Effect of Several Germination Conditions on Total P, Phytate P, Phytase, and Acid Phosphatase Activities and Inositol Phosphate Esters in Rye and Barley

Carmen Centeno,[†] Agustín Viveros,*[,‡] Agustín Brenes,[†] Rosa Canales,[‡] Ana Lozano,[†] and Celia de la Cuadra[§]

Instituto de Nutrición y Bromatología, Facultad de Farmacia, Ciudad Universitaria, 28040 Madrid, Spain, Departamento de Producción Animal, Facultad de Veterinaria, Ciudad Universitaria, 28040 Madrid, Spain, and Centro de Recursos Fitogenéticos CRF-INIA, Aptdo 1045, 28800 Alcalá de Henares, Madrid, Spain

Two assays were conducted to study the evolution of rye and barley phosphatases (phytase and acid phosphatase) and the degradation of its substrates (inositol phosphate esters) during seed germination. In this manner we could obtain a low-phytate, endogenous phosphatase rich ingredient to be used in animal nutrition. In the first assay, the seeds were soaked for 1 and 14 h and germinated for 3 and 5 days with and without the addition of gibberellic acid (GA_3). In the second assay, the seeds were soaked for 1 h and germinated for 1, 3, and 5 days with GA_3. Phytase (up to 5739 and 3151 U kg^{-1}) and acid phosphatase (up to 18288 and 3151 U g^{-1}) activities, and IP_6 (6.09 and 6.01 mg g^{-1}), IP_5 (0.48 and 0.48 mg g^{-1}), and IP_4 (0.13 and 0.06 mg g^{-1}) were detected in ungerminated rye and barley, respectively. The germination process caused a significant increase of Phy and AcPh activities in rye (up to 112 and 213%) and barley (up to 212 and 634%) and a reduction in the phytate phosphorus content (up to 84 and 58%, respectively). Phytate phosphorus content was affected only by soaking time in the case of rye. Finally, during the course of germination, IP_6 and IP_5 were rapidly degraded in rye (88 and 79%) and barley (67 and 52%), and IP_4 was only a short-living intermediate, which was increased during hydrolysis and degraded to IP_3. In conclusion, a marked increase of Phy and AcPh activities in rye and barley with a concomitant decrease in phytate phosphorus content and an increase in the content of lower inositol phosphates were observed during the rye and barley germination.

Keywords: *Rye; barley; germination; gibberellic acid; phosphorus; inositol phosphates; phytase and acid phosphatase activities*

INTRODUCTION

In most plant materials (cereals, grain legumes, and oilseeds), a large proportion of phosphorus is present in the form of phytate (*1*−*3*). Phytate is a complex salt of calcium or magnesium with *myo*-inositol (1,2,3,4,5, and 6 hexakis dihydrogenphosphate) and is considered as the primary storage form of phosphorus and inositol in almost all seeds (*4*). Phosphorus is a vital compound for seed/grain development and successful seedling growth (*5*−*7*). Phosphorus in phytate form is either unavailable to or poorly utilized by monogastric animals and humans (*8*), because of the lack of the phytase enzyme required to hydrolyze the phytate and release the phosphorus. In addition, there is the need to take into account the environmental impact of unused dietary phosphorus excreted in the feces.

Phytases have been isolated and/or characterized in cereals and legumes (*9*, *10*) and microorganisms, particularly fungi (*11*). Phytase (*myo*-inositol hexakisphosphate phosphohydrolases) is a phytate-specific phosphatase that hydrolyses phytate to inositol and free ortophosphate (*11*). Two kinds of phytases have been recognized that initiate the hydrolysis of phytate at either the 3- or the 6-position of the inositol ring. Usually, but not invariably, microbial phytase falls into the first category and plant phytase the second category (*12*). During storage, fermentation, germination, food processing, or digestion in the human gut, phytate is enzymatically hydrolyzed by phytases to a series of lower *myo*-inositol phosphates and phosphate (*10*, *13*). Acid phosphatase is a nonspecific phosphatase that hydrolyzes phosphorylated substrates. Likewise, the positive role of acid phosphatase (pH optimum 2.5, biosynthesized by *A. ficum*, EC 3.1.3.2) in the hydrolysis of phytate and lower phosphate of *myo*-inositol has been reported by Ullah and Phillippy (*14*). Acid phosphatase in *Aspergillus niger* accelerated the hydrolysis of sodium phytate solution (*15*) and complements the breakdown of phytate by attacking the inositol phosphate intermediate (*16*). Enzymes phytase and acid phosphatase have been reported in a number of seeds including cereals and legumes, byproducts, and other feedstuffs (*3*, *17*).

