Effects of Phytic Acid on Protein Digestibility (In Vitro) and HCl-Extractability of Minerals in Pearl Millet Sprouts

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ABSTRACT

Sprouting reduces phytic acid content of pearl millet grain. Protein digestibility (in vitro) and HCl-extractability of P, Ca, Fe, and Zn increased significantly during germination and were found to be dependent upon phytate content of the sprout. Germination at 30°C for 48 hr yielded highest protein digestibility and HCl-extractability of minerals.

Pearl millet (*Pennisetum glaucum*) plays an important role in the agriculture of many developing countries and is a major source of dietary nutrients for many people. The millet grain is a fairly good source of protein (10–15%), but its quality is low (Hoseney et al 1981) and its digestibility is poor (Chauhan et al 1986). Pearl millet grain possesses a relatively better mineral profile than other common grains (Kumar and Kapoor 1984). Like other cereal grains, pearl millet contains a considerable amount of phytic acid (Opoku et al 1981, Mahajan and Chauhan 1987). In several plant foods, phytic acid has hindered the availability of divalent cations (Reinhold et al 1973, Khetarpaul and Chauhan 1991) and inhibited the activity of proteolytic enzymes (Serraino et al 1985). Germination of cereals (Glennie et al 1985) and legumes (Duhan et al 1989) has reduced the level of phytic acid, resulting in better nutritional value of the sprouts (Grewal 1992). This article reports the effect of germination on the phytic acid content of pearl millet and the association of the phytic acid content with protein digestibility (in vitro) and HCl extractability (an index of bioavailability) of important minerals in pearl millet sprouts.

MATERIALS AND METHODS

Pearl millet grain was procured from a local market in a single lot and was cleaned of broken seeds and extraneous matter. The grain was sprouted by spreading 25 g of seeds on filter paper sheets in petri plates and adding 15 ml of distilled water. The petri plates were kept in an incubator at three temperatures for three different time intervals to obtain sprouts about 1.5 cm in length: 1) 25°C for 48, 54, or 60 hr; 2) 30°C for 36, 42, or 48 hr; 3) 35°C for 36, 42, or 48 hr.

Sprouts were separated from ungerminated seeds, oven dried at 65°C for 12 hr to a constant weight, ground to a fine powder using a Cyclotec Mill with 0.5-mm screen (Tecator, Hoganas, Sweden), then stored in air-tight plastic containers for further chemical analysis.

The finely ground sample was extracted with 0.2*N* HCl by shaking at room temperature. Phytic acid in the extract was determined colorimetrically (Haug and Lentzsch 1983). Phytase activity in the sprouts was assayed at pH 5.5 (Lolas and Markakis 1977). Inorganic P (Pi) released from phytate was estimated colorimetrically (Chen et al 1956). Phytase activity was expressed as μmoles of Pi released per minute per gram of pearl millet grain.

Pepsin and pancreatin were used to assess in vitro digestibility (Akeson and Stahmann 1964). Digested protein (N × 6.25) in the incubated mixture, separated by precipitating with trichloroacetic acid, was determined by the micro Kjeldahl method (AOAC 1980). Percent of total protein was calculated.

For determining total minerals (Ca, P, Fe, and Zn), the samples were wet-acid digested using a nitric acid: perchloric acid mixture (5:1 v/v). For HCl-extractable minerals, the samples were extracted with 0.03*N* HCl (the concentration of the acid found in human stomachs) by shaking at 37°C for 3 hr. The clear extract obtained after filtration with Whatman No. 42 filter paper was oven-dried at 100°C and then wet-acid digested. The amounts of Fe and Zn in the acid digest were determined by atomic absorption spectrophotometry (Lindsey and Norwell 1969). Ca was estimated by a titration method (Vogel 1962). P was determined colorimetrically (Chen et al 1956).

The data were subjected to analysis of variance, and correlation coefficients were derived (Panse and Sukhatme 1961).

