Effect of Fermentation with Whey on the HCl Extractability of Minerals from Rice-Dehulled Blackgram Blends

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Various rice-dehulled blackgram blends were developed and fermented with whey at 35 °C for 18 h to find out the effect of fermentation on total minerals and their extractability in 0.03 N HCl, an index of their availability to the human system. Fermentation did not significantly change the total amount of calcium, phosphorus, and iron present in the blends. On the other hand, the HCl-extractability of calcium, phosphorus, and iron was enhanced considerably after whey incorporation and fermentation of cereal-legume blends. The amount of phytate phosphorus also decreased significantly. The higher HCl-extractability of minerals may be partly ascribed to decrease in phytic acid content because a signficant negative correlation occurred between the phytic acid and dietary essential minerals.

Keywords: *Rice-dehulled blackgram blends; whey; HCl extractability; calcium; phosphorus; iron; phytic acid*

INTRODUCTION

Blends of cereals and legumes have an important place in the traditional Indian dietary pattern. Such blends have better protein quality and mineral profiles than cereals or legumes alone, but their utilization for human nutrition is constrained because they contain certain antinutritional factors like phytic acid, polyphenols, and trypsin inhibitor activity.

Phytic acid [myoinositol 1,2,3,4,5,6-hexakis(dihydrogen phosphate)], present in most plant foodstuffs (Reddy et al., 1982; Yadav and Khetarpaul, 1994) as the phytate salt or a complex with protein, chelates with certain metal ions (such as, calcium, zinc, copper, and iron) to form insoluble protein-mineral-phytate complexes. These complexes fail to break down readily and make the minerals, especially divalent cations, unavailable (Davies and Nightingale, 1975; Erdman, 1979). The high polyphenol content in plant food grains (Hahn et al., 1984; Goyal and Khetarpaul, 1993; Saharan, 1994) may also adversely affect the mineral availability.

Because of the aforementioned factors, it is imperative to reduce the level of antinutrients to improve the bioavailability of the minerals in the human system. Fermentation of cereal-legume blends with buttermilk or whey is apparently effective in reducing the content of antinutrients like phytic acid and polyphenols (Saharan, 1994; Sharma, 1994) and, may thus have a beneficial effect on mineral bioavailability. With this perspective, an attempt was made to determine the effects of indigenous fermentation of rice-dehulled blackgram-whey blends on the HCl-extractability and bioavailability of minerals.

MATERIALS AND METHODS

The grains of rice (*Oryza sativa*) and dehulled blackgram (*Vigna radiata* L. Hepber) used in the study were obtained from the market in a single lot. The grains were cleaned of dust, stones, broken and wrinkled seeds, and other foreign materials and were ground in an electric grinder (Cemotec 1090, M/S Tecator, Höganäs, Sweden) with a 1.5-mm sieve size. The buffalo milk used for the preparation of whey was collected from a dairy in a single lot.

Rice-Dehulled Blackgram Blends. Rice flour and dehulled blackgram flour were mixed in the following three proportions: (i) rice + dehulled blackgram (60:40; RBL I); (ii) rice + dehulled blackgram (70:30; RBL II); and (iii) rice + dehulled blackgram (80:20; RBL III). These ratios were selected on the basis of essential amino acid composition, particularly lysine, methionine, and cystine, to achieve cereal-legume blends of good protein quality.

Preparation of Whey. For whey making, buffalo milk was standardized at 6% fat level. One percent citric acid was added to the milk, and the resulting curdled milk was filtered to obtain whey.

Fermentation of Cereal–Legume–Whey Blends. Whey (105 mL) was mixed with each rice-dehulled blackgram blend (100 g) and stirred to obtain a homogeneous mixture. All the rice-dehulled blackgram blends were allowed to ferment with whey in an incubator at 35 °C for 18 h.

Preparation of Samples for Nutritional Analysis. The samples of raw, unprocessed rice, dehulled blackgram, blends of unprocessed rice and dehulled blackgram with and without whey, and cereal-legume blends fermented with whey were oven dried at 65 °C to a constant weight. Dried samples were ground (Cemotec 1090, M/S Tecator, Höganäs, Sweden), filtered through 1.5-mm pore size sieve, and stored at room temperature until further chemical analysis.