Food processes including soaking, germination, and fermentation seem to be relatively simple and nonchemical approaches for reducing antinutritional factors and improving the nutritional quality of grain (*6*, *18*, *19*). These processes have demonstrated, under optimal conditions, their ability to completely reduce the phytate content of cereals and vegetables, accompanied by a large increase in phytase activity (*20*−*24*). In ungermi-

* To whom correspondence should be addressed via e-mail: viverosa@eucmos.sim.ucm.es.
[†] Facultad de Farmacia, Ciudad Universitaria.
[‡] Facultad de Veterinaria, Ciudad Universitaria.
[§] Centro de Recursos Fitogenéticos CRF-INIA.

10.1021/jf010023c CCC: $20.00
Published on Web 06/06/2001

nated seeds very little endogenous phytase activity is detectable while, during germination, phytase activity increases and phytate levels decrease (*25*).

The plant hormones gibberellic acid (GA) and abscisic acid (ABA) are important regulators of the dormancy and germination of seeds. In cereals, GA enhances the synthesis and secretion of enzymes (particularly α-amylases) in the aleurone cells of the endosperm. The scutellum is the main site of de novo GA biosynthesis (*26*). Likewise, several investigations have demonstrated that isolated aleurone layers of barley respond to exogenously applied GA in a manner similar to that of intact aleurone layers (*27*).

Substantial data are available on enzymes responsible for the hydrolysis of the major reserves of the starchy endosperm (starch and protein) and the main components of the cell wall, but less is known about the phosphorus-degrading enzymes. Therefore, the aim of the first assay was to establish the optimal germination conditions of rye and barley to improve the endogenous phosphatases and increase the hydrolysis of phytate and, in the second assay, to determine the different lower phosphate esters of *myo*-inositol produced during the germination process. These studies could help obtain new ingredients rich in endogenous phosphatases to be used in animal nutrition and, thus, limit the amount of excess P in diets and excreta and to reduce the environmental impact of such wastes.

MATERIALS AND METHODS

Materials. Clean and uncontaminated samples of rye and barley were provided by Instituto de Semillas y Plantas de Viveros (INIA, Madrid) from different locations in Spain. Ungerminated and germinated seed samples were freeze-dried and stored at room temperature in airtight containers prior to chemical analysis. Samples were ground in a hammer mill and passed through a 0.5-mm sieve. All samples were analyzed in triplicate.

Germination Procedure. In assay 1, the cereal grains were soaked in a distilled water solution of NaCl (1.5%), alone or with 5 mg kg^{-1} of gibberellic acid (GA3; G-7645 Sigma Chemical), at room temperature for 1 and 14 h with shaking by hand every 30 min. In assay 2, the seeds were soaked in the same solution, with gibberellic acid (5 mg kg^{-1}) for 1 h. Once this was finished, the solution was drained. Duplicated grain samples were germinated at 22 °C for 3 and 5 days (assay 1) and 1, 3, and 5 days (assay 2) in a dark room. The seeds were germinated in trays on moistened filter papers with the water solution, which, as needed, was added during the course of germination.

Determination of Total and Phytate Phosphorus. Total phosphorus concentration was determined spectrophotometrically by the molybdo-vanadate reagent after ashing of the sample with HCL (*28*). Phytate phosphorus was determined according to the indirect method of Haugh and Lantzsch (*29*). In this method, the samples were extracted in 10 mL of 0.2 N HCL. To 0.5 mL of this extract, 1 mL of the ferric solution [0.2 g ammonium iron (III) sulfate 12 H_2O in 100 mL of 2 N HCL and made up to 1L] was added. The tubes were heated in a boiling water bath for 30 min. Once the tubes reached room temperature, 2 mL of 2-2′-Bipyridine solution was added. The decrease of iron determined colorimetrically (519 nm) in the supernatant is a measure for the phytic acid content.

