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Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*)

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Finger millet (*Eleusine coracana*) also known as 'ragi' in India is a source of carbohydrate, protein and mineral that is comparable to other common cereal grains. However, antinutrients like phytate and tannins reduce the nutrient bioavailability which can be improved by suitable processing methods such as germination and fermentation. In our study, the finger millet was germinated (24 h) and then subjected to fermentation (48 h). Major biochemical changes occurred during fermentation (6–18 h) compared to germination (24 h). The processing decreased the pH from 5.8 to 3.8 and increased the total sugars (2-fold), reducing sugars (13-fold) and free amino acids (10-fold). Lactic acid was the predominant organic acid (3.7%). The phytate content decreased by 60% with an increase in HCl-extractable minerals of 47%. The phytate×Ca/Zn molar ratio decreased from 163 to 66.2, indicative of an increased Zn bioavailability. In conclusion, a combination of germination and fermentation is a potential process for decreasing the antinutrient levels and enhancing digestibility. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

Finger millet (*Eleusine coracana*) also known as 'ragi' in India is an important staple food for people belonging to the low socio-economic group. It has a carbohydrate content of 81.5%, protein 9.8%, crude fibre 4.3% and mineral 2.7%, that is comparable to other cereals and millets. Its crude fibre and mineral content is markedly higher than wheat (1.2% fibre, 1.5% minerals) and rice (0.2% fibre, 0.6% minerals); its protein is relatively better balanced; it contains more lysine, threonine and valine than other millets (Ravindran, 1991). However, it also has a high content of antinutrients, such as phytic acid (0.48%), that bind divalent cations and tannins (0.61%) which complex proteins and carbohydrates, thereby decreasing nutrient bioavailablity and present grounds for concern (Geetha *et al.*, 1977; Ravindran, 1991).

The grain is traditionally processed either by germination or fermentation prior to consumption. Germinated finger millet is used to make weaning foods for infants which has reduced viscosity and increased calorie density (Hemanalini *et al.*, 1980). The traditional, naturally fermented finger millet product is called 'Ambali'. Here the thin batter of the finger millet flour is fermented (24 h) and then cooked with rice to a thick porridge, which is again fermented (24 h) to obtain the product (Aliya & Geervani, 1981). The millet is also brewed and consumed as 'Chang' and other beverages (Malleshi & Hadimani, 1993).

Studies on biochemical changes in finger millet due to these processes are limited. The overall nutritive value of finger millet is reported to improve on germination using rat growth studies although improvement was not evident in PER and Ca availability (Hemanalini *et al.*, 1980). The biological value and vitamin B content improved on fermentation of finger millet (Aliya & Geervani, 1981), while a decrease was observed in IVPD (Rajalakshmi & Geervani, 1990).

However, changes in available carbohydrates and protein, organic acid profile, antinutrient levels (phytate and phenols) and the consequent changes in the HCl-extractability of minerals (as an index of their bioavailablity to humans) have not so far been studied. This paper reports the effect of a process that combines both germination and fermentation by endogenous microflora on the above aspects.

MATERIALS AND METHODS

Finger millet Co-13 brown variety, was purchased in bulk from Tamil Nadu Agricultural University, Coimbatore, India. The grains were cleaned, washed, dried and stored at 4°C in air tight containers.

Processing

Dry grains (100 g) were soaked for 12 h, 30°C in distilled water. They were then placed on a cloth which was kept moistened by dipping the edges in water and allowed to germinate for 24 h at 30°C. When the shoots were just visible, they were taken and ground in a blender for 2 min (0.5 mm mesh). The slurry (1:2 w/v grain/H_2O) was allowed to ferment by the natural grain flora at 30°C. Slurry samples were drawn at 0, 6, 12, 18, 24, 36 and 48 h and dried at 65°C to constant weight. Dried samples were flaky and therefore reground mildly in a mortar and pestle to homogenise the flour and stored at 4°C in air-tight containers.

The above processing was carried out in triplicate. The control consisted of the ungerminated grain slurry (raw/0 h) prepared in a similar manner.

(a) The pH was determined in the slurry supernatant after filtration through Whatman No.1 filter paper. The titratable acidity was expressed as lactic acid equivalents by titration against 0.05 N NaOH (Egan *et al.*, 1981).

