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Studies on the Lipolytic Enzymes of *Sesamum indicum* Seed Powder

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Abstract. Optimum conditions for the hydrolysis of simple triglycerides and phosphoglycerides for the activity of the lipolytic enzymes (lipase and phospholipase) extracted from the defatted seeds of *Sesamum indicum* were established for use in laboratory and industry. The enzymes showed optimum activity at 40 °C and pH 7 in aqueous media. N-heptane was found to be the most satisfactory solvent for maximum activities. The activity of lipase extracted from germinated seeds increased with the stage of seed development, but was reverse for the phospholipase activity.

Keywords: lipase/phospholipase activity, solvent media, triglycerides, lipolytic enzymes

Introduction

Sesamum indicum DC. oil is reckoned equal to olive oil in medicinal properties, especially in the treatment of ulcers, psoriasis, prurigo, leucoderma and wounds (Nadkarni, 1982).

Earlier studies on sesame lipids are available (Javed *et al*., 2000; Toro-Vazquez *et al.*, 2000; Yashida *et al*., 1995; Kamal and Appelqvist, 1994) but research concerning its lipolytic enzymes (lipase and phospholipase) has so far not been reported. Enzymes play an important role in *in vivo* synthesis as well as metabolism of a number of organic compounds in the animal and plant kingdom.

In the present studies, enzymes from mature and germinated seeds of sesame were extracted and the enzymatic activity of lipase and phospholipase was investigated at different temperature, pH, aqueous media and organic solvents. The objective was to establish optimum conditions for the hydrolysis of simple triglycerides and phospho-glycerides by lipase and phospholipase, respectively, therefore, these conditions can be applied both in the laboratory and industry. Such investigations were also made earlier on wheat grains, castor bean, oat grains and corn (Banu and Serban, 1970; Berner and Hammond, 1970; Ory, 1969; Ferrigan and Geddes, 1958). Similar studies on *Cassia* sp., *Nicotiana rustica, Zea mays, Carum capticum, Citrullus* sp., of local origin were carried out at PCSIR Laboratories (Waheed *et al*., 2002; Javed *et al*., 1999; Ahmad *et al*., 1993; Aman and Akhtar, 1991; Zaka *et al*., 1989). The present work on sesame is thus an extension of the earlier studies.

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Materials and Methods

Extraction of lipase and phospholipase. Dried seeds of sesame collected from local market, were ground to a fine powder and defatted in a soxhlet extractor with diethyl ether. The defatted seed powder (50 g) was suspended in 200 ml citrate buffer (citric acid 0.1 M and disodium hydrogen phoshphate 0.2 M) of pH 7 in 500 ml conical flask and was shaken at 200 rpm for one h at 40 °C, using a Gallenkamp orbital shaker. 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 condition (Waheed *et al*., 2002).

Preparation of substrate. Olive oil (Italian origin) was purchased from local market and its triglycerides were separated and purified by thin layer chromatography. Ttriglycerides (1 g) were emulsified by blending with 10% gum acacia solution (aqueous media) to determine lipase activity, whereas, 10% egg lecithin (BDH, England) emulsion was used as substrate for the phospholipase activity (Kausar and Akhtar, 1979). 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 was shaken for one h at 40 °C and 200 rpm 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 for 5 min and then centrifuged. The upper layer (3 ml) was reacted with 0.5 ml of 0.1% sodium diethyldithiocarbamate which resulted in golden yellow colour, whose absorbance (A) at a fixed wave length (440 nm) was recorded on a spectrophotometer

(Beckman, model 24, England) against a blank prepared by boiled enzyme powder. A linear standard curve was drawn between the concentration, 80 µg/litre - 800 µg/litre of palmitic acid against the absorbance (A 0.300 - A 0.500) at fixed wavelength (440 nm). The standard curve was used to calculate µ equivalent of fatty acids released per g/h. The activity of lipase or phospholipase was calculated according to Guven *et al*. (1979) as follows.

$$\text{Lipase/phospholipase activity (µU)} = \frac{\text{concentration of fatty acid}}{1000} \times 8$$

Experiments were conducted with citrate buffer solutions to observe the effect of pH (3.0-8.0) on hydrolysis of substrates.

