

Inositol Phosphate, Calcium, Magnesium, and Zinc Contents of Selected Breakfast Cereals¹

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The concentrations in selected breakfast cereals of inositol tris-, tetra-, penta-, and hexakisphosphate (IP3, IP4, IP5, IP6) were determined by HPLC methodology. In addition, the calcium, magnesium, and zinc concentrations were determined by atomic absorption spectrometry. All cereals examined contained each of the inositol phosphate (IP) species. Wheat and oat cereals contained an overall average of 5 and 8 $\mu\text{mol/g}$, respectively, of IP6, representing 46 and 65% of total IP. The mean IP6 content of bran or bran added cereals was 13 $\mu\text{mol/g}$, 65% of the total IP content. Corn, rice, and most multigrain-based cereals contained lower concentrations of IPs. Whole grain or bran cereals to which no source of Ca or Zn was added averaged 10.9 μmol (0.44 mg)/g of Ca, 0.48 μmol (0.032 mg)/g of Zn, and 57.1 μmol (1.4 mg)/g of Mg. Corn and rice cereals contained lower amounts of Ca, Zn, and Mg. Calculated indices of bioavailability, such as phytate/Zn and phytate/Ca molar ratios are most meaningful if based on values for IP6 or IP6 + IP5 rather than total IPs. © 1995 Academic Press, Inc.

Phytate, inositol hexakisphosphate (IP6), is ubiquitous in cereal grains (Reddy *et al.*, 1989; Lásztity and Lásztity, 1990) and, consequently, is present in foods prepared from cereal grains. The potential adverse effect of phytate on dietary mineral utilization by both humans and animals is of concern to nutrition scientists (Morris, 1986; Rosander *et al.*, 1992). One source of phytate in the diet of many in the United States is cereal grain-derived breakfast cereals (Morgan *et al.*, 1986; Fast, 1987). During the processing of cereal grains into foods for human consumption there is the potential for hydrolysis of phytate to lower inositol phosphates by both enzymatic and non-enzymatic means (Reddy *et al.*, 1989; Lásztity and Lásztity, 1990). Studies in animals (Tao *et al.*, 1986; Lönnerdal *et al.*, 1989), humans (Brune *et al.*, 1992; Sandström and Sandberg, 1992), and a cell culture model (Han *et al.*, 1994) have shown that IP5, IP4, and IP3 have either no detrimental effect on mineral uptake or less of a detrimental effect than does IP6.

HPLC methodology is available which can rapidly separate and quantify the different inositol phosphates (Phillippy and Johnston, 1985; Sandberg and Abderinne, 1986; Lehrfeld, 1989) in comparison to the step-gradient ion exchange method (Ellis and Morris, 1982), which does not differentiate between the different inositol phosphate

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formula isomers. The latter methodology will result in higher than actual values for phytate if hydrolysis products are present (Lehrfeld and Morris, 1992) and will consequently yield erroneous estimates of the impact on mineral nutriture of the phytate in such a food.

This note presents the values for inositol tris-, tetra-, penta-, and hexakisphosphate (IP3, IP4, IP5, and IP6) contents of selected breakfast cereals available in the Beltsville, Maryland area. Also included are analyses for the calcium, magnesium, and zinc contents of the same cereals. Specific cereals were chosen to be derived from different grains or milling fractions as could be ascertained from the ingredients list and without regard to manufacturer or sales volume.

MATERIALS AND METHODS

Breakfast cereals were purchased from local grocery stores.⁴ A package from each of two or more lot numbers was purchased for each cereal. In the laboratory one-third to one-half of each package's contents was ground in a micro Wiley mill⁵ to pass a 20-mesh screen. Some cereals were purchased in single serving packages in which case the total content was ground. The <20-mesh material of each lot was analyzed in triplicate for IP contents and in duplicate for Ca, Mg, and Zn concentrations. Purchases were between September 1991 and September 1993.