(b) For organic acids estimation, the slurry was centrifuged at 10 000 *g* for 20 min. The supernatant was analysed using HPLC, CTO-10A SHIMADZU model, Shimpack SCR-101H column, UV detector SPD-10A SHIMADZU, C-R6A CHROMATOPAC integrator using 10 mM perchloric acid as mobile phase at 40°C, as per Andersson & Hedlund (1983).

(c) Soluble protein in the supernatant after centrifugation was estimated using Folin Ciocalteau reagent by the method of Lowry *et al.* (1951).

(d) The dry sample (200 mg) was extracted four times with 20 ml hot 80% ethanol and centrifuged at 10 000 *g* for 20 min. The pooled extract was evaporated and made up to 10 ml. An aliquot was assayed for total sugars by phenol–sulphuric acid method (Dubois *et al.*, 1956), reducing sugars by dinitrosalicylic acid method (Miller, 1959) and free amino acids by ninhydrin method (Magne & Larher, 1992).

(e) The alcohol-insoluble residue was solubilised with 52% perchloric acid. The sugar content of the filtered perchloric acid extract was estimated by the phenol–sulphuric acid method (Dubois *et al.*, 1956). The starch content was obtained using a factor 0.9 (McCready *et al.*, 1950).

(f) Phytic acid was extracted into 20 ml of 0.2 N HCl by continuous shaking of 500 mg dry sample at 200 rpm, for 2 h at 37°C, centrifuged and the supernatant used for analysis. Phytate was estimated colorimetrically using Sigma phytate as standard (Haug & Lantzsch, 1983). Phytic acid was precipitated with an Iron III solution of known concentration. The iron content was estimated using 2′,2′-bipyridyl solution.

(g) Mineral bioavailability: mineral analysis was done by wet acid digestion of the samples using nitric/perchloric acid mixture (2:1) and Ca, P, Fe, Cu, Zn and Mn were estimated in the acid digest (AOAC, 1990) using ICP emission spectroscopy (ARL—3410 ICP with mini torch). HCl extractability of minerals was done by continuous shaking of 1 g of sample with 50 ml of 0.03 N HCl at 150 rpm for 3 h at 37°C; the mixture was filtered through Whatman No.42 and the clear supernatant was oven dried at 100°C and the minerals estimated as above (Chompreeda & Fields, 1984).

$$\% \text{ Mineral extractability} = \frac{\text{Mineral extracted into 0.03 N HCl}}{\text{Total mineral}} \times 100$$

Zinc bioavailability was studied using the phytate/zinc and phytate×Ca/Zn molar ratios (Fordyce *et al.*, 1987).

(h) The dry sample (500 mg) was extracted with 25 ml of 1% HCl in methanol for 24 h at room temperature. The phenolic compounds in the methanol extract was estimated using the Folin Denis reagent with chlorogenic acid as standard according to the procedure of Swain & Hillis (1959).

The values presented are the means of three independent tests.

RESULTS AND DISCUSSION

pH and titratable acidity

The initial pH (5.8) dropped markedly during fermentation (3.87 at 48 h) compared to germination (5.7) (Fig. 1). The maximum drop was from 6 to 12 h of fermentation (5.6–4.6). A similar trend was observed in titratable acidity with the maximum increase occurring from 6 to 12 h fermentation (0.38–1.01%) and it increased further with fermentation time. The results are comparable to the observations of Khetarpaul & Chauhan (1989) on pH and titratable acidity changes during fermentation of pearl millet sprouts. A pH range of 3.6–4.1 is evidently favourable for eliminating undesirable microbial flora (e.g. coliforms) in fermented foods (Hamad & Fields, 1979; Chavan & Kadam, 1989).

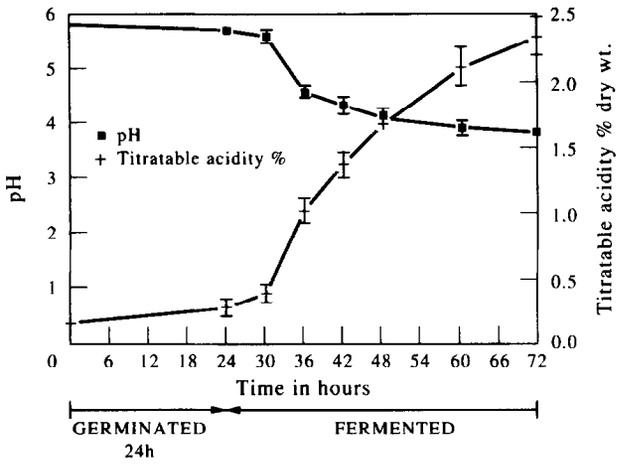

Fig. 1. Changes in pH and titratable acidity during germination and fermentation of finger millet.

