Relation Between Phytic Acid and Trace Metals in Wheat Bran and Soybean

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ABSTRACT

Gel filtration chromatography of extracts of wheat bran suggests that at least 70% of the phytate of wheat bran does not occur as Ca_5Mg-phytate. Apparently iron and zinc are the only soluble metals of wheat bran associated with phytate. No soluble zinc of soybean eluted with phytate, but 30 and 70% of the soluble iron of whole and defatted soybean, respectively, eluted in the phytate fraction. Low-phytate soybean was prepared by treating the finely ground whole soybean with a crude phytase enzyme (a cold water extract of wheat bran). This reduced the phytate-zinc molar ratio from 33 to 13. Rats were fed egg white-glucose diets without or with 12 ppm zinc as raw soybean, autoclaved soybean, low-phytate soybean, $ZnSO_4$, or raw soybean plus $ZnSO_4$. Four-week gains (in grams) were 5 ± 4, 152 ± 13, 182 ± 12, 186 ± 6, 185 ± 14, and 198 ± 11, respectively. Total femur zinc (in micrograms) was 20.8 ± 1, 25.6 ± 2, 39.8 ± 3, 52.4 ± 1, and 66.4 ± 7, respectively. Although the soluble zinc of soybean apparently is not associated with phytate, reduction of phytate improved the bioavailability of the soybean zinc to rats.

For almost a century phytate has generally been thought (Hay 1942, Rose 1912) to exist in most plant materials as phytin (Ca_5Mg-phytate). O'Dell et al (1972b) reported, however, that cereal grain fractions that are rich in phytate contain little calcium. For this reason, they suggested that phytate in corn germ, for example, could not exist as Ca_5Mg-phytate. Phytate may be associated with potassium and manganese, as noted by Ashton and Evans (1962). Morris and Ellis (1976) reported that 60% of the iron in wheat bran could be isolated as monoferric phytate. Oberleas (1973) implies that whether or not a particular metal forms a salt with phytate depends upon the pH of the medium. Consequently the pH of the extraction solvent may determine which trace metals are bound to phytate isolated from plant material. The pH of the extraction solvent varied in the cited studies. Ashton and Evans (1962), for example, used a 2% HCl solution to extract phytate from wheat bran. We found, however, that zinc phytate dissociates when the pH is less than 3 (Morris and Ellis 1980b).

According to Lease (1967), soybean meal contains a substance that bonds more stably with zinc than phytate does. Phytate, however, is generally considered detrimental to the bioavailability of zinc (Davies and Reid 1979, Forbes et al 1979, Oberleas and Prasad 1976, O'Dell et al 1972a, Reinhold 1972) in cereal and soybean. Because published reports conflict on the relationship between phytate and trace metals in plant materials, we undertook the present work with two objectives: 1) to reexamine the relationship of phytate to some of the trace metals of wheat bran and soybean, and 2) to compare the effects of two methods (added $ZnSO_4$ and enzymatic hydrolysis) of reducing the soybean phytate-zinc molar ratio on the bioavailability of the zinc to rats. The effect of phytase treatment of wheat bran was reported previously (Morris and Ellis 1980a).

MATERIALS AND METHODS

Materials

Hard winter wheat bran was obtained from Dixie-Portland Mills (Arkansas City, KS). Essex soybeans were obtained from the University of Maryland agronomy farm. Chemicals were reagent grade from J. T. Baker Chemical Co. (Phillipsburg, NJ). Weanling male rats of the Sprague-Dawley CD strain, 40-50 g, were from Charles River Breeding Laboratories (North Wilmington, MA).

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Preparation of Defatted Soybean

Finely ground soybeans were Soxhlet-extracted for 8 hr with either hexane or chloroform/methanol (2:1). The residue was dried in vacuum at room temperature and refrigerated.

Preparation of Low-Phytate Soybean

Crude phytase enzyme was prepared by suspending wheat bran in 10 volumes of cold water and stirring the mixture for 4 hr at 3° C. The supernatant was collected by centrifugation and then refrigerated. Low-phytate soybean was made by placing 450 g of ground soybean in a 6-L Erlenmeyer flask, adding 1,500 ml of the supernatant from the wheat bran plus 1,500 ml of water, and stirring the mixture 16 hr at 37° C. The entire mixture was then freeze-dried and kept refrigerated. The phytate level of raw soybean, 1.25%, was reduced to 0.5% by the phytase enzyme treatments.