RESULTS AND DISCUSSION

Phytic Acid

Unprocessed pearl millet grain contained a considerable amount of phytic acid (825.7 mg/100 g), which indicates that this antinutrient has a significant bearing on the nutritional value of the grain. Phytic acid content of several cereals has adversely influenced protein and starch digestibility and the availability of essential divalent minerals (Reddy et al 1982). While studying the nutritional value of some newly evolved high-yielding pearl millet varieties, Chauhan et al (1986) found a wide variation in phytic acid content of the millet grain. In vitro digestibility of protein and starch in those varieties was also relatively low.

Sprouting of the millet grains resulted in a significant decrease in phytic acid content at all the temperatures studied (Table I); the longer the period of germination, the greater the extent of decrease. Sprouts had the lowest concentration of phytic acid when the grains were germinated at 30°C for 48 hr. There was inherent phytase in pearl millet grain that became active during germination. However, no significant difference in the activity of phytase was observed (Table I).

TABLE I
Effect of Temperature and Period of Germination on Phytic Acid, Phytase Activity, and Protein Digestibility of Pearl Millet (db)[a]

Temperature (°C)	Period of Germination (hr)	Phytic Acid (mg/100 g)	Inherent Phytase Activity ($\mu mol \cdot min^{-1} g^{-1}$)	Protein Digestibility (%)
25	48	622.8 ± 12.9	1.29 ± 0.61	73.4 ± 5.76
	54	460.8 ± 18.5	1.05 ± 0.15	76.0 ± 5.25
	60	333.7 ± 17.0	0.78 ± 0.15	85.9 ± 3.00
30	36	457.2 ± 10.0	1.60 ± 0.67	73.3 ± 11.5
	42	191.3 ± 6.5	1.47 ± 0.81	78.1 ± 7.27
	48	71.5 ± 16.6	1.38 ± 0.26	79.0 ± 4.72
35	36	478.3 ± 10.0	1.58 ± 0.50	66.7 ± 5.81
	42	204.4 ± 12.2	1.35 ± 0.93	73.4 ± 5.76
	48	108.6 ± 11.1	0.82 ± 0.73	78.4 ± 3.78
Pearl millet grain (control)		825.7 ± 15.0	1.30 ± 0.03	54.2 ± 7.19
CD ($P < 0.05$)		7.11	NS[b]	10.9

[a] Values are mean ± SD of three replicates.
[b] Not significant.

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TABLE II
Total (mg/100 g) and Percent HCl-Extractable Ca, Fe, Zn, and P in Raw and Germinated Pearl Millet (db)[a]

Sample	Period of Germination (hr)	Ca	Fe	Zn	P
Raw millet, mg/100 g[b]		51.4 ± 2.01	16.3 ± 1.11	2.67 ± 0.05	3.02 ± 4.50
HCl-extractable in raw millet, %		32.4 ± 1.51	27.6 ± 1.61	46.8 ± 1.02	39.8 ± 2.13
HCl-extractable in germinated millet, %					
25°C	48	59.4 ± 7.72	33.3 ± 0.02	56.8 ± 2.51	54.6 ± 2.16
	54	61.3 ± 3.3	33.3 ± 0.02	56.8 ± 2.50	59.8 ± 4.73
	60	64.5 ± 5.71	40.4 ± 6.71	58.6 ± 1.32	62.3 ± 5.57
30°C	36	71.4 ± 15.3	34.8 ± 2.96	48.8 ± 2.07	55.6 ± 2.98
	42	74.6 ± 13.0	52.2 ± 8.90	51.9 ± 3.62	65.0 ± 4.93
	48	85.7 ± 18.2	63.2 ± 11.5	63.1 ± 4.06	71.1 ± 3.73
35°C	36	65.7 ± 9.10	34.8 ± 0.01	48.4 ± 2.29	53.3 ± 1.75
	42	68.0 ± 14.7	46.6 ± 0.03	54.0 ± 7.04	58.4 ± 2.62
	48	71.6 ± 16.5	53.1 ± 6.98	56.9 ± 2.68	63.9 ± 2.94
CD ($P < 0.05$)		15.8	10.6	5.69	5.28

[a]Values are means ± SD of three replicates.
[b]Wet-ash procedure.

