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Technical Note

Effect of *Rabadi* Fermentation on HCl-Extractability of Minerals of Wheat

ABSTRACT

Rabadi *fermentation of freshly ground wheat flour–buttermilk mixture brought about a significant increase in HCl-extractability of calcium, iron, copper, zinc, manganese and phosphorus at 30, 35 and 40°C for 6, 12, 18, 24 and 48 h; the longer the period and the higher the temperature of fermentation, the greater was the increase in HCl-extractability of the minerals. The concentrations of non-phytate phosphorus and inorganic phosphorus in the fermented product also increased corresponding to a decreased phytate phosphorus. The level of phytic acid was found to have a significant negative correlation with HCl-extractability of minerals in the fermented products.*

INTRODUCTION

Fermentation is one of the most economical and oldest methods of producing, preserving and improving the nutritive value of several foods. It is also an effective process to improve utilisation of scarce food resources for human nutrition. Fermented foods constitute an important component of human diet all over the world.

Rabadi, an indigenous fermented food of India, is prepared by fermenting cereals and pulses including wheat, barley, maize, pearlmillet, chickpea, mothbean, etc. with country buttermilk. *Rabadi* is a wholesome food possessing several nutritional advantages especially to low income rural populations where buttermilk is available.

Rabadi fermentation has been reported to improve the nutritional value

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of pearlmillet–buttermilk mixture. This increases the level of B-vitamins and enhances the digestibility (starch and protein) and mineral availability (Dhankher & Chauhan, 1987*a*, 1989). The levels of antinutrients like phytic acid and polyphenols are appreciably reduced, too (Dhanker & Chauhan, 1987*b*).

Extractability of minerals in 0·03N HCl, the concentration of acid found generally in the stomach which is an index of availability of the minerals, has been reported to be negatively correlated with the phytic acid in foods (Khetarpaul & Chauhan, 1989). *Rabadi* fermentation has been found to significantly decrease the level of phytic acid in wheat (Gupta, 1989). This paper reports the effect of *rabadi* fermentation on the HCl-extractability of dietary essential minerals from wheat, a staple food in major parts of India and several other countries.

MATERIALS AND METHODS

Materials

Wheat (Var WH-283) grains used in preparation of *rabadi* were procured from the Department of Plant Breeding, Haryana Agricultural University, Hisar, India. The cleaned grains were ground in a cyclone mill (Cyclotec, M/s Tecator, Höganäs, Sweden) using a 0·5 mm sieve size. Buttermilk for carrying out the fermentation was prepared from skim milk powder procured from the National Dairy Research Institute, Karnal, India.

Preparation of buttermilk

In order to obtain curd with desirable consistency, milk was reconstituted by mixing skim milk powder (16·7 g) with distilled water (100 ml). The reconstituted milk was heated and maintained at 40°C for 5 min, inoculated with curd starter (2 g curd for 100 ml milk) and incubated at 37°C for $5\frac{1}{2}$ h. Freshly prepared curd (400 g) was mixed with water (600 ml) to prepare buttermilk. Each day fresh buttermilk was obtained from the freshly prepared curd and was used immediately for *rabadi* fermentation.

Preparation of *rabadi*

Freshly ground wheat flour (50 g) was mixed with buttermilk (1 litre) to prepare *rabadi* in triplicate. The mixture was incubated at 30, 35 and 40°C for 6, 12, 18, 24 and 48 h. The fermented product was cooked for 30 min with continuous stirring and then cooled. The unfermented wheat flour–buttermilk mixture served as the control. The fermented, as well as the unfermented, samples were oven-dried at 60°C for 48 h to a constant weight and finely ground in a 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 with 0·2N HCl for 3 h with continuous shaking in a mechanical shaker at room temperature. 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.

Non-phytate phosphorus was calculated as difference between the total phosphorus and phytate phosphorus. Inorganic phosphorus in the sample was extracted in water by shaking at room temperature for 3 h. Inorganic phosphorus in the extract was determined colorimetrically (Chen *et al.*, 1956).

Total minerals

The sample was wet-digested in diacid mixture (HNO_3 : $HClO_4$, 5:1 v/v) and the minerals including iron, copper, zinc and manganese in acid-digested samples were determined by Atomic Absorption Spectrophotometer (AA 120, Australia) according to the method of Lindsey and Norwell (1969). Calcium and phosphorus in the digested samples were determined titrimetrically (Vogel, 1962) and colorimetrically (Chen *et al.*, 1956), respectively.

HCl-extractable minerals

Minerals including calcium, phosphorus, iron, zinc, copper and manganese from the samples, were extracted in 0·03N HCl by incubating at 37°C in a shaker–waterbath for 3 h (Peterson *et al.*, 1943). After filtration through Whatman No. 42 filter paper, the clear filtrate was oven-dried at 100°C and then wet acid-digested with diacid mixture and the amount of various minerals as mentioned above was determined.

