Assiut J. of Agric. Sci., 39 (5) (17-35)

CHARACTERIZATION OF PHYTASES FROM CEREAL GRAINS AS AFFECTED BY SOAKING AND GERMINATION PROCESSES AND SOME METALS IONS

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Abstract. The properties of phytases of raw, soaked and germinated wheat, barley, sorghum and maize were studied. The optimal pH value was 5.0 for wheat, barley and sorghum phytases and 5.5 for maize germ phytase. The optimal temperature of wheat and barley phytases was 55°C while of sorghum and maize germ it was 50°C. Both optimal pH and temperature of phytases of different cereal grains did not affect by soaking or germination processes. Data revealed that maximal activity of phytase was found to be at 2.0 mM sodium phytate concentration from wheat, barley and sorghum and at 1.5 mM from maize germ. The calculated Michael's constant (K_m) and their corresponding V_{max} values of the extracted phytases from 96 h-germinated studied grains were varied. The enzyme activity was decreased with extending the incubation time more than 2 h for wheat, barley and maize germ phytase, and more than 1 h for sorghum phytase. Ca^{2+} ions addition caused less reduction of phytase activity of all studied cereals, whereas others metal ions had variable effect on reduction of enzyme activity. Wheat and sorghum showed high increase in their phytase activities after 120 h-germination. The same effect was observed after 96 h-germination of barley and maize germ. At above germination times, phytase activity increased by 5.4-, 4.6-, 7.3- and 6.9-folds for wheat, barley, sorghum and maize germ, respectively compared with that before germination.

Keywords: Phytase activity, cereal grains, soaking, germination, inactivation factors.

Introduction

Phytases are a class of phosphatases that catalyze the sequential hydrolysis of phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) IP_6 to less phosphorylated inositol phosphates and, in some cases, to inositol (Wodzinski and Ullah, 1996; Mullaney and Ullah, 2003 and Oh *et al*., 2004). A number of phytases have been isolated from plants (Konietzny *et al*., 1995) and microorganisms (Mullaney and Ullah, 2003). Phytases have been

Received on: 16/11/2008 **Accepted for publication on**: 4/12/2008

Referees: Prof.Dr. Yahia G. Moharam Prof.Dr. Sobhi M. Hassan

classified on the basis of pH optima (acid and alkaline), catalytic mechanisms (Mehta *et al*., 2006) and specificity of hydrolysis of the first phosphate group (3-phytase, 6-phytase and, more recently, 5-phytase) (Barrientos *et al.*, 1994; Mullaney and Ullah, 2003 and Oh *et al*., 2004). Phytic acid is the most abundant inositol phosphate in cells (Reddy *et al*., 1982). Inositol 1, 2, 4, 5, 6- pentaphosphate is formed by microorganisms phytase (EC 3.1.3.8) and inositol 1, 2, 3, 4, 5-pentaphosphate is formed by plant phytase (EC 3.1.3.26) (Cosgrove, 1980). Thus, phytases, the primary enzymes responsible for the hydrolysis of phytic acid, play an important role in the metabolism of inositol phosphates, a class of compounds involved in signal transduction and calcium regulation (Raboy, 2003 and Shears, 1998 and 2001). Phytase activity was detected in many plant species (Dvořáková, 1998). During soaking , germination, malting and bread-making, endogenous phytases activate and hydrolyse IP_6 (Beal and Mehta, 1985; Larssond and Sandberg, 1991& 1995 and Türk and Sandberg, 1992). Among cereal phytases, the highest activity was found in wheat, rye and barley (Lee, 1990; Sandberg and Svanberg, 1991; Konietzny *et al*., 1995; Loewus and Murthy, 2000; Centeno *et al*., 2001 and Centeno *et al*., 2003**)**. Phytic acid occurs naturally in many food derived from plants, principally in cereals and legumes. It is considered to be an important reserve material in the germination and growth of plants (Cosgrove, 1980). Six phosphate groups in the molecule of IP_6 make a strong chelating agent. They can bind minerals such as Ca^{2+}, Mg^{2+}, Fe^{3+}, and Zn^{2+}. Under gastrointestinal pH conditions, insoluble metal-phytate complexes are formed (Platt and Clydesdale, 1987 and Gifford and Clydesdale, 1990) which make the metal unavailable for absorption from the intestinal tract of animals and humans (Kratzer and Vohra, 1986; Nelson *et al.*, 1989). Phytates also reduce the digestibility of protein (Knuckles *et al*., 1989), starch (Kratzer and Vohra, 1986; Nelson *et al*., 1989). and lipids (Yoon *et al*., 1983). The degradation of phytate by phytases is of nutritional importance to increase the nutritive value of human and animal diets (Nyman and Björck, 1989; Sandberg *et al.*, 1999 and Sandberg and Andlid, 2002). Therefore, this investigation was carried out to study the characteristics of phytases extracted from some raw and germinated cereals; wheat, barley, sorghum and maize. Also, the change in the activity of phytases during germination periods was monitored.

