# Distribution of Phytic Acid in Milled Fractions of Scout 66 Hard Red Winter Wheat

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Scout 66 hard red winter wheat was milled in a Buhler pneumatic laboratory flour mill. Break flour, reduction flour, shorts, and bran fractions were collected and analyzed for phytic acid. The analytical method used was as follows: (1) ultrasonication to extract the phytic acid from the various wheat fractions; (2) a commercially available silica-based anion-exchange column (SAX minicolumn) for purification and concentration; and (3) high-performance liquid chromatography (HPLC) with a reversed-phase macroporous polymer column for identification and quantification. Ground whole grain contained 1.03% phytic acid. The phytic acid content of the milled fractions varied from less than 0.08% in the reduction and break flours to 1.05% in the shorts and 4.06% in the bran, with the largest particles containing the highest quantities of phytate.

# INTRODUCTION

Phytic acid [myo-inositol 1,2,3,5/4,6-hexakis(dihydrogen phosphate)] (IP6) is a common component in cereal grains, legumes, various edible nuts, and coffee beans. Phytate frequently is carried through the food-processing chain and consequently can be a significant part of the diet. The mean phytate consumption in the average diet of a human omnivore has been variously estimated to be 290-1293 mg/day. Estimates of the mean phytate intake in a vegetarian diet are considerably higher (2575-4570 mg/day; Held et al., 1988). Phytate in cereals and legumes is generally concentrated in the bran or the germ fractions.

The consumption of cereal brans has markedly increased during this past decade since Burkitt et al. (1974) postulated, on the basis of epidemiological evidence, that the paucity of dietary fiber in the Western diet causes an increase in the incidence of coronary heart disease, bowel cancer, appendicitis, diverticular disease, hemorrhoids, varicose veins, and hiatus hernia. The recent Surgeon General's report (Surgeon General, 1988), which focused on the relationship of diet to the occurrence of chronic disease and the role of diet in preventing disease, should accelerate this trend. Even though the Surgeon General's conclusions regarding cause and effect relationships are much more guarded, he concludes, nevertheless, that Americans should "increase consumption of whole grain foods and cereal products" and reduce consumption of fat. Since phytic acid is found in greatest abundance in the bran and germ fraction of grains, increased grain consumption will concomitantly result in increased consumption of phytate.

Phytate is considered a naturally occurring toxicant (Oberleas, 1973). Recent heightened concern is evidenced by the number of recent monographs (Reddy et al., 1989; Graf, 1986; Cheryan, 1980; Cosgrove, 1980) and review papers (Oberleas, 1983; Graf, 1983; Reddy et al., 1982; Maga, 1982; Erdman, 1979; Cosgrove, 1966) dealing with phytic acid. The concern centers around the undesirable propensity of phytic acid to form insoluble complexes with a number of essential minerals, thus rendering them biologically unavailable, and its ability, in vitro, to partially block the action of a number of digestive enzymes. For example, the activities of pepsin (Knuckles et al., 1989),  $\alpha$ -amylase (Knuckles and Betschart, 1987), and lipase (Knuckles, 1988) are reduced. Activity of trypsin and chymotrypsin at pH 3 and 7.8 is enhanced at low phytate: enzyme ratios and markedly decreased at high phytate: enzyme ratios (Deshpande and Damodaran, 1989).

