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Effect of fermentation on HCl-extractability of minerals from rice–defatted soy flour blend

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Buttermilk fermentation at 25, 30 and 35°C for 12, 18 and 24 h brought about a significant increase in non-phytate and HCl-extractable phosphorus with a corresponding decline in phytate phosphorus of a rice–defatted soy flour blend mixed in a 50 : 50 proportion. The HCl-extractabilities of calcium, iron, zinc, copper and manganese from the rice–defatted soy flour blend also improved. Higher HCl-extractability of minerals may be partly ascribed to the decreased content of phytic acid, as a significant negative correlation between the phytic acid and HCl-extractability of dietary essential minerals was obtained.

INTRODUCTION

Cereals and legumes form an integral part of the Indian dietary pattern. They are generally used together in various recipes, viz, *roti-dal, khichdi, dosa, idli,* etc. Traditional diets based on starchy staple tend to be monotonous and unappetizing. They are, therefore, eaten with sauces and relishes made from a variety of foods including legumes.

Blends of cereals and legumes have an important place in Indian traditional dietary patterns too. No doubt, blends prepared from cereals and legumes such as rice and defatted soy flour will have a better protein quality and mineral profile, but their utilization for human nutrition is constrained due to the presence of a wide variety of inherent antinutritional factors (Gupta, 1987), such as phytic acid, polyphenols, saponins, trypsin inhibitor and haemoglutenins.

Phytic acid, present in considerable amounts in rice and soy flour (Grewal, 1992), chelates with certain metal ions such as calcium, zinc, copper and iron to form insoluble protein–mineral phytate complexes. These complexes are not readily broken down and may pass through the digestive tract unchanged, thereby rendering minerals, especially divalent cations, unavailable (Davies & Nightingale, 1975; Erdman, 1979). Hence, the nutritional value of cereal–legume blends can be significantly enhanced if the level of antinutrients, including phytic acid and polyphenols, is reduced through any of the processing methods.

Previous work from our laboratory (Grewal, 1992; Gupta *et al.*, 1992) has shown that buttermilk fermentation significantly reduced the levels of phytic acid and polyphenols in cereals and legumes including wheat, barley and soybean. An attempt has therefore been made in this study to report the effect of buttermilk fermentation of rice-defatted soy flour blends on HCl-extractability of minerals in 0·03 N HCl, the concentration of the acid found in the gastric content of the human stomach.

MATERIALS AND METHODS

Materials

Rice and defatted soy flour (Vital soy flour, Britannia Industries Ltd, Calcutta, India) were procured from the local market in a single lot. The rice grains were cleaned of broken seeds, dust and other foreign materials and were ground in an electric grinder (Cemotec 1090, M/S Tecator, Höganäs, Sweden) using 1·5 mm sieve size. Skim milk powder, procured from the National Dairy Research Institute, Karnal, India was used for the preparation of buttermilk.

Preparation of rice–defatted soy flour blends

The flour of freshly ground rice and defatted soy flour were blended in a 50 : 50 (w/w) proportion. These ratios were selected on the basis of the composition of essential amino acids, particularly lysine, methionine and cystine, in cereal and legume as compared to their composition in egg protein so as to achieve a cereal–legume blend of good protein quality.

Preparation of buttermilk

Milk was reconstituted by mixing skim milk powder (16·7 g) with distilled water (100 ml). The reconstituted

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milk was heated to 40°C, inoculated with curd (2%) and incubated at 37°C for 5 h for the formation of curd or yoghurt having a pH of 4·6. Freshly prepared curd (400 g) was mixed with double distilled water (600 ml) and stirred to a homogeneous mixture to prepare the buttermilk containing 4·98% solids. Ever day fresh buttermilk was prepared from fresh curd and was used immediately for carrying out the fermentation of rice–defatted soy flour blends.

Fermentation of cereal–legume blend with buttermilk

For carrying out the fermentation, 100 g of the rice-defatted soy flour blend mixed in a 50:50 proportion was mixed with buttermilk (200 ml) and stirred sufficiently so as to obtain a homogeneous mixture. The homogeneous slurry was poured into a conical flask and allowed to ferment at three different temperatures, i.e. 25, 30 and 35°C for 12, 18 and 24 h in an incubator. Each fermentation was carried out in triplicate. The unfermented mixtures containing rich–defatted soy flour blends and buttermilk served as controls.

The fermented as well as unfermented samples were oven-dried at 65°C for 48 h to a constant weight. They were finely ground in a cyclone mill (Cyclotec, M/S Tecator, Höganäs, Sweden) using 0·5 mm sieve size.

Phytate and non-phytate phosphorus

The samples were extracted in 0·3 N HCl with continuous shaking for 3 h in a mechanical shaker at room temperature and phytic acid in the extract was estimated colorimetrically (Haug & Lentzsch, 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.

