Pakistan J. Sci. Ind. Res., Vol. 30, No. 11, November 1987

PROPERTIES OF *MORINGA OLEIFERA* SEED LIPASE

M. Umar Dahot and A.R. Memon

Department of Biochemistry, Institute of Chemistry, University of Sind, Jamshoro

(Received May 20, 1985; revised November 1, 1987)

A thermolabile lipase present in *Moringa oleifera* seed was studied using olive oil emulsion stabilized with 10% gum acacia as a substrate. At pH 5.0 and a temperature 40° lipase activity was found optimum. Manganese chloride stimulated the enzyme activity. Calcium salt had no significant effect on lipolysis whereas mercury, copper, E.D.T.A., sodium deoxycholate, triton X-100, acetone, alcohol and toluene were found inhibitory. The lipase was inactivated completely when it was preincubated at 80° for ten.

Key words: Moringa oleifera, Seed, Lipase

INTRODUCTION

Because of the growing need and importance of enzyme technology in the development of the national economy, agricultural waste material such oilseed cakes and rice bran can be usefully utilized in the development of enzyme preparations. This plan not only underscores the need to encourage the use and exploitation of indigenous materials for enzyme preparations, but also meet the market demands for enzymes required by countries which depend on imported sources.

Lipolytic enzymes are widely used in manufacturing processes in all areas of the world in varied and interesting applications. For instance, detergent powders including fungal and bacterial enzymes containing small amount of amylases in addition to the proteases and lipases are marketed as biological washing powder [1]. The use of lipase in gastrointestinal problems has also been proposed [2]. Lipases are of considerable interest in food and other industries. It is, for instance, observed that uncontrolled lipolysis during milk processing can cause flavour defect and accumulation of free fatty acids in the extraction of cottonseed oil due to the undesirable action of lipases requiring extra operation for their removal prior to marketing. These few examples illustrate the industrial importance of controlled and selective lipolysis and illustrate the considerable industrial importances of lipolytic enzymes.

It is often a difficult problem to study the catalytic activities of lipolytic enzymes due to the physicochemical character of reactions they catalyze, which cause discrepancies in the convential kinetics. Since the enzymes are water soluble but the substrates are not, these reactions are usually carried out by making emulsions of the substrate in order to provide a lipid water interfacial area to which the enzyme binds and which is subscaptible to attack by Lipolytic enzymes [3].

The purpose of this study was two fold; first, the utilization of *Moringa oleifera* seeds as an indigenous source in the preparation of lipolytic enzymes, and, second, to ascertain the type of lipolytic activities present in the crude enzymatic preparations of *M. oleifera* seeds which was reported earlier to exhibit phospholipase B activity [4].

MATERIAL AND METHODS

All reagents were used of analytical grade without further purification.

Plant material. The seeds of *M. oleifera* were collected in dry state during June – August from the Sind University Employees' Colony.

Enzyme preparation. M. oleifera seeds were defatted using diethyl ether in a Soxhlet apparatus for 4-6 hr. and the defatted residue was stored in a vacuum desiccator until it was used.

The soluble and powder enzyme preparation was made according to the method reported earlier [4]. The protein content of soluble enzyme preparation was estimated by the Biuret method [5] using bovine

albumin as a standard and was found to be 4 mg/ml.

Substrate preparation. To study the influence of emulsifying agents such as gum arabic and polyvinyl alcohol on the lipolytic activity of an enzyme preparation from *M. oleifera,* the substrate emulsions were prepared by taking 10 ml of olive oil in an aqueous solution of gum arabic or polyvinyl alcohol in various concentrations adjusting the total volume to 100 ml. They were homogenised for 10 min at maximum speed using a homogenizer (Universal Lab type 309 Poland) at successive intervals of 1 min to prevent overheating. The pH of the prepared emulsion was maintained at 6.8.

Enzyme assay. 150 mg of the enzyme powder preparation or 2.5 ml of soluble enzyme solution were taken in duplicate in a 50 ml conical flask to which 2.5 ml of freshly prepared substrate emulsion was added. Appropriate controls were also taken for blank enzyme and blank substrate: The conical flasks were covered with an aluminium foil and incubated at 37° for one hr, using a shaking water bath incubator operating at 120 revm^{-1}.