The essential minerals of concern in human nutrition which may be affected by phytate are calcium, copper, iron, and zinc (Table I; Janghorbani and Ting, 1990; National Research Council, 1980). Calcium and zinc (Lonnerdal et al., 1989) are not readily absorbed in the presence of phytate. Because the role of dietary calcium in the mature adult in osteoporosis is currently being debated, the ability of phytates to complex calcium and make it unavailable may or may not be significant in this syndrome. However, in children and young adults it probably is of some importance because "inadequate dietary calcium consumption in the first 3-4 decades of life may be associated with an increased risk for osteoporosis in later life" (Surgeon General, 1988). Phytates complex not only with dietary zinc (Sandstrom et al., 1987) but also with endogenous zinc (Flanagan, 1984). Consequently, the ability of phytic acid to contribute to a zinc deficiency is enhanced. It is especially germane in light of Bryce-Smith's (1989) assertion that "a deficiency of the essential micronutrient zinc is endemic in the UK and many other countries". According to Klevay (1987) a deficiency of copper seems to be related to an increase in ischemic heart disease. The nutritional interaction between phytic acid and copper is in dispute. Lee et al. (1988) assert that IP6 enhances copper bioavailability, while Davies and Nightingale (1975) conclude that IP6 retards copper bioavailability. The evidence for iron is less equivocal. Phytate generally reduces iron bioavailability (Brune et al., 1989; National Research Council, 1989). Iron deficiency is responsible for the most prevalent form of anemia; the Surgeon General's (1988) report states that means to enhance iron bioavailability should be sought, and a special priority should be given to study the effect of zinc and iron deficiencies on immunity and the body's ability to fight infections. Morris and Ellis (1982) have pointed out that the ratio of iron to phytate, which can determine the form of the iron-phytate complex, is an important factor in determining whether the iron is bioavailable. Monoferric phytate is soluble and readily absorbed, while diand tetraferric phytate are not readily bioavailable. These iron complexes may have a beneficial effect. Graf and Eaton (1985) have suggested that phytic acid may suppress

 Table I.
 RDA and Total Body Content of Some Essential

 Minerals<sup>a</sup>

essential mineral	RDA, mg	total body content, g	daily turnover, %
calcium	800-1200	980-1200	0.1
copper	1.5-3	0.077	2.6
iron	10-15	4.0	0.25
zinc	10-15	2.3	0.43

<sup>a</sup> Essential minerals for which the daily requirement is less than 25 mg are arbitrarily referred to as trace elements.



Figure 1. Schematic of roller milling of Scout 66 hard red winter wheat by a Buhler pneumatic laboratory flour mill: (a) spirally corrugated rolls with adjustable clearance set as listed micrometers; (b) smooth rolls with adjustable clearance set as listed micrometers; (c) sieves with openings listed in micrometers.

colonic carcinogenesis by preventing iron from catalyzing the generation of hydroxyl radicals.

Thus, we have two apparently conflicting medical imperatives. The bioavailability of a number of essential minerals and trace elements can be improved by reducing the phytate content of the diet. The incidence or severity of several chronic diseases can be reduced or ameliorated by increasing the dietary fiber content of the diet. Dietary fiber and phytate occur together in cereals and legumes. An increase in whole grain foods and cereals will increase dietary fiber and phytate. However, if a bran fraction containing a reduced amount of phytate could be found, then both needs could be met. To this end a typical hard red winter wheat was milled, its bran and shorts were fractionated on the basis of particle size, and the bran fractions were analyzed for phytate and total dietary fiber (TDF). We here report the results of this investigation.

## MATERIALS AND METHODS

Wheat Milling. Scout 66 hard red winter wheat was grown in Manhattan, KS, in 1986. The wheat was tempered to 15.5%moisture overnight and then to 16% for 0.5 h before it was milled in a Buhler pneumatic laboratory flour mill (Buhler, Uzwil, Switzerland) in a milling laboratory maintained at 25 °C and 48% relative humidity. Three break flours (bins 1-3), three reduction flours (bins 4-6), shorts, and bran fractions were collected from the Buhler mill. Straight flour was the combined break and reduction flour fractions (see Figure 1). The shorts and bran were each divided into five subfractions on the basis of particle size by sieving the fractions through a series of screens.

**Fractionation of Shorts and Bran.** A Ro-Tap testing sieve shaker (W. S. Tyler Co., Cleveland, OH) with four 8 in. diameter brass screens in series separated 100 g of bran after 5 min of shaking and 50 g of shorts after 10 min of shaking. Preliminary screenings of shorts and bran were performed to determine the proper combination of screens to yield moderately sized fractions. The screens chosen for shorts were 35, 40, 60, and 80 mesh (495-, 417-, 246-, and 175- $\mu$ m openings, respectively) and for bran were 10, 14, 20, and 35 mesh (1981-, 1397-, 833-, and 495- $\mu$ m openings, respectively). The lower size limit for shorts was 9XX (160  $\mu$ m) and for bran 40 mesh (417  $\mu$ m). The upper size limit for shorts was 30 mesh (589  $\mu$ m). The average particle size for each bran and shorts fraction is the arithmetic mean of the largest and smallest particle sizes in that fraction. For example, the average particle size for on 35-mesh shorts is the arithmetic mean of 589 and 495  $\mu$ m or 542  $\mu$ m.