Effect of temperature. Experiments to study the hydrolysis of substrate were conducted by changing the incubation temperature from 20 °C to 70 °C at 10 °C intervals under the same conditions as mentioned above.

Effect of solvents. Defatted seed powder (1 g) was placed in a 50 ml stoppered conical flask containing 50 µ litre water and 5 ml liquid triglyceride: solvent (1:9) to observe the effect of various organic solventson 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). The mixture was 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 the effect of pH.

Lipase and phospholipase activities in germinated seeds. Seeds of sesame were germinated in an incubator at 30 °C ± 1 °C (Javed *et al*., 1999). 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 section i) from above 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 measured from the standard curve and enzymatic activity was calculated.

Results and Discussion

Enzyme systems play an important role in the synthesis and breakup of a number of organic compounds in animals and plants. The present study is concerned with the lipase and phospholipase enzymes of sesame seed which are involved in the degradation of lipids. These enzymes hydrolyse triglycerides and phosphoglycerides, respectively, and the liberated fatty acids serve as indicator of their activity. The defatted material (meal) of resting and germinated seeds of sesame after treatment of citrate buffer under specific conditions was centrifuged to get lipase and phospholipase enzymes for studying their activities under different parameters.

Fatty acids develop golden yellow colour on treatment with cupric nitrate, triethanolamine and diethyldithiocarbamate solution. In the present study, the absorbance of golden yellow colour was measured by spectrophotometer at 440 nm. Concentration of fatty acids is directly proportional to the development of colour showing the activity of the particular enzyme. The concentration on the basis of absorption was determined with the help of a standard curve drawn between the concentrations of palmitic acid against the absorbance at the same wavelength as mentioned above. The activity of lipase and phospholipase was calculated by Guven's method.

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

The lipase and phospholipase activities of defatted seeds in the pH range of 5.0 to 8.0 were studied by carrying the experiment for 1h (Table 1). Data showed that the activity of lipase in neutral media (pH 7) was maximum (2.53 µU). In case of phospholipase, maximum activity (2.42 µU) was also obtained at pH 7. Optimum pH 7 is also reported for these

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

	Lipase			Phospholipase		
pH	Absorption(A) at 440 nm	Conc. of F.A. (µ equiv./g/h)*	Activity (µ U)**	Absorption (A) at 440 nm	Conc. of F.A (µ equiv./g/h)*	Activity (µ U)**
3	0.324	103	0.82	0.321	90	0.72
4	0.333	142	1.12	0.328	122	0.98
5	0.350	218	1.74	0.348	212	1.70
6	0.368	298	2.38	0.364	282	2.25
7	0.372	316	2.53	0.369	303	2.42
8	0.358	253	2.02	0.354	238	1.89

* = taken from the standard curve (Guven *et al*.,1979); ** = calculated on dry matter basis (Guven *et al*.,1979)

enzymes in other seeds such as groundnut, coconut, maize, wheat and almond (Akhtar *et al*., 1975). It was observed that pH 7 played a vital role for the best 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.

The activities of lipase and phospholipase in the defatted mature seeds were determined under various temperature conditions i.e., 20 °C -70 °C at pH 7 for 1 h. The optimum activity of lipase and phospholipase (2.49 µU, 2.46 µU, respectively) was found to be at 40 °C for both the enzymes (Table 2). The activity decreased when the temperature was increased or decreased from 40 °C. These observations show that these enzymes are more active at 40 °C and are in accordance with the studies of Kenaf seed lipase (Kausar and Akhtar,1979) showing its maximum activity at 40 °C.