Total Minerals. The samples were wet acid digested with a nitric acid and perchloric acid mixture (HNO_3:$HClO_4$, 5:1, v/v) in the digestion chamber. Precautions were taken while handling the acid mixture under the hood and during digestion so that acid fumes were not inhaled. After leaving the sample overnight in the diacid mixture, the sample was cautiously heated for 30 min until the initial vigorous reaction had subsided and then it was heated more strongly for 4 h until most of the nitrous fumes were removed. The heating was continued until white fumes of perchloric acid were evolved, the volume was made to 50 mL with deionized water, and the sample was filtered.

Calcium and iron in the digested samples were determined by atomic absorption spectrophotometry (Lindsey and Norwell, 1969). Phosphorus in the digested samples was determined colorimetrically (Chen et al., 1956).

HCl Extractability of Minerals. The minerals in the samples 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 #42 filter paper was oven dried at 100 °C and wet acid digested. The amounts of the HCl-

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Table 1. Effect of Whey Incorporation on Total Calcium Content and Its HCl Extractability in Blends Containing Rice and Dehulled Blackgram (on Dry Matter Basis)^{a}

blend	total calcium (mg/100 g)	HCl extractability of calcium (%)
rice	43.6 ± 0.12^{a}	83.3 ± 0.03^{a}
dehulled blackgram	121.3 ± 0.11^{b}	48.3 ± 0.07^{b}
blends of rice and dehulled blackgram		
rice + dehulled blackgram (R:BL::60:40-RBL I)	77.3 ± 0.22^{c}	69.2 ± 0.07^{c}
rice + dehulled blackgram (R:BL::70:30-RBL II)	68.4 ± 0.16^{d}	75.4 ± 0.08^{d}
rice + dehulled blackgram (R:BL::80:20-RBL III)	57.1 ± 0.12^{e}	79.0 ± 0.10^{e}
unfermented blends of rice, dehulled blackgram and whey		
RBL I + whey	77.4 ± 0.09^{c}	72.9 ± 0.16^{f} (4)
RBL II + whey	68.5 ± 0.07^{d}	78.7 ± 0.08 (4)
RBL III + whey	57.2 ± 0.05^{e}	82.3 ± 0.08^{h} (5)
fermented blends of rice, dehulled blackgram and whey		
RBL I + whey	77.4 ± 0.10^{c}	77.4 ± 0.20^{i} (10)
RBL II + whey	68.5 ± 0.10^{d}	83.2 ± 0.07^{j} (10)
RBL III + whey	57.2 ± 0.08^{e}	87.3 ± 0.08^{k} (12)
pooled SE (m)	0.08	0.06
pooled CD ($P < 0.05$)	0.23	0.17

[a] Values are means ± SD of three determinations; values in the parentheses indicate percent increase (+) over the control values; superscripts signify the statistical difference at the 5% level.

Table 2. Effect of Whey Incorporation on Total Iron Content and Its HCl Extractability in Blends Containing Rice and Dehulled Blackgram (on Dry Matter Basis)^{a}

blend	total iron (mg/100 g)	HCl extractability of iron (%)
rice	3.66 ± 0.15^{a}	77.5 ± 0.06^{a}
dehulled blackgram	6.36 ± 0.04^{b}	51.8 ± 0.07^{b}
blends of rice and dehulled blackgram		
rice + dehulled blackgram (R:BL::60:40-RBL I)	4.75 ± 0.04^{c}	68.3 ± 0.06^{c}
rice + dehulled blackgram (R:BL::70:30-RBL II)	4.52 ± 0.06^{d}	71.1 ± 0.09^{d}
rice + dehulled blackgram (R:BL::80:20-RBL III)	4.16 ± 0.09^{e}	73.1 ± 0.15^{e}
unfermented blends of rice, dehulled blackgram and whey		
RBL I + whey	4.74 ± 0.06^{c}	70.4 ± 0.07^{f} (3)
RBL II + whey	4.53 ± 0.07^{d}	73.4 ± 0.06^{g} (3)
RBL III + whey	4.15 ± 0.09^{e}	75.2 ± 0.06^{h} (3)
fermented blends of rice, dehulled blackgram and whey		
RBL I + whey	4.76 ± 0.10^{c}	74.1 ± 0.08^{i} (9)
RBL II + whey	4.54 ± 0.09^{d}	77.2 ± 0.06^{j} (9)
RBL III + whey		(9)
pooled SE (m)	0.05	0.04
pooled CD ($P < 0.05$)	0.14	0.13