Analysis. Moisture for tempering wheat was determined in triplicate by a Brabender moisture/volatiles tester, Type SAS (C. W. Brabender Instruments, Inc., Hackensack, NJ) after wheat was cracked in an Enterprise Model 00 (Philadelphia, PA) grain mill. Nitrogen was determined in duplicate according to the semiautomated colorimetric Kjeldahl procedure (AOAC, 1984). The starch contents of the various wheat fractions were estimated according to a polarimetric method (AACC, 1983) in which the samples were dispersed in a concentrated calcium chloride solution and uranyl acetate was used to precipate proteins. Optical rotation was measured at 589 nm with a Perkin-Elmer Model 241, polarimeter. Starch content was calculated by using a specific rotation of 199. Total dietary fiber (TDF) was determined by an enzymatic-gravimetric method (AOAC, 1990). All values are on a dry weight basis.

Extraction of Phytic Acid. Samples of bran fractions (100 mg), reduction flours (200 mg), break flours (200 mg), and shorts (200 mg) were added to a 15-mL polystyrene centrifuge tube containing 5 mL of 0.5 M HCl and stirred to ensure removal of air pockets. The tip of the ultrasonic microprobe (Ultrasonic liquid processor, Model W-385, equipped with a 1/8-in. standard tapered microtip probe; Heat Systems-Ultrasonics Inc., Farmingdale, NY) was inserted halfway into the liquid, and the sample was sonicated for 1.5 min (1-s cycle, 50% duty at energy level 4) (Lehrfeld, 1989). The largest bran flakes (>1981  $\mu$ m) tended to pack on the bottom of the tube during sonication. Incomplete extraction of phytate can result. To prevent the packing of the flakes, the tip of the probe was placed close to the wall of the centrifuge tube and immersed to a depth of two-thirds. The resultant suspension was centrifuged at 1800 rpm (International Model CL clinical centrifuge, International Equipment Co., Needham Heights, MA) for 5 min.

**Concentration and Purification of Phytic Acid.** An aliquot (3 mL) of the above centrifuge was diluted with 20 mL of water and poured onto an Analytichem silica-based, anionexchange (SAX) column (quaternary amine Bond Elut column, containing 500 mg of packing; Analytichem International, Harbor City, CA) that was connected to a glass and plastic vacuum manifold (Visiprep solid-phase extraction vacuum manifold; Supelco, Bellefonte, PA) set at 50–75 mmHg. The resin-bound inositol phosphates and phytic acid were eluted with 2 mL of 2 M HCl into 20-mL vials. Eluted samples were dried in a Savant Speedvac concentrator (Savant Instrument Co., Farmingdale, NY).

Analysis of Samples by Ion-Pair HPLC. The dried residues were resuspended in 2 mL of a 1% tetrabutylammonium hydroxide solution, capped (caps with Teflon inserts), and sonicated in an ultrasonic bath for 5 min. If a precipitate remained after this solubilization step, it was removed by centrifugation for 5 min at 14 000 rpm in a microcentrifuge (Eppendorf Model 5415 centrifuge; Brinkmann, Westbury, NY). Twenty microliters of the clear supernatant was injected onto a macroporous polymer reversed-phase column (PRP-1 5  $\mu$ m, 150 × 4.1 mm; Hamilton Co., Reno, NV) installed in the HPLC system. The system consisted of a Spectra-Physics (San Jose, CA) Model 8100 HPLC equipped with an autosampler (Model 8110) capable of injecting 80 samples unattended, a 20-µL fixed loop injector, and a refractive index (RI) detector (Altex Model 156; Beckman Instruments, Fullerton, CA). The signal from the RI detector was integrated by a mainframe ModComp computer system (Model 32/85; Modular Computer Systems, Ft. Lauderdale, FL). Mobile phase was prepared by mixing 560 mL of methanol and 440 mL of 0.035 M formic acid; 10 mL of tetrabutylammonium

Table II. Extractability of Phytic Acid from Wheat Kernels, Fractured Wheat Kernels, and Bran as a Function of Particle Size

type of sample	phytic acid extracted <sup>a</sup>	phytic acid extracted, <sup>b</sup> %	
whole kernels	0.06-0.11	5.8-10.7	
hand mill 1×	0.29-0.55	28-53	
hand mill 2×	0.73-1.08	71-105	
hand mill 1× (ground) <sup>c</sup>	0.98-1.03	95-100	
hand mill 2× (ground) <sup>c</sup>	0.99-1.07	96-104	
whole bran	3.96-4.08	98-100	
whole bran (ground) <sup>c</sup>	4.01-4.11	<del>99-</del> 101	