Determination of Inositol Tri-, Tetra-, Penta-, and Hexaphosphates. The inositol phosphates were determined by the HPLC method (*30*) with some modifications (*31*). The cereal samples (0.5 g) were extracted under mechanical agitation with 20 mL of 0.5 M HCL for 2 h at room temperature. The chloride extract was centrifuged and filtered through MF-Millipore (0.22 μm). The filtrate was evaporated to dryness under vacuum, dissolved in 15 mL of 25 mM HCL, and passed through a strong anion-exchange (SAX) column (quartenary amine bonded silica, 500 mg; Lida Manufacturing Corp, Kenosha, WI) that was connected to a vacuum manifold (Visiprep, Supelco, Bellafonte, PA). The loaded SAX column was washed successively with 10 and 5 mL of 25 mM HCL. The resin-bound inositol polyphosphates (IP_3, IP_4, IP_5, and IP_6) were then eluted with 10-mL portions of 2 M HCL. The eluent was evaporated to dryness and diluted with 0.5 mL of mobile phase. The inositol tri- (IP_3), tetra- (IP_4), penta- (IP_5), and hexaphosphate (IP_6) were determined by ion-pair C18 reverse-phase HPLC. The mobile phase was 0.012 M formic acid in 51.5% methanol and 0.8% TBA-OH adjusted to pH 4.3 with H_2SO_4. A reverse-phase C18 column (Spherisorb ODS 5 mm, 250 × 4.6 mm) was heated (45 °C) and equilibrated with the mobile phase for 1 h. Analysis was conducted with a Beckman System Gold HPLC instrument. Inositol phosphates were detected by refractive index (Beckman, Model 156). The flow rate was 1.2 mL min^{-1}, and the injection volume was 20 μL. The standards used were inositol triphosphate (IP_3) and sodium phytate (IP_6) (Sigma Chem, Co, St Louis, MO). A mixture of inositol phosphate was prepared by hydrolyzing an aqueous solution of sodium phytate (6 mg mL^{-1}) by autoclaving at 120 °C for 1h. The sodium phytate used consisted of 0.632 mg phytic acid/mg solid. Phosphorus content of sodium phytate was 17.76% determined by ICP atomic emission spectrophotometry. The linearity of phytic acid concentration versus peak area was investigated by 20−μL injections of solutions covering a range from 0.36 to 7.3 mg mL^{-1} phytic acid. Chromatographic analysis was carried out four times on each sample.

Enzyme Activity Measurements. Phytase activity was analyzed according to the method described by Eeckhout and De Paepe (*17*). Finely ground samples (50−100 mg) were weighed in 50-mL volumetric flasks, and the flasks were filled to the mark with sodium phytate solution [1.722 g of sodium phytate (Sigma P3618 from rice), 180 mL of H_2O, and 820 mL of 0.25 M acetate buffer, pH 5.5]. The flasks were shaken for 15 min and incubated in a water bath at 37 °C. After 10- and 70- min incubation, a 2-mL portion of the incubate was transferred to a test tube containing 2 mL of 10% trichloroacetic acid (TCA). The contents of both tubes were filtered, and the reaction was terminated by adding 1 mL of reaction mixture to 1 mL of a freshly prepared color reagent. The color reagent was a mixture of four parts of solution A (15 g of ammonium heptamolybdate 4 H_2O in 55 mL of 36 N H_2SO_4, made up to 1 L) and one part of Solution B (27 g of $FeSO_4 \cdot 7$ H_2O, a few drops of 36 N H_2SO_4, and H_2O to 250 mL). The liberated phosphorus was determined spectrophotometrically (700 nm). In this method, phytase activity was calculated as phytate units $kg^{-1} = (P \times 1000)/(W \times 60)$, where P is μmol of phosphorus liberated by phytase in 60 min, W is sample weight (g), and 60 is the incubation time taken into account (i.e., 70 minus 10 min). From the method described, the phytase unit is defined as that amount of phytase activity that liberates inorganic phosphorus from a 0.0015 M sodium phytate solution at a rate of 1 μmol min^{-1} at pH 5.5 and 37 °C.

Acid phosphatase activity was determined according to the method of Zyla et al. (*32*). In this method, the acid phosphatase activity was measured spectrophotometrically at 405 nm by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl phosphate. Finely ground samples (100−150 mg) were weighed and extracted with 5 mL of 100 mM acetate buffer, pH 4.5, for 20 min. The reaction mixture consisted of 1 mL of substrate (10 mM disodium *p*-nitrophenyl phosphate) and 0.2 mL of enzyme extracted. After 30 min of incubation at 37 °C, the reaction was stopped by the addition of 5 mL of 40 mM NaOH. One unit of acid phosphatase (AcPh) was defined as 1 μmol of *p*-nitrophenol liberated/min under the above conditions.

