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STUDIES OF LIPASE AND PHOSPHOLIPASE ENZYMES OBTAINED FROM THE MEAL OF *CITRULLUS VULGARIS* OF THE CUCURBITACEAE FAMILY

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The lipase and phospholipase extracted from the meal of mature seeds of *Citrullus vulgaris* showed optimum activity at 40°C and pH 7 in aqueous media. *N*-heptane was found to be the most satisfactory solvent media to maximize activities of lipase and phospholipase. The activity of lipase extracted from germinated seeds increased with the stage of seeds development but was found to be reverse for the phospholipase activity.

Key words: *Citrullus vulgaris,* Lipase, Phospholipase.

Introduction

The enzymatic studies of lipase and phospholipase of *Citrullus vulgaris* have been carried out using different temperatures, pH, aqueous media and organic solvents. The objectives were 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.

Citrullus vulgaris (water melon) belongs to family Cucurbitaceae. Research concerning the lipase and phospholipase of *Citrullus vulgaris* has not been previously reported. In the present research, these enzymes from mature and germinated seeds have been extracted to determine their optimum activity on purified triglycerides of olive oil and egg lecithin respectively under different conditions. Enzymes in *in Vivo* systems play an important role both in synthesis and metabolism of a number of organic compounds in the animal and plant kingdoms. Review of the literature reveals that similar investigations have been carried out on castor bean, oat grains, wheat grains and corn (Ferrigan and Geddes 1958; Ory 1969; Banu and Serban 1970; Berner and Hammond 1972). The PCSIR Laboratories have carried out similar studies on *Zea mays, Carum capticum* and *Cassia* species of local origin (Zaka *et al* 1989; Aman and Akhtar 1991; Ahmad *et al* 1993). The present work on watermelon seeds thus is an extension of the earlier studies but the findings are being reported for the first time.

Experimental

Extraction of lipase from the defatted mature and germinated seeds. The seed samples were ground to a fine powder and defatted in a Soxhlet extractor with diethylether. The defatted seeds powder (50 g) was suspended in 0.1 M citrate buffer (citric acid 0.1 M and disodium hydrogen phosphate 0.2 M) of pH 7 for one hour at 40°C. The supernatant containing enzymes was obtained by centrifugation for 15 min at 12,000 rpm. The extract was diluted to 200 ml with citrate buffer and utilized to study enzyme activities under different conditions.

Preparation of substrates. The pure triglycerides of olive oil (1 g) were emulsified by blending with 10% gum accacia solution (aqueous media) to determine lipase activity (Akhtar *et al* 1975) whereas 10% egg lecithin emulsion was used as substrate for the phospholipase activity (Guven *et al* 1979).

i. Effect of pH. Lipase and phospholipase fraction (4 ml) extracted at pH 7 was incubated at 40°C for one hour in the presence of substrate (5 ml) of either 10% triglycerides or lecithin emulsion, citrate buffer (5 ml) at pH 7 and 0.1 M $CaCl_2$ (1 ml) in 50 ml conical flask with a stopper. The released fatty acids after extraction with 5 ml hexane : chloroform (1 : 1 vv^{-1}), were treated with 2.5 ml of CU-TEA reagent in a test tube, shaken for 5 min and then centrifuged. The upper layer (3 ml) was reacted with 0.5 ml of 0.1% sodium diethyl dithiocarbamate to develop a golden yellow colour whose absorbance (A) at a fixed wavelength (440 nm) was recorded. A standard curve of palmitic acid (between the concentration 80 $\mu g\ l^{-1}$ - 800 $\mu g\ l^{-1}$ was drawn against the absorbance (A 0.300 - A 0.500) at fixed wavelength (440 nm). The standard curve was used to calculate μ equiv. of fatty acids released per g h^{-1}. The activity of lipase or phospholipase was calculated using Guven's method. A Beckman spectrophotometer Model 24 was used. Blanks were obtained with boiled enzyme powder/extract by following the above procedure. The pH of the enzyme extracts was adjusted either to acidic or alkaline with 0.1 M solution of citric acid or 0.2 M solution of disodium hydrogen phosphate,