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, copper, zinc and manganese in the digested sample were determined by atomic absorption spectrophotometry (Lindsey & Norwell, 1969). Calcium in the digested samples was estimated by the titration method (Vogel, 1962), whereas, phosphorus was determined colorimetrically (Chen *et al.*, 1956).

HCl-extractability of minerals

The minerals in the fermented sample were extracted with 0·03 N 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. 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 amounts of the minerals.

$$\text{Mineral extractabilty (\%)} = \frac{\text{mineral extractable in 0·03 N HCl}}{\text{total minerals}}$$

Statistical analysis

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

RESULTS AND DISCUSSION

Phytate, non-phytate and HCl-extractable phosphorus

The unfermented rice–defatted soy flour blend contained phytate phosphorus and non-phytate phosphorus constituting 26·3 and 73·6% of total phosphorus, respectively. Fermentation of the blend with buttermilk

Table 1. Effect of temperature and period of fermentation on phytate and non-phytate phosphorus (% of total phosphorus) and HCl-extractability (%) of phosphorus content of fermented rice and defatted soy flour blend

Temperature of fermentation (°C)	Period of fermentation (h)	Phytate phosphorus	Non-phytate phosphorus	Phosphorus-extractability
Control	0	26·3 ± 0·3	73·7 ± 0·03	40·0 ± 4·9
25	12	23·9 ± 0·2	76·1 ± 0·2	42·4 ± 0·3 (+6)
	18	21·1 ± 0·2	78·9 ± 0·2	45·0 ± 0·2 (+14)
	24	18·8 ± 0·2	81·2 ± 0·2	47·6 ± 0·2 (+18)
30	12	22·9 ± 0·2	77·1 ± 0·2	44·1 ± 0·7 (+10)
	18	20·6 ± 0·2	79·4 ± 0·2	48·9 ± 0·1 (+22)
	24	18·2 ± 0·2	81·8 ± 0·2	49·7 ± 0·1 (+24)
35	12	19·3 ± 0·3	80·7 ± 0·3	46·2 ± 0·0 (+15)
	18	16·5 ± 0·2	83·5 ± 0·2	51·2 ± 0·1 (+27)
	24	13·5 ± 0·2	86·5 ± 0·2	53·0 ± 0·1 (+32)
CD ($P < 0{\cdot}05$)		2·29	2·29	1·14

Values are means ± SD of three replicates.
Figures in parentheses indicate increase in extractability of phosphorus expressed as percentage of control values.

Table 2. Effect of temperature and period of fermentation on HCl-extractability (%) of calcium, iron, manganese, zinc and copper of fermented rice and defatted soy flour blend

Temperature of fermentation (°C)	Period of fermentation (h)	Ca	Fe	Mn	Zn	Cu
Control	0	60·0 ± 0·6	72·6 ± 1·0	56·4 ± 0·1	67·7 ± 0·1	28·2 ± 0·0
25	12	65·7 ± 0·5 (+9)	76·4 ± 0·1 (+5)	64·4 ± 0·1 (+14)	74·2 ± 0·0 (+8)	37·7 ± 0·1 (+32)
	18	69·1 ± 0·7 (+15)	79·4 ± 0·1 (+9)	69·2 ± 0·0 (+22)	77·5 ± 0·0 (+14)	38·3 ± 0·0 (+35)
	24	73·2 ± 0·1 (+22)	83·3 ± 0·0 (+14)	72·4 ± 0·1 (+28)	80·3 ± 0·1 (+18)	42·4 ± 0·0 (+50)
30	12	68·2 ± 0·4 (+13)	77·3 ± 1·0 (+6)	66·1 ± 0·1 (+17)	76·7 ± 0·0 (+13)	38·7 ± 0·0 (+37)
	18	72·4 ± 0·5 (+20)	82·1 ± 0·0 (+13)	71·6 ± 0·1 (+26)	79·4 ± 0·0 (+17)	41·7 ± 0·0 (+47)
	24	74·4 ± 0·9 (+24)	84·5 ± 0·0 (+16)	74·8 ± 0·1 (+32)	82·2 ± 0·1 (+21)	42·7 ± 0·1 (+51)
35	12	71·5 ± 0·7 (+19)	78·6 ± 0·1 (+8)	68·7 ± 0·1 (+22)	77·5 ± 0·1 (+14)	40·2 ± 0·1 (+42)
	18	74·3 ± 1·1 (+23)	84·6 ± 4·0 (+16)	72·4 ± 0·0 (+28)	80·3 ± 0·1 (+18)	45·6 ± 0·0 (+59)
	24	76·7 ± 0·5 (+28)	86·4 ± 0·1 (+19)	77·9 ± 0·0 (+37)	83·1 ± 0·1 (+22)	49·3 ± 0·0 (+74)
CD ($P < 0·05$)		0·58	0·69	0·63	0·10	0·10

