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Activity of Lipase and Phospholipase Extracted from the Seed Meal of *Nicotiana tabacum* L.

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Abstract – The activity of the lipase and phospholipase extracted from the seed meal of Nicotiana tabacum L. was studied with the help of Spectrophotometer at different pH, Temperatures and Solvents. Both lipase and phospholipase shown optimum activity at pH 6 and 45°C. Their activities also maximize when n-heptane was used as solvent media.
Key words – *Nicotiana tabacum* L., *Solanaceae*, Lipase, Phospholipase

Introduction

Nicotiana tabacum L. belongs to the genus *Nicotiana* of the plant family *Solanaceae*. This genus contains 45 species and some important species are *N. tabacum, N. glauca, N. alata, N. rustica and N. longiflora* (Chopra, 1970). *Nicotiana tabacum* L. is a native of America and now cultivated in all tropical countries. The juice of the leaves of this plant is sedative, antispamodic and a powerful insecticide (Nadkarni, 1954; Kirtikar *et al.*, 1933).

From a perusal of literature, it was seen that no work on the lipase and phospholipase of *N. tabacum* L. has been reported previously. In present study an attempt was made to extract enzymes from mature seeds to determine their optimum activity using triacylglycerols of Olive oil and egg lecithin as substrates under different conditions of pH, temperatures and solvents. The literature survey has revealed that similar investigations have been carried out on Castor bean (Ory, 1969), Oat grains (Berner *et al.*, 1972), Wheat grains (Ferrigan *et al.*, 1958) and Corn (Banu *et al.*, 1970). The objective of this work was to establish optimum conditions for the hydrolysis of simple triacylglycerols and phosphoglcerides by lipase and phospholipase so that these conditions may be applied both on laboratory and industrial scales.

Experimental

The seeds were ground to a fine powder and defatted by using of Soxhlet apparatus and diethyl ether as solvent. 50 gms of the defatted seed powder was suspended in 0.1 M citrate buffer (citric acid 0.1 M and disodium hydrogen phosphate 0.2 M) of pH 6 and shaken for one hour at 45°C. The supernatant containing enzyme (Zaka *et al.*, 1989) was obtained by centrifugation for 15 minutes at 12,000 rpm. The extract was then diluted to 200 ml with 0.1 M citrate buffer and utilized to study enzyme activities under different conditions. 1gm pure triacylglycerol of Olive oil was emulsified by blending with 10% gum acacia solution in aqueous media was used as substrate to determine lipase activity (Akhtar *et al.*, 1975) whereas 10% egg lecithin emulsion (Guven *et al.*, 1989) was used as substrate for the determination of phospholipase activity.

The hydrolysis of substrate by enzymes extracted from the mature seeds of *N. tabacum* L under different parameters is described below.

Effect of pH – Different experiments were conducted at pH ranging between 3-9 to observe the effect of pH on hydrolysis of the substrates. The pH of the enzyme extracted was adjusted either to acidic or alkaline with 0.1 M solution of citric acid or 0.2 M solution of sodium hydrogen phosphate, respectively. Sodium carbonate (0.1M) and sodium bicarbonate (0.1 M) were used to achieve a pH 9.

Lipase and phospholipase fractions (4 ml each), extracted at pH 6, were incubated at 45°C for one hour in the presence of 5 ml substrate (either 10% triacylglycerols or lecithin emulsion), citrate buffer (5 ml), of pH 6 and 0.1 M calcium chloride (1 ml) in 50 ml stoppered conical flask. The fatty acids released after extraction with 5 ml hexane: chloroform (1:1, v/v) were treated with 2.5 ml of Cu-Tea reagent

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(Guven *et al.*, 1989) in a test tube, shaken for 5 minutes 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) was measured at 440 nm on a spectrophotometer (Beckman, Model 24) against a blank prepared by boiled enzyme powder extract. A standard curve was drawn between the concentration (80 μg/L-800 μg/L) of palmitic acid against the absorbance (A 0.300-A 0.500) at the same wavelength. The standard curve was used to calculate u equivalent of fatty acids released per gm per hour. Using the Guvens method (Guven *et al.*, 1989). The activity of lipase and phospholipase was calculated as follows by the method of Guven *et al.*

$$\text{Lipase/phospholipase Activity} = \frac{\text{Concentration of fatty acids}}{1000} \times 8$$

Effect of temperature – Experiments were conducted to study the hydrolysis of substrate under conditions of different temperatures. The temperature of incubation was changed from 30°C to 70°C at 5°C interval (Kausar *et al.*, 1979; Akhtar *et al.*, 1978). Incubation was carried out for one hour at pH 6.