Extraction of Trace Metals and Gel Filtration Studies

In a typical extraction, 10 g of finely ground wheat bran or soybean was placed in a 250-ml Erlenmeyer flask and 100 ml of cold 1.2*M* ammonium acetate (pH 6.75) was added. The suspension was stirred for 16 hr at 3° C. The supernatant was collected and refrigerated. Of the supernatant, 6 ml was chromatographed on a 2 × 45-cm column of Bio-Gel P-4 filtration medium (Bio Rad Laboratories, Richmond, CA). Elution buffer was the extraction medium (1.2*M* NH_4OAc), and flow rate for elution was about 24 ml/hr. Six-milliliter fractions were collected; the void and inclusion volumes were about 40 and 156 ml, respectively. The presence of ultraviolet-absorbing material was monitored at 280 nm.

Animal Trials

Seventy weanling male rats were allocated at random to seven dietary treatments. The semipurified basal diet, described by Morris and Ellis (1980b), consisted of the following, in grams per kilogram: egg white solids, 200; dextrose, 675; mineral mix, 40 (Briggs and Williams 1963, with zinc salt omitted); non-nutritive fiber, 20; biotin mix (2 mg of biotin per 5 g of mix), 5; vitamin mix, 10; and corn oil, 50. Soybean or soybean preparation, when used as dietary zinc source, was added at the expense of the glucose. Growth and femur zinc were the criteria of zinc bioavailability. The reference zinc compound was $ZnSO_4 \cdot 7H_2O$.

Analytical Procedure

Trace metals were analyzed in a *1N* HCl solution of dry-ashed diet, wheat bran, or soybean by atomic absorption spectrophotometry (Elwell and Gidley 1966). Trace metals in aqueous extracts of wheat bran or soybean or in eluates from gel filtration were analyzed by flame atomic absorption without ashing. Phytate was determined by the procedure of Ellis et al (1977) and inorganic phosphorous by the procedure of Fiske and

Subbarow (1925). Data were treated by analysis of variance and Duncan's multiple range test, and significance was established at $P < 0.05$ unless otherwise noted.

RESULTS

Solubility and Gel Chromatography of Trace Metals

We tested the solubility of trace metals and phytates of wheat bran and soybean at pH 6.7, the pH of a water suspension of the products (Table I). Six 1.2*M* acetate extractions removed about 70, 50, 90, 80, 80, 65, and 98% of the iron, zinc, manganese, calcium, magnesium, phytate, and potassium, respectively, from wheat bran. A single extraction removed about 50, 70, and 75% of the iron, zinc, and phytate, respectively, of raw whole soybeans. Manganese, calcium, magnesium and potassium were not measured in soybean extracts. In comparison to ammonium acetate extracts, only traces of phytate and metals of wheat bran were extractable with cold demineralized water. But the solubilities of phytate, iron, and zinc of soybeans differed very little between cold demineralized water and ammonium acetate.

Figure 1 is a gel filtration chromatogram of an ammonium acetate extract of wheat bran. Iron and zinc were the only soluble trace metals of wheat bran that eluted with phytate. We identified the soluble iron as monoferric phytate (Morris and Ellis 1976). Potassium and inorganic phosphorous (not shown in Fig. 1) were eluted at maximum in fraction 23.

Figure 2 is a chromatogram of an ammonium acetate extract of raw soybean. Raw whole soybean extract showed three iron peaks, whereas the wheat bran extract produced only one peak. Of the soluble soybean iron, 54% eluted near the exclusion limit, 32% with the phytate, and 14% in the low molecular weight fraction. In contrast to the soluble zinc of wheat bran, the soluble zinc of soybean did not elute with the phytate. Figure 3 is a chromatogram of an acetate extract of chloroform-methanol defatted soybean. Only two soluble iron fractions eluted from the soybean defatted with chloroform-methanol. Of the soluble iron, 70% eluted with phytate and 30% eluted in the low molecular fraction. The molar ratio of these two iron peaks was also 7:3 in the raw whole soybean extract. Figure 3 is also typical for autoclaved soybean. The chromatogram of an acetate extract of hexane-extracted soybean did not differ from that of raw soybean (Fig. 2).