Materials and Methods

Materials: Grain samples of wheat (*Triticum aestivum*), Giza 164 cultivar; barley (*Hordeum vulgare*), Giza 124 cultivar; sorghum (*Sorghum bicolor*), Dorado cultivar and maize (*Zea mays*), Giza 2

cultivar were obtained from Field Crops Research Institute, Shandaweel Agricultural Research Center, Sohag, Egypt, during the 2001 season.

Chemicals: Dodecasodium phytate, acetic acid, sodium acetate, calcium chloride, ferric chloride, ferrous sulfate and zinc sulfate were obtained from Sigma, Germany. Imidazol ($C_3H_4N_2$) was purchased from Alderich, Germany.

Methods:

Preparation of samples: **Soaking and germination:** Cereal grains were cleaned, washed and soaked for 12 h except the maize grain for 24 h in tap water at room temperature (25 ± 2°C) as reported by Abdel-Gawad (1993). After soaking, part of soaked samples was used for phytase activity assay. The other part was used for germination in the dark till 168 h for wheat, barley and sorghum and 144 h for maize as mentioned by Bartnik & Szafranska (1987) and Chang (1967). The grains were sprayed every 12 h by sterilized water. The resulted seedlings were dried at 60°C for 48 h and ground in an electric grinder to pass through a 100-mesh (0.15 mm) sieve and stored in closed bottles in a refrigerator at 5°C until analysis.

Defatting of maize germ*:* Phytase activity was assayed in the germ only after separation the germ from the whole raw, soaked and germinated maize grains. The separation of maize germ was carried out manual by cutting the tip cup of seed and then the germ was removed using a small spatula .The collected germ were defatted by colled hexane (1:6 w/v) for 6 h at 25°C. The obtained micelle was filtered under vacuum while the solid residue was re-extracted for 2 h, air desolventized to remove the solvent and stored under refrigeration until used for enzyme assays (Abdel-Gawad and Hamada, 2002).

Analytical methods:

Moisture content: Moisture content of soaked and germinated cereal grains samples that previous dried at 60°C for 48 h was finally performed at 130°C for 1 h (A.O.A.C., 1990).

Extraction of phytase*:* The crude enzyme was extracted as described by Abdel-Gawad and Hamada (2002) by stirring the fine sample in 0.1 M acetate buffer, pH 5.2, using (1 flour: 10 buffer, w/v) at 5 - 10°C for 30 min, then centrifugation for 20 min at 5000 rpm and filtering through four layers of filter cloth. The obtained filtrate was mixed with cold acetone to precipitate the enzyme. The resulted precipitate was redissolved in acetate buffer (pH 5.2), dialyzed overnight against the same buffer and centrifuged as mentioned above. The obtained supernatant was the partial purified phytase**.**

Enzyme assay*:* The activity of partial purified phytase was measured as described by Gibbins

and Norris (1963) and Lolas and Markakis (1977). The reaction mixture contained 4.0 ml buffer (pH from 4.0 to 6.5), 0.2 ml 2 mM dodeca sodium phytate and 0.5 ml enzyme extract. After incubation at the temperature (35 to 65°C) for 60 min, the reaction was stopped by the addition of 0.5 ml 10% TCA. Inorganic phosphate liberated by phytase was determined by measuring the absorbance at 680 nm after 10 min as stated by Chen *et al.* (1956). Potassium dihydrogen phosphate was used as a standard. The activity of phytase is defined as micromole (µM) inorganic phosphorus (P_i) liberated in one minute per 1 g dry sample.

