Characteristics of Phytase and Its Relationship to Acid Phosphatase and Certain Minerals in Triticale[1]

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ABSTRACT

Some properties of triticale phytase were studied using both crude extract in sodium acetate buffer and a partially purified extract. The optimum pH of the enzyme was 5.4 at 45° C. The Michaelis-Menten constant (km) of the partially purified extract was $0.22 \times 10^{-3} M$. The enzyme was slightly activated by magnesium and manganese salts. The activity was inhibited by *p*-chloromercuribenzoate and the salts of iron, copper, silver, nickel, and cobalt. With few exceptions, triticale bran had higher phytase and acid phosphatase activity than flour or whole grain, as well as higher amounts of phytic acid, calcium, magnesium, zinc, and iron. Simple correlation coefficients, determined in all possible combinations, indicated no significant correlations between phytase, phosphatase, phytic acid, calcium, magnesium, zinc, and iron. There were, however, significant correlations between calcium, zinc, and phosphatase; magnesium, zinc, and phytic acid; and iron and phosphatase.

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Phytate is often present as the calcium (Ca) and magnesium (Mg) salt of phytic acid, myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate). It is widely distributed in nature. A large part of the phosphorus (P) in seeds is present as phytate. Phytate is hydrolyzed by the enzyme phytase (myoinositol hexaphosphate phosphohydrolase, EC 3.1.3.8) to inositol and free orthophosphate. This enzyme has a wide distribution in plant and animal tissues, in fungi, and in bacteria (Cosgrov 1966). Phytase activity has been studied in wheat, barley, dwarf bean, cottonseed products, and navy beans (Gibbins and Norris 1963, Lolas and Markakis 1977, Peers 1953, Preece and Gray 1962, Wozenski and Woodburn 1975).

The enzyme has been partially purified from wheat (Peers 1953). Lim and Tate (1973) suggested that the phytase from wheat bran is made up of two distinctive fractions (F1 and F2) with different substrate degradation patterns. A study by Bianchetti and Sartirana (1967) indicated that phytase activity in wheat embryo is regulated by the concentration of inorganic phosphate.

A relatively high content of phytic acid has been found in some of the available lines of triticale (Singh and Reddy 1977). Since triticale has been considered as a promising new cereal grain for both human and animal nutrition, it is important to study the distribution of phytase in various lines of triticales. In this study, characteristics of phytase in triticale were determined. In addition, variations in enzyme activity in the fractions due to milling of the grain into bran and flour and its relation to phytic acid, acid phosphatase, and certain nutritionally important minerals were also studied.

MATERIALS AND METHODS

Triticale lines used in this study were AM 2147, AM 2149, AM 3680, AM 3690, and AM 3696 from Alabama A & M University breeding program, 72-S and 6TA 131 from the Jenkins Foundation, and FS 1045, FS 1795, FS 1897, Rahum, X 2802, and Beagle from CIMMYT Mexico. All triticales were grown at Alabama A & M University farms in 1976 except 72-S, which was obtained from the Washington and Texas crops. A wheat line, Arthur 71, also was grown at Alabama A & M University farms in 1976. Seeds were cleaned, tempered to 13% moisture, and then milled on a Brabender Quadrumat Junior mill. The extraction rates of the flour varied from 45 to 54%. The bran was further milled into a fine powder on a Udy-cyclone sample mill using a 60-mesh screen. The whole grain samples also were prepared by grinding on the Udy-cyclone sample mill.

Determination of phytic acid was by the method of Wheeler and Ferrel (1971) and minerals (Ca, Mg, iron [Fe] and zinc [Zn]) were by atomic absorption spectrophotometer as described earlier (Singh and Reddy 1977). Moisture was determined by AACC methods.

The crude enzyme extract was prepared by constant stirring of 2 g whole grain meal, flour, or bran with 30 ml sodium acetate buffer, pH 5.2, for 30 min at 0–4° C. The mixture was separated by centrifugation at $5,000 \times g$ for 10 min. The supernatant was used for enzyme assay after filtration through cheesecloth.

Table 1 shows the steps to obtain a partially purified crude extract of phytase. Thirty grams of whole meal from X 2802 was extracted with 175 ml sodium acetate buffer for 30 min with constant stirring. The suspension was centrifuged at $5,000 \times g$ to remove the bulk of the solid matter. The supernatant was filtered to give 150 ml of a clear solution. This was poured with stirring into 600 ml ice-cold acetone and allowed to stand 10 min. The precipitate was collected on a Büchner funnel and was washed with 200 ml each of acetone, acetone/ether (1:1), and finally ether to obtain a colorless powder. The acetone powder retained the phytase activity for a longer period ranging from 6 to 8 wk; therefore, for further purification, acetone powder was used.

