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PROPERTIES OF LIPASE (EC 3.1.1.3) FROM DIFFERENT VARIETIES OF MAIZE

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ABSTRACT

Lipase activity was studied in four varieties of Corn (Zea mays) namely: Local yellow (LY), Western yellow (WY), Western white (WW) and Pop corn (POP). Using emulsified olive oil as substrate, lipase was found to be present in the dry seeds of maize. Lipase activity increased with germination and reached it's peak on the first day of germination for WY and LY, third day for POP and sixth day for WW. Lipase activity was tested for its ability to hydrolyze different emulsified oils such as groundnut oil, palm kernel oil, and soybean oil. The highest activity was with soybean oil. This high activity was correlated with high specificity of corn lipase on linoleic acid. Thermal inactivation studies showed that the enzyme was stable up to 50^{o}C and showed rapid inactivation above this temperature. Its optimum temperature was 50^{o}C and the optimum pH, 8.0.

Keywords: Lipase, Enzymes, Maize, Thermal stability, Substrate specificities

INTRODUCTION

Lipase (EC 3.1.1.3) catalyses the hydrolysis of triacylglycerides to a mixture of diacylglycerides and monoacylgycerides. During seed germination, lipases are active in lipid storage organelles, called lipid bodies (oliosomes, oil bodies, spherosomes or oil droplets), where the storage triacylglycerides are localized (Lin *et al.,* 1982). The lipid body should be a prime candidate for subcellular location of lipase, since for lipolysis to occur, enzyme has to come in contact with the substrate (Huang *et al.,* 1983). A unique feature of seed lipases is that they are relatively specific for the characteristic triacylglycerols of a species. Thus corn lipase is most active on triacylglycerols of linoleic and oleic acid, which are the major fatty acid constituents of corn (Lin et al., 1986).

In this work, effort has been made to study lipase in different varieties of maize with a view to understanding their properties.

MATERIALS AND METHODS

Maize: Maize (*Zea mays*); POP, WY, LY and WW were purchased from the local market at Nsukka, Enugu state Nigeria. A grinding buffer was prepared from 0.5 M glycine, 0.6M sucrose, 10 mM KCl, I mM EDTA, I mM β-mercaptoethanol and I mM $MgCl_2$ and the pH of the buffer adjusted to pH 7.6 with NaOH. Different varieties of maize seeds were steeped in water for 24h after which they were spread on moist jute bags and allowed to germinate in the dark. Lipase assay was carried out every 24 h. At the end of the 24h of germination, the plumules and radicals of the germinating seeds were removed and 50 seeds (from each variety) were selected and crushed in the grinding buffer using pestle and mortar. After extraction, the homogenate was filtered using cheese cloth (8 folds). The filtrate was introduced in a centrifuge tube and a second version of the buffer containing 0.5M sucrose instead of 0.6M was layered on top and centrifuged at 10,000 x g for 15 min. The upper layer (lipid bodies) was removed and mixed with the water soluble fraction and kept as the crude enzyme on the basis of a modification of the purification method according to Huang and Lin (1983).

Enzyme Assay: The method of assay was a modification of Williamson *et al.* (1999). Activity with triacylglycerols was measured by the Olive oil emulsion method. The reaction mixture consisted of olive oil emulsion [50 % in 5 % Gum-Arabic solution], 27mM Tris–HCl pH 8.0; 0.013% sodium azide, and enzyme in a total volume of 3.75 ml. After vortex mixing, the mixture was incubated at room temperature for 1h. The reaction was stopped by adding 3.25 ml of 30 %ethanol. Controls were produced using the same reaction mixture but adding ethanol at the beginning of the 1 hour incubation. Thymophthalein (3 drops) was added to the mixture and the free fatty acids released by lipase action were titrated with NaOH (0.05M) until the colour of the solution turned a slight but definite blue. One unit of enzyme activity is defined as the release of 1 µmol of product per minute under the conditions of each assay.

Protein Determination: Protein was determined using the method of Lowry *et al.* (1951).

Lipids Hydrolysis: Emulsion of olive oil, groundnut oil, vegetable oil, palm kernel oil and soybean oil were used for the assay. Oil emulsion was prepared by a mixture of 0.5 ml of oil and 0.5 ml of 5 % Gum Arabic solution. This was vortexed at high speed for 30 seconds.

Thermal Inactivation: 10 ml of the crude enzyme were each added in a conical flask and heated in a

water bath at different temperatures (40, 50, 60, 70 and 80^{o}C) for the time interval between 0 – 8 h.
0.75 ml of the enzyme was withdrawn after every 60 sec and used to assay for residual activity. The method of assay was as earlier stated, except that the period of incubation was 15 min. Each activity was an average of three determinations (in triplicates).

pH Optimum and Stability: The method was a modification of Yamamoto and Fijiwara (1988). Enzyme assay was carried out using the following buffer systems: 0.025M Glycine-NaOH buffer (pH 5.5-9.0); 0.025 M potassium phosphate buffer (pH 5.5-9.0); 0.025 M Tris-HCl buffer (pH 6.5-9.0) and 0.025 M acetate buffer (pH 4.0-6.0) respectively. For stability test, the enzyme was incubated in different buffers (as above) at 29^{o}C for 24h. The residual enzyme activity was determined as earlier stated.

RESULTS AND DISCUSSION

Lipase activity was detected in the dry seeds of maize as has already been reported by Huang and Moreau (1978). However, maximum activity was observed on the first day of germination for WY and LY, on the 3rd for pop corn and on the 6th day for WW. Activity was least in pop corn, and highest in western white (WW) (Figure 1).

