cereals. Finger millet (with dark seed coat) is particularly rich in tannins, which contribute to its poor protein digestibility (Ramachandra et al., 1977). Different methods of processing, such as germination and fermentation, enhance its nutrient profile. Enhancement of biological value (BV), net protein utilization (NPU), and thiamin, riboflavin, and niacin contents have been shown in fermented finger millet (Aliya and Geervani, 1981; Rajyalakshmi and Geervani, 1990). Our earlier paper documented the changes in starch, protein extractability, amino acids, fat, acidity, and pH during natural fermentation of finger millet (Usha Antony et al., 1996a). The microbial profile, heterolactic nature of fermentation, and enzymatic changes were also investigated. (Usha Antony and Chandra, 1997).

Maximum utilization of the nutrient potential of the millet is limited by the presence of phytates, phenols, tannins, and enzyme inhibitors (Ramachandra et al., 1977; Ravindran, 1992). While phytates bind essential minerals and proteins, tannins complex with proteins and enzyme inhibitors reduce digestibility (Reddy and Pierson, 1994). Fermentation is known to alter these components in several foods (Chavan and Kadam, 1989; Reddy and Pierson, 1994; Usha Antony et al., 1996b). However, the extent to which they are modified in finger millet has not been investigated so far. The present study reports the changes in these antinutrients and the accompanying alterations in the *in vitro* mineral, protein, and starch availability.

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MATERIALS AND METHODS

Materials. Finger millet, purchased in bulk from the local market, was used for all experiments. Sodium phytate (dodecasalt hydrate) from wheat (Aldrich) was used as standard for phytic acid estimation and as substrate for phytase assay. Catechin (Sigma) served as standard for total phenols and tannin estimations.

Trypsin (CDH), bovine serum albumin (CDH), pepsin (1:3000, Hi-Media), pancreatin (CDH), and amyloglucosidase (20 000 units/g, Sigma) were used for the assay of trypsin inhibitor activity, soluble protein, in vitro protein digestibility (IVPD) and in vitro starch digestibility (IVSD), respectively.

All other chemicals/reagents used were of analytical grade.

Processing. Finger millet flour (50 g) was made into slurry with distilled water (100 mL) in a 500 mL Erlenmeyer flask, covered with aluminum foil, and allowed to ferment undisturbed at 30 °C in a BOD incubator for 48 h. To simulate household conditions, neither containers nor flour was sterilized. Aliquots of the fermenting slurry were drawn at 0, 12, 18, 24, and 48 h under aseptic conditions and stored at −70 °C until analyses. Fresh slurries were used for the assay of phytase and antitryptic activity. Fermentation was carried out in triplicate.

Analyses. (1) Phytate was estimated by HPLC as described by Camire and Clydesdale (1982). One milliliter of the slurry was extracted into 10 mL of 0.2 N HCl by continuous shaking at 250 rpm at 37 °C for 2 h. After centrifugation at 8000*g* and 30 °C for 10 min, the supernatant was assayed for phytic acid. The HPLC system (CT-10A Shimadzu model) was fitted with a Shimpack ODS-C18 column with an RI (R16A) detector and Chromatopac integrator (Shimadzu). Sodium acetate (0.005 M) served as the mobile phase, with a flow rate of 1.5 mL/min at 30 °C.

(2) Phytase activity was determined by extracting the enzyme as described by Al-Asheh and Duvnjak (1994), from 1 mL of the millet slurry into 10 mL of aqueous $CaCl_2 \cdot 2H_20$, by shaking at 250 rpm at 30 °C for 1 h. After centrifugation at 15000*g* at 5 °C for 15 min, phytase was assayed in the supernatant (Yoon et al., 1996). The reaction mixture consisting of 1000 μL of the enzyme extract, 200 μL of 10 mM sodium phytate, and 200 μL of 0.5 M sodium acetate buffer (pH 5.5), made up to 2 mL with deionized water, was incubated at 37 °C for 1 h. The blank consisted of boiled en-

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zyme. The inorganic phosphate released was measured using ammonium molybdate as described by Heinonen-Lahti (1980).