<sup>a</sup> Milligrams of phytic acid extracted from 100 mg of dry sample; range from six determinations. <sup>b</sup> Percent of total present; range from six determinations. <sup>c</sup> The hand-milled fractions were ground in a VARCO electric dry food grinder for 50 s.

hydroxide (40% w/w solution in water) was added, and the pH was adjusted to 4.3 by the addition of 72% (w/w) sulfuric acid (0.25-0.35 mL). Eluent was pumped through a heated (40 °C) PRP-1 column at a rate of 0.9 mL/min (back pressure was 2700-3200 psi). Six standards were run with each series, covering a range from 0.33 to 6.0 mg/mL phytic acid. Sodium phytate (Aldrich Chemical Co., Milwaukee, WI) was used as the standard. Samples of sodium phytate with different lot numbers had slightly different moisture and IP5 contents (current standard contained 1.5% inositol pentakisphosphate). After adjustment for moisture, 1 mg of sodium phytate was equivalent to 0.625 mg of phytic acid. The correlation coefficient for the standard curve was generally 0.997-0.999. Samples containing more than 0.5% phytic acid had coefficients of variation of less than 3% , whereas samples containing 0.06-0.1% had coefficients of variation (CV = standard deviation/mean) as high as 20%. Coefficients of variation could be as high as 100% for phytic acid values below 0.03%. Large confidence band envelopes occur at these low levels because peak size is approaching the size of the noise peaks. If precision is necessary at these levels, it can easily be obtained by increasing injection size and solute concentration.

## **RESULTS AND DISCUSSION**

Fractionation of Shorts and Bran. The average particle sizes for on 35 mesh, through 35 on 40 mesh, through 40 on 60 mesh, through 60 on 80 mesh, and through 80 mesh for shorts were 542, 456, 332, 211, and 168  $\mu$ m, respectively. The average particle sizes for on 10 mesh, through 10 on 14 mesh, through 14 on 20 mesh, through 20 on 35 mesh, and through 35 mesh for bran were >1981, 1689, 1115, 664, and 456  $\mu$ m, respectively.

**Phytic Acid Methodology.** Each of the fractions was analyzed (six replicates of each fraction) for phytic acid by the ion-pair HPLC method recently reported by Lehrfeld (1989). The method can detect and quantitate not only IP6 but also some of its partially dephosphorylated isomers (the tri-, tetra-, and pentaphosphates of inositol). From a nutrition standpoint the conversion of IP6 to the dephosphorylated isomers can be significant inasmuch as it appears that only phytate and inositol pentaphosphate (IP5) interfere with mineral bioavailability (Sandberg et al., 1989). In the present study this analytical capability revealed that the shorts and bran fractions all contained 91-93% phytic acid and 7-9% inositol pentaphosphate.

Extraction of phytate from wheat fragments is incomplete when the size of the particles is excessively large. To demonstrate this point, wheat kernels, chunks, powdered chunks, flakes, and powdered flakes were sonicated and analyzed (Table II). The protocol reported by Lehrfeld (1989) requires that the cereal samples be ground to pass through a 40-mesh screen. Our experience with these wheat samples demonstrates the reason. The hand mill was used to crack the kernel to facilitate moisture determinations. Kernels were passed through a hand mill once  $(1\times)$  or twice  $(2\times)$ , samples were collected, and phytate determinations were made. Total bran flakes and total bran ground to a fine powder in a Waring blender (semimicro stainless steel container) were analyzed as described. The phytate contents of the flakes and the ground flakes were the same. The bran flakes have a large surface area. Therefore, sonication can efficiently extract the phytic acid even when the flakes are not ground. The cracked wheat, on the other hand, is a mixture of chunks and powder. This causes two problems: it is difficult to get a homogeneous sample, and the sonication does not extract all of the phytic acid from the sample. This accounts for the broad range and lower values for phytic acid in unground hand-milled wheat.