Inositol phosphate analysis. Samples, 0.4–0.6 g, of the ground cereals were transferred to screw cap plastic test tubes, the mass was accurately determined and 6 ml of 0.5 *M* HCl added. The tubes were capped and rotated on a rotator (Glas-Col, Terre Haute, IN) overnight at ambient laboratory temperature. The following morning the tubes were centrifuged and the supernatant solution filtered through No. 41 filter paper. In some instances, a rice cereal and several oat cereals, a gel formed in the tube which did not mix on the rotator and adjustments were made in either the mass of cereal, usually 0.1 g less, or volume of HCl to obtain mixing.

The filtered solutions were analyzed for IP3, IP4, IP5, and IP6 concentration with minor changes from the procedure described by Lehrfeld (1989). Briefly, 2–4 ml of the filtered extract was diluted and passed onto small SAX (Analytichem International, Harbor City, CA) ion exchange columns, washed with 5 ml of deionized water, and then eluted with 2 ml of 2 *M* HCl collected in 30-ml scintillation vials. The HCl was removed to dryness by means of a vacuum-rotary evaporator (Jouan Corp., Winchester, VA). The dried residue was dissolved in 1 ml deionized water and the content of inositol phosphates determined with a Waters (Millipore Corp., Milford, CT) HPLC system using a Hamilton (Hamilton Co, Reno, NV) PRP-1 reversed phase column, 150 × 4.1 mm. The mobile phase was 50% methanol, 0.015 *M* formic acid adjusted to pH 4.3 with H₂SO₄ and 0.1% tetrabutylammonium hydroxide. Flow rate was 1 ml/min at column temperature of 50°C. Detection was by refractive index with a Waters 410 differential refractometer.

⁴ Data reported for commercial brand name foods are factual information only and are limited to the samples analyzed. No warranty or guarantee is made or implied that other samples of these products will have the same or similar composition.

⁵ The mention of a specific equipment or materials manufacturer or trade name is descriptive only and does not constitute endorsement by the U.S. Department of Agriculture.

A reference material certified for inositol phosphate content is not available; however, a quality assurance material was included with each analytical run. Initially, a wheat bran sample was examined which contained no detectable IP3, 0.27 ± 0.07 (mean \pm SD), 2.46 ± 0.22 , and 30.3 ± 0.19 $\mu\text{mol/g}$, respectively, of IP4, IP5, and IP6. The relative standard deviations (RSD) were 26, 9, and 1%. Wheat flour standard reference material No. 1567 (National Institute of Standards and Technology, Gaithersburg, MD) was used as a second material. The IP values ($\mu\text{mol/g}$) for 5 independent analytical runs were IP3 0.015 ± 0.01 , IP4 0.25 ± 0.02 , IP5 0.81 ± 0.05 , and IP6 1.86 ± 0.06 , with RSD of about 70, 8, 6, and 3%, respectively.

Element analysis. Samples of the ground cereals were prepared for element analysis by combination dry-wet ashing described by Hill *et al.* (1986). Concentrations of Ca, Mg, and Zn in the resultant solutions were determined by flame atomic absorption spectrometry using a Perkin-Elmer model 5100 flame atomic absorption spectrometer (Perkin-Elmer Corp., Norwalk, CT) (Hill *et al.*, 1986). Element analysis was monitored for accuracy and validity by analyzing NIST standard reference material No. 1567, wheat flour. Mean \pm SD values (six independent analytical runs) obtained were Ca 180 ± 7 , Zn 10 ± 0.4 , and Mg 345 ± 18 $\mu\text{g/g}$ (dry basis). Certified values \pm uncertainty range are Ca 190 ± 10 and Zn 10.6 ± 1 . This reference material is not certified for Mg content.

RESULTS AND DISCUSSION

Only one of the breakfast cereals examined did not contain detectable amounts of each of the inositol phosphates (Table 1). The mean RSD were IP3 42%, IP4 24%, IP5 10%, and IP6 10%. Although the concentrations were variable within each cereal class, bran or bran added cereals (wheat, corn, or oat brans alone or combinations) tended to contain the highest concentration of IP6, followed by the oat and wheat cereals in that order. The rice and corn cereals were lowest in IP6 content. The mixed grain cereals were also low in IP6, for the most part only 2 to 3 times the IP6 concentration found in the rice and corn cereals. IP6 was the most abundant form in most cereals, puffed wheat and puffed rice being examples of the exception. Of the total inositol phosphates determined, IP6 accounted overall for 46% in wheat cereals and about 62–65% in oat and bran cereals. The sum of IP5 and IP6 account for 76% of the total in wheat cereals and about 89% in oat and bran cereals.