One unit of phytase activity is defined as the amount of enzyme required to liberate one micromole of inorganic phosphate per minute.

(3) Total phenols and tannins were extracted from 1 mL of the slurry into 20 mL of 1% HCl/methanol at room temperature (30 ± 2 °C) for 24 h with occasional swirling.

After centrifugation (8000*g*, at 30 °C, 15 min), the supernatant was estimated for total phenols by reaction with Folin−Denis reagent using catechin as standard, as described by Swain and Hillis (1959), using a Carl Zeiss spectrophotometer (PMQ II).

Tannins in the supernatant were estimated spectrophotometrically using the above-mentioned spectrophotometer (500 nm) after reaction with vanillin−HCl (Price et al., 1978). Values are expressed as catechin equivalents in milligrams per 100 g of dry flour.

(4) Trypsin inhibitor activity was determined by extracting 1 mL of the slurry with 10 mL of 0.1 M phosphate buffer (pH 7.6) by shaking at 200 rpm at 37 °C for 1 h and centrifugation at 8000*g* and 30 °C for 15 min. The extract was assayed for trypsin inhibitor activity as described by Kakade et al. (1969) using a Unicam UV−vis spectrometer (UV4).

One trypsin unit (TU) is defined as an increase of 0.01 absorbance unit at 280 nm. The inhibitory unit (TIU) is defined as the number of trypsin units inhibited per gram of the dry flour under assay conditions.

(5) HCl extractability of minerals (calcium, phosphorus, iron, copper, zinc, and manganese) from the fermented samples (1 mL) into 0.03 N HCl (10 mL) was estimated as described earlier (Sripriya et al., 1997). The minerals were assayed by inductively coupled plasma emission spectroscopy (ARL-3410, ICP with mini torch). Percentage extractability of each mineral was calculated as described by Chompreeda and Fields (1984):

$$(\text{mineral extracted/total mineral}) \times 100$$

(6) Zinc bioavailability was assessed by calculating the phytate/zinc and phytate × calcium/zinc molar ratios and comparing with critical values suggested by Fordyce et al., (1987).

(7) Soluble proteins were analyzed by centrifuging the millet slurry at 8000*g* at 30 °C for 15 min and estimating the protein in the supernatant using Folin−Ciocalteu reagent with bovine serum albumin as standard (Lowry et al., 1951). Values are expressed as milligrams of protein per 100 g of dry flour.

(8) IVPD was determined as described by Lorri and Svanberg (1993). Slurry (2 mL) was mixed with 35 mL of 0.1 M phosphate buffer (pH 2) containing 42.5 mg of pepsin and incubated at 37 °C for 2 h. After centrifugation at 5000*g* at 30 °C for 15 min, the residue was washed in 10 mL of 0.1 M phosphate buffer (pH 2.0) and centrifuged again at 2500*g* at 30 °C for 15 min. The residue was resuspended in 35 mL of 0.1 M phosphate buffer (pH 8.0), containing 42.5 mg of pancreatin and incubated at 37 °C for 1 h. The mixture was centrifuged at 2500*g* at 30 °C for 15 min, the supernatant discarded, and the residue washed twice with 10 mL of 0.1 M phosphate buffer (pH 7.0) by centrifugation. The residue was filtered through Whatman No. 1 filter paper, oven-dried, and analyzed for total nitrogen according to the Kjeldahl method (AOAC, 1990) using a Tecator Kjeltec system 1028. Percentage protein digestibility was calculated as

