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Preliminary Characterization of Phytase Activity in African Oil Bean, Locust Bean and Conophor Nut

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Abstract: Phytase action improves mineral bioavailability by lowering the phytic acid level in legumes and oilseeds. Crude phytases from African oil bean seed (*Pentaclethra macrophylla*), locust bean seed (*Parkia biglobosa*) and conophor nut (*Tetracarpidium conophorum*) were isolated and assayed via estimation of inorganic orthophosphate liberated by the hydrolysis of phytic acid. The phytase activities and corresponding protein concentrations for the three under-exploited oilseeds were: 0.720 μmL^{-1} and 16.85 mgmL^{-1} for *P. macrophylla*; 0.078 μmL^{-1} and 10.65 mgmL^{-1} for *T. conophorμm*; and 0.082 μmL^{-1} and 14.00 μmL^{-1} for *P. biglobosa*; respectively. This implies that the *Pentaclethra* seed phytase has comparatively higher activity with higher protein concentration in the enzyme molecule, followed by the *Parkia* seed phytase andlastly the *Tetracarpidium* seed phytase. The optimum temperatures for the phytase activity were 50°C for oil bean and conophor seeds and 60°C for locust bean seed. The optimum pH was 5.0 for oil bean phytase, 4.0 for conophor nut phytase and7.0 for locust bean phytase, respectively. The results show that locust bean phytase was more thermoactive with an alkaline pH optimum conophor nut and oil bean seeds both have acid phytases in their cotyledons.

Key words: Phytase activity, oil bean, conophor nut, locust bean, temperature, pH

INTRODUCTION

The enzyme phytase catalyzes the stepwise hydrolysis of phytic acid to phosphate and inositol via penta to monophosphate[1]. Phytic acid (myo-inositol hexakisphosphate) is an inhibitor of mineral absorption because the negative charges of the phosphate groups form insoluble salts upon interaction with di- and trivalent cations such as Ca, Fe, Mg and Zn. It is also the principal storage form of phosphorus in plant seeds, especially grains and oilseeds. According to Sandberg[2], mineral content of legumes is generally high but the bioavailability is poor due to the presence of phytate, which is a main inhibitor of Fe and Zn absorption. Phytase is responsible for phytate degradation thereby leading to increased bioavailability of the affected mineral elements in foods. Phytases have been studied extensively in recent years because of the great interest in such enzymes for reducing phytate content in foods and feeding stuffs. Phytase enhances phosphate utilization from phytate and drastically reduces orthophosphate excretion.

Phytases are widespread in nature, occurring in plants, in microorganisms, as well as in some animal tissues. Plant phytases occur mostly in grains, seeds and pollen of higher plants, such as cereals, legumes, oilseeds and nuts; but a low phytase activity is also found in the roots of the plants. In general, legumes and oilseeds exhibit a ten-fold lower phytase activity than cereals. For cereals, phytase activity was found to be mainly associated with the aleurone layer[3], whereas in legumes and oilseeds, phytase activity was reported to be in the cotyledons[4]. All phytase enzymes, including plant phytases, belong to the subfamily of histidine acid phosphatases. However, one major problem commonly encountered in the purification of phytase especially from plants is the separation of phytate-degrading enzymes from contaminating non-specific acid phosphatases[5].

Phytase activity can be determined by measuring the orthophosphate liberated by the action of the enzyme using a method based upon colorimetric measurements of phosphomolybdate in acetone[6]. The phytase enzymes are identified based on pH viz: acid phytase with a pH optimum around pH 5 andalkaline phytase with a pH optimum around pH 8. Oilseed phytases are known to fall into any of these two groups, with temperature optima varying from 35 to 77°C (most around 50-60°C). The present study examines the activity and pH/ temperature characteristics of phytases from three unconventional and under-exploited oilseeds, namely, African oil bean seed, conophor nut andlocust bean seed. These seeds are known to contain high concentrations of phytic acid in their cotyledons[7]. It is expected that a preliminary examination of the nature and behaviour of their respective phytases will give an insight into their usefulness andultimately enhance their wider exploitation.

MATERIALS AND METHODS

Materials source and sample extract preparation: The seeds of African oil bean, conophor and locust bean used

in the study were purchased from an open market at Akure, Ondo State, Nigeria. T he seeds were manually dehulled, sliced to about 1-2 cm thickness with a kitchen knife (for African oil bean seed and conophor nut) anddried at 30°C for 24 h to avoid denaturing enzyme. The dry seed samples were each milled into powder with a laboratory hammer mill and kept at 4°C until ready for use. All chemicals and reagents used were of analytical grade. In the preparation of extract, each 5 g sample was mixed with 50 mL of 0.2 M sodium acetate buffer (pH 5.5) andthe mixed sample solution was filtered through muslin cloth. The filtrate was centrifuged at 3000 rpm for 15 min andthe resulting supernatant was kept at 4°C until use. Egg albumin was used as the protein standard anda phytase enzyme of known activity was used for preparation of enzyme standard solution. Protein concentration was determined using biuret reagent and reading at 540 nm.