[a] Values are means ± SD of three determinations; values in parentheses indicate percent increase (+) over the control values; superscripts signify the statistical difference at the 5% level.

extractable calcium, iron, and phosphorus in the digested samples were determined by the methods just described for estimatino of total minerals:

$$\text{mineral extractability (\%)} = \frac{\text{mineral extractability in 0.03 N HCl}}{\text{total minerals}} \times 100 \quad (1)$$

Phytate and Non-Phytate Phosphorus. The samples were extracted in 0.03 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 and Lentzsch, 1983). Phytate phosphorus was derived with the following formual (Reddy et al., 1982):

$$\text{phytate phosphorus (mg)} = (A \times 28.18)/100 \qquad (2)$$

In eq 2, A is the phytate content (mg). Non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus.

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

RESULTS AND DISCUSSION

Total Minerals. The total mineral content of rice was less than that of dehulled blackgram (Tables 1, 2, and 3). The contents of calcium, iron, and phosphorus in rice were 43.6, 3.6, and 139.7 mg/100 g, respectively. Dehulled blackgram had 121.3 mg of calcium, 6.3 mg of iron, and 378.6 mg of phosphorus per 100 g of sample. With a change in the proportion of blending rice flour and dehulled blackgram flour, a significant ($p < 0.05$) variation was noticed in the total amount of calcium, iron, and phosphorus. In general, the total amount of dietary essential minerals, including calcium, iron, and phosphorus, were significantly higher in blends containing more dehulled blackgram. Thus, RBL I, containing

Table 3. Effect of Whey Fermentation on Total Phosphorus, Phytate Phosphorus, and HCl Extractability in Blends Containing Rice and Dehulled Blackgram (on Dry Matter Basis)[a]

blend	total phosphorus (mg/100 g)	phytate phosphorus (mg/100 g)	HCl extractability of phopshorus (%)
rice	139.7 ± 0.11^{a}	27.0 ± 0.12^{a}	68.8 ± 0.07^{a}
dehulled blackgram	378.6 ± 0.09^{b}	177.0 ± 0.14^{b}	24.1 ± 0.08^{b}
blends of rice and dehulled blackgram			
rice + dehulled blackgram (R:BL, 60:40; RBL I)	241.4 ± 0.09^{c}	85.0 ± 0.21^{c}	52.5 ± 0.08^{c}
rice + dehulled blackgram (R:BL, 70:30; RBL II)	217.4 ± 0.24^{d}	69.8 ± 0.13^{d}	58.4 ± 0.11^{d}
rice + dehulled blackgram (R:BL, 80:20; RBL III)	181.9 ± 0.08^{e}	57.9 ± 0.07^{e}	62.2 ± 0.11^{e}
unfermented blends of rice, dehulled blackgram, and whey			
RBL I + whey	241.5 ± 0.15^{e}	63.9 ± 0.12^{f}	54.2 ± 0.09^{f} (3)
RBL II + whey	217.5 ± 0.07^{d}	55.9 ± 0.11^{g}	61.2 ± 0.10^{g} (5)
RBL III + whey	180.9 ± 0.15^{f}	48.6 ± 0.16^{h}	54.5 ± 0.22^{h} (4)
fermented blends of rice, dehulled blackgram, and whey			
RBL I + whey	241.5 ± 0.10^{c}	53.5 ± 0.09^{i}	59.4 ± 0.11^{i} (13)
RBL II + whey	217.5 ± 0.11^{d}	47.5 ± 0.10^{j}	66.4 ± 0.08^{j} (14)
RBL III + whey	180.9 ± 0.11^{f}	42.9 ± 0.15^{k}	69.9 ± 0.15^{k} (12)
pooled SE (m)	0.07	0.06	0.06
pooled CD ($P < 0.05$)	0.22	0.19	0.19

[a] Values are means ± SD of three determinations; values in parentheses indicate percent increase (+) over the control values; superscripts signify the statistical difference at the 5% level.

rice and dehulled blackgram in 60:40 (w/w) proportion, and a higher amount of total minerals, followed by RBL II (rice:legume 70:30, w/w), and RBL III (rice:legume, 80:20, w/w).

