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Binding of iron, calcium and zinc by fibre of sorghum and ragi

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Iron, calcium and zinc binding by flour, bran and neutral detergent fibre (NDF) of sorghum and ragi were investigated *in vitro*. The percentages of added iron, calcium and zinc bound by sorghum NDF were 54%, 88%, and 67.5%, respectively, and bound by ragi NDF were 59.6%, 88.8%, and 65.6% respectively. Brans were dephytinized by treatment with hydrochloric acid and the effect of phytate hydrolysis of brans on the iron-, calcium- and zinc-binding capacity of brans was tested. Brans treated with hydrochloric acid bound more iron, calcium and zinc than native brans. NDFs of sorghum and ragi were treated with proteases to assess the contribution of fibre-bound protein to mineral-binding capacity. The contribution of fibre-bound protein was 9.6% in sorghum and 19% in ragi for zinc binding. There was no impact of fibre-bound protein on iron and calcium binding. Ascorbic acid, one of the most potent inhibitors of iron binding, could release the bound iron from iron–fibre suspensions of both sorghum and ragi. © 1997 Elsevier Science Ltd

INTRODUCTION

Iron, calcium and zinc, despite their relative scarcity in tissues, perform important functions in health and resistance to disease. Although the dietary intake of these minerals is adequate, deficiencies are noticed in populations of low socioeconomic groups who consume cereals and millets as their staple foods (Simpson *et al.*, 1981; Ismail-beigi *et al.*, 1979). This may be due to lowered bioavailability of these minerals from their diets. Fibre and phytate are known to decrease bioavailability of minerals from cereal and millet diets (Thompson & Weber, 1981; Fernandez & Phillips, 1982; Lee & Garcia-Lopez, 1985). Fibre and phytate bind with minerals forming insoluble complexes, thus reducing their bioavailability (Reinhold *et al.*, 1981; Platt & Clydesdale, 1987; Ha *et al.*, 1989). The mineral-binding capacities of millet fibres like sorghum and ragi, which are consumed as staple foods in the dryland regions of the world, are not known. Hence this study attempts to determine the iron-, calcium- and zinc-binding capacities of sorghum and ragi fibres *in vitro*.

MATERIALS AND METHODS

Sorghum was procured from the National Research Centre for Sorghum, Rajendranagar, Hyderabad, India, and ragi was procured from the Regional Agricultural Research Station, Palem, Andhra Pradesh Agricultural University, Hyderabad, India.

Dehusking

Three kilograms each of sorghum and ragi were pounded separately with addition of enough water (50 ml) to loosen the husk from the grain. The husk was separated by winnowing and used as bran. The dehusked grain was later washed and sun-dried to the original moisture level. Amounts of 275 g of sorghum bran and 325 g of ragi bran were obtained per each kilogram of sorghum and ragi grains dehusked. The bran was dried in an oven at 50°C overnight.

Whole grain and bran were ground into a fine powder to pass through a 60 mesh sieve, and were stored in dry containers in the refrigerator (4°C) until use. Neutral detergent fibre (NDF) was prepared from sorghum and ragi brans by the method of Robertson & Van Soest (1977).

Treatment of bran with hydrochloric acid

Brans were treated with hydrochloric acid to hydrolyse the phytates by the method of Reinhold *et al.* (1981), to assess the mineral-binding capacity of native and treated brans.

Treatment of neutral detergent fibre (NDF) with proteases

NDF was treated with proteases (Reinhold *et al.*, 1981) to remove the fibre-bound protein in order to assess the contribution of fibre-bound protein, if any, to the mineral-binding capacity of NDF.

All the above-treated samples were stored separately in sealed containers at 4°C until used.

Mineral-binding capacity

Iron-, calcium- and zinc-binding capacities of sorghum and ragi samples (i.e. whole flour, bran, bran treated with hydrochloric acid, NDF and NDF treated with proteases) were assessed according to the method of Reinhold *et al.* (1981).

Iron content of the supernatant was determined by reaction with bathophenanthroline sulphonate (Henry *et al.*, 1974). The calcium and zinc contents in the respective supernatants were determined by atomic absorption spectrophotometry (Pye-Unicam, SP9 Model). The difference in mineral concentration between the unexposed control and that exposed to fibre was taken as the mineral binding by the fibre. The effect of pH on iron, calcium and zinc binding by NDFs of sorghum and ragi were determined by adjusting the pH of the reaction mixtures containing fibre with 0.1 N NaOH or 0.1 N HCl within the pH range 4.0–6.5.

Other analyses

Total dietary fiber

Total dietary fibre content of sorghum and ragi was estimated by the rapid enzymatic assay of Asp *et al.* (1983).