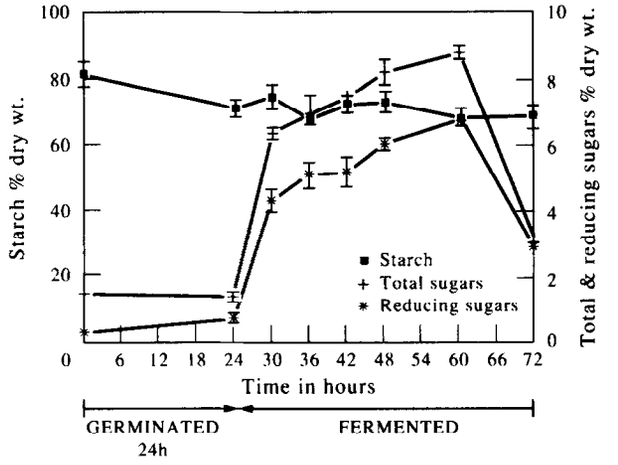

Fig. 3. Changes in starch, total and reducing sugars during germination and fermentation of finger millet.

Soluble proteins and free amino acids

The soluble proteins showed only marginal increase on germination but a marked increase by 10-fold at 18 h fermentation (Fig. 2). This may be due to the increase in microbial enzyme activity and protein hydrolysis during fermentation. The total free amino acids increased rapidly by about 4.5-fold during germination and doubled at 18 h fermentation, reaching a maximum at 36 h of fermentation. The increase in free amino acid content is favourable as the protein quality of food depends not only on its amino acid composition but also on the availability of these amino acids (Hamad & Fields, 1979).

Carbohydrate

Starch content decreased from 81.1 to 71.3 (g/100 g) on germination which further decreased during fermentation (Fig. 3). However, a marked 5-fold increase in total and reducing sugars occurs during the early fermentation time of 0–6 h (Fig. 3). During germination, mobilization and hydrolysis of seed polysaccharides occur (Khetarpaul & Chauhan, 1990). Polysaccharides can be further hydrolysed by fermenting microbes which possess both alpha and beta amylases (Bernfeld, 1962). A sharp decrease in total and reducing sugar at 48 h may be due to microbial utilisation.

Organic acids

Marked increase in organic acids were observed at 0–6 h fermentation, possibly due to microbial activity, converting some of the carbohydrate into organic acids such as lactic, citric and acetic acid (Marfo *et al.*, 1990). Lactic acid appears from 6 h of fermentation and steadily increases up to 48 h to 3.7 g/100 g (Fig. 4). Lactic acid production by heterofermentative organisms is known to be the predominant type of fermentation by endogenous microbial flora of the grains (Khetarpaul & Chauhan, 1989; Nanson & Fields, 1984).

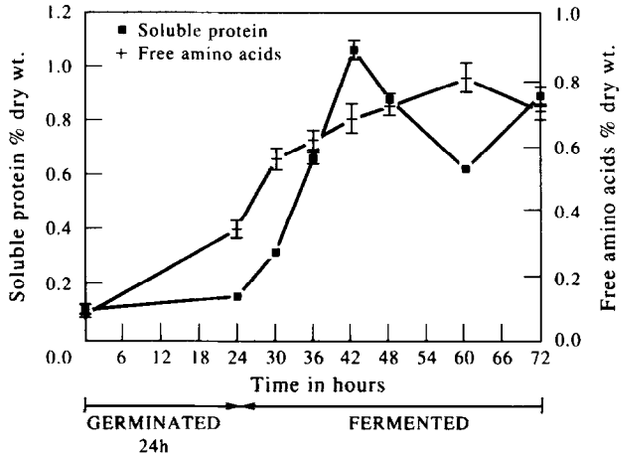

Fig. 2. Changes in soluble proteins and free amino acids during germination and fermentation of finger millet.

