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HCl Extractability of Minerals from Rabadi—a Wheat Flour Fermented Food

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Rabadi was prepared from a precooked wheat flour and buttermilk mixture. This mixture was fermented at different temperatures, i.e., 30, 35, and 40 °C for varying periods, viz. 6, 12, 18, 24, and 48 h. A significant improvement occurred in the HCl extractability of dietary essential minerals including calcium, copper, iron, zinc, manganese, and phosphorus in all of the samples of rabadi fermented at various temperature and time period combinations. The longer the period and the higher the temperature of fermentation, the greater was the increase in HCl extractability of the minerals. The concentration of non-phytate and inorganic phosphorus also increased in the fermented products. Phytic acid had a significant ($P < 0.01$) negative correlation with all extractable minerals in rabadi.

INTRODUCTION

Rabadi, an indigenous fermented food popular in northwestern semiarid regions of India, is commonly prepared by mixing flour of cereal grains like wheat, barley, maize, or pearl millet with buttermilk and allowing it to ferment in an earthen or metallic vessel in the open sun for 4–6 h during the summer months of May and June. It is a lactic acid fermented food in which lactose undergoes acid fermentation naturally and readily.

Previous work done in our laboratory revealed that rabadi prepared by mixing raw pearl millet with buttermilk improved the nutritional value of pearl millet. The levels of B vitamins were increased, and in vitro starch and protein digestibility and bioavailability of minerals (Dhankher and Chauhan, 1987a, 1989) were enhanced. The levels of antinutrients like phytic acid and polyphenols have also been reported to be reduced appreciably (Dhankher and Chauhan, 1987b). Among the cereals, wheat flour is one of the important staple foods of the Indian population, and until now there was no information on the nutritional changes brought about during fermentation of precooked wheat flour used in the preparation of rabadi. Therefore, an attempt has been made to prepare rabadi from precooked wheat flour and buttermilk mixture and to determine the effect of fermentation on HCl extractability of dietary essential minerals.

MATERIALS AND METHODS

Materials. Wheat grains (var. WH-283) 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 used in fermentation was prepared from skim milk powder procured from the National Dairy Research Institute, Karnal, India.

Preparation of Buttermilk. 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 of curd for 100 mL of milk), and incubated at 37 °C for 55 h. Freshly prepared curd (400 g) was mixed with distilled water (260 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. Wheat flour (50 g) and double-distilled water (300 mL) were mixed, autoclaved at 15 psi for 15 min, and cooled. Buttermilk (660 mL) as prepared above was added to the autoclaved/precooked and cooled wheat flour slurry by keeping it near the flame and was mixed thoroughly. The above mixture was fermented at 30, 35, and 40 °C for 6, 12, 18, 24, and 48 h in an incubator. After the expiry of the fermentation period, common salt (8 g) was added to the fermented product. The unfermented mixture was kept as control. The fermented and the unfermented samples were oven-dried at 60 °C for 48 h to a constant weight and finely ground in a Cyclone mill using a 0.5-mm sieve.

Phytate P, Non-Phytate P, and Inorganic P. The samples were extracted with 0.2 N HCl for 3 h with continuous shaking in a mechanical shaker at room temperature. Phytic acid in the extract was estimated colorimetrically (Haug and Lantzsch, 1983). Phytate phosphorus was derived by using the formula (Reddy et al., 1982)

$$\text{phytate P (mg)} = (A \times 28.18)/100$$

where A is the phytate content.

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

Total Minerals. To 1 g of ground moisture-free sample was added 25 mL of diacid mixture (HNO_3:$HClO_4$::5:1 v/v), and the mixture was kept overnight. The next day it was digested by heating until clear white precipitates settled at the bottom. The crystals were dissolved by diluting in double-distilled water, and the contents were filtered through Whatman No. 42 filter paper. The filtrate was made up to 50 mL with double-distilled water and was used for the determination of total iron, copper, zinc, and manganese by atomic absorption spectrophotometry according to the method of Lindsey and Norwell (1969). Total 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, phophorus, iron, copper, zinc, and manganese were extracted from the samples in 0.03 N HCl by incubating at 37 °C in a shaker with water bath 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 amounts of the above-mentioned minerals were determined:

$$\text{mineral extractability (\%)} = \frac{\text{mineral extractable in 0.03 N HCl}}{\text{total mineral}} \times 100$$

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

RESULTS AND DISCUSSION

Total Minerals. Levels of calcium, copper, iron, zinc, manganese, and phosphorus in the precooked unfermented