The fraction of the whole grain incorporated into the cereal and the processing procedure are two important factors determining the inositol phosphate content of breakfast cereals. The concentration of phytate tends to be greatest in the germ and aleurone or pericarp of grains (Lásztity and Lásztity, 1990; O'Dell *et al.*, 1972; Lehrfeld and Wu, 1991). This fact is reflected in the higher concentration of IP6 in the bran and bran-added cereals and in wheat cereals which indicate the whole grain is an ingredient. Phytate is associated with the husk free portion of the oat kernel (Frølich and Nyman, 1988) and the highest IP6 concentrations were found in the whole oat cereals. The low concentration of IP6 in the corn and rice cereals indicate that probably little of the bran and germ of these grains is used in the cereal manufacture. Inositol hexakisphosphate is the major inositol phosphate present in the mature corn, rice, and wheat grain (Ferrel, 1978; de Boland *et al.*, 1975), but represented only 40 to 70% of the total in most of the breakfast cereals. Some hydrolysis of IP6 may occur in the

TABLE 1
INOSITOL PHOSPHATE CONTENT OF SELECTED BREAKFAST CEREALS¹

Manufacturer & Brand ²	Inositol Phosphate			
	IP3	IP4	IP5	IP6
	μmol/g			
Wheat Cereals				
AH,wheatena	1.7±0.05	3.6±0.05	5.1±0.1	4.6±0.1
GM,wheaties	0.6±0.1	1.8±0.2	3.7±0.3	5.1±0.5
K,frosted mini-wheats	0.08±0.02	0.5±0.1	2.2±0.5	6.7±0.7
K,nutri-grain wheat	0.4±0.1	1.6±0.3	3.0±0.2	3.9±0.5
N,shredded wheat	0.1±0.02	0.7±0.4	3.2±0.2	9.7±0.8
Q,puffed wheat	1.1±0.2	1.2±0.2	0.8±0.1	0.4±0.1
RP,wheat chex	1.1±0.2	2.4±0.2	3.6±0.3	3.6±0.3
Mean ³	0.7±0.2	1.7±0.4	3.1±0.5	4.9±1.1
Oat Cereals				
GM,cheerios	0.6±0.1	2.2±0.2	4.6±0.1	5.5±0.4
GM,cheerios, appl-cinn	0.4±0.2	1.4±0.4	2.4±0.2	2.5±0.3
OW,Irish style oatmeal	0.09±0.02	0.9±0.2	3.7±0.7	10.5±1.1
Q,old fashioned oats	0.08±0.03	0.7±0.3	3.0±0.3	10.3±0.4
Q,instant oatmeal	0.03±0.02	0.6±0.2	2.8±0.5	9.3±1.1
Mean ³	0.2±0.1	1.2±0.3	3.3±0.4	7.6±1.6
Bran or Bran Added Cereals ⁴				
GM,fiber one	0.07±0.04	0.8±0.1	4.8±0.9	21.7±3.2
HV,organic oatbran flakes	0.03±0.02	0.4±0.04	1.9±0.1	5.3±0.5
K,all bran	0.8±0.1	3.9±0.6	11.5±1.6	22.6±2.0
K,commonsense oat bran	0.4±0.1	2.3±0.1	5.1±0.2	7.0±0.2
K,cracklin' oat bran	0.5±0.2	2.1±0.2	4.6±0.2	10.7±0.7
K,frosted bran	0.2±0.1	1.2±0.1	3.1±0.1	5.2±0.1
N,100% bran	0.2±0.1	2.3±0.4	8.8±0.1	22.7±2.0
N,shredded wheat 'n bran	0.1±0.02	0.7±0.1	3.1±0.2	11.0±0.8
P,bran flakes	0.8±0.3	3.0±0.7	7.4±0.4	13.2±1.1
Q,crunchy corn bran	0.1±0.04	0.5±0.1	1.1±0.01	1.5±0.3
Q,Quaker oat bran	0.07±0.04	1.0±0.1	5.6±0.8	21.2±1.6
Q,Quaker oats & fiber	0.08±0.03	0.9±0.2	4.2±0.4	18.2±1.6
RP,multi-bran chex	0.8±0.2	2.3±0.4	4.3±0.3	5.7±0.7
Mean ³	0.4±0.08	1.7±0.3	4.9±0.7	12.0±2.1

