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Studies on the Lipolytic Enzymes of *Carica papaya* Seed Powder

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Abstract. The lipolytic enzymes (lipase and phospholipase) extracted from the defatted seeds of *Carica papaya* showed optimum activity at 40 °C and pH 7 in aqueous media. *n*-Heptane was found to be the most satisfactory solvent to maximize activities of lipase and phospholipase. The activity of lipase extracted from germinated seeds increased with the stage of seed development, but the phospholipase activity was noted to decrease.

Keywords: *Carica papaya*, lipase/phospholipase activity, pH/temperature optima, triglycerides

Introduction

The enzymatic studies of lipase and phospholipase of *Carica papaya* have been carried out using different temperatures, pH, aqueous media and organic solvents. The objective was to establish optimum conditions for the hydrolysis of simple triglycerides and phosphoglycerides by lipase and phospholipase so that these conditions can be applied both in the laboratory and industry.

Carica papaya (papaya) locally known as "papita", of the family Caricaceae is used as a common fruit in the Indo-Pakistan sub-continent and also in other countries of the world. The medicinal usefulness of the papaya fruit is well-established for different ailments (Kirtikar and Basu, 1984; Nadkarni, 1982), especially in the treatment of digestive system due to the presence of enzymes. Enzymes *in vivo* play an important role both in the synthesis and metabolism of a number of organic compounds in the animal and plant kingdoms. Review of the literature reveals that *C. papaya* has been studied for its papain, latex, lipase catalyst, flavonoids, proteolytic activity, sugar content, chitinase, cysteine proteinases, pectinesterase and lipids (Caro *et al.,* 2000; Rakhimov, 2000; Mangos *et al.,* 1999; Nguyen and Thanh, 1999; Askari and Qadri, 1998; Esperanza *et al.,* 1998; Albert and Philippe, 1997; Fayyaz *et al.,* 1993; Raie *et al.,*1992; Azarkan *et al.,* 1977), respectively, but studies related to the lipase and phospholipase of *C. papaya* seeds have not been previously reported. In the present studies, these enzymes have been extracted from mature and germinated seeds to determine their optimum activities on purified triglycerides of olive oil and egg lecithin, respectively, under different conditions. Such type of investigations have also been carried out on corn, wheat grains, oat grains and castor bean (Berner and Hammond, 1972; Banu and Serban, 1970; Ory 1969; Ferrigan and Geddes, 1958). The PCSIR Laboratories have carried out similar studies on *Citrullus* sp., *Carum capticum, Zea mays* and *Cassia* sp., of local origin (Javed *et al.,* 1999; Ahmad *et al.,* 1993; Aman and Akhtar, 1991; Zaka *et al.,* 1989). The present work on papaya seeds is thus an extension of the earlier studies but the present findings are being reported for the first time.

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Materials and Methods

Extraction of lipase and phospholipase. The dried seeds of papaya obtained from the fruit available in the local market, were ground to a fine powder and defatted in a Soxhlet extractor with diethylether. The defatted seed powder (50 g) was suspended in 200 ml citrate buffer (citric acid 0.1 M and disodium hydrogen phosphate 0.2 M) of pH 7 for 1 h at 40 °C. The supernatant containing enzymes was obtained by centrifugation (Karl Kolb, Germany) for 15 min at 12,000 rpm. The extract was diluted to 200 ml with citrate buffer and utilized to study the enzyme activities under different conditions (Blain *et al.,* 1976).

Preparation of substrates and determination of enzyme activities. Olive oil (origin: Italy, local market) was taken and its triglycerides were separated and purified by thin layer chromatography. The triglycerides (1 g) were emulsified by blending with 10% gum acacia suspension (aqueous medium) to determine lipase activity, whereas 10% egg lecithin (BDH, England) emulsion was used as substrate for the phospholipase activity (Javed *et al.,* 1999). Hydrolysis of the two substrates by enzymes (lipase and phospholipase), extracted from mature seeds under different parameters, is described below:

Effect of pH. The enzyme extract (15 ml) was incubated at 40 °C for 1 h in the presence of substrates (triglycerides or lecithin emulsion), separately with citrate buffer (pH 7) and calcium chloride (0.1 M). The released fatty acids, after extraction with 5 ml hexane:chloroform (1:1 v/v), were treated with

