Short Communication

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Investigation of Lipase Activity from *Cajanus Cajan* Seeds

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Triacylglycerol is hydrolyzed stepwise to diacylglycerol, monoacylglycerol, glycerol and free fatty acids by lipase (Triacyl glycerol acylhydrolase E.C. 3.1.1.3). Lipase enzyme produced from plants and microorganisms is used in different manufacturing processes throughout the world in varied and interesting applications [1-5]. The distribution and role of lipase activity has been investigated extensively in plant seeds such as castor bean [6-9], oat grain [10-12], wheat grains [13,14], corn [15], *Moringa oleifera* seeds [16], cotton seeds [17], and *Cassia* species seeds [18].

A general survey on lipase activity in seeds of various plants have been carried out in this Laboratory [19]. *Cajanus cajan* seeds is one of them which contain considerable amount of lipase activity. *Cajanus cajan* seeds are used as a pulse and locally it is available in large quantity during Aug. - Sep. The present report therefore, deals with isolation and partial characterization of *Cajanus cajan* seeds lipase in detail.

Dry seeds of *Cajanus cajan* (Pigeon pea) were collected from village Qaim Babar, district Hyderabad, Sindh, Pakistan. The seeds were defatted with diethyl ether in a Soxhlet apparatus. All reagents were of analytical grade and used with out further purification.

Preparation of enzyme solution. Enzyme solution was prepared as reported earlier [16].

Determination of protein. Protein content of the enzyme solution was determined by the method of Lowry *et al*. [20], using bovine serum albumin as a standard and found to be 1.92 mg/ml.

Lipase assay. Lipase activity was determined according to the method described previously [16]. A unit of lipase activity was defined as the amount of enzyme required to release one microequivalent of free fatty acids per hour under the assay conditions.

Figure 1 shows the effect of time on the rate of lipase reaction. The libration of free fatty acids were increased upto 1 hr and then declined. The declination in rate of an enzymatic reaction may be suggested due to the de-activation in rate of reaction were reported by other workers in lipolytic enzyme system [21-23]. A reaction of 1 hr was selected in subsequent experiments because a reasonable change in enzymatic reaction was observed in this time.

The effect of enzyme concentration (*Cajanus cajan* seeds 5-25% solution) on the rate of enzymatic reaction was studied under the standard assay conditions. The rate of enzymatic reaction was increased with increase in enzyme concentration as shown in Fig. 2. Thus in subsequent experiments the concentration of enzyme solution was kept 20%.

Effect of substrate concentration (2.5 to 12.5% olive oil emulsion) on the rate of hydrolysis was investigated, using 20% *Cajanus cajan* seeds enzyme solution under the standard

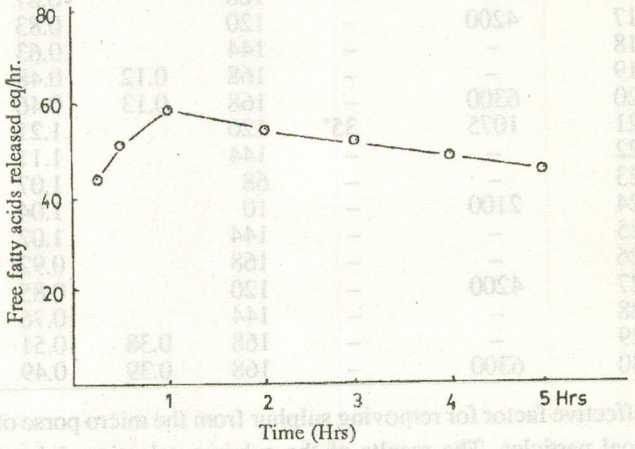

Fig. 1. Effect of time upon the reaction rate of *Cajanus cajan* seeds lipase activity.

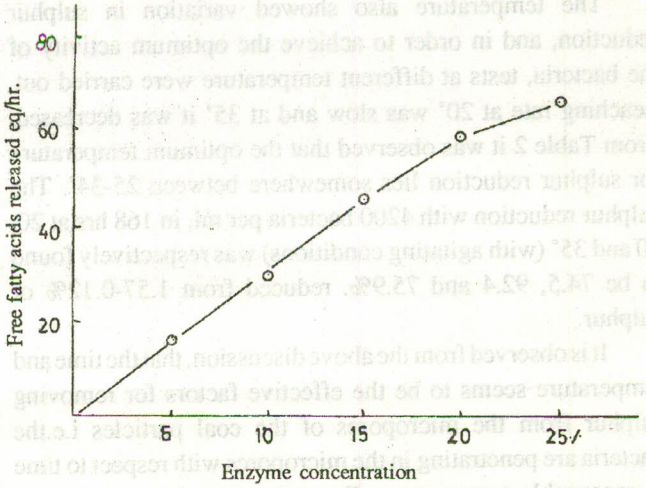

Fig. 2. Effect of enzyme concentration on the activity of lipase at 35°. 2.5 ml of 10% Olive oil emulsion as a substrate was incubated with different concentration of *Cajanus cajan* seeds enzyme solution as described in the method section.