presence of moisture and heat during processing (de Boland *et al.*, 1975). It's more likely, however, that phytase activity promotes IP6 hydrolysis, particularly in wheat-derived cereals (Ferrel, 1978; Lásztity and Lásztity, 1990; Reddy *et al.*, 1989). We produced a low phytate wheat bran by soaking the bran overnight in a water suspension (Morris *et al.*, 1988). The degree of hydrolysis would depend upon the dwell time at proper moisture and temperature conditions. Reviews of breakfast cereal processing may be found in Fast and Caldwell (1991).

Concentrations of the elements Ca, Mg, and Zn are presented in Table 2. The average coefficients of variation for the element analyses were Ca 5.6%, Mg 6.1%, and

TABLE 1—Continued

Manufacturer & Brand ²	Inositol Phosphate			
	IP3	IP4	IP5	IP6
	μmol/g			
Rice Cereals				
K, rice krispies	0.05±0.04 ⁵	0.4±0.1	0.9±0.09	1.2±0.3
Q, puffed rice	0.1±0.05	0.1±0.09 ⁵	0.1±0.1 ⁵	0.05±0.05 ⁵
RP, rice chex	0.03±0.03 ⁵	0.2±0.03	0.8±0.07	1.3±0.3
Mean ³	0.07±0.03	0.2±0.09	0.6±0.3	0.9±0.4
Corn Cereals				
K, corn flakes	trace ⁵	0.06±0.06 ⁵	0.09±0.03 ⁵	0.07±0.1 ⁵
K, frosted flakes	trace	0.04±0.04 ⁵	0.06±0.03 ⁵	0.05±0.05 ⁵
Q, old fashioned grits	trace	0.03±0.02 ⁵	0.3±0.1	2.0±0.2
RP, corn chex	trace	0.04±0.04 ⁵	0.1±0.03 ⁵	0.2±0.2 ⁵
Mean ³	trace	0.04±0.01	0.1±0.05	0.6±0.5
Mixed Grain Cereals ⁶				
GM, golden grahams		0.09±0.01	0.6±0.06	2.5±0.2
GM, multi-grain cheerios	0.3±0.01	1.3±0.4	2.9±0.2	4.5±0.6
K, crispix	0.03±0.01	0.3±0.05	0.7±0.06	0.8±0.2
K, froot loops	0.02±0.01	0.3±0.1	0.8±0.09	1.7±0.3
K, nut & honey crunch	0.04±0.01	0.2±0.04	0.4±0.06	0.8±0.3
K, product 19	0.09±0.03	0.4±0.09	1.0±0.01	1.7±0.07
P, grape-nuts	0.2±0.09	0.3±0.07	1.2±0.3	5.2±0.7
P, grape-nut flakes	0.4±0.2	1.4±0.3	3.1±0.02	4.2±0.2
Q, peanut butter crunch	0.1±0.03	0.6±0.2	1.4±0.2	2.1±0.3
Q, Quaker oat life	0.08±0.03	0.6±0.2	2.5±0.3	7.0±0.7
Q, toasted oatmeal	0.05±0.03	0.6±0.03	2.5±0.1	6.2±0.3
Mean ³	0.1±0.03	0.5±0.1	1.6±0.3	3.3±0.7

1. As is basis, consult Agriculture handbook 8-8 (Douglass et al. 1982) for moisture contents if desired to estimate dry basis.
2. Manufacturer/distributor: AH, American Food Products, Inc.; GM, General Mills, Inc.; HV, Health Valley Foods, Inc.; K, Kellogg Co.; N, Nabisco Brands Inc.; OW, Old Wessex Ltd.; P, General Foods Corp.; Q, Quaker Oats Co.; RP, Ralston Purina Co.
3. Mean±SEM. Values for individual cereals are mean±SD of triplicate analysis of each of two or more different lots.
4. May include one or more of corn, oat or wheat brans or psyllium.
5. One or more aliquots contained no detectable IP, trace $\leq 0.01 \mu\text{mol}$.
6. Derived from two or more of the grains wheat, oats, rice, corn or barley.