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. The *n*-heptane proved to be the best solvent for optimum enzymatic activity for both enzymes as compared to cyclohexane, di-isopropyl ether and cyclo-hexanol. The observed order of activity was *n*-heptane > cyclohexane > di-isopropyl ether > cyclohexanol, values being 2.32 > 1.79 > 0.90 >0.61 µU for lipase and phospholipase 2.16 > 1.70 > 0.75 > 0.48 µU, respectively (Table 3); it was due to straight chain structure and non polar nature of *n*-heptane. This pattern of

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

Temp.	Lipase			Phospholipase		
°C	Absorption(A) at 440 nm	Conc. of F.A. (µ equiv./g/h)*	Activity (µ U)**	Absorption (A) at 440 nm	Conc. of F.A. (µ equiv./g/h)*	Activity (µ U)**
20	0.352	228	1.82	0.350	218	1.74
30	0.363	276	2.21	0.360	262	2.10
40	0.371	311	2.49	0.370	308	2.46
50	0.355	240	1.92	0.352	228	1.82
60	0.326	112	0.90	0.323	98	0.78
70	0.318	77	0.61	0.317	72	0.57

* = taken from the standard curve (Guven *et al*.,1979); ** = calculated on dry matter basis (Guven *et al*.,1979)

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

Solvent	Lipase			Phospholipase		
	Absorption(A) at 440 nm	Conc. of F.A. (µ equiv./g/h)*	Activity (µ U)**	Absorption (A) at 440 nm	Conc. of F.A. (µ equiv./g/h)*	Activity (µ U)**
n-heptane	0.366	298	2.32	0.362	270	2.16
Cyclohexane	0.351	224	1.79	0.348	212	1.70
Di-isopropyl ether	0.326	112	0.90	0.322	94	0.75
Cyclohexanol	0.318	77	0.61	0.314	60	0.48

* = taken from the standard curve (Guven *et al*.,1979); ** = calculated on dry matter basis (Guven *et al*.,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 F.A. (µ equiv./g/h)*	Activity (µ U)**	Absorption (A) at 440 nm	Conc. of F.A. (µ equiv./g/h)*	Activity (µ U)**
5	0.375	328	2.62	0.369	303	2.42
10	0.386	380	3.04	0.358	253	2.02
15	0.396	422	3.37	0.347	205	1.64
20	0.405	464	3.71	0.342	181	1.45
25	0.414	502	4.01	0.332	139	1.11
30	0.418	520	4.16	0.326	112	0.90

* = taken from the standard curve (Guven *et al*., 1979); ** = calculated on dry matter basis (Guven *et al*.,1979)

activity was also observed by Waheed *et al.* (2002) for *Nicotiana rustica*.

The parameters of temperature (40 °C) and pH (7) which showed better activity 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 media was found to be directly proportional to the increase in root length of germinated seeds. The maximum activity of lipolytic enzyme is 4.16 µU at root length of 30 mm. In contrast the activity of phospholipase was inversely proportional to the root length of germinated seeds. The best activity of phospholipase was 2.42 µU at a root length of 5 mm; similar patterns were observed in other studies (Ahmad *et al*., 1993; Aman and Akhtar, 1991) on *Zea mays* and *Carum capticum*.

Conclusion

Lipase and phospholipase of mature and germinated seeds of *Sesamum indicum* exhibit optimum activities at pH 7 and 40 °C in aqueous media. In case of organic solvents, *n*-heptane showed better activities for both the enzymes at pH 7 and 40 °C. The lipase activity is maximum at 30 mm root length, but phospholipase activity was minimum at 30 mm root length. It is concluded that multiple factors are involved for the lipase and phospholipase activity of mature and germinated seeds. The optimum conditions evaluated for the activites of these enzymes can be utilized in the industry to resolve technical processing problems and to reduce the cost and processing time for sesame and other seeds.