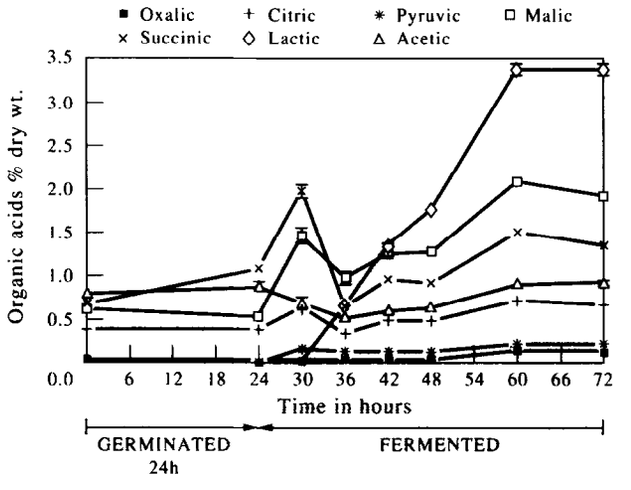

Fig. 4. Changes in organic acid profile during germination and fermentation of finger millet.

Table 1. HCl-extractability of minerals (%) during fermentation of finger millet sprouts

Samples	Minerals					
	Ca	P	Fe	Zn	Cu	Mn
Raw*	47.6 ± 1.2	17.8 ± 0.8	5.9 ± 0.8	57.2 ± 2.6	58.7 ± 4.7	119.3 ± 3.6
Sprouted (24 h)	53.0 ± 0.9	20.0 ± 2.0	8.6 ± 0.0	70.4 ± 2.5	82.9 ± 0.0	131.1 ± 7.0
Fermented						
6 h	56.6 ± 0.9	28.0 ± 0.2	13.4 ± 1.4	73.8 ± 2.1	96.8 ± 1.9	142.8 ± 4.3
12 h	60.8 ± 1.3	29.0 ± 0.4	18.2 ± 1.3	77.8 ± 1.8	97.3 ± 7.5	142.6 ± 5.2
18 h	66.7 ± 1.5	36.6 ± 1.5	22.8 ± 1.5	76.3 ± 3.8	121.0 ± 10.7	161.9 ± 2.2
24 h	65.7 ± 0.6	38.8 ± 2.4	23.5 ± 1.5	71.5 ± 1.8	114.5 ± 4.7	161.2 ± 3.1
36 h	64.0 ± 0.7	36.4 ± 1.1	24.1 ± 0.2	76.8 ± 7.2	113.1 ± 1.9	151.6 ± 4.0
48 h	60.6 ± 1.5	37.8 ± 2.3	21.4 ± 2.8	77.4 ± 2.3	114.5 ± 8.9	144.4 ± 5.0

Values are means of 3 independent processing.
*Total mineral analysis mg/100 g dry matter: Ca—340.15 ± 1.9; P—276.41 ± 7.0; Fe—5.67 ± 1.2; Cu—0.54 ± 0.0; Mn—3.58 ± 0.0; Zn—2.23 ± 0.2.

Mineral bioavailability, phytate and polyphenols

Germination was more effective in increasing the extractability of the trace elements like Cu, Zn and Mn from 0.32, 1.28, 4.27 in the raw grain to 0.45, 1.57, 4.69 (mg/100 g) which further increased to 0.62, 1.73 and 5.20 (mg/100 g), respectively, on fermentation (48 h). Zn extractability was minimum on fermentation, whereas, Ca, P and Fe extractability increased from 162, 49.1, 0.34 (mg/100 g) to 181, 55.3, 0.49 (mg/100 g) on germination which further increased to 224, 107 and 1.33 (mg/100 g), respectively on fermentation (24 h). Fermentation was more effective in increasing the bioavailability of Ca, P and Fe (Table 1).

