Effect of Traditional Food Processing on Phytate Degradation in Wheat and Millets

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For cereal-based vegetarian meals, processing such as soaking cereal flour prior to heating can activate native phytases. This activation will result in improving the zinc bioavailability since degraded products of phytate have a lower affinity for zinc. The effect of increasing the time for soaking wheat batter at 10 °C for 0−48 h and the effect of roti making with millet and sorghum flour batters was investigated. Phytate (IP6) degradation was studied using ion-exchange chromatography on a column of Dowex 1X8 resin (200−400 mesh). A soaking time of 12 h for wheat batter resulted in a 40% decrease in IP6. The decreasing trend for wheat with increased soaking time was significant ($p < 0.01$). Soaking also resulted in an increase of zinc solubility by 38.5%. Degradation of IP6 due to roti making without soaking of batter for all three cereals was 14−19% with a marginal decrease in zinc solubility.

Keywords: *Phytate degradation; traditional methods; zinc solubility*

INTRODUCTION

Phytic acid is a common constituent of most cereal grains and some vegetables and fruits (Chu, 1992). The role of phytate in human nutrition is due to its mineral binding capacity, which reduces bioavailability of certain minerals such as zinc (Sandstrom, 1987). The most abundant form of phytate that exists in plants is myoinositol hexaphosphate (IP6). However, during food processing and digestion, IP6 can be altered to lower degradation products like penta-, tetra-, and triphosphates having lower mineral-binding ability. Plant phytases are found in the seeds of most cereals and legumes. Processing prior to heating may increase phytase activity and therefore favor zinc availability. Further, cooking methods like roasting may bring about chemical degradation of IP6. Limited reports are available about the effect of traditional Indian food processing on phytate degradation and in turn on zinc availability. Hence, as an initial step the effect of roti making of wheat, sorghum, and pearl millet was investigated. The effect of an increase in soaking time of whole wheat batter on the extent of phytate degradation was also examined as a simple means of improving native zinc availability in this traditional food item.

MATERIALS AND METHODS

(a) Preparation of Food Samples. Cereal grains, i.e., wheat, sorghum, and pearl millet, were purchased from a local market and ground in a domestic flour mill to 60 mesh size and stored for the entire experimental period in air-tight containers. Chapati, a nonchemically leavened whole wheat pancake, was prepared with batter soaking times of 0, 12, 24, 36, and 48 h and the temperature maintained at 10 °C. The sorghum and pearl millet flours were used for preparing roti in the traditional way (Subramaniam, 1986). All food samples were prepared in three replicate sets, dried in an oven set at 50 °C until free of moisture, ground to pass a 0.5 mm screen, and stored at 4 °C until further analysis.

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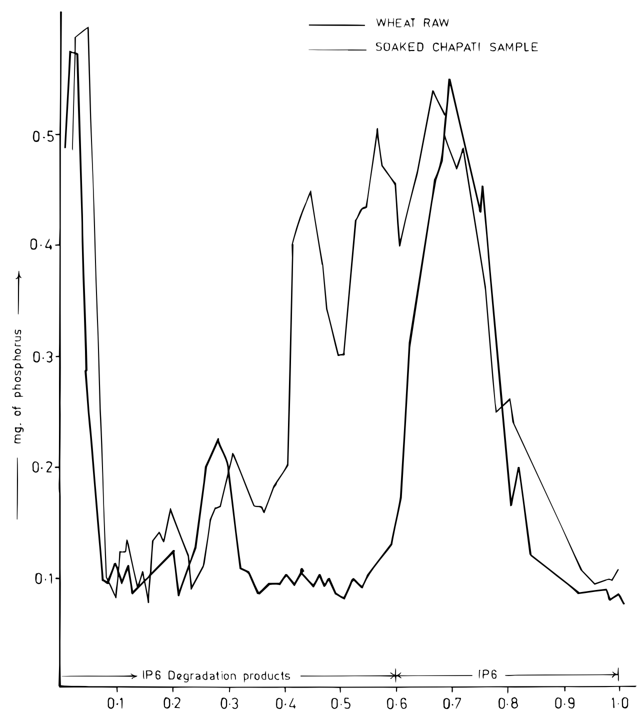

Figure 1. Effect of soaking on phytate components separated by ion-exchange chromatography. The average of three replicates is plotted. Variation between replicates is about 1%. The peak between 0.6 and 1 N HCl indicates IP 6, while peaks below 0.6 N HCl indicate lower esters of phytic acid.