The acetone powder was extracted by mild stirring for 2 hr at 0–4° C with 100 ml sodium acetate buffer and then filtered. The clear solution was brought to 30% saturation with ammonium sulfate [$(NH_4)_2SO_4$]) by slowly dissolving 17.5 g of $(NH_4)_2SO_4$ per 100 ml solution. This solution was centrifuged at $10,000 \times g$ for 20 min and then filtered. The precipitate was discarded. The supernatant contained more than 85% of the phytase activity. The supernatant was brought to 95% saturation with $(NH_4)_2SO_4$. The solution was again centrifuged at $10,000 \times g$ for 20 min and filtered. In this case, more than 90% of the phytase activity was found in the precipitate. The supernatant had an insignificant amount of activity and therefore was discarded. The precipitate was collected, suspended in 100 ml sodium acetate buffer, and dialyzed for 48 hr against three changes of 2 L distilled deionized water. The extract was centrifuged to remove a slight precipitate formed during dialysis. The dialyzed extract was defined as a "partially purified enzyme."

Phytase activity was assayed by measuring the increase in inorganic phosphate by the method of Fiske and SubbaRow (1925). The reaction mixture contained 2 ml sodium phytate (3 m*M*), 2 ml sodium acetate buffer, pH 5.4, and 1 ml enzyme extract The total volume of the incubation mixture was 5 ml. Incubation was done at 45° C for 2.5 hr and enzyme activity was terminated by adding 3 ml 10% trichloroacetic acid (TCA).

Acid phosphatase activity was determined by incubating 0.1 ml phenyl-phosphate (3 m*M*) with 0.3 ml sodium acetate buffer, pH

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4.8 (0.1*M*), and 0.6 ml of the crude enzyme extract (the extract prepared with sodium acetate buffer for the phytase). The mixture was incubated at 30°C for 15 min. The enzyme activity was terminated by the addition of 3 ml 10% TCA. The amount of inorganic P was determined by the method of Fiske and SubbaRow (1925).

RESULTS AND DISCUSSION

Figure 1 shows pH vs. enzyme activity curves for the crude extract and for the partially purified enzyme. Although both preparations had a pH optimum at 5.4, the purified fraction had a relatively sharp optimum near 5.4 with a rapid diminution in activity on either side of this optimum. The crude extract had a relatively broader pH profile extending from 5 to 5.4 (Fig. 1). Various pH values from 5.0 to 5.7 have been reported in the literature as optimum for phytase (Table II). Optimum temperature for enzyme activity was 45°C (Fig. 2). An optimum of 50°C has been reported for navy bean phytase by Lolas and Markakis (1977), 57°C for germinating mung bean by Mandal and Biswas (1970), and 55°C for wheat phytase by Peers (1953).

Progress curves of the hydrolysis of phytate (time vs. velocity) using standard assay mixture at 45°C are presented in Fig. 3. The activity was linearly related to the time of incubation up to 2.5 hr for the crude extract and up to 3.5 hr for the partially purified enzyme.

Effect of substrate concentration on enzyme activity of crude extract and the purified enzyme incubated at 45°C for 2.5 hr is shown in Fig. 4. Apparently, a typical Michaelis-Menten type of curve was not obtained. Activity was inhibited at concentrations above 0.9 m*M* phytate for the crude extract and 0.8 m*M* phytate for the partially purified enzyme extract. Lolas and Markakis (1977) observed a similar relation between substrate and the enzyme for navy bean phytase. Gibbins and Norris (1963) suggested that the inhibition of phytase by high substrate concentrations was indicative of a two-point attachment of phytate to phytase.

From a plot of 1/V and 1/[S], the km of crude extract was $0.18 \times 10^{-3} M$ and of purified enzyme $0.22 \times 10^{-3} M$. For comparison, Michaelis-Menten constants of various phytases reported in the literature are presented in Table III.

The effect of various inorganic salts on partially purified enzyme during standard conditions of assay was evaluated. The results are recorded in Table IV. Evidently, $MnSO_4$ and $MgSO_4$ slightly activated the enzyme reaction. Activation by Mg^{++} has been observed earlier by Peers (1953) for wheat phytase, Gibbins and Norris (1963) for dwarf bean phytase, and Lolas and Markakis (1977) for navy bean phytase. On the other hand, Mn^{++} did not have any effect on wheat phytase (Peers 1953). The enzyme activity was considerably inhibited by the salts of Fe, copper (Cu), silver (Ag), nickel (Ni), and cobalt (Co) (Table IV). In contrast to navy bean phytase (Lolas and Markakis 1977), we noted a substantial