Statistical Analysis. Analysis of variance was performed using the GLM procedure of Statistical Analysis System (*33*) and the significant differences among means were determined by using Duncan's multiple range test. Means square error (MSE) was used as a measurement of the variance not accounted for by the treatments. In assay 2, linear regression was determined between phytate and phosphatase activities.

Table 1. Effect of Soaking, Germination Time, and Gibberellic Acid (GA_3) on Total P, Phytate P, Phytase (Phy), and Acid Phosphatase (AcPh) Activities in Rye[a,b]

		soaking 1 hour				soaking 14 h				
		germination 3 days		germination 5 days		germination 3 days		germination 5 days		
GA_3	US[c]	−	+	−	+	−	+	−	+	MSE[d]
Phy (U kg^{-1})	5739 *f* ± 200	9540 *b* ± 332	12163 *a* ± 309	8380 *c* ± 332	11959 *a* ± 298	6873 *de* ± 183	7129 *d* ± 151	6600 *e* ± 183	6740 *de* ± 200	248
AcPh	18288 *h* ± 277	44793 *d* ± 603	53525 *a* ± 703	37669 *f* ± 105	49114 *c* ± 267	36413 *g* ± 286	50240 b ± 653	37667 *f* ± 105	41116 *e* ± 490	462
total P (%)	0.31 *ab* ± 0.02	0.30 *ab* ± 0.01	0.30 *ab* ± 0.01	0.31 *ab* ± 0.02	0.32 *a* ± 0.02	0.29 *b* ± 0.01	0.29 *b* ± 0.01	0.30 *ab* ± 0.02	0.30 *ab* ± 0.01	0.013
phytate P (%)	0.19 *a* ± 0.01	0.09 *cd* ± 0.01	0.12 *b* ± 0.02	0.04 *f* ± 0.01	0.05 *e* ± 0.01	0.08 *d* ± 0.02	0.11 *bc* ± 0.01	0.03 *f* ± 0.01	0.05 *e* ± 0.01	0.008

[a] Analyzed on the basis of dry matter. [b] Each mean ± standard deviation represents three replications. [c] US, ungerminated seeds. [d] MSE, means square error. [e] *a,b,c,d,e,f,g,h* Means within a row not sharing a common letter are significantly different ($P < 0.05$).

Table 2. Effect of Soaking, Germination Time, and Gibberellic Acid (GA_3) on Total P, Phytate P, Phytase (Phy), and Acid Phosphatase (AcPh) Activities in Barley[a,b]

		soaking 1 h				soaking 14 h				
		germination 3 days		germination 5 days		germination 3 days		germination 5 days		
GA_3	US[c]	−	+	−	+	−	+	−	+	MSE[d]
Phy (U kg^{-1})	1521 *g* ± 80	2369 *f* ± 86	3687 *b* ± 150	3206 *cd* ± 173	4751 *a* ± 221	2595 *e* ± 90	3278 *c* ± 102	3027 *d* ± 75	2242 *f* ± 71	127
AcPh (U g^{-1})	3041 *f* ± 102	12086 *e* ± 309	12401 *e* ± 200	22328 *a* ± 202	19230 *c* ± 192	12453 *e* ± 290	12326 *e* ± 270	20694 *b* ± 388	16650 *d* ± 220	258
total P (%)	0.30 *a* ± 0.01	0.29 *ab* ± 0.01	0.26 *d* ± 0.02	0.28 *b* ± 0.01	0.30 *a* ± 0.02	0.28 *bc* ± 0.01	0.28 *bc* ± 0.01	0.27 *c* ± 0.02	0.29 *ab* ± 0.01	0.014
phytate P (%)	0.18 *a* ± 0.01	0.12 *bc* ± 0.01	0.14 *b* ± 0.01	0.08 *f* ± 0.01	0.11 *cd* ± 0.02	0.12 *bc* ± 0.01	0.14 *b* ± 0.01	0.09 *e* ± 0.01	0.10*d* ± 0.01	0.012

[a] Analyzed on the basis of dry matter. [b] Each mean ± standard deviation represents three replications. [c] US, ungerminated seeds. [d] MSE, means square error. [a,b,c,d,e,f,g] Means within a row not sharing a common letter are significantly different ($P < 0.05$).