(b) Study of Phytate Degradation. Phytic acid and its degradation products were initially extracted in triplicate from food samples (0.5 g) with 3% trichloroacetic acid (10 mL). The supernatant (3 mL) for each food sample was loaded on a column of Dowex 1X8 (200−400 mesh, Cl form). It was subjected to linear gradient elution with 100 mL each of 0−1 N HCl. Fractions of 10 mL each were collected, and 5 mL portions were analyzed for P by the metavanadate method (Jacobsen and Weidner, 1973). Inositol was estimated using 1 mL of eluent according to Nayini and Markaris (1983) to identify the peaks. Standard phytic acid in the form of inositol hexaphosphate (Loba Chem, India) was also subjected to the above procedure.

Table 1. Percent Distribution of Inositol Phosphates[a]

	0.6−1 N HCl (IP6)	0.5−0.6 N HCl	0.4−0.5 N HCl	0.3−0.4 N HCl	0.2−0.3 N HCl	0.1−0.2 N HCl	0−0.1 N HCl
wheat							
raw	63.0	4.1	4.4	4.0	6.0	4.0	14.0
0 h soaked	53.0	10.5	12.0	7.1	5.3	4.1	26.3
12 h soaked	39.4	15.4	11.5	7.2	6.7	3.9	16.1
24 h soaked	42.5	16.0	13.1	6.7	4.5	4.0	12.9
36 h soaked	38.8	21.5	13.6	4.1	1.2	3.3	17.4
48 h soaked	40.9	14.5	12.5	6.2	7.1	5.6	13.1
sorghum							
raw	63.7	5.2	3.3	16.3	13.1	7.2	1.3
cooked	57.8	7.2	7.2	6.9	3.4	4.5	13.1
pearl millet							
raw	66.7	11.0	4.5	3.4	1.5	1.5	11.4
cooked	52.2	13.8	7.3	7.3	3.2	4.0	12.1
phytic acid	96.0		1.0	Tc	0.5	1.5	1.0

[a] Values represent averages of three replications. % C. V. between replicates was in the range 0.02−0.15. Sorghum and pearl millet flours lost their texture due to soaking; hence, they were not studied for the effect of soaking. The trend in degradation of IP6 in wheat after 12 h of soaking was significant ($P < 0.01$).