Phytate phosphorus

Phytate phosphorus was extracted and precipitated from the sample by the method of Wheeler & Ferrel (1971) and Makower (1970) and was colorimetrically determined by the method of Fiske & Subbarow (1925) modified by Morris *et al.* (1931).

Protein

Protein content of sorghum and ragi whole flour, NDF and NDF treated with peptidases was estimated according to the method given by AOAC (1975).

Iron, calcium and zinc

Iron, calcium and zinc contents of sorghum and ragi were estimated after wet digestion using atomic absorption spectrophotometry (Pye-Unicam, SP9 Model).

Statistical analysis

All the experiments were done six times and the results obtained were the means of six concurrent values.

RESULTS

Results of the analysis of total dietary fibre, phytate protein and mineral contents of sorghum and ragi are given in Table 1.

Protein content of sorghum and ragi NDFs was estimated to be 11.8 g per 100 g and 10.5 g per 100 g. Treatment of sorghum and ragi NDFs with peptidases decreased the protein content to 3.6 g per 100 g and 2.1 g per 100 g, respectively. Almost 75–85% of the protein was found to be destroyed by treatment with peptidases in both sorghum and ragi.

Phytate contents of sorghum and ragi brans were estimated to be 212.7 mg per 100 g and 279.2 mg per 100 g, and the treatment with hydrochloric acid was found to decrease the phytate levels to 52.9 mg per 100 g and 66.5 mg per 100 g in sorghum and ragi, respectively. Nearly 70% of the total phytate present in bran was hydrolysed by treatment with hydrochloric acid in both sorghum and ragi.

Effect of pH on iron-, calcium- and zinc-binding capacities of sorghum and ragi NDFs

Iron-, calcium- and zinc-binding capacities of sorghum and ragi NDFs were estimated *in vitro* at simulated duodenal pH conditions (pH 4.5–6.5); the results are depicted in Fig. 1.

Iron, calcium and zinc binding by NDFs of sorghum and ragi were found to be highly pH-dependent. Iron binding by NDFs of sorghum and ragi was minimum at pH 4.0 and maximum at pH 6.5, whereas calcium and zinc binding to NDFs of both sorghum and ragi was found to be minimum and constant from pH 4.0–5.0 and maximum at pH 6.5 within the range tested.

In the absence of fibre, the concentrations of iron, calcium and zinc in the respective reaction mixtures remained constant, within the range of pH tested (pH 4.0–6.5). However, when NDF of sorghum or ragi was added to the respective reaction mixtures containing minerals (iron or calcium or zinc), the concentrations of these minerals were found to decrease in the respective reaction mixtures, as the pH approached 6.5. At pH 6.5 the amounts of iron, calcium and zinc bound by sorghum NDF were 54%, 88% and 67.5%, respectively, and by ragi NDF 59.6%, 88.8% and 46.6%, respectively.

Table 1. Total dietary fiber, phytate, protein and mineral contents of sorghum and ragi whole flour

	Sorghum	Ragi
Total dietary fiber (g per 100 g)	9.5	20.0
Phytate (mg per 100 g)	226	279
Protein (g per 100 g)	11.8	10.5
Iron (mg per 100 g)	2.9	2.6
Calcium (mg per 100 g)	26.0	293
Zinc (mg per 100 g)	2.6	2.4

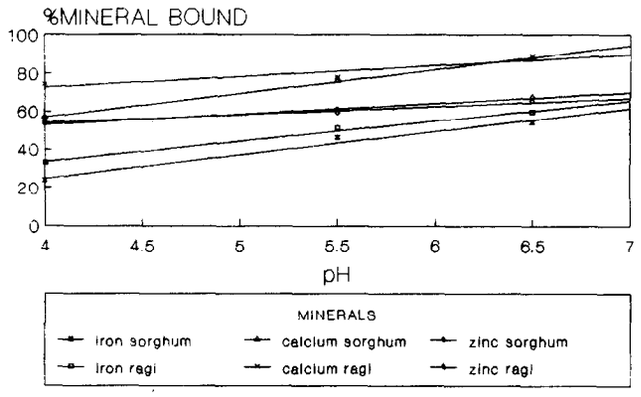


Fig. 1. Effect of pH on mineral binding by sorghum and ragi fibres.

Iron-, calcium- and zinc-binding capacities of sorghum and ragi samples

The percentage of added iron (0.7 μg ml^{-1}), calcium (1.5 μg ml^{-1}) and zinc (1.5 μg ml^{-1}) bound to whole flour, bran, bran treated with hydrochloric acid, NDF and NDF treated with proteases was estimated *in vitro* at pH 6.5 ± 0.05. The results are given in Table 2.

Iron, calcium and zinc bound by whole flour of sorghum was 2.8%, 3.4% and 6.2% less, respectively, than the bran of sorghum, whereas iron, calcium and zinc bound by whole flour of ragi was 20%, 30% and 35% less, respectively, than the bran of ragi.

