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Characteristic Properties of Lipase from Crude Extract of *Caesalpinia bonducella* L. Seeds

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Abstract: Lipase activity was assayed in mature *Caesalpinia bonducella* L. seeds using olive oil emulsion stabilized with 10% gum acacia as a substrate. The maximum lipase activity at pH 7.0 and at 30°C and the pH stability was found in between 6-7.5. Lipase activity was fairly stable up to 60 °C and retaining 90% activity. Whereas lipase activity was completely lost at 90 °C within 10 minutes. *C. bonducella* L. seeds was found most specific towards coconut oil and lipase activity was slightly increased in the presence of calcium but enzyme activity was decreased by the addition of sodium deoxycholate, Tween 80 and Triton X-100.

Key words: *Caesalpinia bonducella* L., seeds, enzyme, Lipase.

Introduction

Lipase (Triacyl-glycerol acylhydrolase °C 3.1.1.3) are versatile group of enzymes which not only hydrolyze the esters of long chain aliphatic acids from glycerol at oil/water interface (Brockman, 1984) but also involved in transesterification reaction. Lipases activity present in food reserve tissues of growing seedlings and especially in those which contains large amount of triacylglycerols and its activity in plants seed increases rapidly after germination. Lipase have preference to hydrolyze triacylglycerol, diacylglycerol and monoacylglycerol to glycerol and fatty acids which are converted into sugars and supports the growth of young plants (Hill and Beevers, 1987). Lipase activity has been determined from castor bean (Maschima and Beevers, 1985), rice bran (Aizono *et al.*, 1973), wheat grains (Tavener and Laidman, 1972) palm seeds (Handerson and Osborne, 1991), corn (Lin and Huang, 1984), *Moringa oleifera* seeds (Dahot and Memon,1987), *Cajanus cajan* (Khan *et al.*, 1991) *Carica papaya* (Foglia and Villeneuve, 1997) *Vernonia anthelmintica* seeds (Afolabi *et al.*, 1991), sunflower seeds (Teissere *et al.*, 1995), rape seed (Hoppe and Theimer, 1997) and *Carissa carandas* fruit (Mala and Dahot, 1995).

In recent years the growing demand of lipolytic enzymes has been increased due to its potential use in the various manufacturing processes of industrial goods such as detergent industry, food industry and in pharmaceutical industry (Boland *et al.*, 1991; Gandhi,1997; Savendsen, 2000).

However, there are many plant seeds, especially of tropical origin, upon which no work has been reported before. *Caesalpinia bonducella* L.seeds is one of them which contains considerable amount of lipolytic activity. *C.bonducella* belongs to family Caesalpiniaceae and locally it is known as Kharpat. *C.bonducella* is used as a medicinal plant. The aim was to isolate and partially characterize the lipase from the crude extract of *C.bonducella* seeds.

Materials and Methods

C.bonducella L. seeds were collected from Tajpur village, District Hyderabad, Sindh. All reagents used were of analytical grade. *C.bonducella* L. seeds were defatted with diethyl ether and (-40 -60°C) residue were stored in a vacuum desicator until it was used. The soluble enzyme preparation and the olive oil substrate emulsion was prepared according to the (Dahot and Memon, 1987). Lipase activity was measured by the olive oil gum arabic emulsion method by Noomrio *et al.* (1990). A unit of lipase activity was defined as the amount of enzyme required to release 1μ mole of free fatty acids per hour under the assay conditions. The protein content of soluble enzyme preparation was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. The protein content in aqueous solution of *C.bonducella* seeds was found to be 2.5 mg ml^{-1}. The pH stability was checked at different pH buffers from 3-10 were reacted with enzyme for 15 min. at 30 °C. The effect of thermostability of lipase activity of *C.bonducella* L. seeds was determined by pre-incubation of enzyme solution at various temperatures ranging from 30-90 °C at pH 7.0 for 10 min.

Results and Discussion

The rate of triacylglycerol hydrolysis by crude lipase preparation of *C.bonducella* seeds during incubation with various time intervals was increased up to 180 min. and then dedined sharply as shown in (Fig.1). This declination may be due to product inhibition (Galliard, 1971) or side product of the reaction, which inhibits the enzyme or the effect of heat on the tertiary structure of the enzyme (Wynn, 1973), or enzyme inactivation due to prolonged incubation (Sonoki and Ikezawa, 1975), or presence of other enzymes in crude sample can not ruled out.

The effect of crude enzyme preparation of *C.bonducella* on the rate of reaction was carried out with different concentrations ranging from 2-12% (Fig.2). The rate of enzymatic reaction was increased with the increase of enzyme concentration up to 12% and then decreased.

The effect of substrate concentration on the lipase activity of *C.bonducella* seeds was investigated using different concentrations (2-12%) of olive oil emulsion. The rate of enzymatic reaction increases up to 10% and then declined in (Fig. 3) and the rate of reaction at higher concentration observed due to the effect of enzyme substrate concentration ratio or enzyme inhibited by the excess of substrate concentration or change of physiochemical characteristics (Khan *et al.*, 1991).