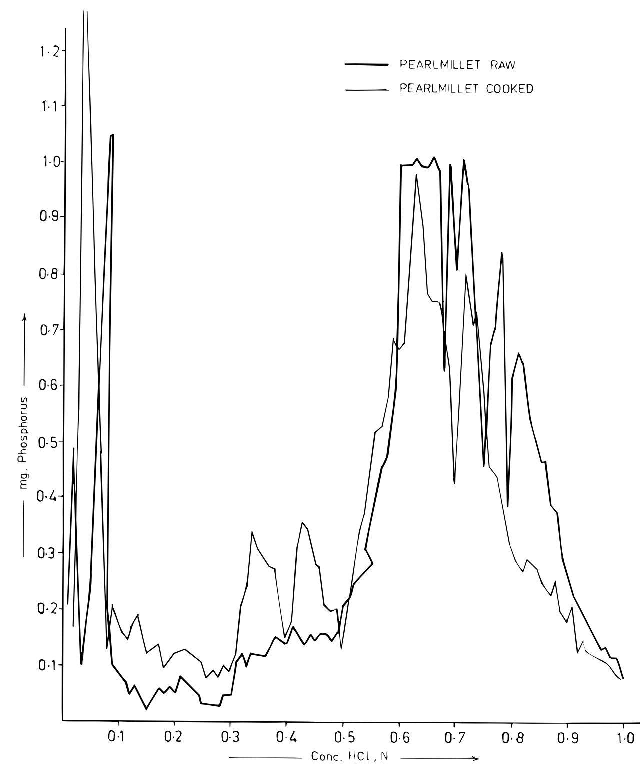

Figure 2. Phytate acid components in pearl millet flour (raw) and pearl millet flour (cooked). Raw pearl millet flour indicates the presence of only inositol hexaphosphate. After cooking, phytic acid is degraded into its lower ester products.

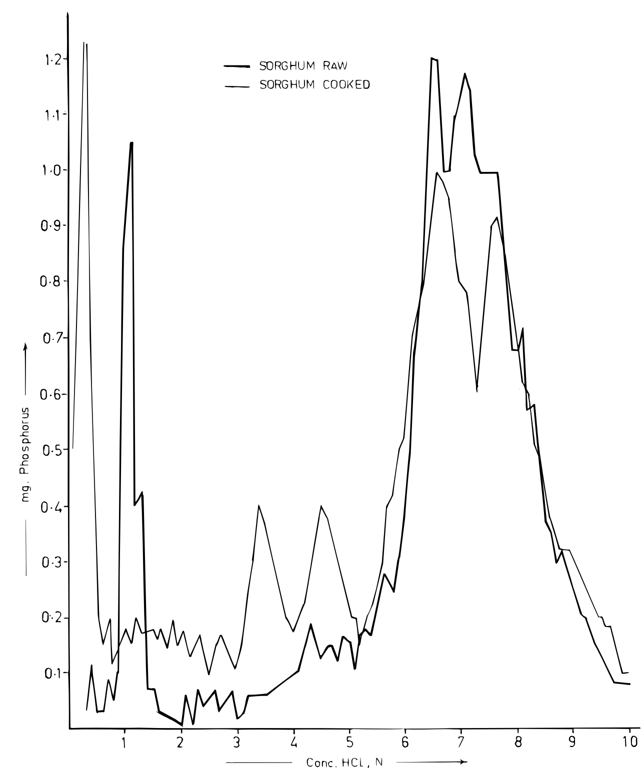

Figure 3. Phytate acid components in sorghum flour (raw) and sorghum flour (cooked). Raw sorghum flour indicates the presence of only inositol hexaphosphate. After cooking, phytic acid in sorghum (cooked) is degraded into its lower ester products.

(c) Assay for Zinc Solubility. The percentage of soluble zinc under simulated gastrointestinal conditions was used as the parameter for changes in zinc availability. It was estimated according to Sandstrom (1987) using 65Zn as the extrinsic tag. In brief, food samples taken from three replicate sets were first suspended in pepsin−HCl buffer (pH 1.2) and kept in an incubator shaker for 2 h at 37 °C. The contents were gradually neutralized using 0.05 M $NaHCO_3$. A mixture of desiccated bile and pancreatin (Sigma, USA) was added, and samples were again kept in an incubator−shaker for 3 h at 37 °C (pH 7.6). Samples were centrifuged, and supernatants were used for taking γ counts on a γ counter (Electronic Corporation of India) with a well-type detector. The percentage of zinc solubility was calculated as (counts per second of supernatant/counts per second of the buffer) 100.

Statistical Methods. Mean and standard deviation were calculated using values of three sets. The effect of soaking time on the IP6 degradation pattern was tested using a nonparametric Theil method for the trend (Sprent, 1993).