Analysis of Milled Fractions of Scout 66 Hard Red Winter Wheat. Various analyses were performed on the singly milled fractions. However, ash, alcohol-soluble carbohydrates, and lipid were not determined because they were not relevant to this study. The values for these components are different for the whole grain and the various milled fractions. For example, the reported averages for ash, alcohol-soluble carbohydrates, and total lipid are 0.48%, 1.4%, 1.08%, respectively, in the flour and 5.78%, 5.10%, and 4.0%, respectively, in the bran (Pomeranz, 1988). Value shifting will probably occur in subfractions of each mill fraction. Total dietary fiber and phytate were directly measured on each of the milled fractions to quantitate the relationship between phytate and fiber. Nitrogen and starch were measured to determine if either could be used as a reliable quantitative marker to estimate the phytate or fiber content in a fraction. The expected relationship between starch and fiber or phytate is an inverse one. As starch increases (adhering endosperm), the amount of phytate and fiber should decrease. A comment is appropriate regarding the precision (reproducibility of measurements) and accuracy (difference between mean and true value) of starch measurements. Polarimetry is a fast and simple method for starch determinations. It gives excellent precision (standard error 1.73%) with all samples and excellent accuracy with samples containing high starch concentrations. However, the starch content of samples such as bran, which contain large amounts of hemicellulose, is consistently underestimated because of the large negative optical rotation of the hemicellulose. Saunders (1970) compared starch values determined by enzymatic, chemical, and polarimetric methods in milled wheat fractions and found that starch in bran and shorts fractions can be underestimated. All values are presented on a dry weight basis. Bran fractions contain more protein than the flour fractions. Protein is reported as  $N \times 5.70$  as suggested by AOAC (Method 979.09) for wheat. Mosse (1990) recommends a N:P value of 5.33.

Analysis of Milled Fractions. The four main fractions produced by the mill (break flour, reduction flour, shorts, and bran) contain widely different quantities of phytate and TDF (Table III). The break flour and reduction flour (65.4% of total) contain less than 0.08% phytic acid and barely a trace of TDF (Pomeranz, 1988). Because phytate at these levels is nutritionally insignificant, no effort was made to reduce the large confidence band envelope. The total bran fraction represents 24.1% of the milled fractions and contains 4.06% phytate and 41.8% TDF. The ratio of phytate to TDF (P/TDF) is 97.1 mg/g. The ratio is useful in two regards. A smaller number indicates a more desirable fiber source, i.e., less phytate per unit of fiber. In addition, the ratio can indicate whether absolute

#### Table III. Analysis of Product from Dry Milling<sup>4</sup>

				phytate/TDF,		
wheat fraction	yield, %	phytate, <sup>b</sup> %	TDF,° %	mg/g	starch,ď %	protein," %
break flour	18.2	<0.08 (15.6)/	tr		83.6	12.88 (4.42)/
reduction flour	47.2	<0.08 (19.1)	tr		84.3	11.57 (4.67)
shorts	10.4	1.05 (3.1)	16.8 (1.2) <sup>f</sup>	62.5	64.6	13.14 (1.49)
bran	24.1	4.06 (1.6)	41.8 (0.49)	97.1	21.3	16.13 (0.53)

<sup>a</sup> Dry basis. <sup>b</sup> Average of six determinations. <sup>c</sup> Average of two determinations. <sup>d</sup> Single determination. <sup>e</sup> Average of two determinations. Protein =  $N \times 5.7$ . <sup>f</sup> Values in parentheses are coefficients of variation.

Table IV. Analysis of Bran Fractions Separated by Screening<sup>a</sup>

av p <b>ar</b> ticle size, μm	yield, <sup>b</sup> %	phytate,° %	TDF, <sup>d</sup> %	phytate/TDF, mg/g	starch, <sup>e</sup> %	protein,/ %
456	3.0 (9.0)#	1.07 (2.8)	14.4 (8.3) <sup>g</sup>	74.3	59.7	17.84 (2.23)#
664	14.2 (13.3)	3.05 (1.4)	33.7 (2.9)	90.5	28.6	17.15 (1.32)
1115	28.6 (0.1)	3.91 (1.8)	47.4 (1.5)	82.5	20.5	17.15 (1.33)
1689	29.5 (3.0)	4.18 (1.7)	49.1 (1.4)	85.1	19.6	15.85 (2.16)
>1981	24.7 (5.3)	4.99 (1.3)	49.3 (0.24)	101.2	17.9	15.28 (5.6)

<sup>a</sup> Dry basis. <sup>b</sup> Average of two determinations. <sup>c</sup> Average of six determinations. <sup>d</sup> Average of two determinations. <sup>e</sup> Single determination. <sup>f</sup> Average of two determinations. Protein =  $N \times 5.7$ . <sup>g</sup> Values in parentheses are coefficients of variation.