$$[(\text{total N} - \text{undigested N})/\text{total N}] \times 100$$

(9) IVSD was estimated as described by Mouliswar et al. (1993). Slurry (2 g in 100 mL) was cooked in a boiling water bath for 15 min. To 50 mL of this slurry was added 30 mL of 0.2 M glycine−HCl buffer (pH 2.0) containing 10 mg of pepsin followed by incubation at 37 °C for 2 h. After neutralization with 0.2 M NaOH, the volume was made up to 100 mL with distilled water. Ten milliliters of this mixture was incubated with 5 mL of 0.5 M phosphate buffer (pH 8.0) containing 15 mg of pancreatin and 15 mg of amyloglucosidase at 37 °C for 2 h. One milliliter aliquots were drawn at half-hour intervals (0, 30, 60, 90, and 120 min) and assayed for reducing sugars released with glucose as standard according to the 3,5-dinitrosalicylic acid method (Miller, 1959).

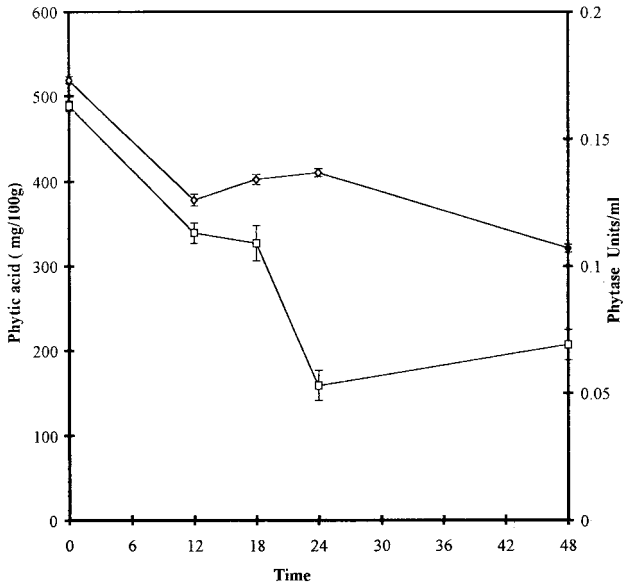

Figure 1. Phytic acid content and phytase activity in fermented finger millet flour: (◇) phytate; (□) phytase.

RESULTS AND DISCUSSION

Phytic Acid and Phytase Activity. Phytates may be hydrolyzed both by grain phytases and by microbial phytases (Reddy and Pierson, 1994). Phytic acid in finger millet decreased significantly ($p < 0.05$) in the first 12 h of fermentation with no further significant change (Figure 1). By 48 h 39% of the phytate was hydrolyzed. Phytase activity reduced from 0.162 to 0.109 unit/mL at 18 h with a sharp decrease by 24 h (Figure 1).

The decrease in phytate was parallel to the phytase activity up to 18 h, when the pH of the fermenting slurry dropped to 5.5 from an initial value of 6.4. The subsequent marked reduction in phytase activity may be attributed to the inhibition of phytase at a lower pH of 4.3. Furthermore, the extraction of phytase is carried out at pH 5.3. At this pH only some phytase components of the grain and microbial phytases may be detected.

Phytate reduction has been reported in several fermented foods made from pearl millet (Dhanker and Chauhan, 1987), maize (Amoa and Muller, 1976), wheat (Gupta et al., 1992), sorghum (Marfo et al., 1990), and cereal−pulse mixtures (Chavan and Kadam, 1989).

Phenols and Tannins. Phenols decreased by 26−29% by 48 h, while tannins showed a more marked reduction of 44−52% (significant, $p < 0.05$). The total phenol content was higher than the tannin content throughout the fermentation (Figure 2). The marked reduction in phenols and tannins in the first 24 h corresponded well with the microbial population, reaching a maximum (10^{10} cfu/g) between 18 and 24 h and remaining stable thereafter (Usha Antony and Chandra, 1997). Release of fiber-bound tannins and polyphenol oxidase activity by fermenting microbes may be respon-