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assay conditions, to find out the optimal substrate concentration as shown in Fig. 3. The rate of reaction initially rose proportionally with increase of substrate concentration upto 10% and then declined. The declination, in rate of reaction at higher concentration observed could be due to the effect of enzyme substrate concentration ratio or enzyme inhibited by the excess concentration of substrate [24-27].

The effect of pH on the relative activity of crude lipase was studied at 35° with 10% olive oil emulsion for 1 hr, using buffers from pH 4.0 to 8.0. The buffers used were 0.2M sodium acetate for pH (4.0 - 6.0) and 0.2M sodium phosphate (6.5-8.0). Lipase has its optimum activity at pH 5.5. At pH 4.5 and 6.0, the activity was lower with 50% of the maximum activity as shown in Fig. 4. The optimum pH (5.5) of *Cajanus cajan* is slightly higher than *Moringa oleifera* [16] and *Cannabinus* [28] seeds lipase (5.0) where as rice bran lipase has been shown to exhibit two pH optima 5.5 and 7.4-7.6 [29].

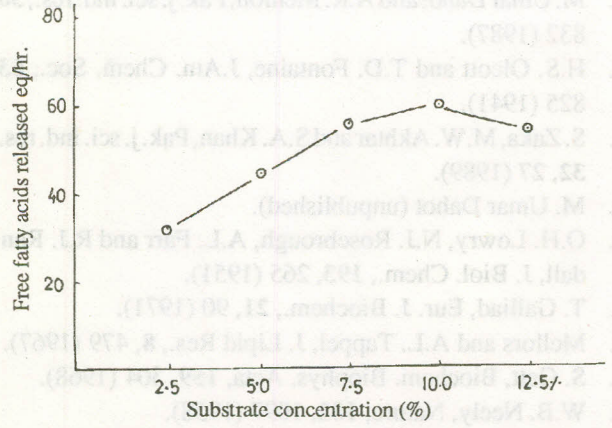

Fig. 3. Effect of substrate concentration on the activity of *Cajanus cajan* seeds lipase. 2.5 ml of 20% *Cajanus cajan* seeds enzyme solution was incubated with different concentration of substrate for 1 hr. at 35° and free fatty acids released were determined as described in Method section.

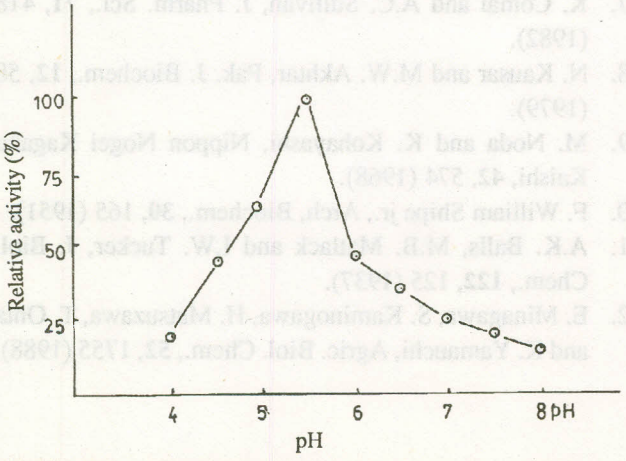

Fig. 4. Effect of pH on the activity of *Cajanus cajan* seeds lipas at 35°. 2.5 ml crude enzyme was added 0.5 ml of buffer of various pH values and 2.5 ml of 10% Olive oil emulsion. The reaction flasks were incubated for 1 hr. at 35° and free fatty acids released were determined as described in Method section. Highest activity was denoted as 100%.

The lipase activity was measured from 20-45° with 0.2M sodium acetate buffer at pH 5.5 using 10% olive oil emulsion as a substrate for 1 hr. The optimum temperature was found to be 30° and further increase of temperature results decrease in rate of reaction as shown in Fig. 5. The declination in rate of reaction could be due to denaturation of enzyme at higher temperature.

