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Improvement in HCl-Extractability of Minerals from Pearl Millet by Natural Fermentation

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ABSTRACT

Natural fermentation of precooked pearl millet flour, carried out at 20, 25 and 30°C for 72 h, brought about a significant increase in non-phytate, inorganic and HCl-extractable phosphorus with a corresponding decrease in phytate phosphorus. HCl-extractability of calcium, copper, iron, zinc and manganese was improved significantly and the improvement was most pronounced at 30°C.

INTRODUCTION

Pearl millet (*Pennisetum typhoideum*) is a staple food for a large section of the population in Asian and African countries. Besides supplying calories and protein in the diet, pearl millet is a good source of essential minerals (Desai & Zende, 1979; Kumar & Kapoor, 1984; Chauhan *et al.*, 1986). However, owing to the presence of certain antinutrients (Chauhan *et al.*, 1986; Mahajan, 1986) including phytic acid and polyphenols, the availability of the minerals from pearl millet in the human system may be low.

Natural fermentation of raw pearl millet flour has been reported to increase the nutritional value of this course grain; digestibility of the proteins and carbohydrates and the HCl-extractability of the minerals (Mahajan, 1986) improves along with reduction in the level of phytic acid (Mahajan & Chauhan, 1987). Cooking prior to fermentation is better than

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cooking the fermented product as the destruction of nutrients, such as the vitamins, formed during fermentation is avoided. Besides, the cooked pearl millet may be a better substrate for the fermenting microflora. Studies in this laboratory have shown that the number and type of microorganisms found in the cooked flour after fermentation (Khetarpaul, 1988) was quite different from those in the raw fermented flour (Mahajan, 1986). There was also a difference in pH and titratable acidity between the raw fermented and precooked fermented pearl millet flour. The method of processing the pearl millet for fermentation may, therefore, influence the extractability of minerals in 0·03M HCl, the concentration of the acid found in gastric contents of human stomach. The solubility of minerals from foodstuffs, subjected to in-vitro gastric simulated conditions, is indicative of their bioavailability from these foodstuffs (Lock & Bender, 1980; Wien & Schwartz, 1985; Kim & Zemel, 1986). The objective of this paper is to report the effect of natural fermentation of precooked pearl millet flour on phytate P, inorganic P and HCl-extractability of important minerals including phosphorus, calcium, iron, zinc, copper and manganese.

MATERIALS AND METHODS

Materials

The pearl millet grains used for fermentation were procured from the local market in a single lot. The grains were cleared of broken seeds, dust and other foreign material and were coarsely ground on the day of fermentation by an electric grinder using a 1·5 mm sieve.

Fermentation

Pearl millet (100 g) was mixed with distilled water (900 ml) in a conical flask and autoclaved at 1·05 kg cm^{-2} pressure for 15 min. After autoclaving and cooling, 10 g freshly ground pearl millet flour was added as inoculum. Fermentation in four replicates was then carried out at 20, 25 and 30°C for 72 h. The autoclaved unfermented pearl millet flour served as control. The fermented, as well as unfermented, samples were oven-dried for 48 h at 65°C to constant weight. The dried product was finely ground in the cyclone mill (Cyclotec, M/s Tecator, Höganäs, Sweden) using a 0·5 mm sieve.

Phytate P, non-phytate P and inorganic P

The samples were extracted in 0·2M HCl with continuous shaking for 2 h in a mechanical shaker at room temperature and phytic acid in the extract was

estimated colorimetrically (Haug & Lantzsch, 1983). Phytate phosphorus was derived by using the following formula (Reddy *et al.*, 1982)

$$\text{Phytate phosphorus (mg)} = \frac{A \times 28{\cdot}18}{100}$$

where A = Phytate content (mg).

Non-phytate phosphorus was calculated as the difference between the total phosphorus and phytate phosphorus.

For determining inorganic phosphorus, a 1-g sample was extracted in 20 ml distilled water by shaking on a rotary shaker for 3 h at room temperature. It was filtered through Whatman No. 42 filter paper and inorganic P in the filtrate was determined colorimetrically (Chen *et al.*, 1956).

Total minerals

The samples were wet acid-digested using a nitric acid and perchloric acid mixture (HNO_3:$HClO_4$:5:1 v/v). The amounts of iron, zinc, copper and manganese in the digested sample were determined by atomic absorption spectrophotometry (AA120, Australia) (Lindsey & Norwell, 1969). Calcium in the digested sample was determined by a titration method (Vogel, 1962) employing hydroxylamine hydrochloride, triethanolamine, polyvinyl alcohol and using calcon as the indicator. The violet colour was titrated against 0·01N EDTA solution to a bluish green end point. Phosphorus was determined colorimetrically (Chen *et al.*, 1956).

HCl-extractable minerals

The minerals in the fermented samples were extracted in 0·03N HCl by shaking the contents at 37°C for 3 h. The clear extract obtained after filtration with Whatman No. 42 filter paper was oven-dried at 100°C and wet acid-digested as mentioned above. The amounts of the extractable phosphorus, calcium, iron, zinc, copper and manganese in the digested samples were determined by the methods described above for estimation of total amount of the minerals.

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0·03N HCl}}{\text{Total mineral}} \times 100$$

Statistical analysis

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

RESULTS AND DISCUSSION

The unfermented pearl millet flour contained phytate P, non-phytate P and inorganic P constituting 57, 43 and 9% of total phosphorus, respectively. Natural fermentation resulted in a significant ($P < 0.05$) decrease in phytate P with a corresponding significant increase in non-phytate P, inorganic P and HCl-extractable P at all temperatures (Table 1). Fermentation at 30°C completely eliminated the phytate P; other temperatures were less effective. Non-phytate P, inorganic P and HCl-extractable P contents were the highest at 30°C followed by those at 25 and 20°C.

