JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of the Activity of Acidic Phytate-Degrading Enzymes in Cereal Seeds

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Five different methods were compared to elucidate the total activity of the acidic phytate-degrading enzymes present in the seeds of rye, wheat, and barley. Phytate-degrading activity was studied at pH 5.0 by quantifying the liberated phosphate. Rye showed the highest acid phytate-degrading activity among the cereals studied. Using an aqueous extract, only 30−50% of the activity was found (rye, 3443 mU g^{-1} of grain; wheat, 1026 mU g^{-1} of grain; barley, 1032 mU g^{-1} of grain) compared to that found by the direct incubation of the dry-milled cereal grains in a buffered phytate-containing solution (rye, 6752 mU g^{-1} of grain; wheat, 2931 mU g^{-1} of grain; barley, 2093 mU g^{-1} of grain). Extending the extraction time resulted in an increase in extractable phytate-degrading activity by, at maximum, 10−15%. Extraction of phytate-degrading activity is strongly enhanced in the presence of Triton X-100 and the protease inhibitor phenylmethylsulfonyl fluoride (rye, 6536 mU g^{-1} of grain; wheat, 2873 mU g^{-1} of grain; barley, 2023 mU g^{-1} of grain), suggesting at least a partial association with membrane structures and a degradation by proteolytic activity during extraction. In addition, it was shown that determining phytate-degrading activity by quantification of the liberated inorganic phosphate is more robust and precise than determining phytate-degrading activity by quantification of the residual phytate.

KEYWORDS: Activity determination; barley; phytase; phytate; rye; wheat

INTRODUCTION

Phytate (*myo*-inositol-1,2,3,4,5,6-hexakisphosphate), the major phosphorus storage compound in plant seeds, can account for up to 80% of seed total phosphorus and represents 0.5−5% of seed dry weight (*1*). Phytate is either unavailable or poorly utilized by humans and monogastric animals due to the very low endogenous phytate-degrading activities present in their digestive tracts. Since phytate is a strong chelating agent, it readily binds divalent metal ions such as calcium, iron, zinc, magnesium, and manganese, making them insoluble and thus unavailable for absorption in humans and animals (*2*−*5*). The intake of large amounts of foods rich in phytate may therefore cause several mineral deficiency symptoms (*6*). Studies in animals (*7*) and in humans (*2*, *8*−*9*) indicate that dephosphorylation of phytate eliminates its negative effect on mineral absorption.

The possibility to degrade phytate during food processing by the phytate-degrading enzyme naturally occurring in the raw materials has been already shown (*10*−*12*). To produce foods with a low phytate content, it is necessary to optimize conditions with respect to phytate hydrolysis. Therefore, besides knowing the properties of the endogenous phytate-degrading enzymes, such as the optimal pH and temperature conditions for phytate degradation, the total phytate-degrading activity present in the raw material has to be known. Phytate-degrading activity of cereals varies over a wide range: in maize and oat, a very low phytate-degrading activity was found, whereas the highest values were observed in rye and triticale (*13*). It is striking that even the phytate-degrading activities reported for one plant species vary considerably (*13*−*16*). The differences could be explained by different varieties used for the studies as well as by differences in the growth conditions, such as location, year, climate. In addition, the different assay conditions for determining phytate-degrading activities may have contributed to the observed differences. Therefore, the aim of this study was to compare the effect of different methods for phytate-degrading activity determination on the measurable phytate degradation using rye, wheat, and barley seeds.

MATERIALS AND METHODS

Chemicals. Cereal grains (rye, barley, and wheat) were obtained from FENACO (Winterthur, Switzerland), and all other chemicals were purchased from Merck (Darmstadt, Germany). Phytic acid dodecasodium salt was obtained from Aldrich (Steinheim, Germany). Ultrasep ES 100 RP18 was purchased from Bischoff (Leonberg, Germany) and AG1 X-4, 100−200 mesh resin was obtained from Bio-Rad (München, Germany. All reagents were of analytical grade.