2.5 ml of Cu-TEA reagent in a test tube, shaken (linear Gallenkamp shaker) for 5 min and then centrifuged. The upper layer (3 ml) was reacted with 0.5 ml of 0.1% sodium diethyldithiocarbamate to develop a golden yellow colour whose absorbance at a fixed wavelength (440 nm) was recorded on a spectrophotometer (Beckman, model 24, England) against a blank prepared by boiled, denatured enzyme powder. A linear standard curve was drawn between the concentrations (80-800 µg/l) of palmitic acid against the absorbance (0.300-0.500) at a fixed wavelength (440 nm). The standard curve was used to calculate µ equiv of fatty acids released per g/h. The activity of lipase or phospholipase was calculated by using the method (Guven *et al.,* 1979) as follows:

$$\text{lipase/phospholipase activity } (\mu U) = \frac{\text{conc of fatty acids}}{1000} \times 80$$

Experiments were conducted with citrate buffer solutions of various pH (5.0-8.5) to determine the effect of pH on hydrolysis of the substrates. The results are reported in Table 1.

Effect of temperature. Experiments to determine the effect of temperature on the hydrolysis of substrates were conducted by changing the incubation temperature from 20-70 °C at 10 °C intervals under the same conditions as mentioned above. The results are reported in Table 2.

Effect of solvents. Defatted seed powder (1 g) was placed in a 50 ml stoppard conical flask containing 50 µl water and 5 ml liquid triglyceride:solvent (1:9) to determine the effect of various organic solvents on lipase activity (Table 3). Lecithin: solvent (1:9) was used to study the effect of solvents on phospholipase activity. The above mixtures were shaken for 2 h at 40 °C as reported earlier (Waheed *et al.,* 2002). The mixture was cooled to room temperature, additional 3 ml solvent was added and mixed thoroughly. The rest of the procedure was conducted as described above for the determination of the effect of pH.

Lipase and phospholipase activities in germinated seeds. Seeds of papaya were germinated in an incubator at 30±1 °C (Aman and Akhter, 1991). Seedlings with roots at root lengths of 5, 10, 15, 20, 25 and 30 mm were dried and crushed separately. The lipase and phospholipase extracted (see effect of pH) from the above mentioned various root lengths were assayed on substrates (triglycerides and lecithin) with buffer solution of pH 7 and an incubation temperature of 40 °C. The fatty acids released were determined from the standard curve and the enzyme activities were calculated.

Results and Discussions

The lipase and phospholipase activities were determined under different conditions of pH, temperatures and solvents. The conditions of pH and temperature, which gave maximum activity of lipase and phospholipase in mature dried seeds in aqueous medium were also applied to germinated seeds.

The lipase and phospholipase activities of defatted seeds in the pH range of 5.0 to 8.5 were studied by conducting the experiment for 1 h (Table 1). The data show that the activities of lipase and phaspholipase in neutral medium (pH 7) were the maximum (5.20 and 5.02 µU, respetively). Optimum pH 7 had also been reported for these enzymes in other seeds such as apple, apricot, and local cultivars of honeydew melon ('sarda' and 'garma') (Akhtar *et al.,* 1975). Other studies were carried out by adjusting the reaction media to pH 7 and varying the reaction temperatures and by changing the solvents in the media.

The activities of lipase and phospholipase in the defatted mature seeds were determined under various temperature conditions, i.e., 20-70 °C at pH 7 for 1 h. The maximum activities of lipase (5.10 µU) and phospholipase (4.89 µU) were observed at 40 °C for both enzymes (Table 2). The activities

Table 1. Lipase and phospholipase activities of mature papaya seeds at different pH

	Lipase			Phospholipase		
pH	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)
5.0	0.346	252	2.02	0.340	225	1.80
5.5	0.370	383	3.06	0.364	352	2.82
6.0	0.384	463	3.70	0.378	428	3.42
6.5	0.399	545	4.36	0.392	505	4.04
7.0	0.418	650	5.20	0.414	628	5.02
7.5	0.405	578	4.62	0.398	540	4.32
8.0	0.377	425	3.40	0.374	405	3.24
8.5	0.359	323	2.58	0.363	345	2.76

decreased when the temperature was increased or decreased from 40 °C. These observations show that these enzymes are more active at 40 °C, being in agreement with the studies on seed lipase of *Hibiscus cannabinus* (Kausar and Akhtar, 1979).