Characteristics of phytase: Phytase properties were carried out using dodecasodium phytate as a substrate as described by Abdel-Gawad and Hamada (2002). The effect of pH on enzyme activity was determined at pH from 4.0 to 6.5 in 0.1M acetate buffer (pH 4.0 to 5.5) and 0.1 M imidazol-HCl buffer (pH 6.0 to 6.5) using 2 mM sodium phytate. Incubation was carried out at 40°C for 60 min. To determine optimal temperature; enzyme assay mixtures were incubated at its optimal pH with the substrate at temperature ranged from 35 - 65°C for 60 min. An optimal substrate concentration for phytase activity was determined at its optimal temperature and pH for 60 min using different sodium phytate level (0 to 4 mM). K_m and V_{max} were calculated from the relation between substrate concentrations and velosity of reaction using Lineweaver- Burck Plot method (Lineweaver and Burk, 1934)**.** The enzyme assays were performed at optimal pH, temperature and substrate concentration for various times (1 - 8 h) to identify an optimal incubation time.

The thermal inactivation of phytase in 0.1 M acetate buffer (at optimal pH) was estimated after heating at a temperature from 30 to 75°C for 10 min in a water bath, cooling, adding substrate at an optimal concentration and the activity was assayed after incubation for 60 min at optimal temperature. The influence of the following salts; $CaCl_2$, $FeCl_3$, $FeSO_4$ and $ZnSO_4$ at a concentration of 10^{-5} and 10^{-2} M on enzyme activity was carried out at optimal pH, temperature and substrate concentration and 60 min incubation. The relative activity of the enzyme was calculated from the following equation:

$$\text{Relative activity} = \frac{\text{Enzyme activity in presence of salts} \times 100}{\text{Enzyme activity in absence of salt}}$$

Effect of germination period on the phytase activity: Phytase was extracted from cereal grains after 24, 48, 72, 96, 120, 144, 168 and

192 h germination and its activity was determined at the optimal pH, temperature, substrate concentration and 60 min incubation.

Results and Discussions

A- Characteristics of cereal phytases:

1. Optimal pH:

The effect of pH on phytase activity of wheat, barley, sorghum and maize germ (Table 1) showed an increase in its activity with increasing the pH up to the optimal values and then decreased. The optimal pH value was 5.0 for wheat, barley and sorghum phytases and 5.5 for maize germ phytase. Soaking or germination processes has no effect on optimal pH of enzyme activity. The highest activity of phytase was noticed after 120 h-germination of wheat and sorghum and 96 h of barley and maize germ. Similar results for the optimal pH values were reported for wheat grain and barley phytases (pH 5.2) by Sayed (1992)**;** Nakano *et al.* (1998) and Greiner *et al.* (2000) and for corn phytase (pH 4.5- 5.6) by Chang (1967), Ostanin *et al.* (1992) and Laboure *et al.* (1993).

2. Optimal temperature:

Phytase activity of raw, soaked and germinated wheat, barley, sorghum and maize germ at different temperature (35 to 65^{o}C) were presented in Table 2. An optimal temperature of phytases was 55^{o}C for both wheat and barley and 50^{o}C for both sorghum and maize germ at the optimal pH of each phytase. Such temperatures of phytase did not alter by soaking or germination treatments. The recorded optimal temperature of wheat bran phytase and semolina phytase was 50^{o} and 55^{o}C, respectively (Kordonowy, 1985)**,** of raw wheat and barley phytases was 55^{o}C (Sayed, 1992 and Greiner *et al.,* 2000) and 50^{o}- 55^{o}C of maize phytase (Chang, 1967 and Laboure *et al.,* 1993).

3. Optimal substrate concentration:

Phytase activity was increased with increasing substrate concentration and the maximal activity was found at 2.0 mM sodium phytate for wheat, barley and sorghum phytase and at 1.5 mM for maize germ phytase (Fig. 1). The activity was gradually decreased with substrate increasing more than the optimal concentration. Nearly the same findings were stated by Sayed (1992) and Chang (1967). According to Gibbins and Norris (1963) and Sayed (1992), when the substrate concentration reached to optimal level, all the active sits of the enzyme molecule became saturated and the activity arrives to the maximal level. Dixon *et al.* (1979) reported that rising the concentration of the substrate above the optimal value reduce from the enzyme activity.

Table 1: Phytase activity of some raw, soaked and germinated cereals at different pH values.