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

Statistical analysis

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

RESULTS

HCl-extractability of minerals of *rabadi*

As a result of fermentation of wheat flour-buttermilk mixture, total content of all the minerals did not change. This was anticipated as nothing was added nor removed which could alter the level of the mineral content (Khetarpaul & Chauhan, 1989).

Rabadi fermentation significantly improved ($P < 0.05$) the HCl-extractability of calcium, iron, manganese, copper and zinc in wheat flour;

TABLE 1
Effect of Temperature and Period of Fermentation on HCl-Extractability (%) of Calcium, Iron, Manganese, Copper and Zinc of *Rabadi* prepared from Raw Wheat Flour and Buttermilk Mixture[a] (on dry matter basis)

Temperature (°C)	*Period of fermentation (h)*	*Ca*	*Fe*	*Mn*	*Cu*	*Zn*
Control	0	65·7 ± 0·23	55·7 ± 2·82	56·8 ± 2·84	27·2 ± 1·21	68·4 ± 3·9
30	6	71·1 ± 0·45	59·5 ± 0·21	63·6 ± 0·47	30·4 ± 0·39	72·0 ± 0·49
	12	74·4 ± 0·24	61·4 ± 0·53	67·2 ± 0·04	32·9 ± 0·32	74·7 ± 0·15
	18	77·2 ± 0·89	63·5 ± 0·15	69·9 ± 0·88	35·2 ± 0·39	77·9 ± 0·07
	24	79·7 ± 0·57	66·0 ± 0·54	71·7 ± 0·76	38·4 ± 0·00	79·6 ± 0·25
	48	82·8 ± 1·19	69·0 ± 1·03	73·9 ± 0·57	41·2 ± 0·54	81·8 ± 0·46
35	6	72·7 ± 0·96	60·5 ± 0·34	65·4 ± 0·47	33·6 ± 0·35	73·7 ± 0·62
	12	75·9 ± 0·51	62·5 ± 0·46	69·8 ± 0·37	36·8 ± 0·07	76·0 ± 0·09
	18	77·8 ± 1·33	64·3 ± 0·23	71·7 ± 0·18	40·0 ± 0·15	78·7 ± 0·58
	24	80·6 ± 1·91	66·9 ± 0·37	74·4 ± 0·12	43·2 ± 0·07	80·4 ± 0·06
	48	84·1 ± 0·54	70·9 ± 0·36	77·6 ± 0·41	46·4 ± 0·30	82·7 ± 0·04
40	6	73·8 ± 0·53	61·4 ± 0·35	66·8 ± 0·11	35·2 ± 0·09	75·1 ± 0·24
	12	76·6 ± 0·89	63·3 ± 0·36	70·8 ± 0·35	38·4 ± 0·00	79·2 ± 0·02
	18	78·1 ± 1·34	65·4 ± 0·34	72·8 ± 0·60	41·6 ± 0·07	81·1 ± 0·23
	24	81·5 ± 1·11	68·1 ± 0·46	75·2 ± 0·33	45·3 ± 0·35	84·8 ± 0·34
	48	86·0 ± 0·57	71·6 ± 0·29	78·0 ± 0·18	48·0 ± 0·15	86·7 ± 0·48
	CD ($P < 0.05$)[b]	2·3203	3·8321	4·2442	1·9386	4·6183

[a] Values are means ± standard deviation of three replicates.
[b] Critical difference at 5% level. Difference of two means within/between the treatments exceeding this value is significant.

the longer the period of fermentation, the higher was the extractability (Table 1). As a result, *rabadi* prepared after 48 h fermentation had the highest extractability in respect of calcium, iron, manganese, copper and zinc. As the temperature was raised from 30 to 35 and 40°C, a marginal increase in the extractability of the minerals at all the periods of fermentation was noticed. After 48 h of fermentation the extractability of calcium and zinc at 40°C was significantly ($P < 0{\cdot}05$) higher than at 30°C, the extractability at 35°C was non-significantly different from either 30°C or 40°C fermented flour. The temperature of fermentation did not significantly ($P < 0{\cdot}05$) affect the extractability of manganese and iron as a result of 48 h of fermentation. HCl-extractability of copper after 48 h of fermentation increased significantly ($P < 0{\cdot}05$) with increase in the temperature. Consequently, the extractability was the highest at 40°C followed by that at 35°C and 30°C. Further, a significant ($P < 0{\cdot}01$) negative correlation was found between the phytic acid and calcium (−0·9960), iron (−0·9890), zinc (−0·9797), copper (−0·9954) and manganese (−0·9892).