Determination of Phytase Activity. Method 1: Extraction of the Soluble Phytate-Degrading Enzymes. Samples (1 g) of dry-milled

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10.1021/jf0204405 CCC: $25.00
Published on Web 01/07/2003

cereal grains were extracted with 20 mL of ice-cold 100 mM sodium acetate buffer, pH 5.0, by shaking for 2 h at 4 °C. The cell debris was removed by centrifugation at 20000*g* for 30 min, and the clear solution was dialyzed (cutoff 20 kDa) against 2 × 2 L of 100 mM sodium acetate buffer, pH 5.0, at 4 °C. Any insoluble material was removed by centrifugation. Enzyme activities were measured at 37 and 45 °C, respectively. The incubation mixture consisted of 350 μL of 0.1 M sodium acetate buffer, pH 5.0, containing 2 μmol of sodium phytate. The enzymatic reactions were started by adding 50 μL of enzyme to the assay mixtures. After an incubation period of 30 min, the liberated phosphate was measured according to the ammonium molybdate method (*17*) with some modifications.

Method 2: Direct Incubation (Quantification of the Liberated Phosphate). Samples (1 g) of dry-milled cereal grains were suspended in 20 mL of 100 mM sodium acetate buffer, pH 5.0, containing 100 μmol of sodium phytate preincubated at 37 and 45 °C, respectively. After certain time intervals at the given temperature, 400 μL portions of the incubation mixtures were removed, and the liberated phosphate was measured by the ammonium molybdate method (*17*) with some modifications.

Method 3: Direct Incubation (Quantification of *myo*-Inositol Phosphates). Samples (0.3 g) of dry-milled rye grains, 0.5 g of dry-milled wheat grains, and 1.0 g of dry-milled barley grains were suspended in 20 mL of 100 mM sodium acetate buffer, pH 5.0, containing 100 μmol of sodium phytate preincubated at 45 °C. The suspensions were incubated for certain time intervals at 45 °C. The reaction was stopped by addition of HCl to a final concentration of 2.4%. *myo*-Inositol phosphates were extracted for 3 h at room temperature. Quantification of *myo*-inositol phosphates was performed by a combination of the AOAC method (*18*) and ion-pair chromatography, as described by Sandberg and Ahderinne (*19*). The slurries were centrifuged at 30000*g* for 30 min. One-milliliter portions of the supernatants were diluted 1:25 with water and applied to a column (0.7 × 15 cm) containing AG1-X8, 100−200 mesh resin. The column was washed with 25 mL of water and then with 25 mL of 25 mM HCl. The *myo*-inositol phosphates were eluted with 25 mL of 2 M HCl. The eluates obtained were concentrated in a vacuum evaporator to complete dryness. The residues were dissolved in 500 μL of water. Twenty-microliter portions of these samples were chromatographed on an Ultrasep ES 100 RP18 (2 × 250 mm). The column was run at 45 °C and 0.2 mL min^{-1}, the eluant consisting of formic acid/methano/water/TBAH (tetrabutylammonium hydroxide) (44:56:5:1.5 v/v), pH 4.25, as described by Sandberg and Ahderinne (*19*). A mixture of the individual *myo*-inositol phosphate esters ($InsP_3$−$InsP_6$) was used as a standard.

Method 4: Direct Incubation after Dialysis (Quantification of the Liberated Phosphate). Samples (1 g) of dry-milled cereal seeds were suspended in 10 mL of 100 mM sodium acetate buffer, pH 5.0, and dialyzed (cutoff 20 kDa) against 2 × 2 L of the same buffer at 4 °C. After dialysis, the suspensions were heated to 45 °C. Thereafter, the same volume 100 mM sodium acetate buffer, pH 5.0, containing 200 μmol of sodium phytate preincubated at 45 °C was added. After certain time intervals at 45 °C, 400 μL portions of the incubation mixtures were removed, and the liberated phosphate was measured by the ammonium molybdate method (*17*) with some modifications.