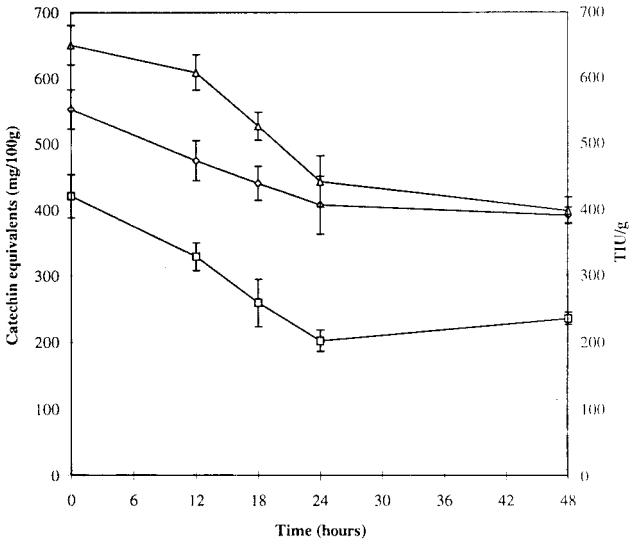

Figure 2. Total phenols, tannins, and trypsin inhibitor activity in fermented finger millet: (◇) total phenols; (□) tannins; (△) TIU.

sible for this marked reduction as also noted in pearl millet *rabadi* fermentation (Dhanker and Chauhan, 1987).

Trypsin Inhibitor Activity. Trypsin inhibitor activity decreased with increasing fermentation time, 32% at 24 h and 39% at 48 h (significant, $p < 0.05$) as in the case of phenols and tannins (Figure 2). Antitryptic activity during fermentation has been studied so far only in legumes and cereal−legume mixtures and not in millets. Antitryptic activity decreased in some foods (Ayyagiri et al., 1989) and was unaltered in others (Padhaye and Salunkhe, 1978).

Mineral Extractability. The HCl extractability of minerals under simulated gastric conditions is an indicator of availability from foods. The mineral extractability in all cases increased to a maximum at 18−24 h (Table 1). The calcium extractability, which was high initially (80%), increased further (100%); the phosphorus and iron extractability doubled; copper increased 4-fold, while zinc and manganese increased by 30.5 and 10%, respectively. Extractability of copper alone decreased at 48 h. The extractability of minerals (calcium, phosphorus, iron, and zinc) showed a significant negative correlation ($p < 0.05$) with the phytate levels (Table 2).

Studies on mineral extractability using the present methodology appear to be restricted to the study of corn meal blends (Chompreeda and Fields, 1984), wheat (Gupta et al., 1991), and pearl millet (Mahajan and Chauhan, 1987, 1988; Dhanker and Chauhan, 1989; Khetarpaul and Chauhan, 1990a). Increased HCl mineral extractability in pearl millet was influenced by temperature and type of fermentation (both natural and pure culture). Apart from the present study, the only other work on finger millet mineral availability is our earlier study on the effect of fermentation on germinated finger millet (Sripriya et al., 1997).

Phytate/Zinc and Calcium × Phytate/Zinc Ratios. A phytate/zinc molar ratio of 10 or less indicates adequate zinc bioavailability and above 20 is associated with clinical evidence of zinc deficiency. The calcium × phytate/zinc ratio is a better indicator of zinc utilization than soluble zinc and phytate/zinc ratio (Fordyce et al., 1987). A significant reduction in the phytate/zinc ratio occurred in finger millet on fermentation for 12 h and fell below 20 only at 48 h (Table 3), indicating enhanced zinc bioavailability from the millet resulting from a substantial reduction in phytate. However, the calcium × phytate/zinc ratio remained above 50 mM/100 g, suggesting poor bioavailability of the mineral. The high calcium content of the millet is probably responsible.