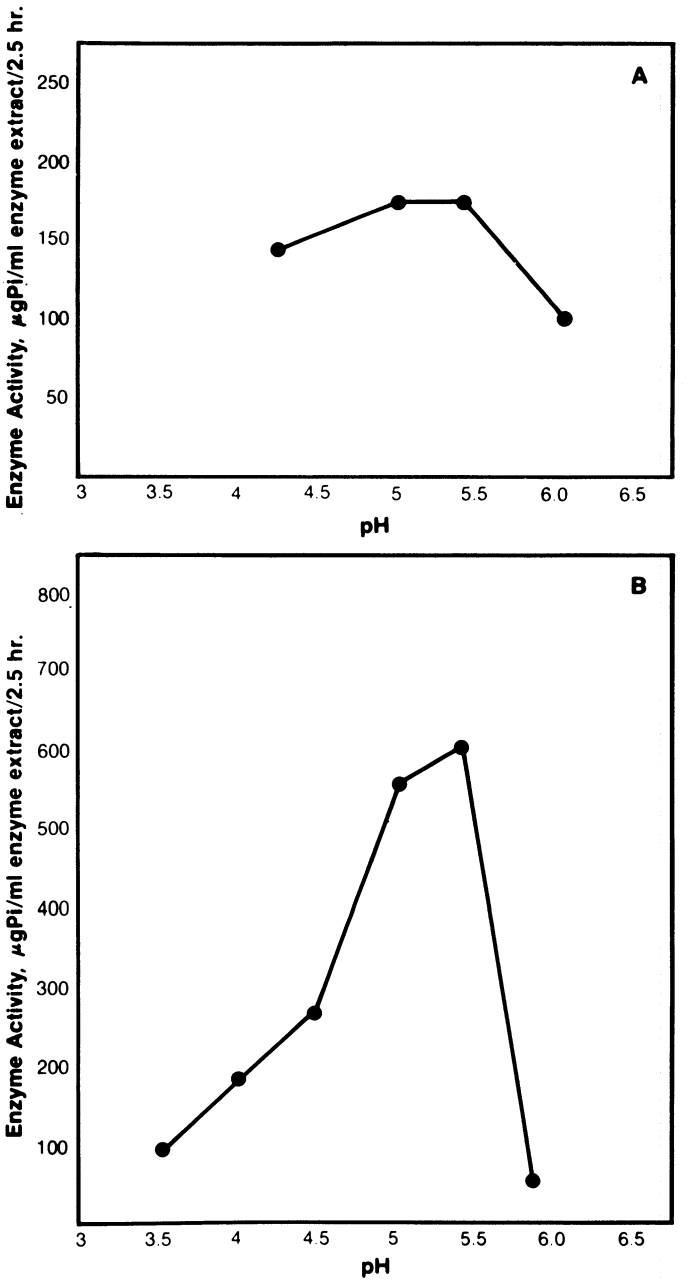

Fig. 1. Activity as a function of pH for phytase (**A**) crude extract and (**B**) partially purified enzyme from X 2802 triticale.

TABLE I
Purification of Triticale Phytase[a]

Fraction	Phytase Activity Units[b]	Protein (mg/ml)	Specific Activity
1. Crude extract (buffer-extracted supernatant fraction left after centrifugation at 5,000 × *g* for 10 min)	1,240	1,400	0.89
2. Acetone powder: Fraction 1 treated with acetone to obtain a colorless powder. The powder was extracted with sodium acetate buffer.	1,280	1,400	0.91
3. Ammonium sulfate, 30% saturation: Fraction 2 brought to 30% saturation with $(NH_4)_2SO_4$. The supernatant (a) and the precipitate (b) were obtained after centrifugation.			
(a) Supernatant	3,120	272	11.47
(b) Precipitate	840	625	1.34
4. Ammonium sulfate, 95% saturation: Fraction 3 (a) brought to 95% saturation. Supernatant (a) contained almost zero activity. Precipitate (b) had most of the activity.			
(a) Supernatant	....	....	....
(b) Precipitate	2,480	252	9.84
5. Partially purified enzyme: Fraction 4 (b) dissolved in sodium acetate buffer and then dialyzed.	2,360	250	9.44

[a] Cultivar used was X 2802 grown at the Alabama A & M University farm.
[b] 1 unit = amount of enzyme releasing 1 μg of inorganic phosphorus from 1.2 m*M* phytate at pH 5.4 and 45°C in 1 hr.