The reaction was stopped by adding chloroforms: methanol (2:1 V/V) and the free fatty acids liberated during hydrolysis were estimated by titrating against 0.05N methanolic solution of sodium hydroxide using thymol blue as an indicator [6]. A unit of lipase activity was defined as the amount of enzyme required to release one micro equivalent of free fatty acids per hour under the assay conditions.

Effect of pH and temperature. The effect of pH on lipase activity was determined through pH 4.0 – 8.5 using acetate, phosphate and glycine buffer.

Similarly the effect of temperature on lipase activity was studied at various temperatures ranging from 25 to 55°.

Heat treatment. The experiments were carried out with a soluble enzyme preparation of *M. oleifera* with preincubation for 10 min at various temperatures ranging from 50 to 80°. The lipase activities were estimated as reported earlier.

Effect of various reagents on lipase activity. The relative activities of the lipase were determined in the presence of the determined amounts of reagents indicated in Table 1.

Table 1. Showing the effect of various reagents in given concentration on lipase activity demonstrated in *Moringa oleifera.*

Reagent added	Amount added	Relative activity	Stimulation or inhibition (%)
Control	–	100	–
Acetone	4%	79	(21)
Alcohol	"	65	(35)
Toluene	"	70	(30)
Triton X-100	"	91	(09)
$MnCl_2$	200τ moles	136	36
$CaCl_2$	200 "	104	04
$HgCl_2$	200 "	25	(75)
$CuSO_4$	200 "	74	(26)
EDTA	200 "	89	(11)
Sodium deoxycholate	200 "	69	(31)

RESULTS AND DISCUSSION

In a previous study [4] has been reported the presence of phospholipase B in a crude enzyme preparation prepared from the seeds of *M. oleifera.* This hydrolysed ovolecithin to glycerylphosphorylcholine and fatty acids. In view of the reports by G.H. de Haas [7] and A.J. Slotboom *et al* [8] that purified lipase preparations from pig pancreas and *Rhizopus arrhizus* are capable of hydrolytic activity removing fatty acids from phosphatidylcholine, it was necessary to determine whether the lipase (EC 3.1.1.3) activity reported in the present study was also responsible for the previously observed phospholipase B activity.

Substrates for lipase activities determination are generally prepared in the form of an emulsion in the presence of emulsified agents [22, 23] (polyvinyl alcohol and gum arabic) and these assist the enzyme to be adsorbed to its surface. There are no data which can be used to discuss how substrates are arranged at the oil-water interface in polyvinyl alcohol/gum arabic emulsified system. It is noticed in the present study that 10% gum arabic provides large surface area for enzymatic reaction as shown in Fig. 1. Thus in subsequent experiments 10% gum arabic was used in the preparation of 10% olive oil substrate emulsion.

Fig. 2 shows the time course curve of lipase activity. The rate of reaction was increased with time and was almost linear upto 1 hr. The lipase activity reached a plateau after 6 hrs incubation and then nearly remained constant upto 18 hr. Therefore, in subsequent experiments the incubation time was 1 hr.

An attempt was therefore made to characterize the

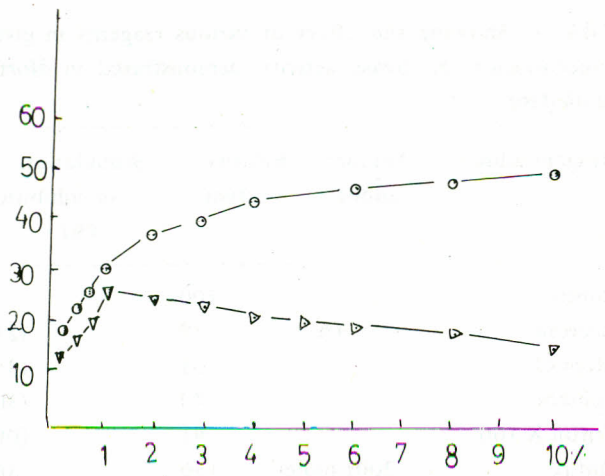

Fig. 1. X-axis Time course of lipase activity. Reaction mixture containing 2.5 ml of enzyme preparation and 2.5 ml of 10% olive oil emulsion were incubated at 37° for different time period at 120 rev m^{-1}.