Preparation of substrate solution and colour-stop mix: For the substrate solution, 7.02 g of anhydrous sodium phytate was dissolved in 900 mL 0.2M sodium acetate buffer (pH 5.5) and the pH adjusted to 5.5 with either acetic acid or NaOH before making it up to 1 litre with the buffer solution. For the colour-stop mix, 250 mL of ammonium heptamolybdate stock solution was mixed with an equal volume (250 mL) of ammonium vanadate stock solution before adding 165 mL HNO_3. The mixture was then cooled to room temperature. Both the substrate solution and the colour-stop mix were prepared fresh everyday during the study. Colour-stop mix was used to stop the activity of the enzyme and to impart colour for spectrophotometric measurements.

Assay of enzyme activity: Determination of phytase activity was based on the estimation of inorganic orthophosphate released on hydrolysis of phytic acid, routinely performed at 37°C following a modification of the method described by Padmanabhan *et al.*[8]. One unit of enzyme activity was defined as the amount of enzyme that liberates 1 μmole of inorganic phosphate per minute under standard assay conditions. About 2 mL of the respective sample extracts were pipetted into test tubes, incubated and equilibrated for 5 min. Then 4 mL of the substrate solution was added into each test tube and mixed thoroughly using vortex. The mixed sample solutions were further incubated for 65 min at 37°C in a water bath and 4 mL of colour-stop mix was added to terminate the activity of the phytase. The mixture was centrifuged for 15 min before absorbance reading of the supernatant at 415 nm with spectrophotometer.

Enzyme activity in relation to pH and temperature: For the effect of pH on enzyme activity, the crude phytase was diluted in 0.2 M sodium acetate buffer (pH 5.5) and the substrate solution was prepared in one of the following buffers: 0.2M glycine (pH 2.0-3.5) and 0.2M sodium acetate (pH 3.5-9.0). All buffers were supplemented with 1 mmole Cach. The substrate solution contained 0.12M phytic acid. Two millim litters of the enzyme preparation was pre-incubated in a water bath at the assay temperature for 5 min andthe enzyme reactions were initiated by adding 4 mL of the substrate solution. The pH of the mixture was adjusted to the desired value before incubation. The mixture was incubated for 60 min at 37°C. The addition of 4 mL of colour-stop mix terminated the reaction. The activity of the enzyme was determined as described above.

For the determination of optimum temperature curves, preparations of the enzyme and substrate solutions as well as their mixing ratios were as described for pH behaviour. However, the pH of the mixtures corresponded to the determined optimum pH of the enzyme. The mixtures were incubated for 60 min at each of the following temperatures: 30, 40, 50, 60, 70 and 80°C. The activity was measured on the basis of inorganic orthophosphate[6,8].

RESULTS AND DISCUSSION

The results of respective activities of the isolated phytases from the under-exploited oilseeds and their corresponding protein concentrations are presented in Table 1. The enzyme activity is significantly ($p<0.05$) higher in the oil bean seed than in locust bean seed and conophor nut. Incidentally, African oil bean seed contains higher phytate and phytate-phosphorus contents than the other two seeds[7]. Although Idriss *et al.*[9] detected no correlation between the concentration of phosphate and the presence of phytate, phosphorus release by phytases is well known[1]. According to Selle *et al.*[10], the majority of total P was present as phytate-phosphorus in a number of plant-based feed ingredients. It is therefore to be expected that the higher phytase activity in the oil bean seed could mean greater degradation of the seed phytates andbetter mineral bioavailability.

A significant proportion of the phosphorus in mature cereal grains and oilseeds is present as phytin-phosphorus[11]. The release of phosphorus from plant phytin during the digestion process is theorized to release other nutrients that may be bound in the phytin complex. It is greatly emphasized that higher phytase activity indicates better nutrient availability. The results of the present study (Table 1) also show that with higher phytase activity there is correspondingly higher protein concentration. The phytase enzyme is a multi-molecular protein unit andthe activity is tied to the amino acid sequences in the molecule.

Figure 1 shows the effect of temperature on the

Table 1: Phytase activities of the oilseeds and corresponding protein concentrations

Oilseed sample	Phytase activity (μmL^{-1})	Protein concentration (mg mL^{-1})
Conophor nut	0.078	10.65
Locust bean	0.082	14.00
African oil bean	0.720	16.85