RESULTS AND DISCUSSION

During the initial steps of standardization of the ion-exchange chromatography technique standard phytic acid gave a single large peak between 0.6 and 1 N HCl (Table 1). This was in agreement with Sandberg and Anderson (1987). The repeatability of the technique was also estimated. Variability between replicate sets for each gradient of HCl was less than 1%. For each food sample 100 fractions of 10 mL each were analyzed, and averages were plotted. Figures 1, 2, and 3 illustrate the pattern of phytic acid and its degradation products for wheat, pearl millet, and sorghum, respectively. The peak obtained under 0.6−1 N HCl fractions represents IP6, while peaks below 0.6 N HCl represent the lower ester forms (i.e., tetra, penta, etc.) of myoinositol.

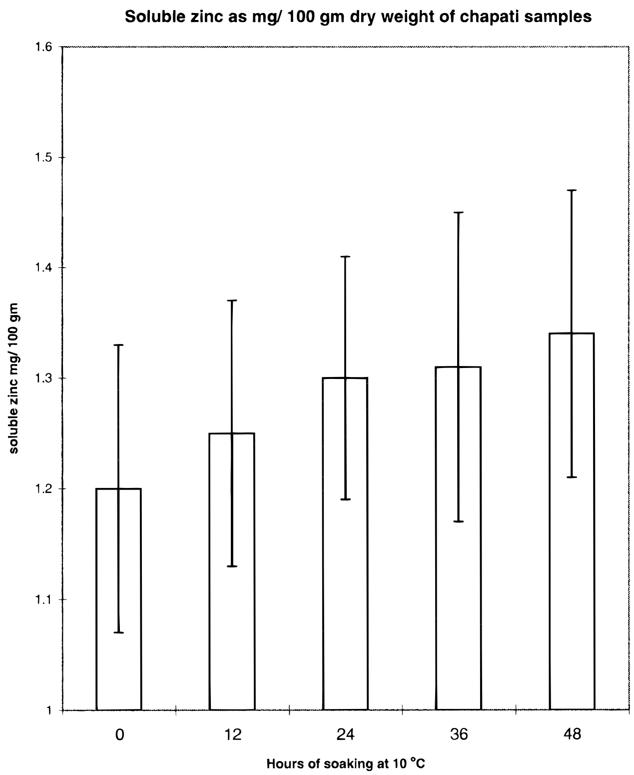

Figure 4.

Table 1 gives the effect of pancake making of cereals on the pattern of phytate distribution. It was seen that there was about 15−20% degradation of IP6 into lower forms for all three cereals. Soaking of wheat batter further helped in reducing the levels of IP6. 12 h. A soaking time of 12 h was found to be sufficient to bring about a 40% reduction in IP6. When the trend in degradation was tested using Theil's method there was significant decrease in IP6 levels and increase in lower forms ($P < 0.01$). There was 38.8% increase in zinc solubility as compared to raw flour in chapati made using a soaking time of 12−48 h (Figure 4). On increasing the soaking time sorghum and pearl milllet flour batters lost the texture required for making roti and hence were not studied.

Bioavailability of dietary zinc is the prime factor that needs to be considered in assessing adequacy of population consuming whole cereal diets. The problem of poor availability has been associated with a higher molar ratio of phytic acid to zinc (Fergusson, 1989; Ellis and Kelsey, 1987). Subsequent research has indicated that the inhibitory effect of phytic acid is maximum in its IP6 form and that degradation of IP6 considerably improves native bioavailability of iron and zinc (Rossander, 1992; Lonnardal, 1988; Sandberg, 1987, 1991). While the majority of studies on phytate degradation pattern have addressed Western populations, there is a need to study the effect of traditional cooking procedures on cereals and legumes consumed in the Indian subcontinent. The present effort was aimed therefore at this very problem. Soaking, malting, and fermentation are known to stimulate phytase activity and enhance iron absorption. In the present study, soaking of wheat batter was also found to be beneficial in terms of 40% reduction in IP6. This outcome may be due to activation of endogenous phytases, which resulted in an increase of soluble zinc by 0.24 mg/100 g dry weight. The present results illustrate that simple means such as soaking wheat flour batter at low temperature for 12 h may prove to be a useful strategy for policy planners as a suggestion for increasing the bioavailability of zinc.

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