Method 5: Extraction of the Soluble Phytate-Degrading Enzymes in the Presence of Triton X-100 and Phenylmethylsulfonyl Fluoride. Samples (1 g) of dry-milled cereal grains were extracted with 20 mL of ice-cold 100 mM sodium acetate buffer, pH 5.0, containing 0.01% Triton X-100 and 0.1 mM phenylmethylsulfonyl fluoride by shaking for 2 h at 4 °C. The cell debris was removed by centrifugation at 20000*g* for 30 min, and the clear solution was dialyzed (cutoff 20 kDa) against 2 × 2 L of 100 mM sodium acetate buffer, pH 5.0, at 4 °C. Any insoluble material was removed by centrifugation. Enzyme activities were measured at 45 °C. The incubation mixture consisted of 350 μL of 0.1 M sodium acetate buffer, pH 5.0, containing 2 μmol of sodium phytate. The enzymatic reactions were started by adding 50 μL of enzyme to the assay mixtures. After an incubation period of 30 min, the liberated phosphate was measured according to the ammonium molybdate method (*17*) with some modifications.

Table 1. Extraction of the Soluble Phytate-Degrading Enzymes (Method 1)[a]

	liberated phosphate (μmol per gram of grain)		
time	rye	wheat	barley
0	0	0	0
5	17.39 ± 0.55	5.23 ± 0.20	5.25 ± 0.23
10	34.24 ± 1.32	10.41 ± 0.24	10.23 ± 0.61
15	51.78 ± 2.27	15.09 ± 0.68	15.53 ± 1.13
20	68.82 ± 3.21	20.40 ± 0.82	20.82 ± 1.22
25	85.63 ± 2.54	25.63 ± 1.25	25.64 ± 1.25
30	103.52 ± 2.72	30.61 ± 0.92	30.62 ± 1.08

[a] Samples (1 g) of dry-milled cereal grains were extracted with 20 mL of ice-cold 100 mM sodium acetate buffer, pH 5.0, by shaking for 2 h at 4 °C. Enzyme activities were measured at 45 °C. After an incubation period of 30 min, the liberated phosphate was measured according to the ammonium molybdate method (*17*) with some modifications. The data are mean values ± standard deviation of five independent experiments.

Table 2. Direct Incubation (Quantification of the Liberated Phosphate) (Method 2)[a]

	liberated phosphate (μmol per gram of grain)		
time	rye	wheat	barley
0	0	0	0
5	33.82 ± 0.94	14.64 ± 0.46	10.35 ± 0.26
10	67.38 ± 1,92	29.22 ± 0.66	20.73 ± 0.68
15	101.27 ± 2.46	44.09 ± 1.76	31.75 ± 0.93
20	135.18 ± 3.61	58.84 ± 2.03	42.07 ± 0.86
25	168.76 ± 5.02	73.11 ± 3.12	52.52 ± 1.29
30	202.51 ± 3.59	88.26 ± 2.65	62.99 ± 1.26

[a] Samples (1 g) of dry-milled cereal grains were suspended in 20 mL of 100 mM sodium acetate buffer, pH 5.0, containing 100 μmol of sodium phytate preincubated at 45 °C. After certain time intervals at 45 °C, 400 μL portions of the incubation mixtures were removed, and the liberated phosphate was measured by the ammonium molybdate method (*17*) with some modifications. The data are mean values ± standard deviation of five independent experiments.

Ammonium Molybdate Method (*17*). Added to the assay mixture were 1.5 mL of a freshly prepared solution of acetone/5 N H_2SO_4/10 mM ammonium molybdate (2:1:1 v/v) and 100 μL of 1.0 M citric acid. Any cloudiness was removed by centrifugation prior to the measurement of absorbance at 355 nm. To calculate the enzyme activity, a calibration curve was produced over the range of 5−600 nmol of phosphate (ϵ = 8.7 cm^2 μmol^{-1}). Blanks were run by addition of the ammonium molybdate solution prior to adding the enzyme to the assay mixture.