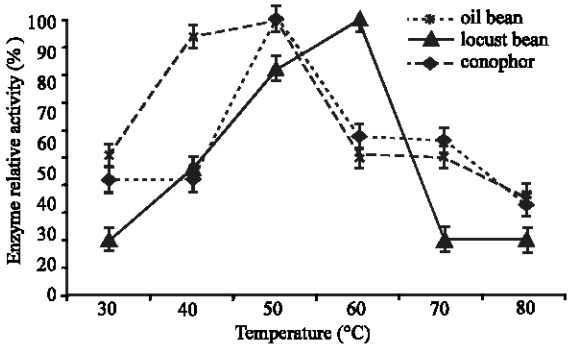

Fig. 1: Thermoactivity profiles of the isolated oilseed phytases

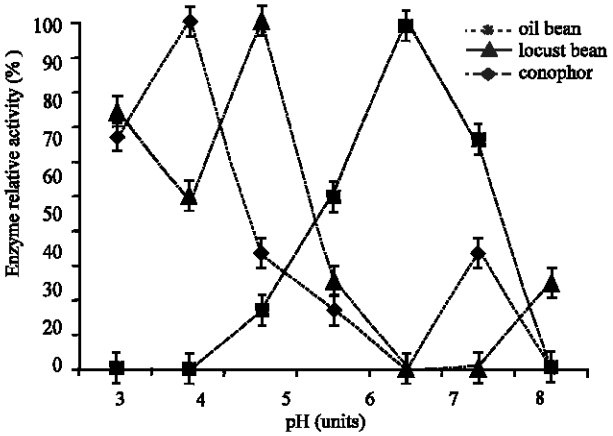

Fig. 2: Effect of pH on the activity of the respective oilseed phytase

activities of the respective phytases. The locust bean phytase was more thermoactive (60°C) than those of African oil bean and conophor nut (both 50°C). Generally, most known phytases have optimum activities at between 50 and 65°C[4,5,12,13]. At 70°C the conophor phytase still maintained an appreciable level of activity, showing the relatively high thermostability of the enzyme. The enzymes that can still maintain activity at elevated temperatures are greatly desired in industrial processes.

The oilseed phytases were optimally active at pH 4.0 (conophor nut), pH 5.0 (African oil bean seed) and pH 7.0 (locust bean seed). Both African oil bean seed and conophor nut have acid phytases, while locust bean seed has an alkaline phytase (strongly active even at pH 8.0)(Fig.2). The majority of plant and microbial phytases are active within the acidic range[1,10,14]. The exhibition of activity in the alkaline region is a great advantage for the locust bean phytase, since the only edible product of the seed, iru, is a product of alkaline fermentation[15].

CONCLUSION

Crude phytases have been isolated from seeds of the African oil bean, conophor and locust bean, which are in the class of lesser-known under-exploited cropseeds. The activity of the respective enzymes has been assayed and the effect of temperature and pH on enzyme activity determined. The phytases are found to be active within temperature range 50- 60°C. However, while African oil bean seed and conophor nut both have acid phytases, the locust bean seed has an alkaline phytase.

REFERENCES

1. Dvorakova, J., 1998. Phytase: Sources, preparation and exploitation. Folia Microbiol., 43: 323-338.
2. Sandberg, A.S., 2002. Bioavailability of minerals in legumes. Br. J. Nutr., 88: S281-285.
3. Gabard, K.A. and R.L. Jones, 1986. Localization of phytase and acid phosphatase isoenzymes in aleurone layers of barley. Physiol. Plant., 67: 182-192.
4. Gibson, D. M. and A. H. J. Ullah, 1988. Purification and characterization of phytase from cotyledons of germinating soybean seeds. Arch. Biochem. Biophys., 260: 503-513.
5. Konietzny, U., R. Greiner and K.D. Jany, 1995. Purification and characterization of a phytase from spelt. J. Food Biochem., 18: 165-183.
6. Heinonen, J. K. and R. J. A. Lahti, 1981. A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic pyrophosphatase. Anal. Biochem., 113: 313-317.
7. Enujiugha, V.N. and O. Ayodele-Oni, 2003. Evaluation of nutrients and some anti-nutrients in lesser-known underutilized oilseeds. Intl. J. Food Sci. Technol., 38: 525-528.
8. Padmanabhan, U., S. Dasgupta, B.B. Biswas and D. Dasgupta, 2001. High affinity association of myo-inositol trisphosphates with phytase and its effect upon the catalytic potential of the enzyme. J. Biol. Chem., 276: 43635-43644.
9. Idriss, E. E., O. Makarewicz, A. Farouk, K. Rosner, R. Greiner, H. Bochow, T. Richter and R. Boriss, 2002. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiol., 148: 2097-2109.

10. Selle, P. H., A. R. Walker and W. L. Bryden, 2005. Total and phytate-phosphorus contents and phytase activity of Australian-sourced feed ingredients for pigs and poultry. Aus. J. Exper. Agric., 435: 475-479.
11. Adeola, O. and J. S. Sands, 2003. Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. J. Anim. Sci., 81: E78-85.
12. Zamudio, M., A. Gonzalez and J. A. Medina, 2001. *Lactobacillus plantarum* phytase activity is due to non-specific acid phosphatase. Lett. Applied Microbiol., 323: 181-184.
13. Lee, W.J., 1990. Phytic acid content and phytase activity of barley malt. J. Amer. Soc. Brew. Chem., 48: 0062.
14. De Angelis, M., G. Gallo, M.R. Corbo, P.L. Mc Sweeney, M. Faccia, M. Giovine and M. Gobbetti, 2003. Phytase activity in sourdough lactic acid bacteria: Purification and characterization of a phytase from Lactobacillus sanfranciscensis CB1. Intl. J. Food Microbiol., 87: 259-270.
15. Oyeyiola, G.P., 1998. Microbiology of the fermentation of iru pete and iru woro obtained from local producers. MIRCEN J. Applied Microbiol. Biotechnol., 42: 279-284.