Table V. Analysis of Short Fractions Separated by Screenings

av particle size, μm	yield, <sup>6</sup> %	phytate, °%	TDF,⁴ %	phytate/TDF, mg/g	starch,• %	protein./ %
168	25.9 (2.9)#	0.06 (16.1)#	4.6 (2.4) <sup>g</sup>	13	83.2	11 17 (3 32)#
211	30.6 (4.6)	0.22 (5.3)	6.1 (2.5)	36	79.6	11.63 (4.41)
332	26.8 (4.6)	1.58 (3.9)	20.2 (6.7)	78.2	53.3	14.48 (1.38)
456	6.6 (7.0)	2.76 (1.9)	34.3 (0.58)	80.5	30.8	15.73 (0.91)
542	10.1 (6.5)	3.11 (1.8)	40.1 (4.4)	77.6	26.6	15.62 (4.20)

<sup>a</sup> Dry basis. <sup>b</sup> Average of two determinations. <sup>c</sup> Average of six determinations. <sup>d</sup> Average of two determinations. <sup>e</sup> Single determination. <sup>f</sup> Average of two determinations. Protein =  $N \times 5.7$ . <sup>g</sup> Values in parentheses are coefficients of variation.

reductions in phytate and TDF are due only to dilution by endosperm tissue or to dilution and selective fractionation. If the ratio remains the same while the absolute values change, then the fluctuations in phytate and TDF are probably due only to a dilution effect. The total shorts represent about 10.4% of the milled fractions and contain 1.05% phytate and 16.8% TDF. The ratio of phytate to TDF is a more desirable 62.5 mg/g. By use of the total shorts instead of the total bran as a dietary fiber source, phytate can be reduced by one-third even though it will be necessary to increase the absolute amount of total shorts used by a factor of 2.5 to achieve an equimolar amount of TDF. Total shorts is a mixture of bran, fine fibrous matter, pieces of germ (mainly scutellum), and flakes of endosperm. The reduction in the value of the P/TDF ratio (total shorts vs total bran) indicates that a selective fractionation has occurred. However, a large component in the reduction of the absolute values is due to dilution by endosperm tissue as deduced from the increased starch value.

Analysis of Bran Fractions. When wheat bran is fractionated by particle size, one finds that the larger particles contain more TDF and phytic acid than smaller particles. The reverse is true for starch and protein (Table IV). As the average particle size decreases from >1981 to 456  $\mu$ m, the amount of phytic acid decreases from 4.99% to 1.07% and the amount of TDF decreases from 49.3% to 14.4%. By a judicious selection of fractions, it is possible to make a composite with increased fiber content and a negligible change in phytic acid content. Consequently, less of the composite will be needed to achieve an equimolar TDF level with total bran and the absolute amount of phytic acid will be reduced. For example, if fractions 1115 and 1689  $\mu$ m are mixed, the new composite will contain 4.05% phytic acid and 48.3% TDF and have a P/TDF ratio of 84.1 as contrasted with values of 4.06%. 41.8%, and 97.1%, respectively, for total bran. An equimolar (TDF) sample of the composite will contain about 13.5% less phytic acid than the total bran equivalent.

The observation of Dokkum et al. (1982) that increased mineral balance values occurred during consumption of bread made with fine bran could be related to the fact that sieved bran fractions contain different amounts of phytic acid. For example, the fine bran (456  $\mu$ m) contains less phytic acid (1.07%) than the coarse bran (>1981  $\mu$ m, 4.99%).