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respectively. Sodium carbonate (0.1 M) and sodium bicarbonate (0.1 M) were used for pH 9. Experiments were conducted at pH 3-9 to observe the effect of pH on hydrolysis of the substrate.

ii. Effect of temperature. Experiments to study the hydrolysis of substrate were conducted by changing the incubation temperature from 20°C to 80°C at 10°C intervals (Kauser and Akhtar 1979) under the same conditions as mentioned above.

iii. Effect of solvents. Defatted seed powder (1 g) was placed in a 50 ml stoppered conical flask and 50 µl water plus 5 ml liquid triglyceride : solvent (1:9) was used to study the effect of various organic solvents (Table 3) on lipase activity. 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 (Blain *et al* 1976) then cooled to room temperature and an additional 3 ml of solvent was added and thoroughly mixed. The rest of the experiment was conducted as indicated in the experiment (i) i.e. effect of pH.

iv. Lipase and phospholipase activities in germinated seeds. Seeds of *Citrullus vulgaris* were germinated in an incubator at 30°C ± 1°C (Aman *et al* 1989). Seeds (1 g) at root lengths of 5, 10, 15, 20, 50 and 30 mm were dried and then crushed. The lipase and phospholipase extracted (see section i) from each root of germinated seeds were assayed on substrates of triglycerides and lecithin at pH 7 and an incubation temperature of 40°C. The fatty acids released were measured from the standard curve and enzymatic activity was calculated as given by Guven *et al* (1979).

Results and Discussion

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

The lipase and phospholipase activities of the meal of mature seeds in the pH range of 3 to 9 were studied by carrying out the experiment for 1 h (Table 1). The data showed that the activity of lipase in neutral media (pH 7) was maximum (3.66 µU). In case of phospholipase, maximum activity (3.37 µU) was also obtained at pH 7. Optimum pH 7 had also been reported for these enzymes in other seeds such as *Carica pappaya* (Akhtar *et al* 1975). It was observed that pH 7 played a vital role for the maximum activity in both lipase and phospholipase.

Other studies were carried out by adjusting the reaction media to pH 7 and varying the reaction temperature and by changing the solvent in the media. It was observed that pH 9 was not attained by the use of citrate buffer solution but by using bicarbonate buffer solutions given under experiment (i).

The activities of lipase and phospholipase in the meal of mature seeds were determined under various temperatures i.e. 20 - 80°C at pH7 for 1 h. The maximum activities of lipase (3.66 µU) and phospholipase (3.30 µU) were found to be at 40°C for both enzymes (Table 2). The activities decreased when the temperature was increased or decreased. These observations were similar to *Hibiscus canabinus* (kenaf seed lipase) at 40°C (Kausar and Akhtar 1978).

A set of experiments was also conducted at pH 7 and 40°C in which different organic solvents were used in the media to determine the most appropriate solvent for hydrolysis of triglyceride and lecithin substrates by lipase and phospholipase of mature seeds. The *n*-heptane proved to be the best solvent for optimum enzymatic activity for both enzymes as compared to cyclohexane, di-isopropyl ether and cyclohex-

Table 1

Lipase and phospholipase activity of mature seeds at different pH

pH	Lipase			Phospholipase		
	Absorption (A) at 440 nm	Conc. of fatty acid (µ equiv $g^{-1}h^{-1}$)*	Activity (µU)**	Absorption (A) at 440 nm	Conc. of fatty acids (µ equiv $g^{-1}h^{-1}$)*	Activity (µU)**
3	0.320	85	0.68	0.308	34	0.27
4	0.327	116	0.93	0.320	85	0.68
5	0.363	276	2.21	0.332	138	1.10
6	0.388	388	3.10	0.354	236	1.89
7	0.404	458	3.66	0.396	422	3.37
8	0.384	370	2.96	0.360	262	2.10
9	0.368	298	2.38	0.322	94	0.75

* Taken from the standard curve; ** Calculated by Guven's method (1979).

anol. The observed order of activity was *n*-heptane > cyclohexane > di-isopropyl ether > cyclohexanol 3.52 > 2.16 > 1.74 > 0.68 μU for lipase and in case of phospholipase it was 3.37 > 2.16 > 1.74 > 0.81 μU respectively (Table 3). The activity of *Zea mays* lipase in different solvents, reported in the literature (Aman *et al*, 1989) was in the same order and supported our observation.