Cereal samples	pH value						
	4.0	4.5	5.0	5.2	5.5	6.0	6.5
	Phytase activity (U/g sample)						
1. Wheat:							
Raw	1.09	1.64	2.18	2.00	1.45	0.73	0.55
Soaked for 12 h	1.83	2.61	3.39	3.13	2.61	1.16	1.00
Germinated for (h)							
24	2.68	3.58	4.17	3.88	3.28	1.79	1.49
48	3.79	4.43	5.38	4.74	4.10	2.21	1.84
72	5.11	6.13	7.49	6.47	5.79	2.72	2.38
96	6.56	8.00	9.47	8.74	7.65	3.28	2.91
120	7.93	10.19	12.46	11.33	9.00	3.77	3.39
144	6.48	7.26	10.32	9.55	7.69	2.64	2.29
168	4.70	5.87	8.22	7.00	5.87	1.95	1.56
2. Barley:							
Raw	1.00	1.45	1.81	1.63	1.27	0.70	0.54
Soaked for 12 h	1.70	2.29	3.00	2.55	2.00	1.27	1.00
Germinated for (h)							
24	2.31	3.34	4.30	3.80	3.00	1.54	1.28
48	3.38	4.16	5.46	4.95	4.16	1.82	1.30
72	5.10	6.20	7.56	7.30	5.40	2.20	1.60
96	6.38	7.76	9.70	9.15	6.66	2.49	1.90
120	5.22	6.38	7.84	7.25	5.52	2.00	1.70
144	4.10	5.27	6.45	5.85	4.39	1.75	1.45
168	3.00	4.22	5.12	4.83	3.32	1.50	0.90
3. Sorghum:							
Raw	0.90	1.26	1.63	1.26	1.08	0.72	0.54
Soaked for 12 h	1.35	1.80	2.25	2.00	1.60	1.13	0.90
Germinated for (h)							
24	2.75	3.90	4.10	3.90	3.44	1.38	1.20
48	3.73	4.40	5.60	5.10	4.20	1.63	1.40
72	4.27	5.46	7.36	7.12	5.94	1.89	1.60
96	5.10	7.53	9.47	8.99	8.00	2.19	1.90
120	5.74	7.99	9.99	9.74	8.99	2.49	2.20
144	4.66	6.47	8.54	8.00	7.50	2.00	1.55
168	3.92	5.22	6.27	5.75	5.22	1.56	1.00
4. Maize germ:							
Raw	1.09	1.27	1.45	1.64	1.80	0.90	0.55
Soaked for 12 h	1.80	2.00	2.20	2.39	2.80	1.60	0.99
Germinated for (h)							
24	2.95	6.30	8.20	8.85	9.48	1.80	1.25
48	4.51	9.00	11.28	12.00	12.80	2.50	1.75
72	6.00	11.29	13.33	14.48	15.60	3.18	2.30
96	7.68	13.75	15.67	16.90	18.55	3.83	2.85
120	6.00	11.00	12.00	15.34	16.34	3.00	2.33
144	4.77	8.50	9.54	11.24	12.95	2.00	1.70

Table(2): Phytase activity of some raw, soaked and germinated cereals at different temperatures and optimal pH value of each.