Table I
Ammonium Acetate Extracts of Wheat Bran and Soybean[a]

Sample	Element	Concentration (mg/g)	Amount Extracted (mg/g) 1 Extraction	Amount Extracted (mg/g) 6 Extractions
Raw wheat bran	Fe	0.144	0.050	0.104
	Zn	0.102	0.026	0.054
	Mn	0.123	0.079	0.112
	Ca	1.160	0.450	0.918
	Mg	6.300	3.250	5.049
	P	13.450[b]	5.000[b]	8.500[b]
	K	12.480	11.850	12.300
Raw soybeans	Fe	0.094	0.048	...
	Zn	0.038	0.027	...
	P[c]	3.52	2.54	...

[a] Each value represents the average of two determinations.
[b] About 90% phytate P.
[c] Phytate P.

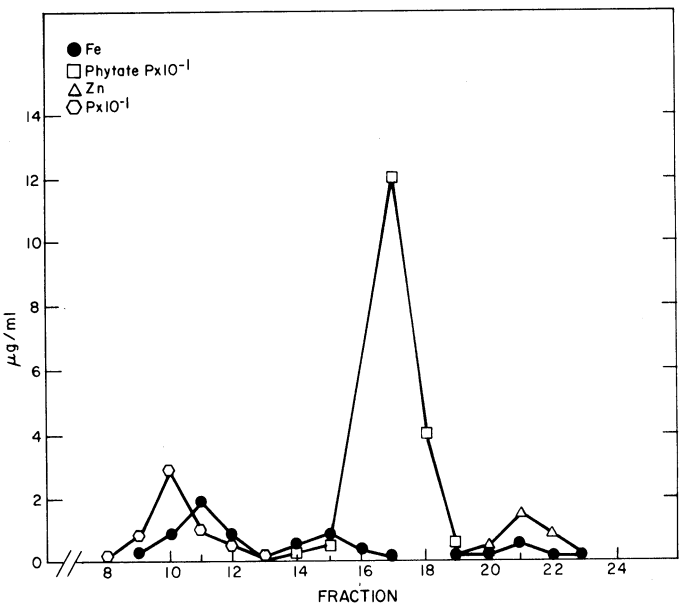

Fig. 2. Typical gel filtration chromatograph of 6 ml of 1.2*M* ammonium acetate extracts of raw whole soybeans or hexane-defatted soybeans.

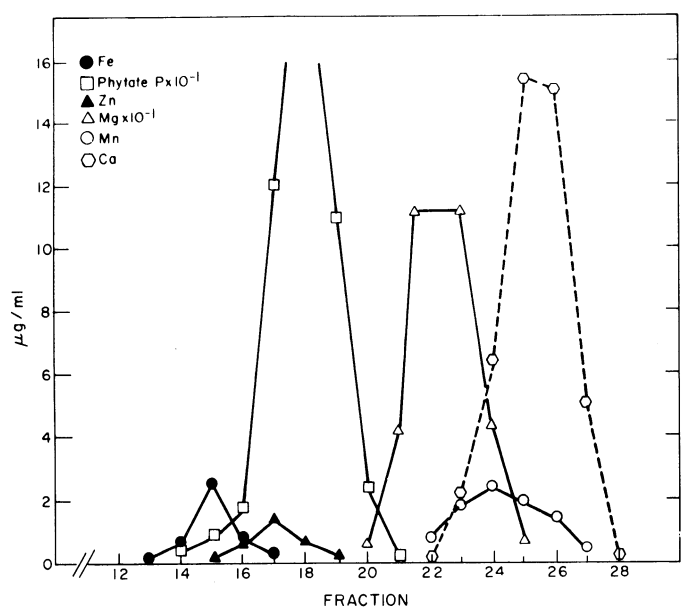

Fig. 1. Typical gel filtration chromatograph of 6 ml of 1.2*M* ammonium acetate extract of raw wheat bran.