Statistical Methods. For statistical comparison, the Student's *t*-test was used.

RESULTS

Five different methods were compared to elucidate the total activity of the acid phytate-degrading enzymes present in the seeds of the cereals rye, wheat, and barley. An incubation temperature of 45 °C was chosen, since this temperature is close to the temperature optimum of the cereal phytate-degrading enzymes and all methods employed still resulted in a linear release of phosphate with time at this temperature (**Tables 1**−**5**). Incubation at 37 °C was performed only for comparison with previous studies.

Rye showed the highest activity of soluble phytate-degrading enzymes with an acidic pH optimum (3443 mU g^{-1} of grain). The activities in wheat and barley were almost identical (wheat, 1026 mU g^{-1} of grain; barley, 1032 mU g^{-1} of grain) (**Table 6**, method 1). Extending the extraction time from 2 h to up to 5 h resulted in a statistically significant increase in extractable phytate-degrading activity by, at maximum, 10−15% depending

Table 3. Direct Incubation (Quantification of *myo*-Inositol Phosphates) (Method 3)[a]

	liberated phosphate (μmol per gram of grain)		
time	rye	wheat	barley
0	0	0	0
5	28.49 ± 4.82	11.02 ± 2.37	8.13 ± 2.03
10	57.03 ± 8.12	22.29 ± 4.38	16.54 ± 3.51
15	84.35 ± 13.71	33.75 ± 6.07	23.46 ± 4.97
20	116.02 ± 12.76	44.86 ± 6.82	33.19 ± 7.62
25	143.78 ± 23.75	56.27 ± 11.22	41.27 ± 10.63
30	170.97 ± 24.31	66.58 ± 10.84	49.48 ± 11.54

[a] Samples (0.3 g) of dry-milled rye grains, 0.5 g of dry-milled wheat grains, and 1.0 g of dry-milled barley grains were suspended in 20 mL of 100 mM sodium acetate buffer, pH 5.0, containing 100 μmol of sodium phytate preincubated at 45 °C. The suspensions were incubated for certain time intervals at 45 °C. The reaction was stopped by addition of HCl to a final concentration of 2.4%. *myo*-Inositol phosphates were extracted for 3 h at room temperature. Quantification of *myo*-inositol phosphates was performed by a combination of the AOAC method (*18*) and ion-pair chromatography, as described by Sandberg and Ahderinne (*19*). The data are mean values ± standard deviation of five independent experiments.

Table 4. Direct Incubation after Dialysis (Quantification of the Liberated Phosphate) (Method 4)[a]

	liberated phosphate (μmol per gram of grain)		
time	rye	wheat	barley
0	0	0	0
5	33.13 ± 0.68	14.47 ± 0.48	10.09 ± 0.31
10	65.37 ± 1.39	28.92 ± 0.92	20.45 ± 0.59
15	98.35 ± 2.23	43.43 ± 1.33	30.38 ± 1.58
20	131.58 ± 3.17	57.97 ± 2.38	40.47 ± 1,63
25	164.98 ± 4.98	72.43 ± 2.51	50.64 ± 1.91
30	197.08 ± 3.52	87.31 ± 3.52	60.28 ± 1.88

[a] Samples (1 g) of dry-milled cereal seeds were suspended in 10 mL of 100 mM sodium acetate buffer, pH 5.0, and dialyzed against the same buffer. After dialysis, the suspensions were heated to 45 °C. Thereafter, the same volume of 100 mM sodium acetate buffer, pH 5.0, containing 200 μmol of sodium phytate preincubated at 45 °C, was added. After certain time intervals at 45 °C, 400 μL portions of the incubation mixtures were removed, and the liberated phosphate was measured by the ammonium molybdate method (*17*) with some modifications. The data are mean values ± standard deviation of five independent experiments.