Table 3. Changes in Total P, Phytate P, Phytase (Phy) and Acid Phosphatase (AcPh) Activities during the Germination of Rye and Barley[a]

germination time (days)	phytate P (%)	total P (%)	(phytate P/total P) × 100	(Phy) (U kg^{-1})	(AcPh) (U g^{-1})	ratio AcPh/Phy
			Rye			
US[c]	0.19 ± 0.01[b]	0.30 ± 0.01	63 ± 1.2	5245 ± 183	17936 ± 240	3.4
1 d	0.17 ± 0.01	0.31 ± 0.02	55 ± 0.9	6524 ± 190	24923 ± 210	3.8
3 d	0.06 ± 0.01	0.29 ± 0.01	21 ± 0.7	8229 ± 300	56099 ± 700	6.8
5 d	0.03 ± 0.01	0.31 ± 0.01	10 ± 0.6	8188 ± 260	40962 ± 606	5.0
			Barley			
US	0.19 ± 0.01	0.30 ± 0.02	63 ± 0.9	1436 ± 90	3151 ± 97	2.2
1 d	0.18 ± 0.01	0.30 ± 0.01	60 ± 1.3	2150 ± 101	3425 ± 111	1.6
3 d	0.12 ± 0.01	0.32 ± 0.01	38 ± 1.7	3300 ± 142	13075 ± 200	4.0
5 d	0.08 ± 0.01	0.33 ± 0.01	24 ± 1.1	4257 ± 185	15025 ± 225	3.5

[a] Analyzed on the basis of dry matter. [b] Each mean ± standard deviation represents three replications. [c] US, ungerminated seeds.

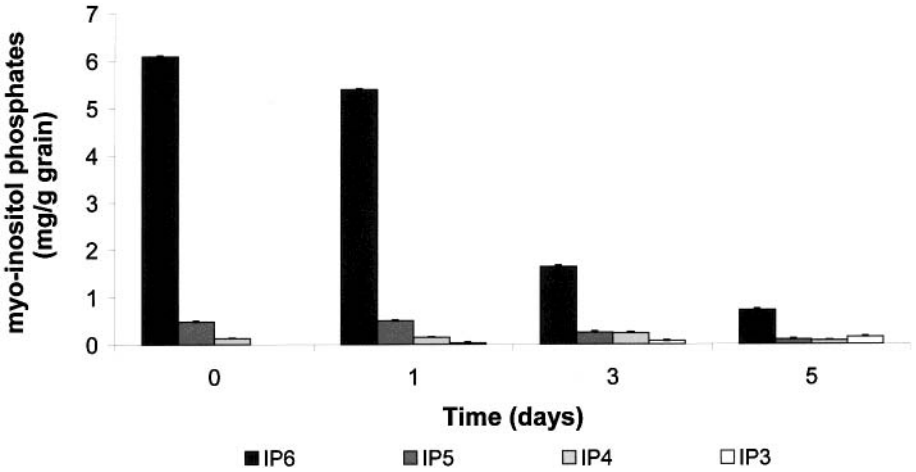

Figure 1. Changes in *myo*-inositol hexa- (IP6), penta- (IP5), tetra- (IP4), and tri- (IP3) phosphates during germination of rye.

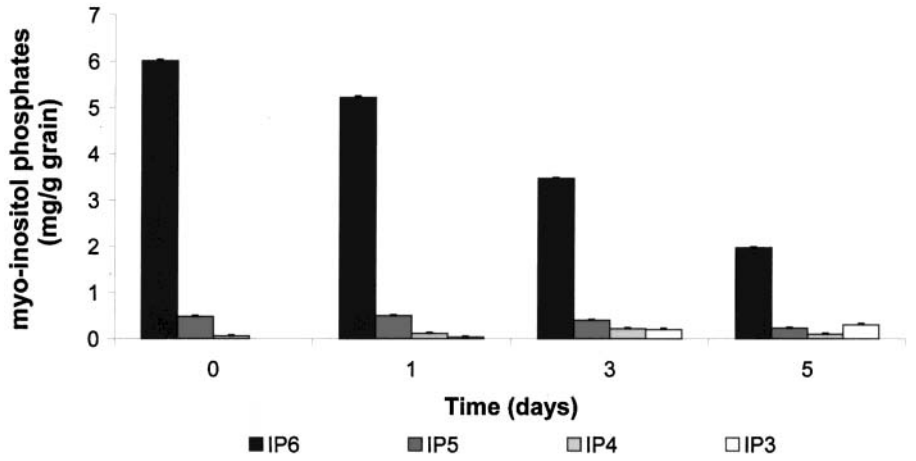

Figure 2. Changes in *myo*-inositol hexa- (IP6), penta- (IP5), tetra- (IP4), and tri- (IP3) phosphates during germination of barley.

