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STUDIES OF LIPASE AND PHOSPHOLIPASE PROCURED FROM THE MEAL OF *CARUM CAPTICUM*

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The lipase extracted from the meal of mature seeds of *Carum capticum* show optimum activity at 40° and pH 5 in aqueous media, whereas the phospholipase activity is maximum at pH5 under the same conditons. N-heptane was found to be the most satisfactory media solvent to maximize activiteis of lipase and phospholipase. The activity of lipase, extracted from germinatd seeds, increases proportionally with stage of seeds development but the reverse was observed for phospholipase activity.

Key words: Lipase, Phospholipase and *Carum capticum*.

Introduction

Numerous studies have been carried out to enhance our understanding of plant lipid biochemistry e.g. classification of lipids [1], Lipid changes during germination [2], the distribution of fatty acids in triglycerides [3], the positional and geometrical isomers of fatty acids [4], the analysis and evaluation of non-saponified material such as fatty alcohols [5], hydrocarbons, sterols etc. A complete investigation of lipids of matured and germinating seeds require the extraction of lipase and phospholipase and analysis of their hydrolysis behaviour on triglycerides. These studies help us to understand the metabolism of hydrolysis of triglycerides of resting seeds and the triglycerdies produced during the process of germination.

The enzymatic studies of lipase and phoslpholipase of *C. capticum* have been carried out using different condtions of pH, temperature, aqueous media and different organic solvents. The object is to establish optimum conditons for the hydrolysis of simple triglycerides and phosphoglycerides by lipase and phospholipase so that these condtions may be applied on laboratory as well as on industrial scale [6].

Carum capticum, a species of Carum genus, belongs to the Umbelliferae family. No work on the lipase of *C. capticum* has been reported previously. In the present work, an attempt has been made to extrtact lipase and phospholipase from mature and germinated seeds to determine the optimum activity of the lipase on purified triglycerides of olive oil under different conditions of pH, temperature and solvents. Exmination of the literature has revelaed that this type of investigation has been carried out on castor bean [7], oat grains [8], wheat grains [9] and corn [10] etc. The phospholipases have been classified as A, B, C and D. Phospholipase A, B and C breaks down the bond between glycerol and carboxylic acid at α, β and γ positions whereas D breaks down the bands between phosphoric acid and choline in lecithin. Phospholipase D is wide spread in plants e.g. spinach leaves, beets, savoy cabbage and many others [11].

Experimental

Extraction of lipase and phospholipase from the defatted resting and germinated seeds. The seed samples were ground to a fine powder and defatted in a Soxhlet extractor with diethyl ether. The defatted seed powder (50 g) was suspended in 0.1 M citrate buffer of pH 5 (200 ml) and shaked for 1 hr. at 40°. The supernatant containing enzymes [12] was obtained by centrifugation for 15 min. at 12,000 rpm and used to study enzyme activities under different conditions. The extract was diluted to 200 ml with citrate buffer.

Preparation of substrates. The pure triglycerides of olive oil (1 g) [13] was emulsified by blending with 10% gum accacia solution (aqueous media) to determine lipase activity whereas 10% egg lecithin emulsion [14] was used as substrate for the phospholipase activity.

The hydrolysis of substrates by enzymes extracted from resting seeds under different parameters are given below:

(i) *Effect of pH.* Lipase and phospholipase fraction (4 ml) extracted at pH 5 was incubated at 40° for 1 hr. in the presence of 5 ml of substrate of either 10% triglyceride or lecithin emulsion, 5 ml of citrate buffer at pH 5 and 1 ml of 0.1 M $CaCl_2$ in 50 ml conical flask with a stopper. 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 [14] in a test tube shaken for 5 min. and then centrifuged. Upper layer (3 ml) was reacted with 0.5 ml of 0.1% sodium diethyl dithio-carbamate to develop a golden yellow colour whose absorbance (A) at a fixed wave length 440 nm was noted . A standard curve was drawn between the concentration (80 µg/L-800 µg/L) of palmetic acid against the absorbance (A 0.300 - A 0.500) at fixed wavelength 440 nm, with the help of this standard curve

µ eq. of fatty acids released per litre were determined. Using the Guven's method [14], the activity of lipase or phospholipase was calculated. A Beckman Spectrophotometer Model 24 was used. Blanks were obtained with enyzyme powder/extracted boiled and then 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 0.2M solution of disodium hydrogen phosphate, respectively. Sodium carbonate (0.1 M) and sodium bicarbonate (0.1 M) were used for pH 9. Experimentas were conducted at pH 3, 4, 5, 6, 7, 8 and 9 to observe the effect of pH on hydrolysis of the substrates.