Table 5. Extraction of the Soluble Phytate-Degrading Enzymes in the Presence of Triton X-100 and Phenylmethylsulfonyl Fluoride (Method 5)[a]

	liberated phosphate (μmol per gram of grain)		
time	rye	wheat	barley
0	0	0	0
5	32.81 ± 0.71	14.48 ± 0.39	10.13 ± 0.21
10	65.03 ± 1.57	28.87 ± 1.04	20.55 ± 0.76
15	98.09 ± 2.21	42.98 ± 1.76	29.71 ± 1.29
20	129.97 ± 2.78	57.18 ± 2.84	40.33 ± 1.24
25	165.63 ± 2.56	71.85 ± 1.88	51.26 ± 1.55
30	194.43 ± 2.11	85.43 ± 1.84	60.32 ± 1.01

[a] Samples (1 g) of dry-milled cereal grains were extracted with 20 mL of ice-cold 100 mM sodium acetate buffer, pH 5.0, containing 0.01% Triton X-100 and 0.1 mM phenylmethylsulfonyl fluoride, by shaking for 2 h at 4 °C. Enzyme activities were measured at 45 °C. After an incubation period of 30 min, the liberated phosphate was measured according to the ammonium molybdate method (*17*) with some modifications. The data are mean values ± standard deviation of five independent experiments.

on the cereal under investigation. On the other hand, much higher extractable phytate-degrading activities were found in the presence of the nonionic detergent Triton X-100 and the protease inhibitor phenylmethylsulfonyl fluoride (rye, 6536 mU g^{-1} of grain; wheat, 2873 mU g^{-1} of grain; barley, 2023 mU g^{-1} of grain) (**Table 6**, method 5).

Table 6. Phytate-Degrading Activity (Methods 1–5)[a]

	phytate-degrading activity (mU g^{-1} of grain)				
cereal	method 1	method 2	method 3	method 4	method 5
rye	3443 ± 27a	6752 ± 78b	5731 ± 951b	6570 ± 65b	6536 ± 72b
wheat	1026 ± 15c	2931 ± 29d	2232 ± 491d	2899 ± 31d	2873 ± 26d
barley	1032 ± 17c	2093 ± 23d	1635 ± 383d	2025 ± 24d	2023 ± 24d

[a] Values are based on regression over the 30 min sampling period. Activity (units) was expressed as 1 μmol of phosphate liberated per minute. Means followed by a different differ ($P < 0.05$).

The phytate-degrading activities determined by direct incubation of the dry-milled cereal grains in a buffered phytate-containing solution and quantification of the liberated inorganic phosphate (rye, 6752 mU g^{-1} of grain; wheat, 2931 mU g^{-1} of grain; barley, 2093 mU g^{-1} of grain) (**Table 6**, method 2) were very similar to the phytate-degrading activities obtained by performing extraction in the presence of Triton X-100 and phenylmethylsulfonyl fluoride (rye, 6536 mU g^{-1} of grain; wheat, 2873 mU g^{-1} of grain; barley, 2023 mU g^{-1} of grain) (**Table 6**, method 5). Dialysis of the suspended flour prior to incubation did not have a significant effect on the phytate-degrading activities determined (rye, 6570 mU g^{-1} of grain; wheat, 2899 mU g^{-1} of grain; barley, 2025 mU g^{-1} of grain) (**Table 6**, method 4).

Besides quantification of the liberated phosphate, quantification of the decrease in phytate could be used for determination of phytate-degrading activity. Direct incubation of the dry-milled cereal grains in a buffered phytate-containing solution and quantification of the degraded phytate by reversed-phase high-performance liquid chromatography (**Table 6**, method 3) resulted in only 76−85% of the phytate-degrading activities determined under identical conditions by quantification of the liberated inorganic phosphate (**Table 6**, method 2). The efficiency of the *myo*-inositol phosphate isolation was determined to be 96−102% by using a mixture of the individual *myo*-inositol phosphate esters ($InsP_3$−$InsP_6$) as a standard. In addition, the data suggest that the phosphate analysis performed is more precise than the phytate analysis; the deviation from the mean value was determined to be 16−23% with phytate analysis, but only 1−3% with phosphate analysis.