Soluble Protein and IVPD. Soluble proteins also showed an increase (Table 4) and corresponded with the earlier data of increase in protein extractability and free amino acids at 24 and 48 h of fermentation of finger millet (Usha Antony et al., 1996). Enhanced protein availability and utilization from fermented cereals and cereal−legume mixtures have been demonstrated using both *in vitro* assays, and bioassays(Chavan and Kadam, 1989; Aliya and Geervani, 1981). Similarly in finger millet, protein digestibility increased significantly ($p < 0.05$) from 47.6 to 70.1% (23% increase within 24 h), followed by a slight increase at 48 h (not significant, $p < 0.05$; Table 4). A high correlation coefficient (significant, $p < 0.05$) between reduction in phytate, phenols, tannins, and antitryptic activity and increase in IVPD was observed (Table 5). The IVPD increase observed in the present study was higher at 24−48 h than that in pearl millet, wherein a 24% increase was achieved in 72 h (Khetarpaul and Chauhan, 1990b). Lorri and Svanberg (1993) also noted an increase in the IVPD of fermented finger millet.

IVSD. IVSD was enhanced considerably at 12, 18, and 24 h (Figure 3). It is of interest that even at 0 min, the sugar released was higher in the fermented sample. On analyses of these data in terms of percentage starch digested at the end of 2 h, the unfermented millet had a value of 93.9 ± 2.6%, while the 12, 18, and 24 h samples showed 103.3 ± 6.4, 104.1 ± 3.4, and 103.0 ± 3.7%. In the case of 48 h fermented millet only 82.8 ±

Table 1. HCl Extractablity of Minerals during Fermentation of Finger Millet Flour (Milligrams per 100 g of Dry Matter)[a]

fermentation time (h)	calcium	phosphorus	iron	copper	zinc	manganese
0	250.9 ± 15.5 (80.1)	127.8 ± 10.8 (27.4)	1.71 ± 0.14 (26.2)	0.26 ± 0.10 (25.7)	1.11 ± 0.05 (55.0)	4.54 ± 0.00 (46.1)
12	292.9 ± 24.4 (93.6)	175.2 ± 10.0 (37.5)	2.16 ± 0.036 (33.1)	0.86 ± 0.02 (85.2)	1.49 ± 0.08 (78.8)	5.03 ± 0.01 (51.0)
18	345.1 ± 20.5 (110.2)	211.5 ± 23.6 (45.3)	2.67 ± 0.58 (40.9)	0.84 ± 0.06 (83.2)	1.66 ± 0.01 (82.2)	5.17 ± 0.01 (52.4)
24	316.3 ± 24.1 (101.0)	248.6 ± 21.0 (53.2)	3.44 ± 0.07 (52.7)	1.051 ± 0.11 (104.1)	1.64 ± 0.17 (81.2)	5.58 ± 0.02 (56.6)
48	316.4 ± 12.8 (101.1)	262.8 ± 8.6 (56.3)	3.56 ± 0.16 (54.5)	0.62 ± 0.12 (61.4)	1.71 ± 0.12 (84.7)	5.02 ± 0.02 (50.9)

[a] Values in parentheses are percentage of the raw millet extracted. Mineral content of finger millet (mg/100 g of dry weight): Ca, 313.1 ± 8.1; P. 467.2 ± 16.8; Fe, 6.53 ± 0.99; Cu, 1.01 ± 0.11; Zn, 2.02 ± 0.25; Mn, 9.86 ± 0.11.

Table 2. Correlation Coefficient between HCl Mineral Extractability and Phytate Content in Fermenting Finger Millet

mineral	correl coef[a]	mineral	correl coef[a]
calcium	−0.665*	copper	−0.512
phosphorus	−0.782*	zinc	−0.865*
iron	−0.707*	manganese	−0.465

[a] Asterisks indicate correlation coefficients significant at 5% level.

Table 3. Phytate/Zinc and Calcium × Phytate/Zinc Molar Ratios (Millimolar per 100 g of Dry Matter) in Fermenting Finger Millet

fermentation time (h)	phytate/zinc	calcium × phytate/zinc
0	29.9	233.5
12	21.8	170.1
18	23.2	180.9
24	23.9	186.7
48	18.5	144.4

[a] Values are the mean of three independent estimations.