The parameters of temperature (40°C) and pH 7 which showed maximum activity for the enzymes from resting seeds were also applied to germinated seeds at root length of 5 to 30 mm (Table 4). The activity of lipase in aqueous media was found to be directly proportional to the increase in root length of germinated seeds. The maximum activity of lipolytic enzyme was 5.13 μU at root length of 30 mm. In contrast, the activity of phospholipase was inversely proportional to the root length of germinated seeds. The maximum activity of phospholipase was 3.44 μU at a root length of 5 mm. Similar patterns were observed by Aman and Akhtar (1991) and Ahmad *et al* (1993) who worked on *Zea mays* and *Carum capticum* respectively.

Conclusion

Lipase and phospholipase of mature and germinated seeds of *Citrullus vulgaris* exhibit maximum activities at pH 7 and 40°C in aqueous media. In case of organic solvents media, *n*-heptane shows the maximum activities for both enzymes at pH 7 and 40°C. The lipase activity is maximum at maximum root length, but phospholipase activity is minimum at maximum root length. It may be concluded that multiple factors are involved for the lipase and phospholipase activity of mature and germinated seeds. The study provides useful information both on an industrial scale and in the development of a biotechnological approach of the technical processing problems of *Citrullus vulgaris* and perhaps of other seed crops.

Table 2
Lipase and phospholipase activity of mature seeds at different temperatures

Temp. °C	Lipase			Phospholipase		
	Absorption (A) at 440 nm	Conc. of fatty acid (μ equiv $g^{-1}h^{-1}$)*	Activity (μU)**	Absorption (A) at 440 nm	Conc. of fatty acids (μ equiv $g^{-1}h^{-1}$)*	Activity (μU)**
20	0.366	290	2.32	0.358	252	2.01
30	0.396	422	3.37	0.388	388	3.10
40	0.404	458	3.66	0.394	413	3.30
50	0.394	413	3.30	0.386	378	3.02
60	0.362	270	2.16	0.354	236	1.89
70	0.328	121	0.97	0.320	85	0.68
80	0.308	34	0.27	--	--	--

* Taken from the standard curve; ** Calculated by Guven's method (1979).

Table 3
Lipase and phospholipase activity of mature seeds in the presence of different solvents

Solvents	Lipase			Phospholipase		
	Absorption (A) at 440 nm	Conc. of fatty acid (μ equiv $g^{-1}h^{-1}$)*	Activity (μU)**	Absorption (A) at 440 nm	Conc. of fatty acids (μ equiv $g^{-1}h^{-1}$)*	Activity (μU)**
N-heptane	0.400	440	3.52	0.396	422	3.37
Cyclohexane	0.362	270	2.16	0.360	262	2.10
Di-isopropyl ether	0.350	218	1.74	0.350	218	1.74
Cyclohexanol	0.320	85	0.68	0.324	102	0.81

* Taken from the standard curve; ** Calculated by Guven's method (1979).

Table 4
Lipase and phospholipase activity of germinated seeds of different root lengths

Root length (mm)	Lipase			Phospholipase		
	Absorption (A) at 440 nm	Conc. of fatty acid (μ equiv $g^{-1}h^{-1}$)*	Activity (μU)**	Absorption (A) at 440 nm	Conc. of fatty acids (μ equiv $g^{-1}h^{-1}$)*	Activity (μU)**
5	0.406	467	3.73	0.398	430	3.44
10	0.418	520	4.16	0.384	370	2.96
15	0.426	556	4.45	0.372	316	2.53
20	0.433	588	4.70	0.360	262	2.10
25	0.440	620	4.96	0.354	236	1.89
30	0.445	642	5.13	0.350	218	1.74

* Taken from the standard curve; ** Calculated by Guven's method (1979).

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