Cereal samples	Temperatures (°C)						
	35	40	45	50	55	60	65
	Phytase activity (U/g sample)						
1. Wheat:							
Raw	1.27	2.18	2.73	3.25	3.80	2.18	0.54
Soaked for 12 h	2.35	3.39	4.17	5.22	5.70	3.65	1.00
Germinated for (h)							
24	3.60	4.17	6.26	7.46	8.40	5.00	1.49
48	4.95	5.38	7.92	9.81	11.39	6.96	1.89
72	6.13	7.49	9.54	12.94	14.00	7.83	2.38
96	7.65	9.47	11.66	14.98	17.13	9.84	2.91
120	9.45	12.46	15.48	18.12	20.75	11.30	3.39
144	7.68	10.32	13.38	15.70	17.58	9.56	2.66
168	5.00	8.22	9.80	11.38	13.80	7.00	1.58
2. Barley:							
Raw	1.08	1.81	2.17	2.70	3.26	2.17	0.54
Soaked for 12 h	2.00	3.00	3.82	4.00	4.84	3.56	1.00
Germinated for (h)							
24	3.34	4.30	6.10	6.94	8.20	4.30	1.28
48	4.68	5.46	7.00	8.59	10.41	5.46	1.56
72	5.95	7.56	9.73	11.35	12.97	7.56	1.89
96	7.50	9.70	11.37	13.32	14.98	9.43	2.22
120	6.67	7.84	10.44	12.48	13.64	8.13	2.00
144	5.27	6.45	9.66	10.54	12.30	7.00	1.75
168	4.22	5.12	7.23	9.64	10.85	5.13	1.20
3. Sorghum:							
Raw	1.08	1.63	1.80	1.98	1.68	1.00	0.54
Soaked for 12 h	1.58	2.25	2.48	2.70	2.25	1.58	0.90
Germinated for (h)							
24	2.75	4.10	5.74	6.89	5.52	2.52	1.14
48	3.50	5.60	7.24	9.11	7.00	4.20	1.40
72	4.27	7.36	8.55	10.20	8.00	4.98	1.60
96	5.35	9.47	10.20	11.66	9.23	5.83	1.90
120	6.25	9.99	11.99	14.50	10.50	6.75	2.20
144	5.44	8.54	10.88	13.47	9.33	5.18	1.80
168	4.44	6.27	7.84	10.18	7.57	4.20	1.30
4. Maize germ:							
Raw	1.45	1.80	2.20	2.73	2.20	1.00	0.55
Soaked for 12 h	2.20	2.80	2.99	3.39	2.80	1.60	0.99
Germinated for (h)							
24	5.90	9.48	10.11	10.74	8.85	6.53	1.45
48	7.77	12.80	13.54	14.30	12.00	9.00	2.50
72	10.43	15.60	16.50	17.67	15.60	12.20	3.20
96	12.79	18.55	19.50	20.50	18.55	15.40	3.80
120	10.00	16.34	17.67	19.00	16.34	13.33	3.00
144	8.52	12.95	16.00	17.00	13.97	10.90	2.00

From a plot of 1/[V] vs 1/[S], the Michael's constant (K_m) value was calculated (Lineweaver and Burk, 1934). It was 0.13, 0.17, 0.10 and 0.091 mM for phytase extracted from the 96 h-germinated wheat, barley, sorghum and maize germ, respectively. Meanwhile, the corresponding V_{max} values were 2.27, 2.00, 1.30 and 1.50 mM of P_i liberated /min/ml of the enzymes of the previous germinated cereals respectively as shown in Fig. 2. The K_m of wheat phytase in this study was lower than that recorded by Nakano *et al.*(1999) for two fractions of wheat phytases (0.48 and 0.77 mM). Greiner *et al.* (2000) obtained 0.072 and 0.190 mM K_m values for constitutive barley phytase and the phytase induced during germination of barley, respectively. In contrast, the K_m value of phytase from 96 h-germinated maize germ (0.091 mM) was closed to that reported by Chang (1967) for phytase from 4 days-germinated corn**.** Sayed (1992) found the following V_{max} values; 0.25, 0.48 and 0.31 mM of P_i liberated/ min/ ml enzyme for wheat grains, barley and triticale phytases, respectively. In rice bran phytase, Abdel-Gawad and Hamada (2002) reported V_{max} value of 0.88 mM P_i /min/ml enzyme.

4. Optimal incubation time:

The effects of incubation time on phytase activity of 96 h-germinated wheat, barley, sorghum and maize germ at optimal conditions are shown in Fig. 3. Results showed that, the maximal activity of the wheat, barley and maize germ phytases was at two hours of incubation. This time was one hour for sorghum phytase. The enzyme activity decreased with extended the optimal incubation time of the germinated cereal phytases. The same observation was stated by Abdel-Gawad and Hamada (2002) for rice bran phytase and Rizk (1991) for broad bean and sunflower meal phytases.

5. Thermal inactivation:

As shown in Fig. 4, preheating of enzyme extracts of sorghum and maize germ to 50^{o}C and wheat and barley to 55^{o}C for 10 min did not depress enzyme activity. Over these temperatures the activity of phytases was gradually decreased. The preheating phytase of wheat, barley and sorghum to 70^{o}C and of maize germ to 75^{o}C was practically inhibited. According to Chang (1967) at 60^{o}C the activity of phytase of germinated corn seeds was inhibited to about 40% and was completely inhibited at 80^{o}C. Chen *et al.* (2001) found that the residual sorghum phytase activity after 30 min of preheating at 70^{o}C and 80^{o}C was about 90% and 18%, respectively compared with that at 37^{o}C. Also, Abdel-Gawad and Hamada (2002) noticed that preheating rice bran phytase up to 55^{o}C for 10 min before assay caused a loss to its activity.