Effect of solvent – Defatted seed powder (1 gm) was placed in a 50 ml stoppered conical flask containing 50 μl water and 5 ml of triglyceride: solvent (1:9 w/v) to observe the effect of different organic solvents on lipase activity and Lecithin: solvent (1:9 w/v) was used to study the effect of solvents on phospholipase activity. Both the above mixtures for the two enzymes were shaken separately for two hours at 40°C [Blain *et al.*, 1976] and cooled to room temperature. 3 ml of solvent was more added in the mixture and mixed thoroughly. The remaining experimental conditions were same as those mentioned in the Effect of pH experiment.section described earlier.

Results and Discussion

Enzymes play an important role in the *vivo* synthesis and breakdown of a number of organic compounds in both the animals and plants. The present study was concerned with the lipase and phospholipase enzymes of *Nicotiana tabacum* L., which were involved in degradation of lipids. These enzymes hydrolyse triglycerides and phosphoglycerides, respectively and the librated fatty acids serve as an indicator of their activity.

Table 1. Lipase Activity of *Nicotiana tabacum* L. Seeds at Different pH

pH	Concentration of F.A. (μ equiv./L)[a]	Activity *(μU)[b]
3	80	0.64
4	110	0.88
5	280	2.24
6	458	3.66
7	390	3.12
8	310	2.48
9	240	1.92

a: Taken from the standard curve.
b: Calculated by Guven's method.
*: Activity equals to μ equiv./gm/hr.

Table 2. Phospholipase Activity of *Nicotiana tabacum* L. Seeds at Different pH

pH	Concentration of F.A. (μ equiv./L)[a]	Activity (μU)[b]
3	30	0.24
4	90	0.72
5	206	1.65
6	398	3.18
7	310	2.48
8	225	1.80
9	110	0.88

The activities of lipase and phospholipase at different pH are reported in Tables 1 and 2. These tables reflect that the maximum activity of lipase is 3.66 μU and of phospholipase is 3.18 μU at pH 6 respectively. This indicates that the enzymes are more active in slightly acidic media. Akhtar *et al.* (1975) reported the maximum activity of lipase of different fruits and vegetables at pH 6 and 7 respectively.

Determination of activities of the two enzymes under various temperatures i.e. 30°C to 80°C with an

Table 3. Lipase Activity of *Nicotiana tabacum* L. Seeds at Different Temperatures

Temperature °C	Concentration of F.A. (μ equiv./L)[a]	Activity (μU)[b]
30	210	1.68
35	325	2.60
40	390	3.12
45	460	3.68
50	405	3.24
55	240	1.92
60	110	0.88
65	90	0.72
70	50	0.40

Table 4. Phospholipase Activity of *Nicotiana tabacum* L. Seeds at Different Temperatures

Temperature °C	Concentration of F.A. (μ equiv./L)[a]	Activity (μU)[b]
30	180	1.44
35	270	2.16
40	340	2.72
45	415	3.32
50	240	1.92
55	115	0.92
60	105	0.84
65	70	0.56
70	40	0.32

increase of 5°C at pH 6 revealed maximum activities as 3.68 and 3.32 μU, respectively (Tables 3, 4) at 45°C. The activities were affected by changing the temperature and it was noted that the activity decreased either the temperature was decreased or increased from 45°C. This observation was also supported by the reported work for cottonseed (Rakhimov *et al.*, 1976) and rice bran (Aizono *et al.*, 1976).

The studies were also made to examine the effect of various organic solvents on lipase and phospholipase activities of the seeds of *N. tabacum* L. The n-heptane was found to be the best solvent for maximum enzymatic activity (Tables 5, 6) of both the enzymes as compared to cyclohexane, di-isopropyl ether and cyclohexanol. The order of activity turned to be *n*-heptane>cyclohexane>di-isopropyl ether>cyclohexanol (2.88>2.24>1.68>1.00 μU respectively) for lipase with a similar order of activity for phospholipase (2.72>1.68>1.44>0.76 μU respectively).

The present investigation, thus, shows that the lipase and phospholipase enzymes of *N. tabacum* L. exhibit maximum activities at pH 6 and 45°C in *n*-heptane. It is concluded that multiple factors are involved in controlling the lipase and phospholipase activities of the seeds. The results reported here provide information that should be useful, both on an industrial scale and in the development of processing of *N. tabacum* L. and possibly of other seed crops.

Table 5. Lipase Activity of *Nicotiana tabacum* L. Seeds in the Presence of Different Solvents

Solvents	Concentration of F.A. (μ equiv./L)[a]	Activity (μU)[b]
n-Heptane	360	2.88
Cyclohexane	280	2.24
Di-isopropyl ether	210	1.68
Cyclohexanol	125	1.00

Table 6. Phospholipase Activity of *Nicotiana tabacum* L. Seeds in the Presence of Different Solvents

Solvents	Concentration of F.A. (μ equiv./L)[a]	Activity (μU)[b]
n-Heptane	340	2.72
Cyclohexane	210	1.68
Di-isopropyl ether	180	1.44
Cyclohexanol	95	0.76

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