Although the amounts of iron, calcium and zinc bound by sorghum samples were less than for ragi, the differences between the amounts of these minerals bound to whole flour, bran and whole flour NDF were less in ragi than sorghum. The amount of calcium bound was more than iron and zinc in almost all the samples tested.

The difference in the amount of iron bound by native brans and in brans treated for phytate hydrolysis was very little. It was 2.8% in sorghum and ragi, indicating that phytates are not a major interfering factor for iron binding by sorghum and ragi fibres.

The amount of calcium bound by sorghum and ragi brans was 12.3% and 3.4% less, respectively, than their brans treated for phytate hydrolysis, and the amount of zinc bound by sorghum and ragi brans was 19.6% and 6.3% less, respectively, when compared to their brans treated for phytate hydrolysis, indicating that phytate is a major interfering factor for calcium and zinc binding by sorghum and ragi brans.

The treatment of sorghum and ragi NDFs with proteases did not alter the iron- and calcium-binding capacities of sorghum and ragi NDFs. The amounts of iron and calcium bound to sorghum and ragi NDFs and NDFs treated with proteases were the same, indicating that there is no contribution of fibre-bound protein to iron and calcium binding. However, the amounts of zinc bound to NDFs of sorghum and ragi were 9.6% and 19% more than NDFs treated with proteases (Table 2), indicating that the contribution of fibre-bound protein to zinc binding was 9.6% in sorghum and 19% in ragi.

Effect of ascorbic acid on iron binding by fibre

Ascorbic acid, one of the potential inhibitors of iron binding, was added to the iron–fibre complexes at 0.44 M concentration in order to assess its effect on iron binding by flour, bran and NDFs of sorghum and ragi. Before ascorbic acid addition, the amounts of iron bound to sorghum flour, bran and NDF were 34.1%, 45.5% and 54.0%, respectively. The amount of iron bound to the samples was completely released when ascorbic acid was added at 0.44 M concentration to the iron–fibre complexes.

DISCUSSION

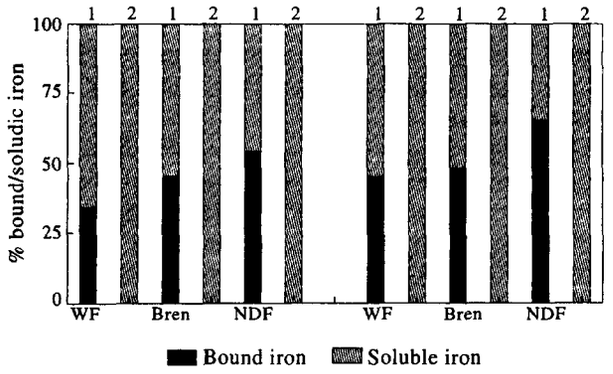
Millets like sorghum and ragi form an important source of iron, calcium and zinc in Indian diets, especially for people living under poor socio-economic conditions. However, fibre and phytate present in these foods bind with these minerals, thus reducing their bioavailability (Leigh & Miller, 1983). Binding substantially decreases the effective mineral concentrations in the lumen leading to lowered uptake by the mucosa.

Iron, calcium and zinc binding by sorghum and ragi NDFs increased with increase in pH from 4.0 to 6.5 (Fig. 2). Mercedes Torre *et al.* (1995) reported that ion-exchange and adsorption are the two physicochemical mechanisms by which fibre binds with minerals. When

Table 2. *In vitro* iron-, calcium- and zinc-binding capacities of sorghum and ragi samples

Sample	Iron bound (%)		Calcium bound (%)		Zinc bound (%)	
	Sorghum	Ragi	Sorghum	Ragi	Sorghum	Ragi
Whole flour	34.1	45.4	58.0	71.7	32.7	53.1
Bran	45.5	48.2	73.9	75.1	45.7	59.3
Bran treated with HCl	48.3	51.0	86.2	78.5	65.3	65.6
NDF	54.0	59.6	88.0	88.8	67.5	65.6
NDF treated with proteases	54	59.6	88.0	88.8	57.9	46.6

$n = 6$.



1. Before ascorbic acid addition WF = Whole flour
2. After ascorbic acid addition NDF = Neutral detergent fibre

Fig. 2. Effect of ascorbic acid on iron binding by sorghum and ragi fibres.

translated to the physiological environment, mineral binding with fibre starts in the small intestine (pH 4.5) and reaches a maximum in the duodenum (pH 6.0–6.5). Reinhold *et al.* (1975) reported that binding of iron to wheat bran increased up to pH 6.5 and decreased above pH 6.5, due to instability of iron in solutions above pH 6.5. Thompson & Weber (1979) observed an increase in the amount of iron and zinc bound by wheat bran, corn bran, soy bran, oat hulls, rice bran and cellulose, as the pH increased from pH 0.65 to 6.8. Rendleman (1982) reported that cellulose, starch, hemicellulose and pectin have little affinities for calcium ions at the neutral pH range of 5–8 at 37°C.