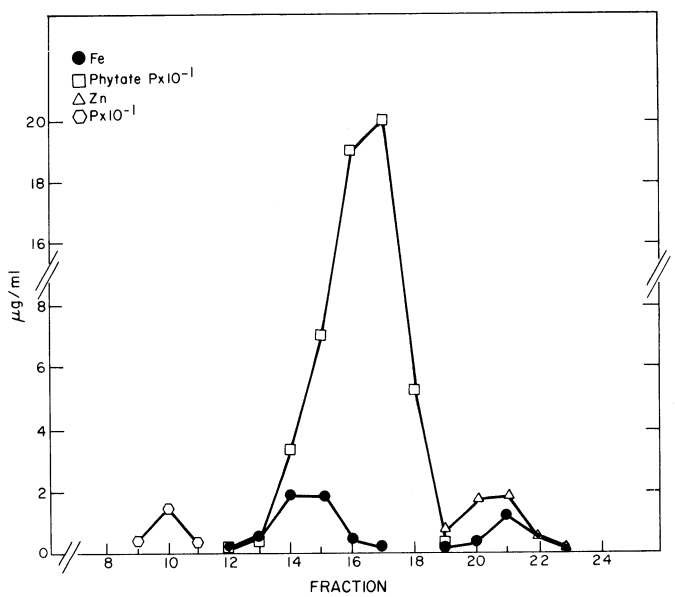

Fig. 3. Typical gel filtration chromatograph of 6 ml of 1.2*M* ammonium acetate extracts of MeOH-$CHCl_3$-defatted soybeans or autoclaved soybeans.

Bioavailability of Zinc

In this experiment we compared how two methods (added $ZnSO_4$ and enzymatic hydrolysis) of reducing the phytate-zinc molar ratio of raw soybean affected the bioavailability of the zinc to rats. We measured gain in body weight and femur zinc (Table II). The growth response of rats fed either 12 ppm dietary zinc as raw soybean or 6 ppm dietary zinc as $ZnSO_4$ was equal. No significant difference was found in growth response among rats when $ZnSO_4$, autoclaved soybean, or low-phytate soybean (phytate-zinc molar ration 13) was the zinc source at 12 ppm dietary zinc. However, when $ZnSO_4$ was added to the diet to reduce the phytate-zinc molar ratio to 13, the growth response and femur zinc of these rats were significantly greater than those of rats fed $ZnSO_4$, autoclaved soybean, or low-phytate soybean as a zinc source. Total femur zinc among rats significantly decreased in the following order of zinc source: raw soybean and $ZnSO_4$, $ZnSO_4$, low-phytate soybean, autoclaved soybean, and raw soybean (Table II). Bone zinc response to raw soybean as zinc source was equivalent to 50% of the response to $ZnSO_4$.

DISCUSSION

Calcium phytate (with a calcium-phytate molar ratio of 3 or greater) is insoluble in 1.2*M* ammonium acetate buffer.[2] However, we extracted about 70% of the phytate with an acetate buffer at the pH of a water suspension of the natural product; apparently the phytate in wheat bran is not in the form of Ca_5Mg-phytate. Gel chromatography of the acetate extract also indicated that calcium was not associated with the phytate of wheat bran. Furthermore, the total amount of calcium in wheat bran would be adequate to complex with only 10% of the phytate. O'Dell et al (1972a) also reported that the aleurone layer of wheat does not contain enough calcium to account for the phytate chiefly as calcium phytate. They suggested that a mixed potassium-magnesium salt would be more likely. But gel chromatography of an acetate extract of wheat bran showed that neither magnesium or potassium was associated with the soluble bran phytate. Published reports on phytates reviewed by Oberleas (1973) indicated that phytate of plant material might be associated with protein. Morris and Ellis (1976) suggested and May et al (1980) confirmed that monoferric phytate is the major fraction of iron in bran. We have not thoroughly studied the ionic strength required to release the phytate from bran, but we found that ammonium acetate solution less than 0.5*M* extracted only a small portion of the iron and phytate from wheat bran. Possibly, therefore, the salt-extractable phytate, including monoferric phytate, is bound by cationic groups of protein or other cellular components. The zinc complex of wheat bran has not been identified. If zinc of wheat bran is complexed to phytate, as suggested by gel chromatography, it probably is a monozinc phytate because it is soluble in 1.2*M* ammonium acetate. Dizinc and trizinc phytates form cloudy suspensions in an acetate solution.

[2] Unpublished data.