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Table I. Effect of Temperature and Period of Fermentation on HCl Extractability (Percent) of Calcium, Iron, Manganese, Copper, and Zinc of Rabadi Prepared from Autoclaved Wheat Flour[a] (on Dry Matter Basis)

temp, °C	period of fermentation, h	Ca	Fe	Mn	Cu	Zn
control	0	68.2 ± 0.43	56.4 ± 0.50	61.6 ± 0.85	28.8 ± 0.00	72.6 ± 1.77
30	6	72.6 ± 0.20	60.1 ± 1.21	66.8 ± 0.89	35.2 ± 0.30	76.0 ± 0.33
	12	74.9 ± 0.06	62.3 ± 0.22	70.8 ± 0.42	38.4 ± 0.13	80.5 ± 0.53
	18	78.3 ± 0.35	65.0 ± 1.08	72.6 ± 1.13	40.0 ± 0.15	83.5 ± 0.22
	24	80.1 ± 0.60	68.2 ± 0.25	74.0 ± 0.21	44.8 ± 0.19	86.1 ± 0.54
	48	82.8 ± 0.64	72.9 ± 0.39	75.8 ± 0.29	46.4 ± 0.15	87.7 ± 1.32
35	6	73.4 ± 1.19	62.2 ± 0.16	69.4 ± 1.49	38.4 ± 0.15	77.4 ± 0.28
	12	75.7 ± 1.63	63.2 ± 0.59	71.7 ± 2.84	41.6 ± 0.15	80.8 ± 0.42
	18	79.3 ± 1.91	66.2 ± 0.38	73.9 ± 0.74	43.2 ± 0.07	83.7 ± 0.48
	24	81.3 ± 1.41	68.8 ± 1.91	77.3 ± 1.01	46.4 ± 0.07	86.3 ± 0.44
	48	83.2 ± 0.40	73.4 ± 0.23	79.9 ± 0.86	48.0 ± 0.13	88.2 ± 0.67
40	6	74.9 ± 0.55	63.9 ± 0.34	71.2 ± 0.00	40.0 ± 0.15	82.1 ± 0.44
	12	76.3 ± 0.79	66.3 ± 0.59	74.9 ± 0.35	43.9 ± 0.06	85.4 ± 0.44
	18	80.7 ± 1.52	68.4 ± 0.34	77.2 ± 0.85	45.8 ± 0.07	87.3 ± 0.40
	24	82.5 ± 1.30	70.3 ± 0.28	79.5 ± 0.84	48.0 ± 0.19	89.4 ± 0.24
	48	85.6 ± 0.83	73.1 ± 0.37	83.0 ± 0.56	54.4 ± 0.19	91.4 ± 1.71
CD ($P < 0.05$)[b]		2.43	3.43	3.93	1.27	3.21

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

Table II. Effect of Temperature and Period of Fermentation on Phytate P, Inorganic P, and HCl Extractability of P of Rabadi Prepared from Autoclaved Wheat Flour[a] (on Dry Matter Basis)

temp, °C	period of fermentation, (h)	phytate P, g/100 g	phytate P, % of total P	inorganic P, g/100 g	extractable P, %
control	0	0.08 ± 0.003	9.38 ± 0.25	0.48 ± 0.05	53.4 ± 0.34
30	6	0.07 ± 0.003	8.78 ± 0.24	0.49 ± 0.02	54.6 ± 0.15
	12	0.06 ± 0.001	7.72 ± 0.32	0.50 ± 0.02	56.0 ± 0.12
	18	0.05 ± 0.001	6.60 ± 0.22	0.52 ± 0.05	57.1 ± 0.09
	24	0.04 ± 0.003	5.27 ± 0.14	0.54 ± 0.06	58.7 ± 0.05
	48	0.03 ± 0.001	3.99 ± 0.12	0.55 ± 0.03	63.9 ± 0.39
35	6	0.06 ± 0.001	7.62 ± 0.21	0.51 ± 0.03	56.6 ± 0.14
	12	0.05 ± 0.001	6.33 ± 0.32	0.52 ± 0.00	58.2 ± 0.18
	18	0.04 ± 0.001	5.39 ± 0.22	0.54 ± 0.01	59.9 ± 0.38
	24	0.03 ± 0.001	3.98 ± 0.21	0.55 ± 0.02	61.8 ± 0.02
	48	0.02 ± 0.001	2.79 ± 0.31	0.56 ± 0.03	65.5 ± 0.08
40	6	0.06 ± 0.001	6.86 ± 0.22	0.52 ± 0.02	57.5 ± 0.12
	12	0.04 ± 0.002	5.39 ± 0.23	0.53 ± 0.04	59.3 ± 0.13
	18	0.04 ± 0.002	4.70 ± 0.23	0.55 ± 0.01	61.0 ± 0.09
	24	0.03 ± 0.002	3.38 ± 0.32	0.56 ± 0.02	64.3 ± 0.21
	48	0.02 ± 0.001	2.04 ± 0.21	0.57 ± 0.06	67.0 ± 0.06
CD ($P < 0.05$)[b]		0.004	0.40	0.01	0.84