Phytate complexes with essential elements such as Zn, Fe and Ca and reduces their bioavailability which can be enhanced by degradation of phytate (Moeljopawiro *et al.*, 1987). Lopez *et al.* (1983) observed that natural lactic acid fermentation decreased the phytic acid in corn meal due to phytase production by the microbes. Phytase activity was also found during germination of wheat, barley, rye and oats which hydrolyse phytate to phosphate and myoinositol phosphates (Larsson & Sandberg, 1992). The increase in HCl extractable minerals may be attributed to reduction in phytate and presence of enhancers such as organic acids and ascorbic acid (Indumadhavi & Agte, 1992). Finger millet is the richest source of Ca among cereals (340 mg/100 g) of which only 162 mg/100 g is bioavailable in the raw grain. Processing improved its bioavailability up to 227 mg/100 g. Iron availability improved from 0.34 to 1.4 mg/100 g due to processing. There is high prevalence of iron deficiency anaemia in a vegetarian population like in India, mainly due to the poor bioavailability of iron caused by the inhibitors such as phytates, tannins and fibre in plant foods (Indumadhavi & Agte, 1992).

Zn is a coenzyme for an estimated 200 enzymes, many of which affect protein synthesis and thus growth (O'Dell, 1984). The phytate/Zn and phytate×Ca/Zn molar ratios are indicators of Zn bioavailability. In finger millet, processing has resulted in the decrease of phytate/Zn molar ratio from 19.2 to 7.8 (Table 2). Molar ratios of 10 or less indicate adequate Zn bioavailability and above 20 are associated with clinical or chemical evidence of Zn deficiency (Fordyce *et al.*, 1987). The phytate×Ca/Zn ratio decreased from 163 to 66.1 on processing of finger millet, where ratios above 50 are associated with inadequate Zn bioavailability. The high calcium content of finger millet (340 mg/100 g), has accentuated the inhibitory effect of phytate on Zn bioavailability in terms of phytate×Ca/Zn ratio. However, processing of finger millet has considerably reduced the molar ratios and enhanced the Zn bioavailability.

Total phenols decreased on germination from 1.43 to 1.28 g/100 g and increased on fermentation to 1.86 g/100 g (Fig. 5). Khetarpaul & Chauhan (1991) reported a similar increase in polyphenols during fermentation of pearl millet flour due to microbial activity, which may hydrolyse the condensed tannins to lower molecular weight phenols. The galloyl (trihydroxybenzene) groups are mainly responsible for inhibiting the iron absorption (Brune *et al.*, 1991).

Table 2. Changes in molar ratios of phytate, Ca and Zn during fermentation of finger millet sprouts

Samples	Molar ratio*	
	Phytate/Zn	Phytate×Ca/Zn
Raw	19.2	162.8
Sprouted (24 h)	18.1	153.3
Fermented		
6 h	16.6	140.6
12 h	14.5	123.1
18 h	12.2	103.3
24 h	11.0	93.1
36 h	9.0	76.4
48 h	7.8	66.2

*Molar ratio expressed in terms of mmoles/100 g dry wt.

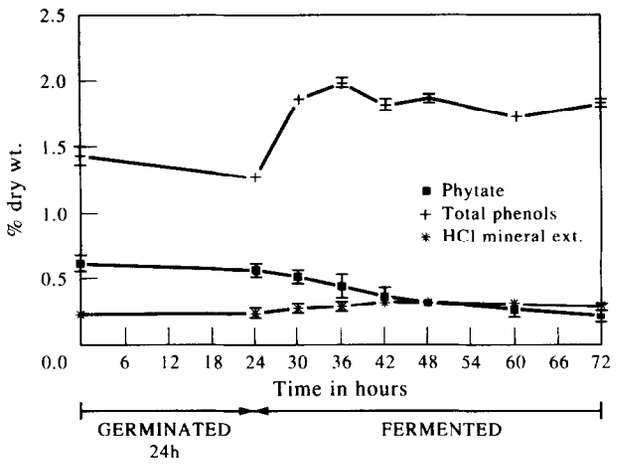

Fig. 5. Changes in phytate, HCl extractable minerals and total phenols during germination and fermentation of finger millet.

CONCLUSION

Major biochemical changes were observed when germination (24 h) was followed by fermentation (6–18 h) compared to germination (24 h) alone. While germination (24 h) was effective in starch and protein hydrolysis, fermentation was more effective in reducing pH and phytate and increasing the mineral bioavailability, free sugars and amino acids.

The results indicate that a combination of germination followed by fermentation is a potential process for developing a food product of improved nutritive value and digestibility from finger millet. Further investigations on the *in vitro* protein and starch digestibility, growth and toxicological analyses by animal feed studies are required to draw firm conclusions.

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