DISCUSSION

Phytate-degrading activity was measured by quantifying the liberation of phosphate from phytate or by quantifying the decrease in phytate during the incubation period. With all assays performed, it was shown that the phytate was hydrolyzed at a constant rate over at least 2 h (data not shown). Thus, the assays performed are, in general, suitable for phytate-degrading activity determination.

In agreement with published data (*13*−*16*), the highest phytate-degrading activity was found with rye. At 37 °C, the extractable phytate-degrading activity for rye, wheat, and barley was reported to be 2430−5520, 490−660, and 200−600 mU g^{-1}, respectively (*14*, *16*). To compare our data with the published ones, we conducted the phytate-degrading enzyme activity assay at 37 °C for each cereal under investigation (method 1). At 37 °C, only 71%, 65%, and 58% of the phytate-degrading activity at 45 °C was found with rye, wheat, and barley, respectively. Thus, the total phytate-degrading activity

at 37 °C in rye, wheat, and barley results in 2440, 660, and 600 mU g^{-1}, respectively, which is in good agreement with the data found in the literature.

Comparing the total phytate-degrading activity resulting from a direct incubation of the cereal flours (method 2) with the total extractable phytate-degrading activity (method 1) showed significantly higher activity by direct incubation. This is also in agreement with the literature data. At 37 °C, the phytate-degrading activity for rye, wheat, and barley was reported to be 4100-6100, 900−2100, and 400−1520 mU g^{-1}, respectively (*13*, *15*). At 37 °C, the phytate-degrading activity in rye, wheat, and barley was determined to be 4745, 1854, and 1202 mU g^{-1}, respectively.

From the data, in could be concluded that only 51%, 35%, and 49% of the total phytate-degrading activity of rye, wheat, and barley, respectively, is extractable under the conditions used. Very similar results have been previously reported by Schindler et al. (*16*). Theoretically, the difference could be also explained by liberation of phosphate from phosphorylated compounds other than phytate present in the cereal flour. Since 60−90% of the total phosphate in plant seeds occur as phytate phosphate and phytate was given in excess to the assay mixtures, it could be assumed, however, that the enzymatic hydrolysis of phosphorylated compounds other than phytate do not contribute, to a significant extent, to the phosphate liberated during the phytate-degrading activity assay. This assumption was confirmed, because dialysis of the suspended flour prior to incubation did not have a significant effect on the phytate-degrading activities determined.

Prolonged extraction times did not result in an increase in extractable phytate-degrading activity, but the extraction of phytate-degrading activity is strongly enhanced by the simultaneous presence of Triton X-100 and the protease inhibitor phenylmethylsulfonyl fluoride in the extraction buffer. This suggests breakdown of phytate-degrading enzymes by proteases during extraction and at least a partial association of the phytate-degrading enzymes with membrane structures.

HPLC ion-pair chromatography has been developed for separation and quantitative determination of phytate and lower *myo*-inositol phosphates. This method is capable of detecting directly the reduction in phytate during enzymatic hydrolysis, but with respect to the determination of phytate-degrading activity, the method is too time-consuming, and significant amounts of lower *myo*-inositol phosphates in the assay mixtures must have been generated until the reduction in phytate could be quantified with adequate accuracy.

In summary, direct incubation of the dry-milled cereal grains in a buffered phytate-containing solution and quantification of the liberated phosphate was found to be the best method to determine total acid phytate-degrading activity in the cereals under investigation.

ACKNOWLEDGMENT

The authors thank E. Haller for her excellent technical assistance.

LITERATURE CITED

(1) Reddy, N. R., Pierson, M. D., Sathe, S. K., Salunkhe, D. K., Eds. *Phytates in Cereals and Legumes*; CRC Press Inc.: Boca Raton, FL, 1989.