References

Ahmad, I., Raie, M.Y., Akhtar, M.W. 1993. Studies of lipase and phospholipase procured from the meal of *Carum capticum*. *Pakistan Journal of Scientific and Industrial Research* **36:** 248-251.

Akhtar, M.W., Parveen, H., Kausar, S., Chughtai, M.I.D. 1975. Lipase activity in plant seeds. *Pakistan Journal of Biochemestry* **8:** 77-82.

Aman, T., Akhtar, M.W. 1991. Isolation and characterization of *Zea mays* (Neelum) root phospholipase. *Science International, Lahore* **3:** 61- 64.

Banu, C., Serban, L. 1970. Enzymic changes in dehydrated products: Lipase activity in some oleaginous seeds. *Industrial Aliment (Bucharest)* **21:** 367-369.

Berner, D.L., Hammond, E.G. 1970. Specificity of lipase from several seeds. *Lipids* **5:** 572-573.

Blain, J.A., Akhtar, M.W., Patterson, J.D.E. 1976. Enzyme activity in organic solvents. *Pakistan Journal of Biochemistry* **9:** 41-45.

Chopra,G.L. 1970. *Pedaliaceae in Angiosperms*, pp. 333-335, 9th edition, Unique Publishers, Lahore, Pakistan.

Ferrigan, M., Geddes, W.F. 1958. Distribution of lipase in the commercial mill products from hard red spring wheat. *Cereal Chemistry* **35:** 422-427.

Guven, K.C., Bergisadi, N., Guler, E. 1979. A modification of Duncombes method and its application to the lipolytic assay of Heparin. *Fette, Seifen, Anstrichmittel* **81:** 152-154.

Javed, M.A., Ahmad, Ahmad, I., Ali, H. 1999. Studies of lipase and phospholipase enzymes obtained form the meal of *Citrullus vulgaris* of the *Cucurbitaceae* family. *Pakistan Journal of Scientific and Industrial Research* **42:** 345-348.

Javed, M.A., Akhtar, N., Jabbar, A. 2000. Fatty acid and lipid composition of *Sesamum indicum* DC. *Pakistan Journal of Scientific and Industrial Research* **43:** 23-25.

Kamal, E.A., Appelpvist, L.A. 1994. Variation in fatty acid composition of the different acyl lipids in seed oils of four *sesamum* species. *Journal of American Oil Chemist Society* **71:** 135-139.

Kausar, N., Akhtar, M.W. 1979. Isolation and characterization of *Hibiscus cannabinus (kenat)* seed lipase. *Pakistan Journal of Biochemistry* **12:** 58-64.

Nadkarni, A.K. 1982. *Sesamum indicum*, In: *The Indian Materia Medica* **I** *part* II, pp. 1126-1129, 3rd edition, Popular Prakshan Bombay, India.

Ory, R.L.1969.Acid lipase of the caster bean. *Lipids* **4:** 177-185.

Toro-Vazquez, J.F., Briceno-Montelongo, M., Dibildox, Alvarado, E., Charo-Aionso, M., Reyes-Hernandez, J. 2000. Crystallization kinetics of palm stearin in blends with sesame seed oil. *Journal of American Oil Chemist Society* **77:** 297-310.

Waheed A., Mahmaud S., Ahmad A. 2002. Activity of lipase and phospholipase extracted from the seed meal of *Nicotiana rustica* of the family *Solanaceae*. *Proceedings of Pakistan Academy of Sciences* **39:** 75-78.

Yashida, H., Shigezaki, J., Takagi, S., Kajimoto, G. 1995. Variation in the composition of various acyl lipids, tocopherols and lignans in sesame seed oil roasted in microwave oven. *Journal of The Science of Food and Agriculture* **68:** 407-415.

Zaka, S. Akhtar, Khan, M.W., Shafiq, A.1989. Phosphatide Acyl hydrolase and triglycerides acylhydrolase activities in the developing seeds of *Cassia* species. *Pakistan Journal of Scientific and Industrial Research* **32:** 27-32.