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Studies on The Lypolitic Enzymes of Carum Roxburghianum Seed Meal

Shahid Mahmud*, Amran Waheed, and Razia Khanum

Applied Chemistry Research Centre, PCSIR Laboratories Complex, Ferozepur Road, Lahore-54600, Pakistan

Abstract – The lipase and phospholipase activities of meals of resting seeds of *C. roxburghianum* were studied at different temperatures, solvents and pH. Both the enzymes showed the maximum activities at 40°C and in *n*-heptane used as solvent. However, lipase showed maximum activities at two different pH, one at pH 5 (acidic) and other at pH 8 (alkaline) whereas phospholipase showed only one pH optimum at pH 8. During the course of germination, the lipase showed an increase whereas reverse was the case with phospholipase.
Keywords – Lipase, phospholipase, enzymes, carum.

Introduction

Umbelliferae is a very important family in plant kingdom. It is widely distributed throughout the world. It is stated that 176 species belong to 56 genera of this family occur in different parts of Pakistan (Bhatty, 1977). Among these only a few are cultivated as crops while rest of species are wild. Since old times these plants were used in foods and feed, (Chopra, 1970) in cosmetics and even in medicines (Kirtikar and Basu, 1984).

Carum roxburghianum belongs to the plant family *Umbelliferae* (Bhatty, 1977). In the present investigations, lipase and phospholipase of *Carum roxburghianum* were studied under different conditions of pH, temperature and in different organic solvents. The object was to establish optimum conditions for hydrolysis of simple triglycerides and phosphoglycerides so that these may be applied to both on laboratory and industrial scales. The enzymes were extracted from mature and germinated seeds, to determine their optimum activity on purified triglycerides of olive oil and egg lecithin respectively under different conditions of pH, temperature and solvents. An examination of literatures shows that similar investigations has also been carried out on caster bean (Dry, 1969), oat grains (Berner and Hammond, 1972), wheat grains (Ferrigan and Geddes, 1958), corn (Banu and Serban, 1970), *Citrullus colocyvthus* (Javed *et al.*, 1999) and *Nicotiana tabacum* (Waheed *et al.*, 2002), but no work has been reported on the lipase and phospholipase of *Carum roxburghianum*.

*Author for correspondence
Fax: +92-42-923-0705; E-mail: shahidmahmud@hotmail.com

Experimental

Extraction of lipase and phospholipase – The powdered seeds were defatted in a soxhlet extractor with diethyl ether. The defatted seed powder (50 g) was suspended in 0.1M-citrate buffer of pH 5 (200 ml) and shaken for 1 hr. at 40°C. The supernatant containing enzymes (Zaka *et al.*, 1989) was separated by centrifugation for 15 min. at 12,000 rpm and was used to study the enzyme activities under different conditions. The extract was diluted to 200 ml with citrate buffer.

Preparation of Substrate – The pure triacylglycerols of olive oil (1 g) was made into emulsion by blending with 10% gum acacia solution (in water) (Akhter *et al.*, 1975) to determine lipase activity whereas 10% egg lecithin emulsion (in water) (Guven *et al.*, 1989) was used as substrate for the phospholipase activity.

Lipase and Phospholipase Activity – The hydrolytic effect of enzymes on their substrates under different conditions is given below.

Effect of pH (1) – Lipase and phospholipase extract (4 ml) of pH 5, 5 ml substrate of 10% triglycerides or lecithin emulsion, 5 ml citrate buffer of pH 5 and 1 ml of 0.1M $CaCl_2$ were added into a 25 ml conical flask fitted with a stopper. The flask was incubated at 40°C for 1 hr. in a water bath shaker working at the rate of 200 vibrations per minute. The fatty acids were released which were then extracted with 5 ml hexane chloroform (1:1 v/v). The extract thus obtained was treated with 2.5 ml of copper nitro triethanol amine (cu-TEA) reagent (Dry, 1969). It was shaken for 5 min. and then centrifuged. The upper layer (3 ml) was treated with 0.5 ml of 0.1% sodium

diethyl dithiocarbamate 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 known concentrations (80 µg/1 - 800 µg/1) of a fatty acid (palmitic acid) against their absorbencies (0.300A - 0.500A) at a fixed wavelength (440 nm). With the help of this standard curve the concentrations of fatty acids released by the enzyme as µ mole per minute per gram were calculated and then using the Guven's method ((Guven *et al.*, 1989), the activity of lipase or phospholipase was calculated. Blanks were taken after boiling the enzyme extract and then following the above procedure. Different experiments were conducted at pH 3, 4, 5, 6, 7, 8 and 9 in order to observe the effect of pH as shown in Table 1. A Beckman spectrophotometer Model 24 was used.

Effect of Temperature (2) – In order to study the effect of temperature on the enzymatic hydrolysis of substrates, different experiments were conducted at 20°C, 30°C, 40°C, 50°C, 60°C, 70°C and 80°C (Berner and Hammond, 1972). The results are shown in Table 2.