Zn 7.5%. Excluding cereals that contain an exogenous source of Ca, wheat cereals averaged 7.9 μmol (0.3 mg)/g and oat and bran cereals 11 to 12 μmol (0.5 mg)/g of Ca while other cereals contained about 2 μmol (0.1 mg)/g or less. Both Mg and Zn concentrations reflected the grain component(s) used as the cereal ingredients. Bran and bran-added cereals were highest in Mg, averaging almost 70 μmol (1.7 mg)/g, wheat and oat cereals averaged 45 μmol (1.1 mg)/g, the whole grain cereals being highest, and rice, corn, and mixed grain cereals averaged 5 to 25 μmol (0.1 to 0.6 mg)/g. Zinc concentration averaged 0.6 μmol (39 μg)/g in bran and bran-added cereals and 0.43 μmol (28 μg)/g in wheat and oat cereals. Some cereals contained less than 10 μg (15 μmol)/g of Zn and a serving of these breakfast cereals, approximately

TABLE 2
CALCIUM, MAGNESIUM, AND ZINC CONTENT OF SELECTED BREAKFAST CEREALS¹

Manufacturer & Brand ²	Element		
	Ca	Mg	Zn
	μmol/g		
	Wheat Cereals		
AH,wheatena	8.61±1.0	55.0±1.4	0.542±0.005
GM,wheaties	65.9±6.9 +	42.3±4.4	0.400±0.076
K,frosted mini-wheats	7.72±0.53	36.9±1.2	1.35±0.076 +
K,nutri-grain wheat	7.27±0.74	36.1±1.5	2.89±0.32 +
N,shredded wheat	9.41±0.43	45.8±3.5	0.395±0.047
Q,puffed wheat	6.39±0.29	51.7±3.4	0.430±0.071
RP,wheat chex	21.0±3.9 +	43.9±3.9	0.420±0.044
Mean ³	7.9±0.52	45±2.7	0.4±0.27
	Oat Cereals		
GM,cheerios	47.8±2.4 +	52.1±3.8	2.94±0.20 +
GM,cheerios apple-cinnamon	31.8±2.9 +	27.3±1.3	0.245±0.01
OW,Irish style oatmeal	11.3±1.3	53.6±2.5	0.492±0.022
Q,old fashioned oats	10.6±0.89	50.0±3.0	0.459±0.024
Q,instant oatmeal	163±11 +	50.6±2.5	0.478±0.082
Mean ³	11±0.35	47±4.9	0.42±0.058
	Bran or Bran Added Cereals ⁴		
GM,fiber one	40.9±2.0 +	93.1±15	0.729±0.069
HV,organic oatbran flakes	10.4±0.18	36.1±2.9	0.282±0.006
K,all bran	112±5.9 +	148±11	2.97±0.15 +
K,commonsense oat bran	11.8±0.33	53.7±1.4	2.68±0.085 +
K,cracklin' oat bran	13.0±0.29	69.1±4.2	1.28±0.13 +
K,frosted bran	6.43±0.17	36.0±0.92	2.55±0.059 +
N,100% bran	18.0±1.6	107±2.0	4.35±1.4 +
N,shredded wheat 'n bran	10.1±0.43	53.7±0.80	0.502±0.021
P,bran flakes	13.5±0.29	41.5±3.9	1.93±0.057 +
Q,crunchy corn bran	18.0±0.41 +	17.0±1.9	1.79±0.072 +
Q,Quaker oat bran	19.7±1.9	95.2±0.54	0.754±0.010
Q,Quaker oats & fiber	13.4±0.40	76.0±5.4	0.624±0.022
RP,multi-bran chex	8.22±0.83	54.9±1.3	2.38±0.18 +
Mean ³	13±1.3	68±9.9	0.58±0.086