(2) Andersson, H.; Nävert, B.; Bingham, S. R.; Englyst, H. N.; Cummings, J. H. The effects of breads containing similar amounts of phytate but different amounts of wheat bran on calcium, zinc and iron balance in man. *Br. J. Nutr.* **1983**, *50*, 503−510.

(3) Brune, M.; Rossander, L.; Hallberg, L.; Gleerup, A.; Sandberg, A.-S. Iron absorption from bread in humans: inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *J. Nutr.* **1992**, *122*, 442−449.

(4) Morris, E. R. Phytate and mineral bioavailability. In *Phytic acid: Chemistry and application*; Graf, E., Ed.; Pilatus Press: Minneapolis, MN, 1986; pp 57−76.

(5) Nävert, B.; Sandström, B.; Cederblad, A. Reduction of phytate content of bran by leavening in bread and its effect on zinc absorption in man. *Br. J. Nutr.* **1985**, *53*, 47−53.

(6) Torre, M.; Rodriguez, A. R.; Saura-Calixto, F. Effects of dietary fiber and phytic acid on mineral availability. *Crit. Rev. Food Sci. Nutr.* **1991**, *1*, 1−22.

(7) Lönnerdal, B.; Sandberg, A.-S.; Sandström, B.; Kunz, C. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr.* **1989**, *119*, 211−214.

(8) Sandström, B.; Sandberg, A.-S. Inhibitory effects of isolated inositol phos-phates on zinc absorption in humans. *J. Trace Elements Electrolytes Health Dis.* **1992**, *6*, 99−103.

(9) Sandberg, A.-S.; Rossander, L.; Türk, M. Dietary *Aspergillus niger* phytase increases iron absorption in humans. *J. Nutr.* **1996**, *126*, 476−480.

(10) Sandberg, A.-S. The effect of food processing on phytate hydrolysis and availability of iron and zinc. In *Nutritional and Toxicological Consequences of Food Processing*; Friedman, E., Ed.; Plenum Press: New York, 1991; pp 499−508.

(11) Giovanelli, G.; Polo, R. Formation of fermentation products and reduction in phytic acid in wheat and rye flour breadmaking. *It. J. Food Sci.* **1994**, *6*, 129−141.

(12) Agte, V.; Joshi, S.; Khot, S.; Paknikar, K.; Chiplonkar, S. Effect of processing on phytate degradation and mineral solubility in pulses. *J. Food Sci. Technol.* **1998**, *35*, 330−332.

(13) Viveros, A.; Centeno, C.; Brenes, A.; Canales, R.; Lozano, A. Phytase and acid phosphatase activities in plant foodstuffs. *J. Agric. Food Chem.* **2000**, *48*, 4009−4013.

(14) Pointillart, A. Phytates, phytases: leur importance dans l'alimentation des monogastriques. *INRA Prod. Anim.* **1994**, *7*, 29.-39.

(15) Eeckhout, W.; De Paepe, M. Total phosphorus, phytate-phosphorus and phytase acrtivity in plant foodstuffs. *Anim. Feed Sci. Technol.* **1994**, *47*, 19.29.

(16) Schindler, B.; Lantzsch, H.-J.; Rudolf, U.; Drochner, W. Beitrag zur Bestimmung der Aktivität pflanzlicher Phytase. *Proc. Soc. Nutr. Physiol.* **1997**, *6*, 39.

(17) Heinonen, J. K.; Lahti, R. J. A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic phosphatase. *Anal. Biochem.* **1981**, *113*, 313−317.

(18) Association of Official Analytical Chemists. Phytate in foods, anion-exchange method no. 986.11. In *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, VA, 1990; pp 800−801.

(19) Sandberg, A.-S.; Ahderinne, R. HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. *J. Food Sci.* **1986**, *51*, 547−550.

Received for review April 16, 2002. Revised manuscript received September 4, 2002. Accepted September 19, 2002.

JF0204405