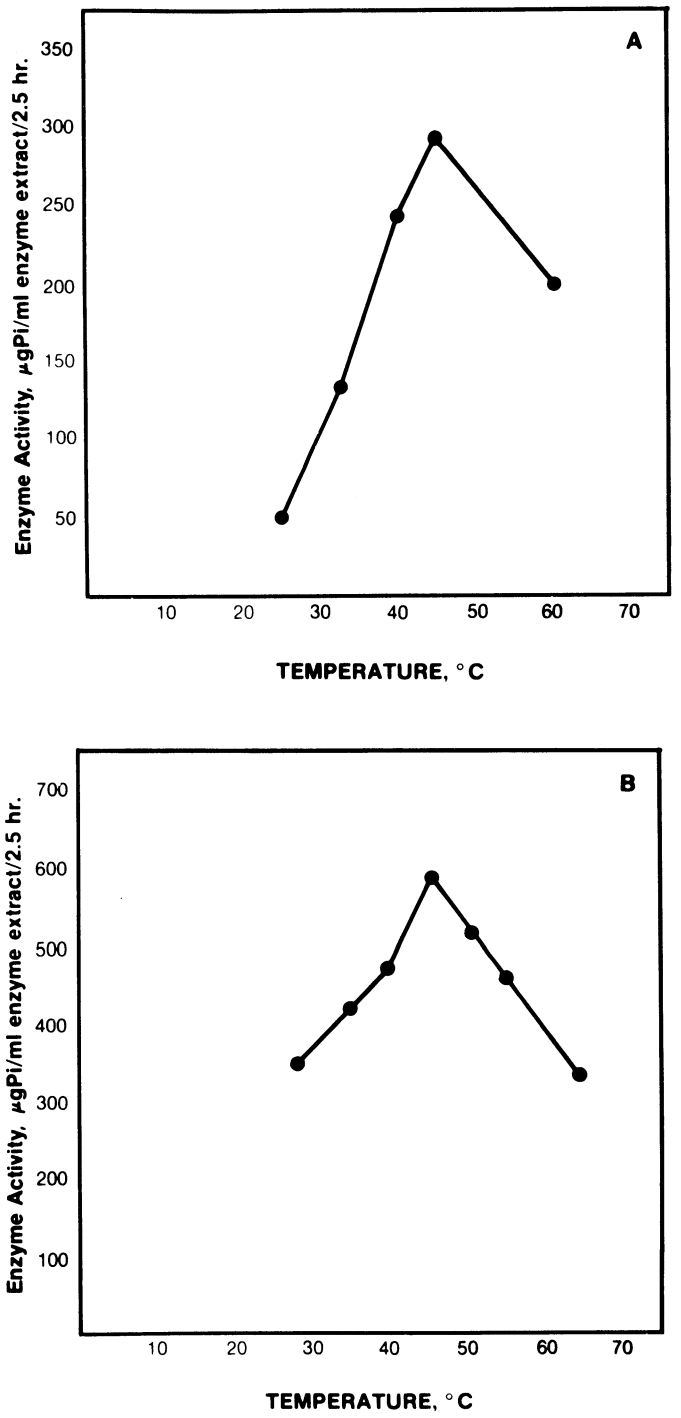

Fig. 2. Effect of temperature on phytase activity of (**A**) crude extract and (**B**) partially purified enzyme of X 2802 triticale.