A set of experiments was also conducted at pH 7 and 40 °C in which different organic solvent suspensions were used in the media to determine the most appropriate solvent for hydrolysis of triglycerides and lecithin substrates by lipase and phospholipase of mature seeds. *n*-Heptane proved to be the best solvent for optimum enzyme activity for both the enzymes as compared to cyclohexane, di-isopropylether and cyclohexanol. The observed order of activity was *n*-heptane > cyclohexane > di-isopropylether > cyclohexanol: 4.80 > 3.84 > 2.62 > 1.62 µU for lipase while in the case of phospholipase it was 4.52 > 3.64 > 2.92 > 1.48 µU, respectively (Table 3). The higher activities of these enzymes in *n*-heptane may be due to its straight chain structure.

The parameters of temperature (40 °C) and pH 7, which showed maximum activities for the enzymes from mature seeds were also applied to germinated seeds at root lengths of 5 to 30 mm (Table 4). The activity of lipase, carried out in aqueous medium, was found to be directly proportional to the increase in root length of the germinated seeds. The maximum activity of the lipolytic enzyme was 6.78 µU at the root length of 30 mm. In contrast, the activity of phospholipase was inversely

Table 2. Lipase and phospholipase activities of mature papaya seeds at different temperatures

Temp (°C)	Lipase			Phospholipase		
	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)
20	0.368	375	3.00	0.362	338	2.72
30	0.407	590	4.72	0.396	527	4.22
40	0.416	638	5.10	0.411	612	4.89
50	0.404	572	4.58	0.394	517	4.14
60	0.385	465	3.72	0.371	388	3.10
70	0.373	401	3.21	0.349	268	2.14

Table 3. Lipase and phospholipase activities of mature seeds of papaya in the presence of different solvents

Solvents	Lipase			Phospholipase		
	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)	Absorption at 440 nm	Conc of fatty acids (µ equiv/g/h)	Activity (µU)
n-Heptane	0.409	600	4.80	0.403	565	4.52
Cyclohexane	0.387	480	3.84	0.383	455	3.64
Di-isopropylether	0.360	328	2.62	0.365	355	2.92
Cyclohexanol	0.337	202	1.62	0.334	185	1.48

Table 4. Lipase and phospholipase activities of germinated seeds of papya at different root lengths

Root length (mm)	Lipase			Phospholipase		
	Absorption at 440 nm	Conc of fatty acids (m equiv/g/h)	Activity (µU)	Absorption at 440 nm	Conc of fatty acids (m equiv/g/h)	Activity (µU)
5	0.419	655	5.24	0.444	796	6.37
10	0.422	673	5.38	0.431	724	5.79
15	0.427	700	5.60	0.395	522	4.18
20	0.438	763	6.10	0.386	475	3.80
25	0.444	796	6.37	0.373	401	3.21
30	0.453	848	6.78	0.359	325	2.80

proportional to the root length of germinated seeds. The maximum activity of phospholipase was 6.37 µU at the root length of 5 mm. Similar patterns were observed by other workers (Ahmad *et al.,* 1993; Aman and Akhtar, 1991) who worked on C*arum capticum* and *Zea mays,* respectively.

Conclusion

Lipase and phospholipase of mature and germinated seeds of *Carica papaya* exhibited maximum activities at pH 7 and 40 °C in aqueous medium. In the case of organic solvents, *n*-heptane showed the maximum activities for both the enzymes at pH 7 and 40 °C. The lipase activity was maximum at the maximum root length, but phospholipase activity was minimum at the maximum root length. It is concluded that multiple factors are involved for the lipase and phospholipase activities of mature and germinated seeds. The study provides useful information for further work on an industrial scale and on the resolution of the technical processing problems of papaya, and perhaps of other seed crops.

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