(ii) *Effect of temperature.* Experiments to study the hydrolysis of substrates under conditon of different temperatures were condcuted at seven different temperatures by changing the temperature [15, 16] in incubation from 20° to 80° at 10° intervals under the same condtions as mentioned above.

(iii) *Effect of solvents.* Defatted seed powder (1g) was placed in a 50 ml stoppered conical flask and 50 µl water plus 5 ml liquid triglyceride: solvlent (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 shaked for 2 hrs. at 40° [16, 17]. The mixture was cooled at room temperature and a furhter 3 ml solvent was added and the mixture thoroughly mixed. The rest of the expriment was conducted as indicated above in experiment No.1 i.e. effect of pH.

(iv) *Lipase and phospholipase activities in germinated seeds.* Seeds of *C. capticum* were germinated in an incubator at 30 + 5° [18, 19]. Seeds (1g) at root lengths of 5, 10, 15, 20, 25 and 30 mm were picked up separately, dried and then crushed. The lipase and phospholipase procured (see section I) from each root length of germinated seeds were assayed on substrates of triglycerides and lecithin at pH 5 and an incubation temperature of 40°. The fatty acids released were measured from the standard curve and enzymatic activity was calculated as given by Guven.

Results and Discussion

Enzyme in *vivo* systems play an important role in the synthesis and break up of a number of organic compounds in the animal and plant kingdoms. The present study is concerned with lipase and phospholipase, enzymes which are involved in the degradation of lipids. Th meal of Carum after the extraction of oil, has been used for the procurement of lipases and phospholipases under condtions described in exprimental. The acitivities of these enymes have been investigated under different conditions using triglycerides and lecithin (Phosphoglycerides) as substrate. Lipases and phospholipases hydrolyse triglycerides and phosphoglycerides, respectively, resulting in the liberation of fatty acids. The concentration of liberated fatty acids reflects the activity of lipase under different exprimental conditions. The reasonably high quantity of meal given under experimental for the assay is required otherwise positive results would not be achieved due to the presence of very small quantity of enzymes in the meal.

The absorbancy of fatty acids liberated from substrate is measured by spectrophotometer at visible wave length of 440°nm against a blank sample which is prepared by boiling the mixture to destroy enzymes as given in experiment No.1. The concentration of the quantity of fatty acids released as a results of the hydrolysis of substrates due to the presence of lipase and phospholipase present in the meal is measured from the standard curve. The activity of the enzymes was calculated by the application of the following formula

$$\text{Lipase/phospholipase acitivity } (\mu U) = \frac{\text{Determined standard curve}}{1000} \times 8$$

The lipase and phospholipase activities have been determined under different conditions of pH, temperature and solvents. The conditions of pH and temperature which gave maximum activity of lipase and phospholipase in resting seeds was also applied to germinated seeds.

The lipase and phospholipase activities of the meal of resting seeds in the pH range of 3 to 9 were studied by carrying out the expriments for 1 hr. (Table 1). The data shows that the activity of lipase in acidic media is maximum (4.8 µU) at pH 5 and in alkaline media. It is maximum (2.2 µU) at pH 8. In the case of phospholipase, maximum acitivity (4.5 µU) was obtained at pH 5. More than one pH optima have also been reported for these enzymes in castor bean (20). It has been determined that pH 5 plays a vital role for the maximum acitivity in both lipase and phospholipase.

TABLE 1. LIPASE AND PHOSPHOLIPASE ACTIVITY OF RESTING SEEDS AT DIFFERENT PH.

pH	Lipase			Phospholipase		
	Absorption (A) (at 440nm)	Conc. of F.A. (µ equir./L)*	Activity (µU)+	Absorption (A) (at 440 nm)	Conc. of F.A. (µ equir./L)	Activity (µU)
3	0.349	150.0	1.2	0.326	100.0	0.80
4	0.401	400.0	3.2	0.395	375.0	3.00
5	0.450	600.0	4.0	0.449	562.5	4.50
6	0.413	450.0	3.6	0.401	400.0	3.20
7	0.355	175.0	1.4	0.340	150.0	1.20
8	0.369	275.0	2.2	0.332	125.0	1.00
9	0.340	150.0	1.2	0.326	100.0	0.80

* Taken from standard curve, [14]

+ Calculated by K.C. Guven's method [14].

Other studies were carried out by adjusting the reaction media to pH 5 and varying the reaction temperature and by changing the solvent in the media. It is also worth to mention here that pH 9 was not attained by the use of citrate buffer solution, but by using a bicarbonate buffer solutionas given under expriment No.1.