The effect of pH on lipase activity of *C.bonducella* seeds was determined in the range of 3.0 to 8.5 using universal buffer (Britton and Robinson type). Lipase activity was maximum at pH 7.0 (Fig.4). Lipase activity of *C.bonducella* seeds belongs to neutral type, which is similar to rape seeds (Hoppe and Theimer, 1997), *Hibiscus canabinus* seeds (Kausar and Akhtar, 1979), sunflower cotyledons (Huang and Moreau,

Table 1: Effect of various metal ions/reagents in 5mM concentration on *C.bonducella* L. seed lipase activity.

Reagent added	Relative %	Activation/ [Inhibition](%)
Control	100.00	-
Triton X-100	52.80	(47.20)
Tween-80	40.53	(59.47)
Sodium deoxycholate	10.13	(89.87)
Manganese	13.06	(86.94)
Calcium	106.40	6.40

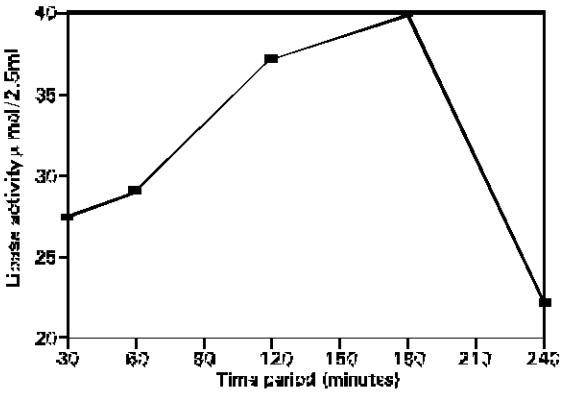

Fig. 1: Effect of time period on lipase activity of *C. bonducella* L. seeds

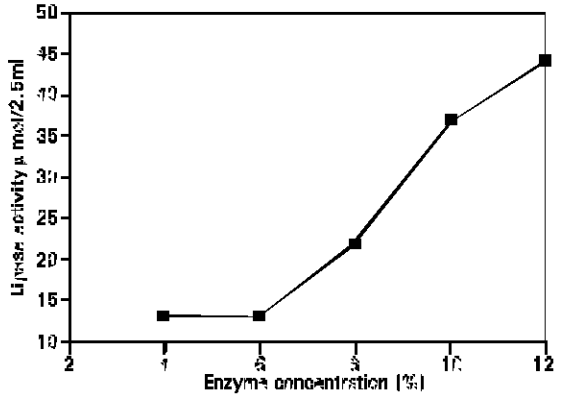

Fig. 2: Effect of enzyme concentration on lipase activity of *C. bonducella* L. seeds

1978), *Juglansregia, Allium cepa, Pisum sativum, Citrus decumana, Cucumis melo, Zea mays and Prunus amygdlus* seeds (Akhtar *et al.*, 1975).

The effect of pH on stability of enzyme was determined in the presence of buffer solution at different pH values and the lipase activity of *C.bonducella* L. seeds was found stable over the pH range of (6-7.5) shown in (Fig.5).

The effect of temperature on the lipase activity using olive oil as substrate at pH 7.0 is presented in Fig. 6. The optimum temperature for *C.bonducella* L. seeds lipase activity was found to be 30 °C. These results are in agreement with that of Khan *et al.* (1991), Dahot *et al.* (1989) for *Cajanus cajan* L. seed lipase and for *Carissa carandas* fruit lipase (Mala and Dahot, 1995).

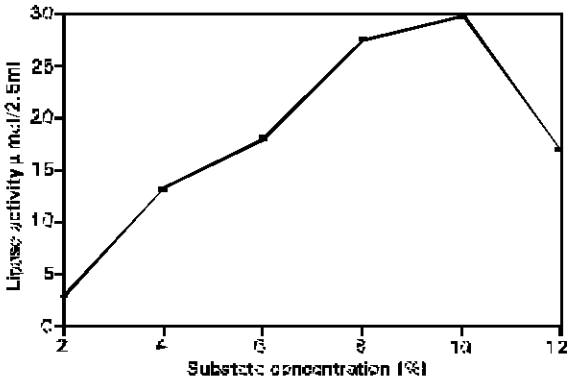

Fig. 3: Effect of substrate concentration on lipase activity of *C. bonducella* L. seeds.