Values are means ± SD of three replicates.
Figures in parentheses indicate increase in extractability of minerals expressed as percentage of control values.

resulted in a significant decline in phytate phosphorus with a corresponding significant increase in non-phytate and HCl-extractability of phosphorus (Table 1). The phosphorus extractability increased to varying extent, i.e. 6–32% over the control values, depending upon the temperature and period of fermentation. The higher the temperature and longer the duration of fermentation, the greater was the extractability of the rice defatted soy flour blend. Maximum phosphorus extractability was noted when the blend was fermented at 35°C for 24 h. The improvement in phosphorus extractability corresponded with a proportional increase in non-phytate phosphorus at all the temperatures and periods of fermentation. This showed that hydrolytic reduction of phytic acid during buttermilk fermentation may be contributing towards the extractable phosphorus. Correlation coefficients showed a significant ($P < 0·01$) negative correlation between phytate and extractable phophorus. Thus, the higher the non-phytate phosphorus, the greater was the extractable phosphorus in the blend. The reduction in phytate phosphorus during buttermilk fermentation may be due to the hydrolysis of phytic acid by phytase, elaborated by fermenting microflora (Lopez *et al.*, 1983). Cleavage of phosphorus from phytic acid may explain the improved HCl-extrability of phosphorus in the fermented blend. Natural as well as *rabadi* fermentation has been reported earlier to increase the HCl-extractability of phosphorus with a corresponding decrease in phytic acid content of pearl millet flour (Khetarpaul & Chauhan, 1989) and soybean (Grewal, 1992).

HCl-extractable minerals

The HCl-extractability of calcium of the defatted soy flour blend increased with an increase in the temperature and period of fermentation when compared to the unfermented blend (Table 2). The percent enhancement in extractability of calcium ranged from 9 to 22, 13 to 24 and 19 to 28% over the control value when fermentations were carried out at 25, 30 and 35°C for different time periods, respectively. An improvement in extractability of iron, manganese, zinc and copper also occurred when the blend was fermented. The unfermented blend had almost 73% iron extractability. Fermentation at 25°C for 12 h brought about a marginal increase in iron extractability. As the temperature and period of fermentation was raised, further improvement in iron extractability was noted. The extent of the increase varied from 5 to 19% when rice–defatted soy flour was fermented for 12, 18 and 24 h at different temperatures.

A two- to three-fold enhancement in manganese extractability occurred when the blend was fermented for 18 and 24 h at different temperatures. Similarly fermentation at 35°C for 24 h raised the zinc extractability from 68 to 83%. A significant improvement in copper extractability was observed; the increase varied from 32 to 74% in the fermented blends.

Buttermilk fermentation improved the HCl-extractability of minerals, an index of their bioavailability to the human system. Higher HCl-extractabilities of calcium, iron, copper, zinc, phosphorus and manganese from the fermented cereal–legume blend may be partly ascribed to the decreased content of phytic acid, which had a significant negative correlation with the mineral extractability (Table 3). Decrease in phytic acid content possibly through hydrolysis by phytase of the fermenting microflora (Khetarpaul and Chauhan, 1991), may indicate that the divalent cations are freed from the phytate-mineral complex, which may account for their increased HCl-extractability in the fermented blend. Improvement in the HCl-extractability of minerals through sequential culture fermentation of pearl millet by yeast and lactobacilli was reported previously (Khetarpaul & Chauhan, 1991). Fermentation has also

Table 3. Correlation coefficients of HCl-extractability (%) of calcium, phosphorus, iron, manganese, copper and zinc with phytic acid content of fermented rice and defatted soy flour blend prepared in a 50:50 proportion.

Minerals	Correlation coefficients
Extractable Ca	0·8137
Extractable P	0·9734
Extractable Fe	0·9524
Extractable Mn	0·9421
Extractable Cu	0·9495
Extractable Zn	0·9185

Values are significant at the 1% level.

been reported to increase the HCl-extractability of minerals in corn and soy bean (Chompreeda & Fields, 1984; Grewal, 1992).

Thus, lactic acid fermentation of rice–defatted soy flour by buttermilk is a potential method for improving the HCl-extractability of calcium, copper, iron, zinc and manganese. The availability of minerals from plant foods such as cereals and legumes is limited due to the presence of antinutrients. Therefore, consumption of fermented cereal–legume blends may help to ameliorate the prevalent mineral deficiencies caused by their limited bioavailability and may lead to a better mineral status of the vegetarian population of developing countries.

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