RESULTS

The changes in total phosphorus, phytate phosphorus content, phytase (Phy), and acid phosphatase (AcPh) activities of rye and barley during different germination conditions are summarized in Tables 1, 2, and 3. In ungerminated seeds (assays 1 and 2), phytate phosphorus content, as a percentage of total phosphorus content, for the two cereals studied (rye and barley) was similar (62%). Likewise, in both assays, phosphatase activities were detected in ungerminated seeds, observing that rye contained higher Phy and AcPh activities respectively (up to 5739 U kg^{-1} and 18288 U g^{-1}) than barley (up to 1521 U kg^{-1} and 3151 U g^{-1}).

On the other hand, the results showed (assay 2) that ungerminated seeds contained IP_6, IP_5, and IP_4 (Figures 1 and 2). The analysis detected greater concentrations of total inositol phosphates in rye (6.70 mg g^{-1}) that in barley (6.55 mg g^{-1}). IP_6 was the major inositol phosphate in both cereals (6.09 and 6.01 mg g^{-1}, respectively), representing more than 90% of the total *myo*-inositol phosphates. The concentrations of *myo*-inositol pentaphosphate (IP_5) was only 8% of total phytate, whereas IP_4 was 2 and 1% for rye and barley, respectively. IP_3 was not detected in ungerminated seed.

The germination process, depending on different experimental conditions (assays 1 and 2), caused a

significant increase of Phy and AcPh activities in rye (up to 112 and 213%) and barley (up to 212 and 634%) and a significant reduction of phytate phosphorus content (up to 84 and 58% respectively). In rye and barley (assay 2), linear regression coefficients showed an increase in Phy ($R^2 = 0.84$, $P < 0.0001$ and $R^2 = 0.90$, $P < 0.0001$, respectively) and AcPh ($R^2 = 0.73$, $P < 0.0001$ and $R^2 = 0.92$, $P < 0.0001$, respectively) with a concomitant decrease in phytate phosphorus content. Likewise, the enzyme activities and the phytate phosphorus content during the germination were affected by the soaking time. In fact, 14 h of soaking resulted in lower Phy and AcPh activities in rye (35 and 11%) and barley (20 and 6%) respectively, and a lower phytate phosphorus content, only in the case of rye (10%), than 1 h soaking.

In addition, when we compared, in both assays, the influence of germination time on parameters studied, we observed higher Phy and AcPh activities (up to 6 and 27%) in germinated rye during 3 days in comparison to those in 5 days. In contrast to rye, germinated barley during 5 days had greater Phy and AcPh activities (up to 29 and 60%) than those in 3 days (Tables 1 and 2). Likewise, in rye and barley, the lower values of phytate phosphorus content were found with germinated seeds during 5 days (up to 0.03 and 0.08% respectively). It is interesting to remark, in the case of rye, that the progressive decrease observed in the phytate phosphorus content between the third and fifth day of germination was not accompanied by an increase in Phy and AcPh activities. However, in barley, a gradual degradation of phytate phosphorus content was always related with an increase of phosphatase activities. The lower values of R^2 obtained in rye compared to those obtained in barley could explain these differences.

The effect of addition of GA_3 in the germination process on the different parameters analyzed is shown in Tables 1 and 2. The inclusion of GA_3 in germinated rye resulted in higher Phy and AcPh activities (21 and 24%, respectively) than when this hormone was not added. However, in barley, Phy and AcPh activities were greater (25%) and lower (10%), respectively, when GA_3 was present. Moreover, a higher phytate phosphorus content was observed when GA_3 was added in germinated rye (38%) and barley (20%).

When we considered all the germination conditions as a whole (Tables 1 and 2), the greatest Phy and AcPh activities in rye were found ($P < 0.05$) with 1 h of soaking, 3 days of germination, and the addition of GA_3 (12163 U kg^{-1} and 53525 U g^{-1}, respectively). However, the lowest phytate phosphorus content was obtained ($P < 0.05$) with 1 (0.04%) or 14 h (0.03%) of soaking, 5 days of germination, and without addition of GA_3. In barley, the greatest enzyme activities were found ($P < 0.05$), with 1 h of soaking and 5 days of germination, with addition of GA_3, in the case of Phy (4751 U kg^{-1}), and without this hormone in the case of AcPh (22328 U g^{-1}). Likewise, the lowest phytate P content in this cereal (0.08%; $P < 0.05$) was found with 1 h of soaking, 5 days of germination, and without addition of GA_3.