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

wheat flour–buttermilk mixture were 785.0, 0.6, 5.6, 2.1, 2.2, and 811.0 mg/100 g, respectively. As expected, these values for total mineral content were not changed due to fermentation, as nothing was deleted or added. Earlier studies also showed no change in the total mineral content of fermented pearl millet (Khetarpaul and Chauhan, 1989; Mahajan and Chauhan, 1988).

HCl Extractability of Minerals. Temperature and period of fermentation had an enhancing effect on the HCl extractability of calcium, iron, copper, zinc, manganese, and phosphorus. The longer the period of fermentation, the higher was the extractability (Table I). As a result, rabadi prepared after 48 h of fermentation had the maximum extractability with respect to calcium, iron, copper, zinc, and manganese. As the temperature was raised from 30 to 35 and 40 °C, a marginal increase in the extractability of minerals at all periods of fermentation was observed.

The values of calcium extractability ranged from 74.9 to 85.6% after fermentation at 40 °C for 6, 12, 18, 24, and 48 h. When the fermentation period of the wheat flour-buttermilk mixture was prolonged form 0 to 48 h at different temperatures, the calcium extractability was raised from 68.2 to 85.6%.

The extractability of copper, manganese, and zinc was improved in all of the fermented products. The unfermented wheat flour–buttermilk mixture had about 29% copper extractability, and as a result of fermentation for varying periods, i.e., 6–48 h at 30, 35, and 40 °C, improvement ranged from 35.2 to 46.4%, from 38.4 to 48%, and from 40 to 54.4%, respectively. Hence, higher temperatures and longer periods of fermentation produced more improvement in copper extractability. At different temperatures, there were nonsignificant improvements in zinc extractability when fermentation was carried out for different time periods. Lower temperatures and shorter fermentation periods minimized the zinc extractability.

Rabadi fermentation at different temperatures and time periods improved the HCl extractability of minerals, an index of their bioavailability to the human system (Mahajan and Chauhan, 1988). Higher extractability of calcium, iron, zinc, copper, and manganese from the fermented rabadi may be partly due to the decreased content of phytic acid as evidenced by the reduction in phytate phosphorus (Table II) by fermentation, possibly through hydrolysis by phytase, an enzyme reported in the fermenting microflora (Irving and Cosgrove, 1974; Nayini and Markakis, 1984). This enzyme may be responsible for releasing these metallic ions in free form and thus may account for their increased HCl extractability in the fermented product (Dhankher and Chauhan, 1987b). The existence of significant ($P < 0.01$) negative correlations between the level of phytic acid and HCl extractability of minerals further strengthens this argument. Improvement in HCl extractability of minerals as a result of fermentation has also been reported earlier in corn and soybean (Chompreeda and Fields, 1984) and pearl millet (Khetarpaul and Chauhan, 1989).

Phytate, Inorganic, and HCl-Extractable P. The unfermented precooked wheat flour–buttermilk mixture contained phytate P, constituting 9.38% of total P. As a result of fermentation, the content of phytate P decreased with a corresponding increase in inorganic P (Table II). Due to rabadi fermentation, HCl extractability of phosphorus was enhanced at all temperatures and time periods. However, at 40 °C phosphorus extractability was the highest. A significant negative correlation was found between phytate and inorganic as well as extractable phosphorus.

The reduction in phytate P during rabadi fermentation may be due to hydrolysis of phytic acid by phytase elaborated by fermenting microflora (Lopez et al., 1983). Cleavage of phosphorus from the phytic acid may explain the increased levels of inorganic P and higher HCl extractability of phosphorus in the fermented product. Natural fermentation has been reported earlier to increase the HCl extractability of phosphorus with the corresponding decrease in phytic acid content of pearl millet flour (Mahajan and Chauhan, 1987).

In conclusion, HCl extractability of dietary essential minerals, an index of their bioavailability to the human system, was improved significantly as a result of fermentation of a wheat flour–buttermilk mixture, and hence the nutritional quality of the wheat flour was enhanced.

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