Analysis of Shorts Fractions. Phytic acid TDF and protein content of shorts parallel particle size (Table V). Large particles contain a greater percentage of these components than the smaller particles. The reverse is true for starch. As expected, when the amount of starch goes up, the amount of phytate and TDF goes down. From the similar values for the P/TDF ratios for particles 332, 456, and 542  $\mu$ m it would appear that the main cause for the reduction of phytate and TDF is a dilution effect caused by an increase in the amount of adhering endosperm. Shorts with average particle size 542  $\mu$ m contain 3.11% phytic acid and 40.1% TDF, while shorts with average particle size of 168  $\mu$ m contain only 0.06% phytate and 4.6% TDF. As pointed out earlier, total shorts can serve as an improved TDF source. Alternatively, shorts with an average size of 542  $\mu$ m can substitute for total bran on an almost equal weight basis with a marked reduction in phytate. This fraction contains 96% of the TDF and only 76% of the phytate in an equal weight of total bran.

From the results of Morris and Ellis (1982) it appears that the ratio of phytate to iron in the complex determines whether the mineral is bioavailable or not. Consequently, it does not appear necessary to remove all of the phytate to improve the bioavailability of the other trace element. Perhaps a slight modification as demonstrated here with the addition of some exogenous phytase (Sandberg and Anderson, 1988) might sufficiently reduce the phytate content so that it no longer interferes with mineral absorption.

## CONCLUSION

We have demonstrated that simple sieving of bran and shorts can give fractions with widely different protein, starch, phytate, and TDF contents. Phytate contents of bran and shorts ranged from 0.06% to 4.99%, while the TDF contents ranged from 4.6% to 49.3%. Because the phytate:TDF ratios of the bran fractions varied from 74.3 to 101.2, it is possible by judiciously mixing select fractions to obtain composites with TDF contents comparable to total bran and yet containing 19–33% less phytate. Use of these bran mixtures as nutritional supplements may provide needed fiber, with its concomitant health benefits, without adversely reducing mineral bioavailability through binding to phytate. Comparable fractions from commercial milling should be available by simple sieving of bran and shorts already available from process streams.

# LITERATURE CITED

- AACC. Approved methods of the AACC; American Association of Cereal Chemists: St. Paul, MN, 1983; Method 76-20, final approval May 1969, reviewed Oct 1982.
- AOAC. Official methods of analysis of the AOAC; Association of Official Analytical Chemists: Arlington, VA, 1984; Method 7.025, final action 1984.
- AOAC. Official methods of analysis of the AOAC; Association of Official Analytical Chemists: Arlington, VA, 1990; Method 985.29, first action 1985, final action 1986.
- Brune, M.; Rossander, L.; Hallberg, L. Iron absorption: no intestinal adaptation to a high phytate diet. Am. J. Clin. Nutr. 1989, 49, 542-545.
- Bryce-Smith, D. Zinc deficiency—the neglected factor. Chem. Br. 1989, 25, 783-786.
- Burkitt, D. P.; Walker, A. R. P.; Painter, N. S. Dietary fiber and disease. J. Am. Med. Assoc. 1974, 229, 1068.
- Cheryan, M. Phytic acid interactions in food systems. In CRC Critical Reviews in Food Science and Nutrition; CRC Press: Boca Raton, FL, 1980; pp 297–336.
- Cosgrove, D. J. The chemistry and biochemistry of inositol polyphosphates. Rev. Pure Appl. Chem. 1966, 16, 209-224.
- Cosgrove, D. J. Inositol Phosphates—Their Chemistry Biochemistry and Physiology: Elsevier: New York, 1980.
- Davies, N. T.; Nightingale, R. The effects of phytate on intestinal absorption and secretion of zinc and whole body retention of zinc, copper, iron and manganese in rats. Br. J. Nutr. 1975, 34, 243–258.
- Deshpande, S. S.; Damodaran, S. Effect of phytate on solubility, activity and conformation of trypsin and chymotrypsin. J. Food Sci. 1989, 54, 695–699.
- Dokkum, W. V.; Wesstra, A.; Schippers, F. A. Physiological effects of fibre-rich types of bread 1. The effect of dietary fibre from bread on the mineral balance of young men. Br. J. Nutr. 1982, 47, 451-460.
- Erdman, J. W., Jr. Oilseed phytates: Nutritional implications. J. Am. Oil Chem. Soc. 1979, 56, 736-741.
- Flanagan, P. R. A model to produce pure zinc deficiency in rats and its use to demonstrate that dietary phytate increases the excretion of endogenous zinc. J. Nutr. 1984, 114, 493-502.
- Graf, E. Applications of phytic acid. J. Am. Oil Chem. Soc. 1983, 60. 1861–1867.
- Graf, E. Chemistry and applications of phytic acid: An overview. In *Phytic Acid: Chemistry and Applications*; Graf, E., Ed.; Pilatus Press: Minneapolis, MN, 1986; pp 1–21.
- Graf, E.; Eaton, J. W. Dietary suppression of colonic cancer fiber or phytate? *Cancer* 1985, 56, 717-718.
- Held, N. A.; Buergel, N.; Wilson, C. A.; Monsen, E. R. Constancy of zinc and copper status in adult women consuming diets varying in ascorbic acid and phytate content. *Nutr. Rep. Int.* 1988, 37, 1307-1317.
- Janghorbani, M.; Ting, B. T. G. Stable isotope methods for studies of mineral/trace element metabolism. J. Nutr. Biochem. 1990, 1, 4–19.