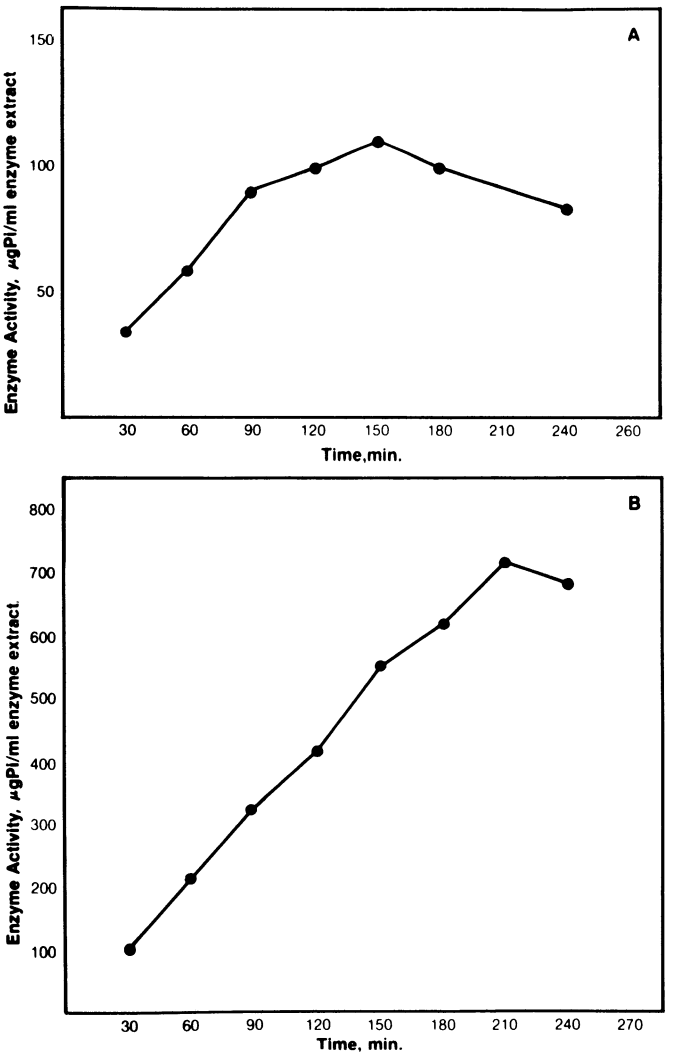

Fig. 3. Effect of incubation time on phytase activity of (**A**) crude extract and (**B**) partially purified enzyme of X 2802 triticale.

TABLE II
Optimum pH for Phytase Activity

Phytase Source	pH	Reference Source
Triticale (both crude and partially purified extracts)	5.4	...[a]
Navy bean	5.3	Lolas and Markakis (1977)
Wheat flour	5.15	Peers (1953)
Wheat bran	5.0	Nagai and Funahashi (1962)
Dwarf bean	5.2	Gibbins and Norris (1963)
Corn	5.6	Chang (1967)
Mung beans (germinating)	7.5	Mandal et al (1972)
Lettuce seeds (germinating)	5.0	Meyer (1958)

[a]Authors' results on cultivar X 2802 grown at Normal, AL.

TABLE III
Michaelis-Menten Constant (km) of Phytases

Phytase Source	km	Reference or Source
Triticale (crude extract)	$0.18 \times 10^{-3} M$	...[a]
Triticale (partially purified)	$0.22 \times 10^{-3} M$	...[a]
Corn	$0.99 \times 10^{-3} M$	Chang (1967)
Wheat, whole grain (partially purified)	$0.33 \times 10^{-3} M$	Peers (1953)
Wheat bran	0.57×10^{-3}	Nagai and Funahashi (1962)
Dwarf bean	$0.15 \times 10^{-3} M$	Gibbins and Norris (1963)
Mung bean (germinating)	$0.65 \times 10^{-3} M$	Mandal and biswas (1970)
Navy bean	$0.018 \times 10^{-3} M$	Lolas and Markakis (1977)

[a]Authors' results on cultivar X 2802 grown at Normal, AL.

TABLE IV
Effect of Metal Salts on Triticale Phytase Activity[a]

Metal Salt	Relative Activity[a] (%)		
	$10^{-5}M$	$10^{-4}M$	$10^{-3}M$
$FeSO_4$	45	31	27
$FeCl_3$	53	27	18
$CuSO_4$	35	18	10
p-Chloromercuribenzoate	72	55	52
$MnSO_4$	105	110	117
$MgSO_4$	102	109	110
$AgNO_3$	73	63	70
$Ni(NO_3)_2$	62	31	10
$CoCl_2$	82	73	63

[a]The activity of the partially purified phytase extracted from cultivar X 2802 in absence of metal salt was taken as 100%.

TABLE V
Phytase Activity in Various Fractions of Triticales and a Wheat[a]

Sample[b]	Phytase Activity (mgPi/g/2.5 hr)		
	Flour	Whole Grain	Bran
AM 2147	13.14	15.48	16.20
AM 2149	11.47	15.45	12.50
AM 3680	12.80	12.84	14.25
AM 3690	13.10	9.51	13.10
AM 3696	11.97	13.35	15.45
FS 1045	17.10	13.08	17.70
FS 1795	19.00	11.40	17.85
FS 1897	15.45	16.65	20.10
X 2802	17.85	13.08	16.55
Beagle	7.15	12.84	15.30
Rahum	15.54	14.28	18.00
6TA 131	5.95	15.45	11.85
72-S(T)	12.12	11.45	15.45
72-S(W)	8.35	9.54	15.30
Wheat (Arthur 71)	5.54	9.54	12.80
Average	12.44	12.93	15.51
LSD (0.05)	0.16	0.13	0.24
LSD (0.01)	0.22	0.18	0.34

[a]Data expressed on 14% moisture basis. Each value is the mean of three replicates.
[b]Crops grown in 1976 at Normal, AL, except cultivar 72-S, which was obtained from Texas [72-S(T)] and Washington [72-S(W)].