The effect of thermostability of lipase was determined by pre-incubation of enzyme solution at various temperatures between 30-90° for 10 mins. The remaining lipase activity was determined by standard assay conditions and results are presented in Fig. 6. Lipase activity of *Cajanus cajan* seed crude enzyme preparation was found thermolabile and it is fairly stable upto 45° but 97% of its activity lost at 90°.

Table 1 shows the effect of metal ions on the lipase activity. Slight inhibition was noted with Ca^{2+} and Mg^{2+} while Zn^{2+} small increase (11.0%) in the rate on lipase

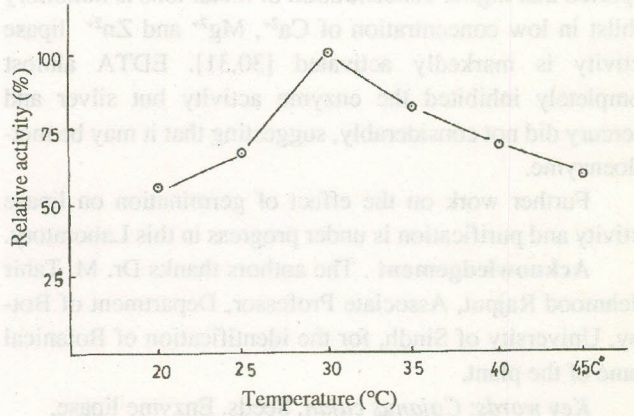

Fig. 5. Effect of temperature on the activity of lipase. 2.5 ml of crude enzyme was added to 2.5 ml of 10% Olive oil emulsion in presence of 0.2M sodium-acetate buffer pH 5.5. The reaction flasks were incubated at various temperature for 1 hr. and free fatty acids released were determined as described in Method section. The highest activity was denoted as 100%

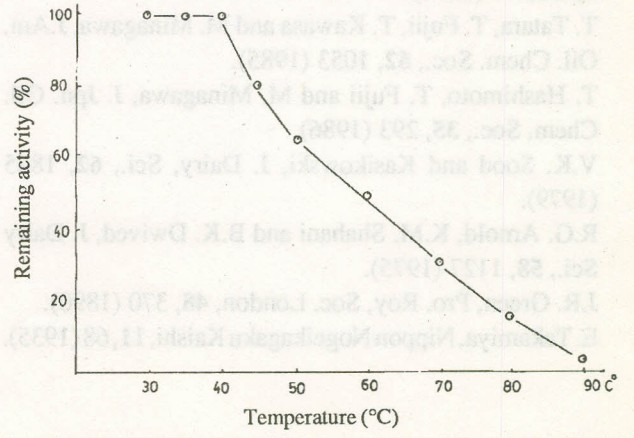

Fig. 6. Inactivation of *Cajanus cajan* seed lipase activity at different temperature. Samples of crude enzyme were incubated with 0.2H sodium-acetate buffer pH 5.5 at different temperature (30-90°) for 10 mins and the remaining lipase activities were then determined as described in Method section.

TABLE 1. EFFECT OF METAL IONS ON THE *CAJANUS CAJAN* SEEDS LIPASE ACTIVITY.

Metal ions $5x10^{-3}$ M	Activity units	% Relative activity*	% Activation /(inhibition)
Control	74	100	-
$CaCl_2$	63	85.14	(14.86)
$MgCl_2$	44	59.45	(40.55)
$ZnCl_2$	82	110.81	10.81
E.D.T.A.	02	2.70	(97.79)
$AgNO_3$	12	16.21	(83.79)
$Hg(NO_3)_2$	09	12.16	(87.84)

The enzyme was incubated at 30° for 10 mins. in the presence of metal ions in buffer pH 5.5. Subsequently substrate was added and incubation was continued for one hour.
*Expressed as % of activity with no addition

action, may be suggested that the concentration of these metal ions is already present optimal in *Cajanus cajan* seeds. It is reported that higher concentration of metal ions is inhibitory whilst in low concentration of Ca^{2+}, Mg^{2+} and Zn^{2+} lipase activity is markedly activated [30,31]. EDTA almost completely inhibited the enzyme activity but silver and mercury did not considerably, suggesting that it may be metalloenzyme.

Further work on the effect of germination on lipase activity and purification is under progress in this Laboratory.

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Key words: *Cajanus cajan*, Seeds, Enzyme lipase.

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