Finally, to examine the action of the rye and barley phosphatases on inositol phosphates content (assay 2), the hydrolysis products were separated by HPLC method (Figures 1 and 2). The germination strongly reduced the concentration of total inositol phosphates in rye (84%) and barley (60%), however, these changes mainly occurred after 1 day of germination. During the first day, a slight decrease in IP_6 was observed in rye (11%) and barley (13%) that was accompanied respectively by an increase of IP_5 (4 and 4%) and IP_4 (15 and 100%). At the end of this period, small amounts of IP_3 were detected in rye (0.03 mg g^{-1}) and barley (0.04 mg g^{-1}). Between day 1 and day 5 of germination, IP_6 and IP_5 were rapidly degraded in rye (87 and 80%) and barley (62 and 54%) with a clear increase of IP_3 (400 and 650%, respectively). IP_4 showed an increase in rye and barley until 3 days of germination (85 and 267%, respectively) to be strongly hydrolyzed (67 and 55%, respectively) in the last 2 days.

DISCUSSION

The different values found in the literature on the content of phytate phosphorus of feedstuffs are related to cultivars, processing conditions, and analytical methods used. In our assays, the values of phytate phosphorus content found in ungerminated rye and barley agree with those shown by Ravindran et al. (*4*) and Eeckhout and De Paepe (*17*). In addition, Eeckhout and De Paepe (*17*) and Viveros et al. (*3*) found a high correlation between total P and phytate P content in cereals and cereal byproducts. Likewise, the ungerminated rye and barley contained high amounts of IP_6, representing 90% of total *myo*-inositol phosphate, whereas the other *myo*-inositol phosphates were found only in small (IP_5 and IP_4) or trace amounts (IP_3). When we compared the phytate phosphorus content obtained by the HPLC method (assay 2) and iron precipitation method (assays 1 and 2), we observed values lower in the former, even if calculated as the sum of inositol tri- to hexaphosphate. This difference could be explained by the fact that the iron precipitation method caused coprecipitation with other phosphorus compounds, increasing the values (*34*, *35*).

Sandberg and Ahderine (*34*), Phillippy et al. (*36*), Lehrfeld (*30*, *37*), Sandberg and Svanberg (*38*), De la Cuadra et al. (*39*); Ayet et al. (*40*); Burbano et al. (*31*), Khun et al. (*41*), Kasim and Edwards (*42*); Greiner et al. (*10*) and Trugo et al. (*43*) have presented data on inositol phosphate content in some food and feed ingredients. The IP_6 contents obtained in our ungerminated seeds are similar to those determined by Sandberg and Svanberg (*38*) and Kasim and Edwards (*42*) for rye and Lehrfeld (*37*) for barley. However, Kasim and Edwards (*42*) obtained lower values for barley. When considering other classes of inositol phosphates (IP_5, IP_4, and IP_3), the values found in the literature differed by considerable margins in comparison to our data (*37*, *38*, *42*). However, the amounts of inositol phosphates decreased in descending pattern from IP_5 to IP_3 as in the literature in both cereals.

On the other hand, the enzyme Phy has been detected in a number of ungerminated cereals (*3*, *17*, *44*−*46*). In our data, as other authors reported, ungerminated rye exhibits higher Phy and AcPh activities than those exhibited by barley seeds (*3*, *10*, *47*). In addition, Viveros et al. (*3*) found that Phy and AcPh were positively correlated with phytate P in barley and rye seeds. However, Barrier-Guillot et al. (*48*) in wheat and Eeckhout and De Paepe (*17*) in wheat and barley reported that no relationship could be established between endogenous phytase activity and total phosphorus or phytate phosphorus contents. This lack of correlation has also been suggested in other cereals by Sauveur (*49*).

In our assays, a marked increase in Phy and AcPh activities was observed during the germination of rye and barley. Knowledge about the Phy and AcPh activities in germinated seeds, including cereals, is shown by different authors (*5*, *10*, *22*, *44*, *50*−*52*). In the case of rye and barley, Bartnik and Szafranska (*51*) found an increase in Phy activity in the germination process. Greiner et al. (*10*) also detected Phy and AcPh activities in germinated rye, observing that AcPh activity was about four times greater than that of Phy.