Protein Digestibility

Percent protein digestibility (in vitro) of pearl millet grain improved following germination at all temperatures; the longer the period of germination, the greater the protein digestibility of the sprouts (Table I). The improvement in protein digestibility during germination may be attributed to modification and degradation of storage proteins of the grain. Sprouting causes mobilization of proteins with the help of activated proteases, leading to the formation of polypeptides, oligopeptides, and amino acids. Furthermore, hydrolytic reduction of phytates during germination (Table I) may also partly account for the improved protein digestibility of millet sprouts because phytates are known to inhibit proteases (Serraino et al 1985). Phytic acid binds to protonated basic residues of protein at acidic pH, forming a binary protein-phytate complex. At alkaline pH, in the presence of cations, phytic acid forms ternary protein-mineral-phytate complexes that inhibit enzymatic degradation of the protein. Protein digestibility was significantly ($P < 0.05$) negatively correlated (−0.7376) with phytic acid content of the millet sprouts. Germination was reported earlier to enhance protein digestibility of several grains, including horse gram and moth bean (Subbulakshmi et al 1976), chickpea and black gram (Jood et al 1989), and mung bean (Kataria et al 1989).

HCl-Extractability of Minerals

Germination did not significantly alter ($P < 0.05$) the concentration of total P, Ca, Fe, and Zn in the pearl millet grain, but it increased HCl-extractability of these minerals (Table II). That may be an indication of their bioavailability to the human digestive system (Lock and Bender 1980).

HCl-extractability of P in the grains improved significantly following germination at all the temperatures. Longer germination periods resulted in higher P extractability. After 48-hr germination, P extractability was the highest at 30°C. Pi, cleaved from phytate by phytase, might account for higher extractability after germination. A significant negative correlation between level of phytic acid and HCl-extractability of P was observed ($r = -0.8684$, $P < 0.01$).

Sprouting, at all temperatures, also enhanced HCl-extractability of Ca, Fe, and Zn in pearl millet grain. The enhancement was greater at longer periods of germination (Table II). Extractability of Ca and Fe increased by 100%, or more, after 48-hr germination at 30 and 35°C. The increase was less at 25°C. There was about a 50% increase in Zn extractability after 48-hr germination at 30°C. Temperatures of 35 and 25°C were relatively less effective in improving the extractability of Zn. Divalent cations may be present as mineral-phytate chelates in ungerminated grains, which may explain the lower extractability of those minerals in HCl. Hydrolytic diminution of phytic acid during germination may account for enhanced extractability of divalent cations in pearl millet sprouts. There were significant negative ($P < 0.01$) correlations between concentration of phytic acid and HCl extractability of Ca ($r = -0.8094$), Fe ($r = -0.9435$), and Zn ($r = -0.9584$).

Sprouting of pearl millet at 30°C for 48 hr appears to be most beneficial in reducing phytic acid and improving protein digestibility (in vitro) and in HCl-extractability of P, Ca, Fe, and Zn. However, additional work, using other pearl millet cultivars, should be performed to confirm these findings. Consumption of such a nutritionally improved food may be helpful in raising the nutritional status of people in developing countries where pearl millet is widely grown and commonly consumed.

LITERATURE CITED

AKESON, W. C., and STAHMANN, M. A. 1964. A pepsin-pancreatin digest index of protein quality evaluation. J. Nutr. 83:257-259.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1980. Official Methods of Analysis of the AOAC, 13th ed. The Association: Washington, DC.

CHAUHAN, B. M., SUNEJA, N., and BHAT, C. M. 1986. Nutritive value and fatty acid composition of some high yielding varieties of bajra. Bull. Grain Technol. 21:441-442.

CHEN, P. S., TOSHIBARA, T. Y., and WARNER, H. 1956. Microdetermination of phosphorus. Anal. Chem. 28:1756-59.