Effect of Solvents (3) – Defatted seed powder (1g) was placed in a 50 ml stoppered conical flask and 50 µl water plus 5ml 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 hours at 40° (Dry, 1969). The mixture was cooled to room temperature and a further 3 ml solvent was added and the mixture was thoroughly mixed. The rest of the experiment was conducted as indicated above under the effect of pH.

Table 1. Lipase and Phospholipase activities of resting seeds at different pH.

pH values	3	4	5	6	7	8	9
Lipase Activity (µ U/g/hr*)	1.0	3.0	4.2	3.1	1.0	2.0	1.0
Phospholipase Activity (µ U/g/hr*)	0.6	2.6	4.0	2.7	1.6	0.8	0.4

*Calculated by Guven's method

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

Temperatures (°C)	20	30	40	50	60	70	80
Lipase Activity (µ U/g/hr*)	3.0	4.0	4.2	4.1	2.0	0.7	0.1
Phospholipase Activity (µ U/g/hr*)	2.7	3.8	4.0	3.6	1.7	0.5	-

*Calculated by Guven's method

Table 3. Lipase and phospholipase activities of resting seeds in different solvents.

Solvents	N-heptane	Cyclo hexane	Di-isopropyl ether	Cyclo hexanol
Lipase Activity (µ U/g/hr*)	3.0	1.7	1.3	0.3
Phospholipase Activity (µ U/g/hr*)	2.7	1.4	1.1	0.1

*Calculated by Guven's method

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

Root length (mm)	5	10	15	20	25	30
Lipase Activity (µ U/g/hr*)	4.0	4.8	5.2	5.5	5.7	5.9
Phospholipase Activity (µ U/g/hr*)	3.8	2.7	2.0	1.6	1.5	1.3

*Calculated by Guven's method

Lipase and phospholipase activities in germinated seeds – Seeds of *C. roxburghianum* were germinated in an incubator at 30°C (Banu and Serban, 1970). Seeds (1 g) at root lengths of 5, 10, 15, 20, 25 and 30 mm were picked up separately, dried and then crushed. The lipase and phospholipase extracted from each root length of germinated seeds were assayed on substrates of triglycerides and lecithin at pH 5 and incubation temperature of 40°C. The fatty acids released were measured from the standard curve and enzymatic activity was calculated as given by Guven. The results are shown in Table 4.

Results and Discussion

Carum roxburghianum seeds have been studied for lipid classes (Waheed *et al.*, 2003). The lipase and phospholipase activities of *C. roxburghianum* 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 experiments for 1 hr (Table 1). The data shows that maximum lipase activities were 4.2 U/g at pH5 (acidic media) and 2.0 U/g at pH8 (alkaline media). In the case of phospholipase, maximum activity (4.0 µ U) was obtained at pH 5. More than one pH optima have also been reported for these enzymes in cassia seeds (Zaka *et al.*, 1989). It has been determined that pH 5 plays a vitalrole for the maximum activity in both lipase and

phospholipase.

Other studies were carried out by adjusting the reaction media to pH 5 and varying the reaction temperature in the media. The activity of lipase and phospholipase in the meal of resting seeds were determined under the various temperature conditions *i.e.* 20°C - 80°C at pH 5 for 1 hr. The maximum activity of lipase (4.2 μ U) and phospholipase (4.2 μ U) was found to be at 40° for both the enzymes (Table 2). The activities decrease either if the temperature is increased or decreased because the enzyme remains active within a limied range of temperature. These observations also are supported by previous workers (Khan and Akhtar, 1979) who worked on *Hibiscus cannabinus* showing maximum activity at 37°C.

A set of experiments was also conducted at pH 5 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 substrate 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-isoprophyl ether and cyclohexanol. The order of activity has been determined as follows.

n-heptane > cyclohexane > di-isopropyl ether > cyclohe xanol *i.e.* 30.0 μ U > 1.7 μ U > 1.3 μ U > 0.3 μ U for lipase activity, respectively. Similarly, in case of phospholipase 2.7 μ U > 1.4 μ U > 1.1 μ U > 0.1 μ U respectively (Table 3). The activity in different solvents has also been carried out by previous workers (Blain *et al.*, 1976,). Blain, Akhtar and Petterson showed similar results in *n*-heptane to be the more suitable solvent.

The parameters of temperature (40°) and pH (Berner. and Hammond, 1975) which showed maximum activity for the enzymes from resting seeds have also been applied to germinated seeds at root length 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 5.9 μ U/g/h at root length of 30 mm. In contrast, the activity of phospholipase, it was inversely proportional to the root length of germinated seeds. The maximum activity of phospholipase is 3.8 μ U at root length of 5 mm. This is also supported by Tehseen Aman and coworkers (Aman *et al.*, 1989) who worked on maiz seeds showing maximum activity at root length of 30 mm and 5 mm for lipase and phospholipase respectively.

In summing up, the lipase and phospholipase of resting seeds of *C. roxburghianum* exhibit maximum activities at pH 5, 40°C in the presence of *n*-heptance in the media. In the case of germinated seeds, pH 5, 40°C 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 study provide useful information for industrial work and biotechnological research.

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