TABLE VI
Phosphatase Activity in Various Fractions of Triticales and a Wheat[a]

Sample[b]	Phosphatase Activity (μg/g/30 min)		
	Flour	Whole Grain	Bran
AM 2147	962	1,730	2,110
AM 2149	960	1,540	1,920
AM 3680	1,730	1,211	2,690
AM 3690	770	1,150	1,540
AM 3696	960	1,340	1,920
FS 1045	1,150	1,344	1,730
FS 1795	963	1,730	1,340
FS 1897	961	1,540	1,920
X 2802	962	1,730	2,113
Beagle	770	960	1,340
Rahum	959	2,110	2,300
6TA 131	963	1,150	1,540
72-S(T)	964	1,920	2,690
72-S(W)	380	961	1,340
Wheat (Arthur 71)	382	1,152	2,114
Average	922	1,438	1,907
LSD (0.05)	61	64	65
LSD (0.01)	84	89	90

[a]Data expressed on 14% moisture basis. Each value is the mean of three replicates.
[b]Crops grown in 1976 at Normal, AL, except cultivar 72-S, which was obtained from Texas [72-S(T)] and Washington [72-S(W)].

reduction in the activity of phytase by $CoCl_2$.

A number of triticales grown at Alabama A & M University farm in 1976 were examined for phytase activity using the crude enzyme extracts. For comparison, one commonly grown wheat was also included in the study. The results presented in Table V clearly indicate a significant difference in phytase activity of triticales. Among triticale lines originated at Alabama A & M University,

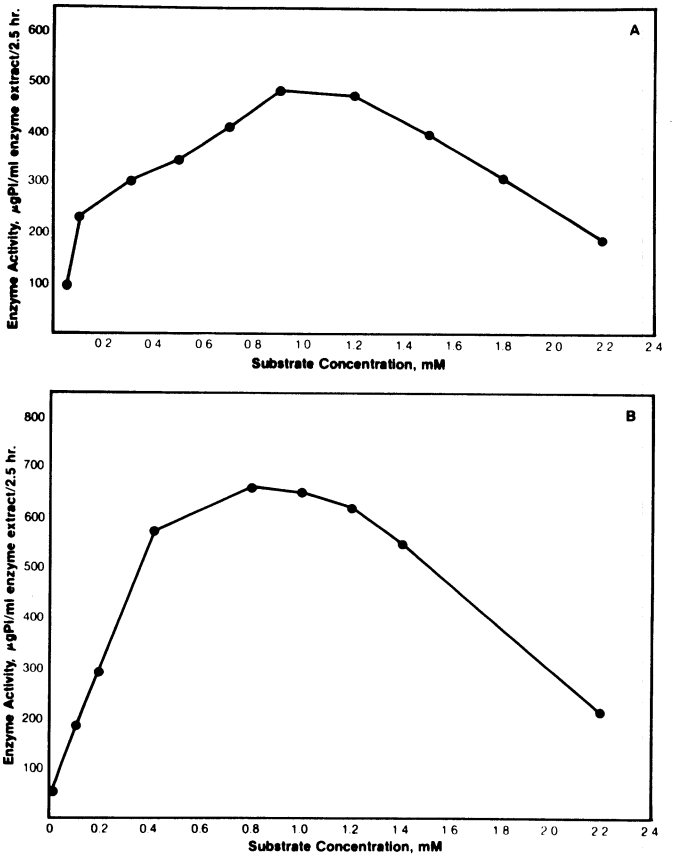

Fig. 4. Effect of substrate concentration on phytase activity of X 2802 triticale: (**A**) crude extract and (**B**) partially purified enzyme.

TABLE VII
Phytic Acid in Various Fractions of Triticales and a Wheat[a]

Sample[b]	Phytic Acid (mg/g)		
	Flour	Whole Grain	Bran
AM 2147	1.28	2.28	2.10
AM 2149	1.50	3.08	1.20
AM 3680	1.65	2.68	2.50
AM 3690	1.55	3.03	0.70
AM 3696	1.25	3.07	1.30
FS 1045	1.58	2.90	1.40
FS 1795	1.90	3.60	0.90
FS 1897	1.83	3.30	1.30
X 2802	2.08	2.88	1.90
Beagle	1.90	3.23	2.40
Rahum	2.05	3.13	2.30
6TA 131	1.67	2.95	1.00
72-S(T)	1.87	3.35	0.90
72-S(W)	1.81	3.38	2.90
Wheat (Arthur 71)	1.35	2.25	2.20
Average	1.69	3.04	1.67
LSD (0.05)	0.02	0.05	0.04
LSD (0.01)	0.03	0.07	0.06

[a]Data expressed on 14% moisture basis. Each value is the mean of three replicates.
[b]Crops grown in 1976 at Normal, AL, except cultivar 72-S, which was obtained from Texas [72-S(T)] and Washington [72-S(W)].