Likewise, in the course of germination, IP_6 and IP_5 were rapidly degraded, and IP_4 was only a short-living intermediate which was increased during hydrolysis and degraded slowly to IP_3. Similar results were obtained by Greiner et al. (*10*) who observed a similar pattern of phytate degradation with phytase extracted from rye. In addition, although a gradual degradation of phytate phosphorus content was observed during the germination in both cereals, this reduction was not always, in the case of rye, related to an increase in Phy and AcPh activities. Bartnik and Szafranska (*51*) and Sandberg (*20*) also reported, in cereals, an increase in phytase activity after first hours of germination that was not related with a reduction of phytate phosphorus. However, Lee (*44*), Kikunaga et al. (*5*), and Oloffs et al. (*52*) observed an increase during germination of phytase activity in rye and barley with a concomitant decrease of phytate phosphorus content, which demonstrated a good inverse correlation between the levels of both phytase activity and inorganic phosphorus, and phytate breakdown. In contrast, Fretzdorff and Weiper (*50*) observed in rye that, during the early stages of germination (3 days), phytase activity did not change, and phytate content was reduced. This anomalous behavior found among the different authors could be justified by the presence of inhibitors in the grain (*51*), or that during germination both acidity and temperature are far from optimal for the efficacy of these enzymes.

It is interesting to note, in our results, the great efficacy of germination process in rye (up to 84%) by breakdown phytate phosphorus. Sandberg and Svanberg (*38*) demonstrated that soaking for 30 min in optimal conditions for rye phytase (pH 5.0 and 55 °C) completely degraded the inositol hexa- and pentaphosphate. Greiner et al. (*10*) and Greiner and Konietzny (*53*) reported that rye enzyme exhibits a broad affinity for various phosphorylated compounds, showing a marked reduction in *myo*-inositol phosphates (IP_6 and IP_5) when added to black bean.

The action of GA_3 on the phosphatase activities of germinated seeds is not well understood, there is little existing information and it is contradictory. Moreover, most authors have used degermed grains or aleurone layers instead of whole seeds in their investigations. In our results, the addition of GA_3 to the germination process produced, generally, an increase in Phy and AcPh activities. Obata and Suzuki (*54*) and Ashford and Jacobsen (*55*) showed that the activities of phosphatases in the aleurone layer of huskless barley were absolutely dependent upon the presence of GA_3. Katayama and Suzuki (*56*) reported that incubation with 10 ppm GA_3 remarkably stimulated release of AcPh and phytate degradation into Pi and *myo*-inositol within the aleurone tissue. Apparently the presence of GA stimulated activation and secretion, but not de novo synthesis, of AcPh in barley seeds (*57*) by two GA_3-induced mechanisms: one for enzyme formation and the other for enzyme secretion (*54*).

On the other hand, in our assays, the germinated seeds with GA_3 contained more phytate phosphorus than those germinated without this hormone. This different response obtained by adding GA_3 on phytate P content could be explained by the interference between both endogenous and exogenous gibberellins, being the response already saturated in germinated seeds by endogenous hormone. It seems that the growing embryo causes a progressive loss of responsiveness in the aleurone layer by GA_3 (*56*). Likewise, enzymes were shown to differ in their responses to different doses of GA_3 (*58*). Eastwood and Laidman (*59*) also suggested that the stimulation of phytate degradation by GA_3 is caused by removing the inhibition of phosphorus on phytase action.

In conclusion, a marked increase of Phy and AcPh activities in rye (up to 112 and 213%) and barley (up to 212 and 634%) with a concomitant decrease in phytate phosphorus content (up to 84 and 58% respectively) and an increase in the content of lower inositol phosphates [inositol penta- (IP_5), tetra- (IP_4), and triphosphate (IP_3)] were observed during several germination conditions in rye and barley. This germination step can be used successfully to make low-phytate ingredients with high endogenous phosphatase activities. The utilization of these phosphatase-rich ingredients, such as germinated rye and barley, in animal feeds could increase the availability of phosphorus in the feeds for monogastric animals which could lower the cost of feed formulation and result in reduction of the phosphorus pollution in areas of intensive animal agriculture. The possible use in the diet of these germinated products requires additional investigation. Such studies are now in progress.

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Received for review January 2, 2001. Revised manuscript received April 5, 2001. Accepted April 5, 2001. This research was supported by a grant from the Spanish Ministry of Education and Science (AGF98-0889).

JF010023C