Table 4. Soluble Protein Content and IVPD of Fermented Finger Millet Flour[a]

fermentation time (h)	soluble proteins[b] (mg/100 g)	IVPD[b] (%)
0	99.1 ± 1.4	47.6 ± 6.5
12	127.6 ± 2.7*	50.8 ± 6.0
18	108.6 ± 8.1	58.3 ± 5.4
24	114.3 ± 5.3*	70.1 ± 7.4*
48	139.1 ± 8.0*	73.2 ± 6.5*

[a] Dry matter basis. [b] Significant difference from the 0 h value ($p < 0.05$) is indicated by an asterisk.

Table 5. Correlation Coefficient between Antinutrients and IVPD in Finger Millet Fermentation[a]

antinutrient	IVPD	antinutrient	IVPD
phytate	−0.661*	tannins	−0.894*
phenols	−0.921*	trypsin inhibitor activity	−0.995*

[a] Asterisks indicate correlation coefficients significant at 5% level.

3.6% was digested. In the 48 h fermented millet, both the initial glucose released and the rate of release were markedly reduced, probably due to the lower starch content. Starch decreased from 59.4% in the unfermented millet to 52% after 48 h of fermentation (Usha Antony et al., 1996). In view of the slow release of glucose, the 48 h fermented millet merits further research attention for its use in the management of hyperglycemia. Simultaneously, the additional benefits of enhanced protein and mineral availability are an advantage.

Conclusion. Phytates, phenols, tannins, and trypsin inhibitors have traditionally been labeled as antinutrients, with much emphasis on their negative nutrient-binding properties. Nevertheless, currently much attention has been focused on their antioxidant and anticarcinogenic properties (Thompson, 1993). The present study documents the changes in these components in finger millet fermented for 48 h.

The phytates, phenols, tannins, and trypsin inhibitor activity decreased markedly on fermentation. A concomitant enhancement in mineral availability (calcium, phosphorus, iron, and zinc), soluble protein, IVPD, and IVSD was observed. All of these were marked at 24 h, with minimal changes in the next 24 h. This has nutritional significance in view of the fact that essential

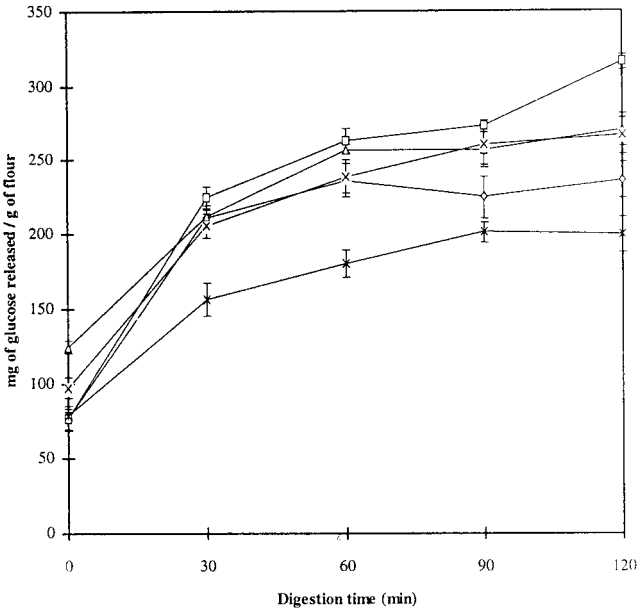

Figure 3. IVSD of fermented finger millet flour: (◇) 0 h; (□) 12 h; (△) 18 h; (×) 24 h; (∗) 48 h.

minerals such as Ca, Fe, and Zn as well as protein tend to be limited in rice-based diets consumed by the lower economic strata. Supplementing fermented finger millet could add substantially to the nutrient intake of such groups. The slow release of starch, higher protein availability, and retention of a portion of phytates, phenols, tannins, and trypsin inhibitors suggest finger millet's potential as a therapeutic and health food.

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