30 g, provides less than 5% of the recommended dietary allowance, RDA, for males and females over the age of 18 (National Research Council, 1989). However, most breakfast cereals are consumed with milk which would improve the Zn and Ca intakes considerably. On the other hand, a serving of most of the whole grain cereals will provide about 10% of the RDA for Mg and the bran and bran-added cereals will supply two to three times this percentage. The average amount of Zn per serving will be about 1 to 2.4 mg, for the adult male these amounts are 6 to 16% of the RDA. The cereals containing an exogenous source of Ca and/or Zn will, of course, provide higher percentages than in these examples.

TABLE 2—Continued

Manufacturer & Brand ²	Element		
	Ca	Mg	Zn
	μmol/g		
	Rice Cereals		
K, rice krispies	2.38±1.1	11.7±0.98	0.185±0.019
Q, puffed rice	1.18±0.28	6.81±0.69	0.148±0.011
RP, rice chex	1.97±0.03	12.1±0.56	0.170±0.017
Mean ³	1.8±0.33	10±1.7	0.17±0.023
	Corn Cereals		
K, corn flakes	0.92±0.04	3.54±0.59	0.028±0.007
K, frosted flakes	0.62±0.02	2.53±0.34	0.020±0.001
Q, Quaker old fashioned grits	0.44±0.02	11.4±2.3	0.084±0.10
RP, corn chex	0.76±0.04	3.04±0.64	0.025±0.005
Mean ³	0.7±0.10	5.1±2.1	0.04±0.015
	Mixed Grain Cereals ⁵		
GM, golden grahams	12.1±1.6	17.7±5.1	0.102±0.010
GM, multi-grain cheerios	41.4±1.5 +	36.7±2.4	0.288±0.018
K, crispix	2.74±0.11	9.47±0.13	1.46±0.089 +
K, froot loops	2.57±0.18	11.4±0.88	2.47±0.043 +
K, nut & honey crunch	1.45±0.30	8.09±0.88	0.051±0.006
K, product 19	3.77±0.21	13.8±0.47	8.48±0.22 +
P, grape-nuts	9.77±0.51	37.6±4.7	1.41±0.071 +
P, grape-nut flakes	8.78±0.48	41.5±5.1	1.46±0.16 +
Q, peanut butter crunch	2.89±0.17	18.4±2.0	1.39±0.029 +
Q, Quaker oat life	81.0±3.2 +	41.4±1.8	0.395±0.020
Q, toasted oatmeal	19.0±0.92	35.2±0.68	2.48±0.047 +
Mean ³	7.01±1.96	24.7±4.13	0.209±0.080

1. See footnote 1, Table 1.

2. See footnote 2, Table 1.

3. Mean \pm SEM of cereals with no added source of element, (+ after value indicates that ingredients list specifies added source of element). Values for individual cereals are mean \pm SD for duplicate analysis of each of two or more different lots.

4. May include one or more of corn, oat or wheat brans or psyllium.

5. Derived from two or more of the cereal grains wheat, oats, rice, corn or barley.

The nutritional contribution of breakfast cereals to mineral nutriture is not complete, however, unless the possible adverse impact of the phytate is included in the consideration. As noted in the introduction, animal models and limited human data show that the hydrolysis products of phytate, IP5, and inositol phosphates of lesser degree of phosphorylation, have a less adverse effect on dietary mineral utilization than does phytate, IP6. Phytate/Zn molar ratios, one estimate of the efficacy of a food to provide utilizable Zn (Morris, 1986; Brune *et al.*, 1992), of the cereals examined in this study are in some instances almost twofold greater when calculated from the total inositol phosphates compared to IP6 alone. In contrast to the detrimental effect on mineral

absorption, phytate may provide a beneficial antioxidant role in the etiology of carcinogenesis (Graf and Eaton, 1993). The most accurate estimate, therefore, of the actual IP6 concentrations in foods as-eaten will provide the best estimates of adequate mineral nutriture and dietary benefits from cereal products.

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