AM 2147 had the highest phytase activity in flour, whole grain, and bran. Among CIMMYT lines, FS 1795 had the highest phytase activity in flour and FS 1897 had a significantly higher activity in bran and whole grain than other lines. There were variations due to location also as was evident from the results obtained with triticale 72-S grown in Texas and Washington.

A comparative study on the distribution of enzyme indicated that bran, in general, had higher phytase activity than flour or whole grain (Table V). Peers (1953) studied the distribution of phytase in various anatomical parts of wheat grain and suggested that phytase was more dispersed throughout wheat grain than its substrate, phytate. In the wheat grain (Cappelle Deprez, a soft variety) studied by Peers (1953), maximum phytase activity was found in aleurone layers. This seems to be consistent with our results obtained on a soft wheat (Arthur 71) where bran (containing epidermis, testa, and aleurone layers) had a comparatively higher activity than the endosperm flour. Lim and Tate (1973) found that wheat bran phytase had two fractions F_1 and F_2 with the pH optima of 5.6 and 7.2. In our studies, however, we did not attempt to fractionate this enzyme. In general, hard wheats had a higher phytase activity than soft wheats but the variation in activity between species and between different varieties of one species (ie, *Triticum vulgare*) was not great (Peers 1953). On the contrary, analysis of variance indicated a significant variation due to variety in triticale (Table V).

The data on the distribution of phosphatase activity are presented in Table VI. Bran had higher phosphatase activity compared with flour or whole grain. Unlike phytase activity, phosphatase activity was intermediate in whole grain.

Table VII contains data on phytic acid concentration of grain, flour, and bran of various triticales and wheat. In general, milling of the grain resulted in a considerable reduction of phytic acid in the flour. The bran had relatively higher amounts of phytic acid than the flour. In earlier studes on several commercial lines of triticale, Singh and Reddy (1977) obtained similar results. It was recognized then that the phytic acid concentration in triticale is influenced by variety and location. It is apparent from the data obtained during this study on wheat (Arthur 71) and triticale (6TA 131) that the amount of phytic acid in grain, flour, and bran from the crop grown in 1976 was not the same as the one grown in 1975. A similar trend also was found for the data on mineral compositions. There were significant variations in mineral contents due to cultivars (Table VIII). Such variations due to cultivars of triticale have been reported earlier from this laboratory (Singh and Reddy 1977) as well as others (Kozak and Tarkowski 1977, Lorenz et al 1974). The amount of Ca, Mg, Zn, and Fe was highest in bran and least in flour (Table VIII). This is consistent with earlier studies made by Singh and Reddy (1977) on triticales grown in Alabama and Lorenz et al (1974) on triticale grown in Colorado.

Simple correlation coefficients, determined in all possible combinations, indicated no significant correlations between phytase, phosphatase, phytic acid, Ca, Mg, Zn, and Fe (Table IX). Apparently, phosphatase activity cannot be used as an index for phytase activity in triticale grain. Positively significant correlations were observed between Ca,Zn, and phosphatase; Mg, Zn, and phytic acid; and Fe and phosphatase. In our earlier studies (Singh and Reddy 1977), positive and significant relations also were noted between Mg and Zn in grain, flour, bran, and middlings; and between phytic acid and Mg in grain of triticale. Although data on simple correlation coefficients are presented only for whole grain, similar trends were evident for bran and flours. The data on phytase and phytic acid distribution in triticale are of practical significance. Even through phytase is distributed in all milling fractions, its activity may not coincide with the amount of phytic acid.

TABLE VIII
Calcium, Magnesium, Zinc, and Iron Contents of Various Triticales and a Wheat[a]

	Calcium (μg/g)			Magnesium (μg/g)			Zinc (μg/g)			Iron (μg/g)		
Cultivars[b]	Flour	Whole Grain	Bran	Flour	Whole Grain	Bran	Flour	Whole Grain	Bran	Flour	Whole Grain	Bran
AM 2147	285	491	925	145	919	2,019	11	26	61	101	148	184
AM 2149	224	357	1,356	143	1,006	2,506	6	29	82	91	153	188
AM 3680	258	411	1,100	204	1,038	2,113	6	37	69	111	174	184
AM 3690	242	256	1,138	149	1,163	2,238	7	18	73	56	118	188
AM 3696	205	382	1,681	132	938	2,263	5	29	74	101	168	180
FS 1045	266	434	1,469	178	1,238	2,088	4	39	61	107	167	183
FS 1795	282	503	1,200	189	1,306	2,638	6	36	69	103	156	166
FS 1897	264	514	1,056	232	1,525	2,613	4	44	70	78	153	178
X 2802	281	539	1,400	245	919	2,488	11	45	64	108	161	181
Beagle	235	424	1,306	180	1,163	2,325	4	37	74	99	155	189
Rahum	246	507	1,363	219	1,506	2,244	7	44	66	119	159	177
6TA 131	203	379	925	189	1,175	2,138	5	31	62	77	153	173
72-S(T)	249	493	1,613	258	1,325	2,931	10	44	99	109	158	194
72-S(W)	278	425	1,138	260	1,275	2,950	9	38	90	107	120	193
Wheat (Arthur-71)	222	374	700	165	1,038	1,650	4	23	38	86	148	182
Average	249	433	1,138	193	1,169	2,347	7	35	70	97	153	183
LSD (0.05)	0.78	1.26	3.67	5.96	1.27	1.31	0.27	0.34	0.31	0.20	0.32	0.31
LSD (0.01)	1.08	1.77	5.10	8.27	1.76	1.81	0.38	0.47	0.43	0.28	0.45	0.43