- Klevay, L. M. Hypertension in rats due to copper deficiency. Nutr. Rep. Int. 1987, 35, 999-1005.
- Knuckles, B. E. Effect of phytate and other myo-inositol phosphate esters on lipase activity. J. Food Sci. 1988, 53, 250-252.
- Knuckles, B. E.; Betschart, A. A. Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. J. Food Sci. 1987, 52, 719–721.
- Knuckles, B. E.; Kuzmicky, D. D.; Gumbmann, M. R.; Betschart, A. A. Effect of myo-inositol phosphate esters on in vitro digestion of protein. J. Food Sci. 1989, 54, 1348-1350.
- Lee, D. Y.; Schroeder, J., III; Gordon, D. T. Enhancement of Cu bioavailability in the rat by phytic acid. J. Nutr. 1988, 118, 712-717.
- Lehrfeld, J. High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. *Cereal Chem.* 1989, 66, 510–515.
- Lonnerdal, B.; Sandberg, A. S.; Sandstrom, B.; Kunz, K. Inhibitory effects of phytic acid and other phosphates on zinc and calcium absorption in suckling rats. J. Nutr. 1989, 119, 211-214.
- Maga, J. A. Phytate: Its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. J. Agric. Food Chem. 1982, 30, 1-9.
- Morris, E. R.; Ellis, R. Phytate, wheat bran, and bioavailability of dietary iron. In Nutritional Bioavailability of Iron; Kies, C., Ed.; ACS Symposium Series 203; American Chemical Society: Washington, DC, 1982; pp 121-141.
- Mosse, J. Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. J. Agric. Food Chem. 1990, 38, 18-24.
- National Research Council (U.S.). Subcommittee on the Tenth Edition of The RDAs. In *Recommended Dietary Allowances*, 10th ed.; National Academy Press: Washington, DC, 1989; pp 174-246.
- Oberleas, D. Phytates. In *Toxicants Occurring Naturally in Foods*, 2nd ed.; National Academy of Sciences: Washington, DC, 1973; pp 363-371.
- Oberleas, D. Phytate content in cereals and legumes and methods of determination. Cereal Foods World 1983, 28, 352-357.
- Pomeranz, Y. Chemical composition of kernel structures. In Wheat Chemistry and Technology; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1988; pp 97-158.
- Reddy, N. R.; Sathe, S. K.; Salunkhe, D. K. Phytates in Legumes and Cereals. Adv. Food Res. 1982, 28, 1–92.
- Reddy, N. R.; Pierson, M. D.; Sathe, S. K.; Salunkhe, D. K. Phytates in Cereals and Legumes; CRC Press: Boca Raton, FL, 1989.
- Sandberg, A. S.; Andersson, H. Effect of dietary phytase in the stomach and small intestine of humans. J. Nutr. 1988, 118, 469-473.
- Sandberg, A. S.; Carlsson, N. G.; Svanberg, U. Effects of inositol tri-, tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. J. Food Sci. 1989, 54, 159-161.
- Sandstrom, B.; Almgren, A.; Kivisto, B.; Cederblad, A. Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole wheat. J. Nutr. 1987, 117, 1898-1902.
- Saunders, R. M.; Potter, A. L.; Connor, M.; McCready, R. M.; Walker, H. G., Jr. Analysis of starch in wheat milling fractions. *Cereal Chem.* 1970, 47, 147–152.
- Surgeon General (U.S.) Surgeon General's Report on Nutrition and Health, Summary and Recommendations; U.S. Department of Health and Human Services: Washington, DC, 1988.

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