6. Effect of metal salts:

Results of the effect of metal salts on the % of relative activity of phytases are summarized in Table 3. Ca^{2+} ions caused less reduction of phytase activity comparing with other metals. Fe^{3+} ions at concentration of 10^{-5} M caused a reduction in phytase activity of wheat, barley, sorghum and maize germ by 23, 31, 28 and 87%, respectively. This reduction was increased to 41, 54, 54 and 93%, respectively in presence of 10^{-2} M Fe^{3+}. Replacing Fe^{2+} ions instead of Fe^{3+} at the same concentrations caused less effect on phytase activity of the studied samples. Addition Zn^{2+} ions at 10^{-5} M concentration reduced the enzyme activity to 35, 15, 38 and 84% for wheat, barley, sorghum and maize germ, respectively. Such losses were increased to 47, 36, 57 and 95%, respectively when 10^{-2} M Zn^{2+} added. The reduction in the maize germ phytase by Fe^{3+}, Fe^{2+} and Zn^{2+} ions at concentrations of 10^{-5} and 10^{-2} M was more than that occurred for phytases from other cereals. Maize germ phytase showed only a noticeable increase in its activity when 10^{-5} M Ca^{2+} ions was present. Results of Nagai and Funahashi (1962) showed that no increase in the activity of wheat bran phytase by addition of Ca^{2+} ions. The study on barley phytase activity by Greiner *et al.* (2000) indicated that Ca^{2+} and Mg^{2+} ions had no significant effect, whereas Mn^{2+} and Co^{2+} caused slight reduction, Fe^{2+}, Fe^{3+} and Zn^{2+} ions had strong inhibitory effects. The reduction of phytase activity in the presence of Fe^{2+} and Fe^{3+} was attributed to the formation of insoluble ferric phytates.

Table(3): Effect of some metal salts on the relative activity of phytases extracted from 96 h-germinated cereal grains.

Metal salts	Relative activity*							
	Wheat	Barley	Sorghum	Maize germ	Wheat	Barley	Sorghum	Maize germ
	10^{-5} M				10^{-2} M			
$CaCl_2$	94	98	85	105	83	89	57	53
$FeCl_3$	77	69	72	13	59	46	46	7
$FeSO_4$	81	108	96	21	70	87	72	9
$ZnSO_4$	65	85	62	16	53	64	43	5

*The relative activity without metal salts addition was taken as 100%.

B. Effect of germination period on phytase activity:

Fig. 5 illustrated the germination period effect of wheat, barley, sorghum and maize germ on their phytase activities. Wheat and sorghum showed highest increase in phytase activity up to 120 h-germination (reached to 20.75 and 14.5 U/g sample) then decreased to 13.8 and 10.18 U/g sample, respectively after 168 h-germination. Barley and maize germ showed the maximal phytase activity at 96 h-germination and thereafter their activities were lowered. Phytase activity of wheat, barley, sorghum and maize germ increased by 5.4-, 4.6-, 7.3- and 6.9-folds after 120, 96, 120 and 96 h-germination, respectively. In other study, phytase activity of wheat, barley, oat and rye increased by 4.5-, 6.0-, 9.0- and 2.5-folds after 3 days germination (Bartnik and Szafranska, 1987). Germination for 5 days increased the phytase activity in rye and barley up to 112 and 212, respectively (Centeno *et al.*, 2001).