[a] Data expressed on 14% moisture basis. Each value is the mean of three replicates.
[b] Crops grown in 1976 at Normal, AL, except cultivar 72-S, which was obtained from Texas [72-S (T)] and Washington [72-S (W)].

TABLE IX
Simple Correlation Coefficients between Phytase, Phosphatase, Phytic Acid, and Minerals (Whole Grain)

	Calcium	Magnesium	Zinc	Iron	Phosphatase	Phytic Acid
Phytase	0.2904	−0.0307	0.1312	0.4902	0.2450	−0.0613
Calcium		0.2890	0.7863**	0.4152	0.5299*	0.1882
Magnesium			0.5079*	−0.1453	0.0868	0.6152*
Zinc				0.4493	0.3897	0.1185
Iron					0.5432*	−0.1150
Phosphatase						−0.0266

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1976. Approved methods of the AACC. Methods 44-15A, approved April 1967. The Assocation: St. Paul, MN.

BIANCHETTI, R., and SARTIRANA, M. L. 1967. The mechanism of the repression by inorganic phosphate of phytase synthesis in the germinating wheat embryo. Biochim. Biophys. Acta 145:485.

CHANG, C. W. 1967. Study of phytase and fluoride effects in germinating corn seeds. Cereal Chem. 44:129.

COSGROV, D. J. 1966. The chemistry and biochemistry of inositol polyphosphate. Rev. Pure Appl. Chem. 16:209.

FISKE, C. H., and SUBBAROW, J. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 6:375.

GIBBINS, L. N. and NORRIS, F. W. 1963. Phytase and acid phosphatase in the dwarf bean (*Phaseolus vulgaris*). Biochem J. 86:67.

KOZAK, L., and TARKOWSKI, C. 1977. Zawartosc Skladnikow mineralnych w ziarniakach triticale, pszenicy i zyta. Rocz Nauk Roln. A-102-2:66.

LIM, P. E., and TATE, M. E. 1973. The phytase. II. Properties of phytase fractions F1 and F2 from wheat bran and the myo-inositol phosphates produced by fraction F_2. Biochim. Biophys. Acta. 302:316.

LOLAS, G. M., and MARKAKIS, P. 1977. The phytase of navy bean (*Phaseolus Vulgaris*). J. Food Sci. 42:1094.

LORENZ, K., REUTER, F. W., and SIZER, C. 1974. The mineral composition of triticales and triticale milling fractions by x-ray fluorescence and atomic absorption. Cereal Chem. 51:534.

MANDAL, N. C., and BISWAS, B. B. 1970. Metabolism of inositol phosphates. 1. Phytase synthesis during germination of cotyledons of mung beans (*Phaseolus aureus*). Plant Physiol. 45:4.

MANDAL, N. C., BURMAN, S., and BISWAS, B. B. 1972. Isolation, purification and characterization of phytase from germinating mung bean. Phytochemistry. 11:495.

MEYER, A. M. 1958. The breakdown of phytin and phytase activity in germinating lettuce seeds. Enzymologia. 19:1.

NAGAI, Y., and FUNAHASHI, S. 1962. Phytase (*myo*-inositol hexaphosphate phosphohydrolase) from wheat bran. Part I. Purification and substrate specificity. Agric. Biol. Chem. 26:794.

PEERS, G. F. 1953. The phytase of wheat. Biochem. J. 53:102.

PREECE, I. A., and GRAY, H. J. 1962. Studies on phytin. 2. Preliminary study of some barley phosphatase. J. Inst. Brew. 68:66.

SINGH, B., and REDDY, N. R. 1977. Phytic acid and mineral compositions of triticales. J. Food Sci. 42:1077.

WHEELER, E. L. and FERREL, R. E. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem. 48:312.

WOZENSKI, J., and WOODBURN, M. 1975. Phytic acid (*myo*inositol hexaphosphate) and phytase activity in four cottonseed protein products. Cereal Chem. 52:665.

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