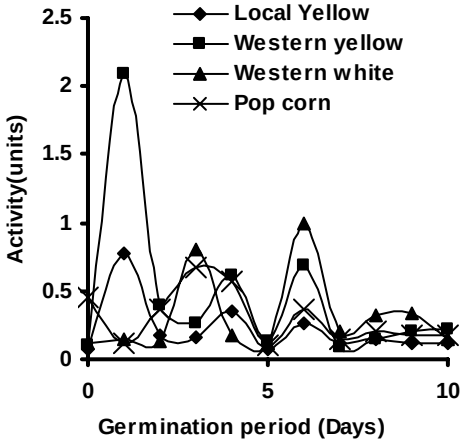

Figure 1: Variation of lipase activity with the period of germination for four varieties of maize.

In early seedling growth of oil seeds, the reserve triacylglycerides in the storage tissues are rapidly mobilized. The triacylglycerides are hydrolyzed to fatty acids are metabolized by β-oxidation. The acetate generated is processed in the glyoxylate cycle (Muto and Bevers, 1974; Huang and Mareau 1978; Lin *et al.,* 1982: Lin *et. al.,* 1983). Generally, lipolysis is most active in day 2 – 6 of germination but gradually drops as the storage triacylglycerides were depleted. Earlier reports on maize lipase indicate that there was no activity in the dry ungerminated seeds and that the peak of activities is between 5 – 6 days of germinations (Lin *et al.,* 1986). Lipase from a specific plant species displays highest specific activity with the major endogenous seed storage triacylglycerides. Thus castor bean lipase is most active on triricinolein, but is also fairly active on all other triacylglycerides containing saturated and unsaturated fatty acids. Corn lipase has highest activity on all other triacylglycerides of linoleic acid and oleic acid, rape seed lipase on trierucin and elm lipase on tricaprin (Lin *et al.*, 1986; Hope and Theimer, 1996). Such specificity is of physiological significance since each plant species produces very specific lipases for much more efficient catalysis as the fatty acid composition in each species is genetically determined and well defined. In this work, corn lipase showed highest activity with soybean oil (Figure 2) as the substrate. Soybean oil contains a high proportion of linoleic acid (50 – 55 %) and oleic acid (19 – 25 %).

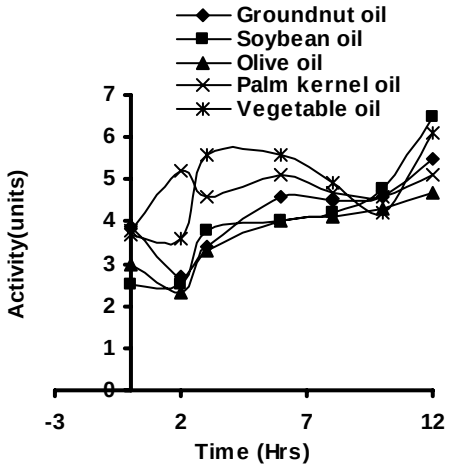

Figure 2: Lipase activity in the different substrates

Also groundnut oil with oleic acid content of 25 – 45 % is hydrolyzed by corn lipase. This is explained on the basis of specificity of the enzyme. There is only a few published reports on thermal inactivation of plant lipases. Hou *et al.* (1999), reported that fatty acid esterase from yam is stable below 50^{0}C. Lipase from micro-organism, *Pseudomonas* spp were shown to be thermostable, retaining sufficient activity when incubated at 50^{0}C for 24h. Inactivation studies of corn lipase showed that the enzyme is highly stable with a monophasic inactivation curves at various temperatures studied (Figure 3). This stability depends on the pH of the medium and the purity of the enzyme. For these organisms, the optimum temperature was 65^{o}C (Nisho *et al.,* 1987). The optimum temperature for maize lipase was 65^{o}C (Figure 4). The pH optimum of the enzyme was 8.0 and the enzyme was stable in the alkaline region of 8 – 9.5 suggesting that the enzyme is an alkaline enzyme.

In conclusion, during germination, the enzyme activities change as the day of germination progresses due to the mobilization of triacylglycerides from the storage tissue.
There is a linear correlation in change in activity with days of germination until a peak is reached which

eventually marks the depletion of triacylglycerides in the storage tissues. The fact that lipase activity in the scutellum appears due to *de novo* synthesis of lipase in post-germinative growth of maize kernel supports this linear proposition.

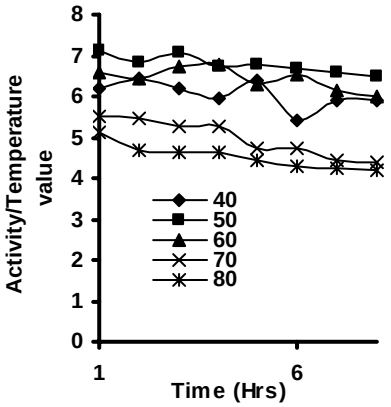

Figure 3: Heat inactivation of Lipase at different temperatures of 40^0 C, 50 ^{0} C 60^{0}C, 70^{0}C and 80^{0}C

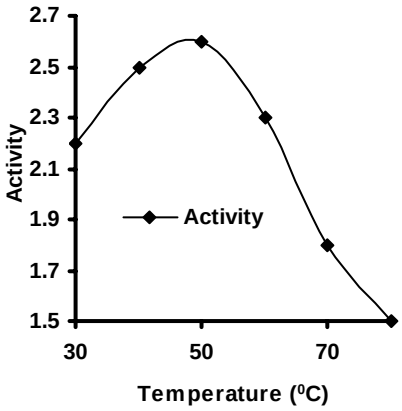

Figure 4: Temperature optimum for lipase activity

Maize lipase has the highest activity in Western white (WW); and show high specificity when oils of high percentage linoleic acid were used.

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