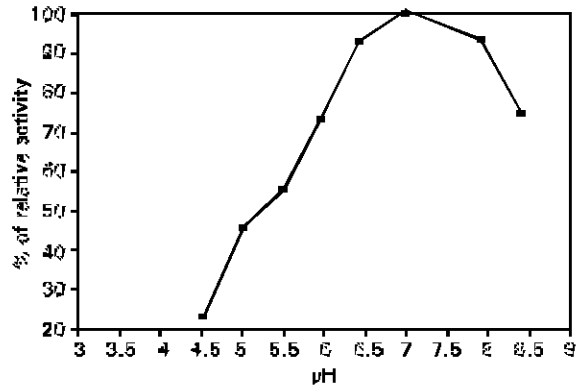

Fig. 4: Effect of pH on lipase activity of *C. bonducella* L. seeds

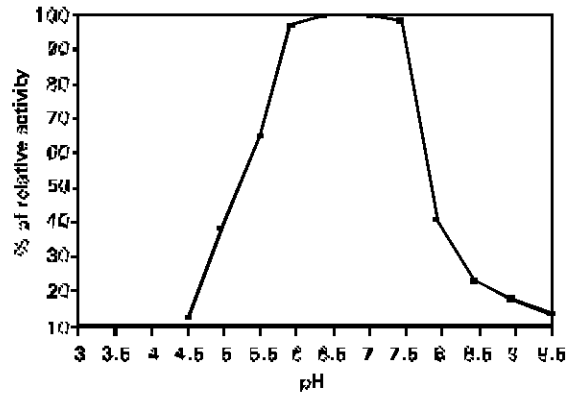

Fig. 5: Effect of pH stability on lipase activity of *C. bound cella* L. seeds.

Lipase activity is fairly stable up to 60 °C and retains nearly 90% activity. Whereas, lipase activity was completely lost at 90 °C within 10 min. The rapid loss in lipase activity at temperature above 60 °C may be due to deactivation of enzyme (Fig.7).

The lipase activity of *C.bonducella L.* seeds against different

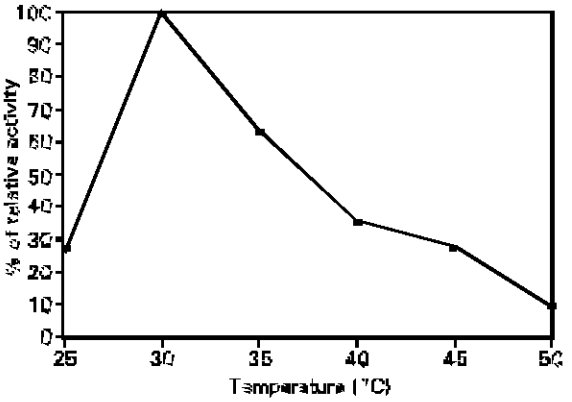

Fig. 6: Effect of temperature on lipase activity of *C. bonducella* L. seeds

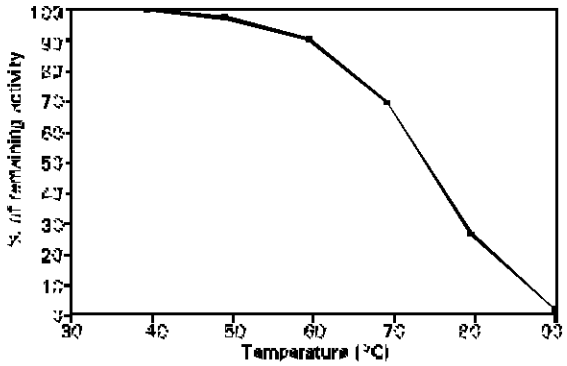

Fig. 7: Thermostability of lipase activity of *C. bonducella* L. seeds

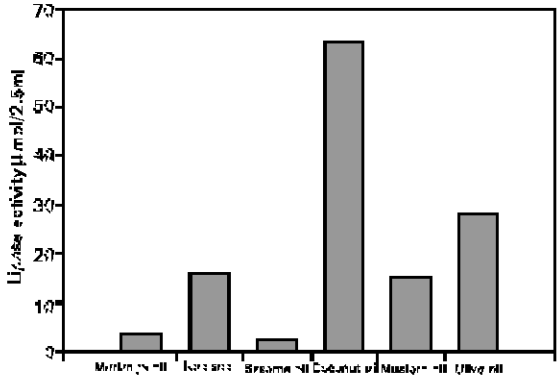

Fig. 8: Effect of substrate specificity lipase activity of *C. bonducella* L. seeds

substrates at pH 7.0 was mostly specific towards coconut oil (Fig.8). The specificity of *C.bonducella* L. seed lipase is similar to rice bran (Aizono *et al.*, 1973), *Cucumis melo* (Akhtar and Kausar, 1978), and *Hibiscus canabinus* seed (Kausar and Akhtar, 1979).

The data of effect of the various metal ions, reagents and detergents on lipase activity of *C.bonducella* L. seed were presented in Table 1. The small increase (6.40%) in the rate of lipase action in the presence of Ca^{2+} may be due to the presence of already optimum concentration of Ca^{2+} in the crude sample of *C.bonducella* L. seeds. The lipase activity in the presence of high concentration of Ca^{2+} showed inhibitory effect whilst in low concentration. Similar results have been reported by Mala and Dahot, (1995). Sodium deoxycholate, Mn^{2+}, Tween 80, and Triton X-100 inhibited the lipase activity. The results are in agreement with the results of other workers in case of lipase activity of *Cajanus cajan L.* seeds (Dahot *et al.*, 1989; Noomrio *et al.*, 1990; Khan *et al.*, 1991), rape seed (Rosnitschek and Theimer, 1980) and rice bran (Matsuda and Hirayama, 1979).

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