DUHAN, A., CHAUHAN, B. M., PUNIA, D. and KAPOOR, A. C. 1989. Phytic acid content of chickpea and blackgram: Varietal differences and effect of domestic processing and cooking methods. J. Sci. Food Agric. 49:449-455.

GLENNIE, C. W., CILLIERS, J. J. L., and GEYER, H. L. 1985. Changes in phytate related compounds in grain sorghum during germination. Nutr. Rep. Int. 32:349-355.

GREWAL, R. B. 1992. Nutritional improvement of soybean through fermentation and its utilization in traditional foods of India. Ph.D. thesis, CCS Haryana Agricultural University: Hisar, India.

HAUG, W., and LENTZSCH, H. J. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34:1423-1426.

HOSENEY, R. C., VARIANO-MARSTON, E., and DENDY, D. A. V. 1981. Sorghum and millet advances. Cereal Sci. Technol. 4:71.

JOOD, S., CHAUHAN, B. M., and KAPOOR, A. C. 1989. Protein digestibility (in vitro) of chickpea and black gram as affected by domestic processing and cooking. Plant Foods Hum. Nutr. 39:149-154.

KATARIA, A., CHAUHAN, B. M., and PUNIA, D. 1989. Antinutrients and proten digestibility (in vitro) of mung bean as affected by domestic processing and cooking. Food Chem. 32:9-17.

KHETARPAUL, N., and CHAUHAN, B. M. 1991. Effect of natural fermentation on antinutrients and in vitro digestibility of starch and protein in pearl millet flour. J. Sci. Food Agric. 55:189-195.

KUMAR, V., and KAPOOR, A. C. 1984. Trace mineral composition of different varieties of cereals and pulses. Indian J. Nutr. Diet. 21:137.

LINDSEY, W. L., and NORWELL, M. A. 1969. A New DPTA-TEA soil test for zinc and iron. Agron. Abstr. 61:84.

LOCK, S., and BENDER, A. E. 1980. Measurement of chemically

available iron in foods by incubation with human gastric juice in vitro. Br. J. Nutr. 43:413.
LOLAS, G. M., and MARKAKIS, P. 1977. The phytase of navy beans (*Phaseolus vulgaris*). J. Food Sci. 42:1094-1106.
MAHAJAN, S., and CHAUHAN, B. M. 1987. Phytic acid and extractable phosphorus of pearl millet flour as affected by natural lactic acid fermentation. J. Sci. Food Agric. 41:381-386.
OPOKU, A. R., OHENHEN, S. D., and EJIOFOR, N. 1981. Nutrient composition of pearl millet grains and malt. J. Agric. Food Chem. 29:1247-1248.
PANSE, Y. G., and SUKHATME, P. V. 1961. Statistical Methods of Agricultural Workers, 2nd ed. Indian Council of Agricultural Research: New Delhi.
REDDY, N. R., SATHE, S. K., and SALUNKHE, D. K. 1982. Phytates in legumes and cereals. Adv. Food Res. 28:1-92.
REINHOLD, J. G., NASR, K., LAHIMGRAS AD-EH, A., and HADEYAL, M. 1973. Effect of purified phytate and phytate rich bread upon metabolism of zinc, calcium, phosphorus and nitrogen in man. Lancet 1:283.
SERRAINO, M. R., THOMPSON, L. O., SAVOIE, L., and PARENT, G. 1985. Effect of phytic acid on the in vitro rate of digestibility of rape seed protein and amino acids. J. Food Sci. 50:1689.
SUBBULAKSHMI, G., GANESH KUMAR, K. G., and VENKATARAMAN, L. V. 1976. Effect of germination on the carbohydrate, proteins, trypsin inhibitor, amylase inhibitor and hemagglutinin in horse gram and moth bean. Nutr. Rep. Int. 13:19-31.
VOGEL, A. 1962. Text Book of Quantitative Inorganic Analysis, 3rd ed. Longmans: Harlow, England.

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