Table II
Bioavailability of Soybean Zinc to Rats[a]

Zinc Source	Added Zn (ppm)	Phy/Zn[b]	Four-Week Gain (g)	Femur Zinc Concentration (ppm)	Femur Zinc Total (μg)
Basal	...	...	5 ± 4 a	74 ± 11 a	7.9 ± 1 a
$ZnSO_4$	6	...	146 ± 14 b	77 ± 8 a	20.7 ± 2 b
	12	...	185 ± 14 c	191 ± 12 c	52.4 ± 1 e
Raw soybean	12	33	152 ± 13 b	79.2 ± 8 a	20.8 ± 1 b
Autoclaved soybean	12	30	182 ± 12 c	93.8 ± 8 a	25.6 ± 2 c
Low-phytate soybean	12	13	186 ± 6 c	129 ± 12 b	39.8 ± 3 d
Raw soybean and $ZnSO_4$	30[c]	13	198 ± 11 d	215 ± 16 d	66.4 ± 7 f

[a] Means with common letter are not significantly different ($P < 0.05$). Each value represents the mean ± SD for 10 rats.
[b] Phytate-zinc molar ratio; no detectable phytate in basal diet ingredients.
[c] Raw soybean 12 ppm; $ZnSO_4$ 18 ppm.

About 54% of the soluble iron of raw or hexane-defatted soybean eluted with the exclusion volume when the acetate extract was passed over the P-4 gel column. But no iron eluted in the exclusion volume when the soybean was defatted with a polar solvent, methanol-chloroform. These results suggest that the major soluble iron fraction of soybean may be associated with a polar lipid. We did not, however, characterize any of the iron fractions from soybean. Gel chromatography indicated that the fraction that eluted with the phytate might be monoferric phytate. We found no evidence that the extractable zinc of soybean, in contrast to that of wheat bran, was associated with phytate. Ammonium acetate (1.2*M*) extracts of both soybean and wheat bran contained phytate and zinc, but when the soybean extract was chromatographed by gel filtration, the zinc was not associated with the phytate. Possibly, the native zinc of bran might be present as zinc phytate. Lease (1967) reported that soybean contains a substance that formed a more stable bond with zinc than phytate did.

Oberleas (1973) found that dietary contents of phytate and zinc expressed as a molar ratio, phytate/zinc, might be a satisfactory means of predicting whether the zinc of a phytate-containing diet would have poor bioavailability. Recently Davies and Olpin (1979) and also Morris and Ellis (1980b) showed that the phytate-zinc molar ratio gives a fair degree of accuracy in predicting the bioavailability of zinc to rats. Our present data indicated that zinc of raw soybean with a phytate-zinc molar ratio of 33 is poorly available to the rat. But when the phytate-zinc molar ratio was enzymatically reduced to 13, growth response was similar to that of rats fed $ZnSO_4$ as a zinc source. The femur zinc values of the rats fed soybean (phytate/zinc = 13) were significantly less than values of rats fed $ZnSO_4$ as zinc source but significantly greater than values of rats fed raw soybean as zinc source. Supplementing the raw soybean diet with $ZnSO_4$ to reduce the phytate-zinc molar ratio to 13 enhanced growth, and the response of femur zinc was significantly greater than the response to $ZnSO_4$ alone as the zinc source. The strong effect on growth and femur zinc could not be attributed entirely to decreasing the phytate-zinc ratio, because total dietary zinc was also increased. Davies and Reid (1979) reported similar results on the growth response of rats fed soya-based, textured-vegetable protein. Morris and Ellis (1980a) also observed similar results with wheat bran. Forbes and Parker (1977) found that zinc in soy protein products was less readily available than zinc of sources such as zinc carbonate, but soy protein did not affect the availability of zinc from other sources in the diet.

Allred et al (1964) reported that autoclaving decreased the phytic acid content of isolated soybean protein so that it bound less zinc than the native original protein did. We found no evidence that the water-soluble zinc of whole soybean was associated with phytate. We found only a 12% decrease in the phytic acid of soybean that was dry autoclaved for 30 min, but the bioavailability of the zinc increased significantly. A 12% decrease in phytate would not be expected to significantly affect the bioavailability of zinc to rats. Because soybean meal, when digested, released a water-soluble substance that formed a more stable complex with zinc than did phytate (Lease 1967), possibly autoclaving improves digestability of soybean and increases the yield of the complexing substance. We found, however, that autoclaving did not significantly affect bioavailability of the zinc of wheat bran to rats (Morris and Ellis 1980a). Although the soluble zinc of soybeans apparently is not associated with phytate, reduction of phytate in soybean improved the bioavailability of the soybean zinc to rats. Apparently, however, phytate is not solely responsible for the poor bioavailability of the zinc of soybean to rats.