On addition of whey and fermentation, the calcium, iron, and phosphorus contents of the rice-dehulled blackgram blends did not change significantly. There was only a slight contribution of calcium (0.071%), iron (0.001%), and phosphorus (0.11%) from whey. Thus, whey incorporation led to a nonsignificant increase in the total mineral content of rice-legume blends (Tables 1, 2, and 3).

HCl-Extractability of Minerals. The addition of whey to the raw blends of rice and dehulled blackgram brought about a slight, but significant, change in the extractability of calcium and iron. An increase of 4−5% over the control value was observed in the extractability of calcium when whey was added to rice-dehulled blackgram blends. The extractability of iron in the unfermented rice-legume-whey blends was in the range 70.4−75.2%. The extractability of calcium and iron was the highest in blends containing whey and less legume.

After fermentation at 35 °C for 18 h, the HCl-extractability of minerals improved significantly ($p < 0.05$) in various rice and dehulled blackgram blends. Improvement to the extent of 10−12% in HCl-extractability of calcium and 9% in that of iron occurred over the control value in the fermented blends.

Fermentation with whey improved the HCl-extractability of dietary essential minerals. The higher HCl-extractability of calcium and iron from the fermented cereal-legume blend may be partly ascribed to the decreased content of phytic acid (Sharma, 1994), which had a significant negative correlation ($p < 0.01$) with the extractability of minerals. The decrease in phytic acid content may indicate that the divalent cations are freed from the phytate-mineral complex. The latter may account for increased HCl-extractability of minerals of the fermented blend.

These results are in accord with those of fermented pearlmillet and buttermilk mixture (Dhankher, 1985), lactic fermentation of pearlmillet flour (Khetarpaul and Chauhan, 1990), and buttermilk fermented cereal-legume blends (Goyal, 1991).

Phytate and HCl-Extractable Phosphorus. From the total extractable phosphorus in rice, 19.3% was present in the form of phytate P and 80.6% constituted non-phytate P (Table 3). In the dehulled blackgram, a considerable amount (46.7%) of phosphorus was present in the form of phytate P. With an increase in the amount of legume in the rice-dehulled blackgram blends, the amount of phytate P increased with a corresponding decline in non-phytate P. Phytate P ranged from 31.8 to 35.2% in various rice-dehulled blackgram blends; the maximum amount of phytate P was in RBL I, followed by RBL II and RBL III. The extractability of phosphorus from the raw, unprocessed RBL blends varied from 52.5 to 62.2%.

Whey incorporation resulted in an increase in extractability of phosphorus, with a proportional decrease in phytate P in all the rice-legume blends. The extractability of phosphorus improved by 3 to 4% (Table 3).

Fermentation with whey further lowered the levels of phytate P in all the rice-dehulled blackgram blends with a simultaneous increase in extractability of phosphorus (12−14%); (Table 3). Thus, the hydrolytic reduction of phytic acid during whey fermentation may be contributing towards the extractability of phosphorus. Correlation coefficients showed a significant negative correlation between phytate and extractable phosphorus. Hence, the lower the phytate P, the more extractable was phosphorus in a blend.

The reduction in phytate P during whey fermentation may be due to the phytate hydrolysis by phytase elaborated by fermenting microflora (Lopez et al., 1983). Cleavage of phosphorus from phytic acid may explain the improved extractability of phosphorus in a fermented blend. Natural as well as *rabadi* fermentation has been reported earlier to increase the HCl-extractability of phosphorus with a corresponding decrease in the phytic acid content of pearlmillet flour (Khetarpaul and Chauhan, 1989) and soybean (Grewal, 1992).

Whey incorporation did not change the mineral content of the blends significantly. On the other hand, whey addition brought about a significant improvement in the HCl-extractability of minerals, which was further enhanced on fermentation. A corresponding decrease in phytate P and enhancement in HCl-extractability of calcium, iron, and phosphorus were noticed in the present study. Therefore, consumption of fermented cereal-legume blends may help to ameliorate prevalent mineral deficiencies caused by their limited bioavailability from the unprocessed cereals and legumes and may lead to better mineral status of the vegetarian population of the developing countries.

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