TABLE 2

Effect of Temperature and Period of Fermentation on Phytate P (% of total P), Non Phytate P (% of total P), Inorganic P (mg/100 g) and HCl-Extractability of P(%) of *Rabadi* prepared from Raw Wheat Flour and Buttermilk Mixture[a] (on dry matter basis)

Temperature (°C)	*Period of fermentation (h)*	*Phytate P*	*Non-phytate P*	*Inorganic P*	*Extractability*
Control	0	10·06 ± 0·23	89·94 ± 0·23	383·2 ± 2·98	41·0 ± 0·31
30	6	9·38 ± 0·12	90·62 ± 0·12	393·6 ± 3·97	42·6 ± 0·16
	12	8·40 ± 0·33	91·60 ± 0·33	420·2 ± 2·56	43·9 ± 0·09
	18	6·90 ± 0·22	93·02 ± 0·22	433·7 ± 13·08	44·7 ± 0·07
	24	5·63 ± 0·33	94·37 ± 0·33	452·9 ± 5·69	45·9 ± 0·14
	48	4·53 ± 0·22	95·47 ± 0·22	471·4 ± 4·01	47·5 ± 0·12
35	6	8·27 ± 0·12	91·73 ± 0·12	408·0 ± 0·91	43·4 ± 0·16
	12	6·71 ± 0·09	93·29 ± 0·09	423·9 ± 0·67	44·4 ± 0·25
	18	5·62 ± 0·23	94·38 ± 0·23	453·2 ± 1·16	45·3 ± 0·19
	24	4·42 ± 0·61	95·58 ± 0·61	469·1 ± 4·80	47·8 ± 0·07
	48	3·15 ± 0·22	96·85 ± 0·22	485·0 ± 2·49	52·8 ± 0·14
40	6	6·90 ± 0·63	93·10 ± 0·63	428·2 ± 0·50	48·2 ± 0·33
	12	5·72 ± 0·22	94·28 ± 0·22	450·4 ± 2·20	49·7 ± 0·12
	18	4·78 ± 0·21	95·22 ± 0·21	467·5 ± 3·40	51·3 ± 0·25
	24	3·65 ± 0·32	96·35 ± 0·32	490·5 ± 6·38	52·8 ± 0·27
	48	2·63 ± 0·22	97·37 ± 0·22	503·4 ± 3·40	54·7 ± 0·15
	CD ($P < 0{\cdot}05$)[b]	0·6978	0·6978	10·4512	0·8546

[a] Values are means ± standard deviation of three replicates.

[b] Critical difference at 5% level. Difference of two means within/between the treatments exceeding this value is significant.

Phytate P, inorganic P and HCl-extractability of P of *rabadi*

Phytate phosphorus decreased whereas non-phytate P, inorganic P and HCl-extractability of P increased gradually with an increase in the period of *rabadi* fermentation of wheat flour, i.e. from 6 to 48 h (Table 2). As a result, phytate phosphorus was the lowest and non-phytate phosphorus, inorganic phosphorus and extractability of phosphorus were the highest after 48 h fermentation at all the temperatures. Phytate P in wheat flour–buttermilk mixture formed 10% of the total phosphorus. This percentage of phytate phosphorus was reduced to about 4·5% after 48 h fermentation at 30°C; the value was still significantly ($P < 0{\cdot}05$) lowered when the fermentation was carried out at 35°C. Raising the temperature further from 35 to 40°C did not make any significant difference. Non-phytate phosphorus showed a reverse trend, i.e. the highest at 40°C and the lowest at 30°C. As the temperature was raised from 30 to 35°C and 40°C, HCl-extractability of phosphorus and the amount of inorganic phosphorus showed an increasing trend; the highest values were observed at 40°C. A significant ($P < 0{\cdot}01$) negative correlation occurred between the phytic acid and inorganic P(−0·9999) and extractable P(−0·9830).

DISCUSSION

A significant improvement in the HCl-extractability of minerals including calcium, phosphorus, iron, zinc, copper and manganese, an index of their bioavailability during *rabadi* fermentation, may perhaps be ascribed to a decrease in the level of phytic acid of wheat. The decrease in the phytic acid content, as evidenced by reduction in phytate phosphorus (Table 2) may be due to hydrolytic action of phytase, an enzyme reported in wheat grains and the fermenting microflora (Lopez *et al.*, 1983). As a result of such hydrolysis inorganic phosphorus is liberated from phytic acid and the chelated divalent cations may be released in free form, thereby increasing the extractability of the minerals in the fermented product. Existence of significant ($P < 0{\cdot}01$) negative correlation between the level of phytic acid and HCl-extractability of minerals further strengthens this argument. Fermentation has been reported to enhance HCl-extractability of minerals in corn and soybean (Chompreeda & Fields, 1984) and pearl millet (Khetarpaul & Chauhan, 1989).

Rabadi fermentation from wheat is, therefore, an effective method of improving the HCl-extractability and possibly the bioavailability of minerals, thus improving the nutritional quality of wheat flour. Therefore, this indigenous fermented product may be useful in preventing and ameliorating mineral deficiencies and improving the nutritional status of populations consuming such food.

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