The mineral-binding capacity of whole flour was 20–30% less than that of bran and NDF, in both sorghum and ragi. This is due to the higher concentration of fibre in the bran and NDF. Reinhold *et al.* (1984) reported that the amounts of iron bound by tortillas and maize meal were almost 20% less than bran and NDF.

Brans treated for phytate hydrolysis bound more calcium and zinc than their native brans, indicating that phytate is major interfering factor for calcium and zinc binding by sorghum and ragi brans. However, sorghum and ragi brans treated for phytate hydrolysis bound iron as effectively as the native brans. Simpson *et al.* (1981) suggested that phytates in wheat bran may not be the cause of the inhibitory effect of bran on iron absorption in man. This conclusion was based on two observations. First, it has been reported that half the iron in bran is in the form of monoferric phytate, and it has been shown that iron absorption is the same from meals with ferric chloride and monoferric phytate added. Second, a marked reduction of phytate content of bran by endogenous phytase does not abolish the inhibitory effect of bran on iron absorption. Rendleman (1982) reported that dephytinized wheat bran bound more calcium than non-dephytinized wheat bran. He suggested that, when the calcium and phytate molar ratios are greater than 1, phytate may affect calcium absorption. Ismail-beigi *et al.* (1979) reported that more zinc was bound by dephytinized tanok (wholemeal wheat bread) when compared to native tanok.

Earlier studies by McCance & Widdowson (1942) and Reinhold *et al.* (1973) have shown that phytate is the major factor which binds minerals such as calcium and zinc. However, Sullivan *et al.* (1966) reported that phytate is digestible and, like other digestible chelators, would ultimately liberate bound metal as digestion proceeds. On the other hand, metals bound by the indigestible residue, mainly fibre, remain unavailable for absorption. Although fibre may be attacked by the bacteria of the large gut with release of metals, absorption can no longer occur in this region and the metals will be lost in the faeces. Consequently, it is the fibre content of the foodstuffs that largely determines the availability of bivalent metals.

Our results indicate that there was no contribution of fibre-bound protein to iron and calcium binding in either sorghum or ragi. However, the contribution of fibre-bound protein to zinc binding was 9.6% in sorghum and 19% in ragi. Reinhold *et al.* (1975) reported that treatment of wheat flour with pancreatin decreased its ability to bind zinc. Rendleman & Grobe (1982) reported that proteins contribute as much as 7% of total zinc binding by wheat bran.

The iron bound by the sorghum and ragi samples was completely released when ascorbic acid was added at 0.44 M concentration to the iron–fibre complexes. Most of the dietary non-haem iron is bound by dietary fibre in the duodenum (pH 6.0–6.5), thus rendering it unavailable for absorption. It is presumed that fibre-bound iron would be released by the surges of gastric acid which enter the duodenum, or it may also be released by chelators such as ascorbic acid when taken in sufficient amounts. Reinhold *et al.* (1981) reported that ascorbic acid could inhibit iron binding by wheat and maize fibres only when added at concentrations greater than 0.3 mmol litre^{-1}.

It has been reported that mineral nutriture may suffer as a result of increased dietary fibre consumption (Southgate, 1987). One mechanism by which dietary fibre may influence mineral availability is cation-exchange. Dietary fibre contains acidic groups such as uronic acids and acidic side-chains on closely associated protein. Dietary fibre sources vary dramatically in their ability to bind minerals and this point must be noted when dietary fibre sources are included in the diet. It is important to know the properties of fibre sources because of their possible physiological effects. The binding of trace minerals by fibre requires further elucidation of the influence of physicochemical properties. The capacity of various fibre components of a particular food to bind with the minerals and the effect of wet and dry heat processing methods of food preparation on the binding of dietary fibre to minerals need to be investigated.

Since inclusion of complex carbohydrates in the diet is now being advocated as a preventative measure for a

number of health complications, knowledge of physicochemical properties of these complex carbohydrate sources and their judicious use is essential to strike a balance between their positive and negative effects.

Measurements of mineral binding to fibre offer an important tool for studying mineral–fibre interactions. However, the extrapolation of these *in vitro* binding study results to *in vivo* effects on availability must be done carefully. An *in vitro* system can at best only approximate *in vivo* conditions. Preparation of fibre from the foods may cause chemical and physical alterations in the fibre thus influencing its behaviour. Substances added to the *in vitro* system, such as buffers, may affect binding.

This work shows that nutritionally significant minerals such as iron, calcium and zinc vary in their affinity for fibre. This is influenced by pH, type of fibre used (NDF), phytates and proteins, as seen for zinc binding by sorghum and ragi.

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