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LITERATURE CITED

ALLRED, J. B., KRATZER, F. H., and PORTER, J. W. G. 1964. Some factors affecting the in vitro binding of zinc by isolated soya-bean protein and by casein. Br. J. Nutr. 18:575.

ASHTON, W. W., and EVANS, C. J. 1962. Phosphorus compounds of oats. III. Composition of phytin from oats and other cereals. J. Sci. Food Agric. 13:168.
BRIGGS, G. M., and WILLIAMS, M. A. 1963. A new mineral mixture for experimental rat diets and evaluation of other mineral mixtures. Fed. Proc. 22:261.
DAVIES, N. T., and OLPIN, S. E. 1979. Studies on the phytate:zinc molar contents in diets as a determinant of Zn availability to young rats. Br. J. Nutr. 41:591.
DAVIES, N. T., and REID, H. 1979. An evaluation of the phytate, zinc, copper, iron and manganese contents of, and Zn availability from, soya-based textured-vegetable-protein meat-substitutes or meat-extenders. Br. J. Nutr. 41:579.
ELLIS, R., MORRIS, E. R., and PHILPOT, C. 1977. Quantitative determination of phytate in the presence of high inorganic phosphate. Anal. Biochem. 77:536.
ELWELL, W. T., and GIDLEY, J. A. F. 1966. Atomic Absorption Spectrophotometry, 2nd ed. Pergamon Press: New York.
FISKE, C. H., and SUBBAROW, Y. S. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.
FORBES, R. M., and PARKER, H. M. 1977. Biological availability of zinc as influenced by whole fat soy flour in rat diets. Nutr. Rep. Int. 15:681.
FORBES, R. M., WEINGARTNER, K. E., PARKER, H. M., BELL, R. R., and ERDMAN, J. W. 1979. Bioavailability to rats of zinc, magnesium and calcium in casein-, egg- and soy-protein containing diets. J. Nutr. 109:1652.
HAY, J. G. 1942. The distribution of phytic acid in wheat and a preliminary study of some of the calcium salts of this acid. Cereal Chem. 19:326.
LEASE, J. G. 1967. Availability to the chick of zinc phytate complexes isolated from oil seed meal by an in vitro digestion method. J. Nutr. 93:523.
MAY, L., MORRIS, E. R., and ELLIS, R. 1980. Examination by Mossbauer spectroscopy of the chemical identity of iron phytate in wheat. J. Agric. Food Chem. 28:1004.
MORRIS, E. R., and ELLIS, R. 1976. Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. J. Nutr. 106:753.
MORRIS, E. R., and ELLIS, R. 1980a. Bioavailability to rats of iron and zinc in wheat bran: Response to low-phytate bran and effect of the phytate/zinc molar ratio. J. Nutr. 110:2000.
MORRIS, E. R., and ELLIS, R. 1980b. Effect of dietary phytate/zinc molar ratio on growth and bone zinc response of rats fed semipurified diets. J. Nutr. 110:1037.
OBERLEAS, D. 1973. Toxicants Occuring Naturally in Foods, 2nd ed. National Academy of Science: Washington, DC.
OBERLEAS, D., and PRASAD, A. S. 1976. Factors affecting zinc homeostasis. Page 155 in: Prasad, A. S., ed. Trace Elements in Human Health and Disease. Vol. 1, Zinc and Copper. Academic Press: New York.
O'DELL, B. L., de BOLAND, A. R., and KOIRTYOHANN, S. R. 1972b. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. J. Agric. Food Chem. 20:718.
O'DELL, B. L., BURPO, C. E., and SAVAGE, J. E. 1972a. Evaluation of zinc availability in foodstuffs of plant and animal origin. J. Nutr. 102:653.
REINHOLD, J. G. 1972. Phytate concentrations of leavened and unleavened Iranian breads. Ecol. Food Nutr. 1:187.
ROSE, R. A. 1912. A resume of literature on inosite-phosphoric acid, with special reference to the relation of that substance to plants. Biochem. Bull. 3:21.

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