The acitivity of lipase and phospholipase in the meal of resting seeds were determined under the various temperature condtions i.e. 20°–80° at pH 5 for 1 hr. The maximum activity of lipase (4.80 μU) and phospholipase (4.4μU) was found to be at 40° for both enzymes (Table 2). The activites decrease either if the temperature is increased or decreased. These observation also are supported by previous workers [15].

A set of experiments was also conducted at pH 5 and 40° 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 resting seeds. The *n*-heptane has been shown to be the best solvent for optimum enzymatic activity for both enzymes as compared to cyclohexane, di-isopropyl ether and cyclohexanol. The order of activity has been determined as follows: n-heptane > cyclohexane > di-isopropyl· ether cyclohexanol i.e. 3.22 μU > 1.92 μU > 1.48 μU > 0.52 μU for lipase activity respectively. Similarly, in case of phospholipase 3.00 μU > 1.60 μU = 1.60 μU > 0.8 μU respectively (Table 3).

The activity in different solvents has also been carried out by previous workers [17].

The parameters of temperature (40°) and pH (5) which showed maximum activity for the enzymes from resting seeds have also been applied to germinated seeds at root lenght of 5 to 30 mm (Table 4). The activity of lipase, carried out in aqueous media was found to be directly proportional to the increased root length of germinated seeds. The maximum activity of lipolytic enzyme is 6.0 μU at root length of 30°. In contrast, the acitivity of phospholipase, was inversely proportional to the root length of germinated seeds. The maximum activity of phospholipase is 4.2 μU at root length of 5 mm. This is also supported by Tehseen Aman [18, 19] who worked on maiz seeds.

In summing, lipase and phospholipase of resting seeds of *C. capticum* exhibit maximum activities at pH 5, 40° in the presence of *n*-heptane in the media. In the case of germinated seeds, pH 5, 40° and aqueous media was used to determine lipase and phospholipase activities. It was found that the lipase activity was maximum at maximum root length but phospholipase was minimum at maximum root length. It is concluded that multiple factors are involved for the lipase and phospholipase activity of resting and germinated seeds. The results obtained in this tudy provide information that should be useful, both on industrial scale and with an object to develop a biotechnology approach to the investigation of technical problems in the processing of Carum and perhaps other seed crops.

TABLE 2. LIPASE AND PHOSPHOLIPASE ACTIVITY OF RESTING SEEDS AT DIFFERENT TEMPERATURES.

Temp. (°C)	Lipase			Phospholipase		
	Absorption (A) (at 440nm)	Conc. of F.A. (μ equir./L).	Activity (μU)	Absorption (A) (at 440 nm)	Conc. of F.A. (μ equir./L)	Activity (μU)
20	0.413	450.0	3.60	0.395	375.0	3.0
30	0.432	525.0	4.20	0.419	475.0	3.8
40	0.450	600.0	4.80	0.439	550.0	4.4
50	0.446	587.5	4.70	0.395	375.0	3.0
60	0.369	275.0	2.20	0.350	200.0	1.6
70	0.329	112.5	0.90	0.319	75.6	0.6
80	0.306	25.0	0.20	—	—	—

TABLE 3. LIPASE AND PHOSPHOLIPASE ACTIVITY OF RESTING SEEDS IN THE PRESENCE OF DIFFERENT SOLVENTS.

Solvents	Lipase			Phospholipase		
	Absorption (A) (at 440nm)	Conc. of F.A. (μ equir./L).	Activity (μU)	Absorption (A) (at 440 nm)	Conc. of F.A. (μ equir./L)	Activity (μU)
N-Heptane	0.401	402.5	3.22	0.395	375.0	3.00
Cyclohexane	0.360	240.0	1.92	0.350	200.0	1.60
Di-isopropyl-ether	0.347	185.0	1.480	0.350	200.0	1.60
Cyclohexanol	0.317	65.0	0.52	0.326	100.0	0.80

TABLE 4. LIPASE AND PHOSPHOLIPASE ACTIVITY OF GERMINATED SEEDS OF DIFFERENT ROOT LENGTHS.

Root length	Lipase			Phospholipase		
	Absorption (A) (at 440nm)	Conc. of F.A. (μ equir./L).	Activity (μU)	Absorption (A) (at 440 nm)	Conc. of F.A. (μ equir./L)	Activity (μU)
5	0.454	612.5	4.90	0.432	525.0	4.2
10	0.468	662.5	5.30	0.417	462.5	3.7
15	0.475	700.0	5.60	0.401	400.0	3.2
20	0.481	725.0	5.80	0.390	362.5	2.9
25	0.484	737.5	5.90	0.379	312.5	2.5
30	0.490	750.0	6.00	0.372	287.5	2.3

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