TABLE 1
Effect of Natural Fermentation on Phytate, Non-phytate and Inorganic P (% of total P) and HCl-Extractability (%) of Cooked Pearl Millet Flour[a]

Treatments	*Phytate P*	*Non-phytate P*	*Inorganic P*	*P extractability*
Control:				
Autoclaved unfermented flour	57·1 ± 0·63	42·9 ± 0·0	9·3 ± 0·10	34·8 ± 0·29
Fermentation:				
20°C	21·5 ± 0·18	78·5 ± 0·20	27·5 ± 0·07	57·0 ± 0·62
25°C	9·6 ± 0·05	90·4 ± 0·07	30·4 ± 0·12	62·7 ± 0·60
30°C	0·0 ± 0·0	100·0 ± 0·0	47·6 ± 0·61	84·3 ± 0·36
CD ($P < 0.05$)[b]	0·57	0·57	0·51	0·78

[a] Values are means ± SD of four replicates (on dry matter basis).
[b] Critical difference at 5% level. Differences of two means within/between the temperature treatments exceeding this value are significant.

Calcium extractability was significantly ($P < 0.05$) improved by natural fermentation at all temperatures (Table 2). HCl-extractability of calcium in the fermented product at 30°C was significantly ($P < 0.05$) higher than that at 20 and 25°C; the extractabilities at the latter temperatures were, of course, not significantly different from one another.

The fermentation significantly improved ($P < 0.05$) the HCl-extractability of iron in pearl millet flour at all temperatures (Table 2). The extractability increased more than three-fold by fermentation at 30°C; the fermentations at 20 and 25°C were relatively less effective and there was no significant ($P < 0.05$) difference in iron extractability at these temperatures.

HCl-extractability of zinc increased at all the fermentation temperatures (Table 2). The extractability at 30°C was significantly ($P < 0.05$) higher than that at 20°C; the differences between the extractability at 20 and 25°C and that between 25 and 30°C were not significant.

TABLE 2
Effect of Natural Fermentation on HCl-Extractability (%) of Ca, Fe, Zn, Cu and Mn of Cooked Pearl Millet Flour[a]

Treatments	*Ca*	*Fe*	*Zn*	*Cu*	*Mn*
Control:					
Autoclaved unfermented flour	44·8 ± 3·49	20·5 ± 2·95	44·5 ± 1·81	37·4 ± 0·46	55·7 ± 0·00
Fermentation:					
20°C	75·0 ± 0·41	52·3 ± 1·70	58·8 ± 2·15	84·5 ± 1·20	75·4 ± 0·00
25°C	77·8 ± 2·27	52·2 ± 4·75	61·5 ± 0·00	85·7 ± 0·00	81·7 ± 0·00
30°C	82·6 ± 5·10	72·3 ± 4·39	64·6 ± 0·00	86·6 ± 0·00	81·7 ± 0·00
CD ($P < 0·05$)[b]	6·53	7·74	3·30	6·51	2·23

[a] Values are means ± SD of four independent replicates (on dry matter basis).
[b] Critical difference at 5% level. Differences of two means within/between the temperature treatments exceeding this value are significant.

Copper extractability during natural fermentation was more than doubled at all temperatures. Variation in the temperatures of fermentation did not cause a significant change in copper extractability.

A significant increase in manganese extractability was noticed when the pearl millet flour was fermented at 20, 25 and 30°C. The extractability at 20°C was significantly less than that at 25 or 30°C; the extractabilities at 25 and 30°C were the same.

HCl-extractability of the minerals increased significantly but to a varying extent as a result of natural fermentation of pre-cooked pearl millet flour at 20, 25 and 30°C. Decrease in the level of phytic acid (Khetarpaul, 1988) by fermentation possibly through hydrolysis by phytase of the pearl millet grain or the fermenting microflora (Lopez *et al.*, 1983; Dhankher & Chauhan, 1987), may release these divalent cations in free form and may account for their increased HCl-extractability in the fermented product. Cleavage of phosphorus from the phytic acid may explain the increased level of inorganic phosphorus and higher HCl-extractability of phosphorus in the fermented pearl millet flour. Phytic acid in the fermented products had a significant negative correlation with extractable zinc ($P < 0·01$), iron ($P < 0·05$), manganese ($P < 0·05$), phosphorus ($P < 0·05$) and calcium ($P < 0·01$) which indicates that the increase in HCl-extractability of these minerals is, perhaps, due to the decrease in the level of phytic acid in the fermented pearl millet flour. Improvement in HCl-extractability of minerals through natural fermentation of the raw pearl millet flour has been reported earlier (Mahajan, 1986). Fermentation has also been shown to enhance the HCl-extractability of minerals in corn and soybean (Chompreeda & Fields, 1984) and *rabadi* (Dhankher, 1985)—fermented pearl millet food.

Natural fermentation at 30°C was most effective in improving the HCl-extractability of dietary essential minerals including phosphorus, calcium, iron, zinc, copper and manganese. Phytate phosphorus decreased with a corresponding significant increase in non-phytate phosphorus, HCl-extractable phosphorus and inorganic phosphorus. Better microbial growth at 30°C, as compared with that at 20 and 25°C, may be responsible for higher microbial metabolic activity in enhancing the HCl-extractability of the minerals to a greater extent.

Natural fermentation is thus an effective method of improving the HCl-extractability of minerals from cooked pearl millet flour. Consumption of such a fermented product may lead to better mineral status of the population confronted with various mineral inadequacies due to limited bioavailability of these minerals from the food grains.

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