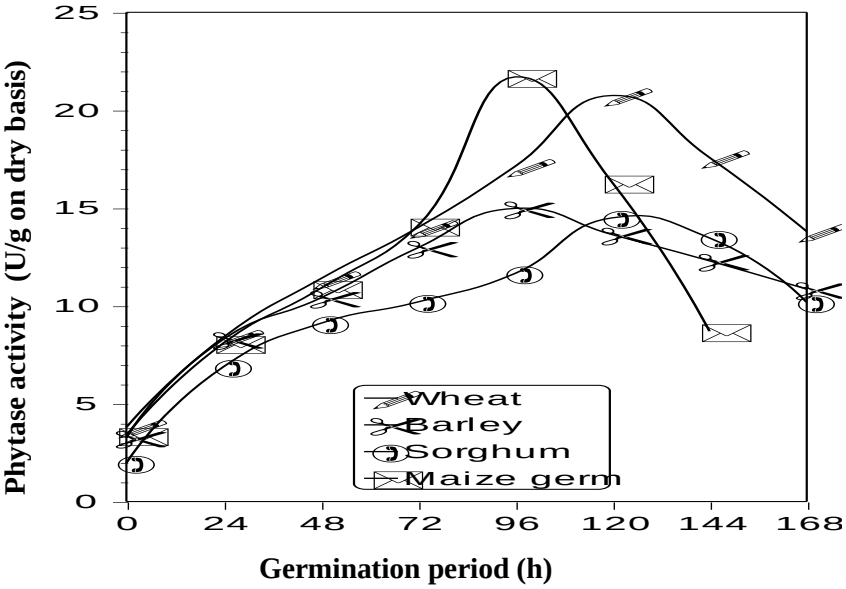

Fig.(5): Effect of germination on phytase activity of some cereals.

Conclusion: The phytase properties of raw, soaked and germinated wheat, maize, barley and sorghum were determined. The optimal pH was 5.0 for wheat, barley and sorghum and 5.5 for maize phytase. The optimal temperature was 55 ºC for wheat and barley and 50 ºC for maize phytase. Maximal phytase activity was found at 2.0 mM sodium phytate for wheat, barley and

sorghum and at 1.5 mM for maize. The activity was increased with incubation time extended up to 2 h for wheat, barley and maize phytase and up to 1 h for sorghum phytase, and then it was decreased. Ca^{2+} ions addition at 10^{-5} and 10^{-2} M caused less reduction of phytase activity of the studied cereals, whereas others metal ions had variable effect on reduction of enzyme activity. The reduction of phytase activity was enhanced with increasing the metal ions concentration from 10^{-5} to 10^{-2} Mol. Phytase activity showed the highest value after 120 h-germination for wheat and sorghum and after 96 h for barley and maize germ.

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خصائص إنزيمات الفيتيز المستخلصة من الحبوب وتأثرها بعمليات النقع والإنبات وبعض أيونات المعادن

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تم دراسة خصائص إنزيمات الفيتيز المستخلصة من حبوب القمح، الشعير، الذرة الرفيعة، وجنين الذرة الشامية الخام، المنقوعة والمنبتة. فكانت قيم الـ pH المثلي هي (5.0) لفيتيز كل من القمح، والشعير، والذرة الرفيعة و (5.5) لفيتيز جنين الذرة الشامية، وكانت درجة الحرارة المثلي لفيتيز القمح والشعير 55ºم بينما كانت 50ºم لفيتيز الذرة الرفيعة وجنين الذرة الشامية. وقد اتضح أن كل من درجات الـ pH و الحرارة المثلي لإنزيمات الفيتيز من جميع الحبوب تحت الدراسة لم تتأثر بعمليات النقع والإنبات.

وأظهرت النتائج أن أقصى نشاط لفيتيز كل من القمح، والشعير، والذرة الرفيعة قد تحقق عند تركيز 2.0 مللي مول من فيتات الصوديوم بينما كان عند تركيز 1.5 مللي مول لفيتيز جنين الذرة الشامية ، وقد تباينت قيم ثابت ميخائيل K_m ، وقيم V_{max} المقابلة لإنزيمات الفيتيز المستخلصة من جميع الحبوب والتي تم إنباتها لمدة 96 ساعة . كما اتضح أن النشاط الإنزيمي ينخفض كلما زاد وقت التحضين عن ساعتان لفيتيز القمح ، والشعير، وجنين الذرة الشامية و إذا زاد عن ساعة لفيتيز الذرة الرفيعة. تبين أن إضافة أيونات الكالسيوم كان أقل تأثيراً في خفض نشاط الفيتيز في كل العينات تحت الدراسة، بينما تباين تأثير إضافة باقي أيونات المعادن الاخري في خفض النشاط الإنزيمي .

كما أتضح أن أعلى زيادة في نشاط الفيتيز كانت عند إنبات حبوب القمح والذرة الرفيعة لمدة 120 ساعة ، وعند إنبات الشعير والذرة الشامية لمدة 96 ساعة حيث زاد النشاط الإنزيمي بمقدار 5.4 ، 4.6 ، 7.3 ، 6.9 مرة عنه قبل الانبات لكل من القمح، والشعير، والذرة الرفيعة، وجنين الذرة الشامية على التوالي.