Y-aix Microequivalents of free fatty acids released per hour.

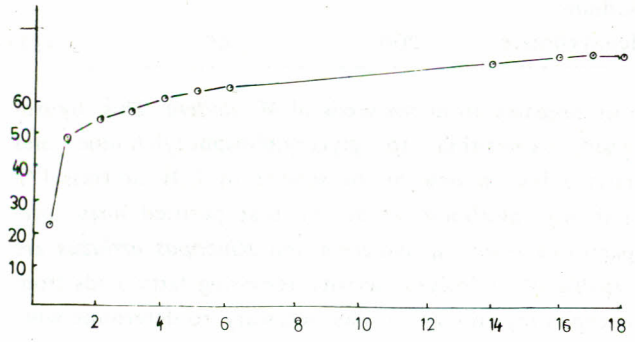

Fig. 2. X-axis Time course of lipase activity. Reaction mixture containing 2.5 ml of enzyme preparation and 2.5 ml of 10% olive oil emulsion were incubated at 37° for different time period at 120 rev m^{-1}.

Y-axis Microequivalents of free fatty acids released.

lipase activity present in the crude enzymatic preparation of *M. oleifera* seeds, by using heat treatment techniques which can be employed to demonstrate the presence of more than one enzyme [9-12]. It was observed that the preincubation of an enzyme at different temperatures ranging from 50-80° resulted in the loss of lipase activity as shown in Fig. 3 and the enzymatic activity was completely lost at 80°. This differs from a previous study [4] in which phospholipase B activity was found to be heat stable even at 98° when it retained 53% of its activity. This suggests the presence of two separate hydrolytic activities, one of them due to lipase, which is heat labile and the other due to phospholipase B which is heat resistant.

The effect of sodium deoxycholate on the crude enzymatic preparation of *M. oleifera* lipase was observed to be inhibitory. A similar observations was made in the

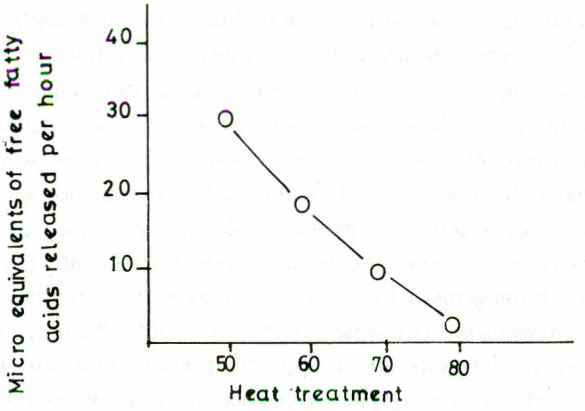

Fig. 3. Effect of heat treatment on Olive oil emulsion by *Moringa oleifera* enzyme, after pre-incubation of the enzyme preparation at different temperatures.

study of phospholipase B activity. It is possible that this is due to modification in the micellar size of the lipid substrate, and the enzyme protein is unlikely to be modified by the presence of sodium deoxycholate [13, 14, 15, 16].

The influence of various reagents on the enzymatic activity of lipase was studied and tabulated in Table 1. The rate of hydrolysis of lipase has been found to be inhibited to varying degrees by the presence of organic solvents which have been used either in the purification of enzymes or have been employed as preservatives against microbial action [17]. The small increase (4%) in the rate of lipase action in the presence of $CaCl_2$ may suggest that the concentration of Ca^{2+} present in the seeds of *M. oleifera* is already optimal because lipase action in the presence of high concentration of Ca^{2+} is reported to be inhibitory whilst in low concentration of Ca^{2+} lipase activity is markedly activated [17, 18]. However, the requirement of Ca^{2+} in this case seems to be obligatory, since when EDTA is present in the reaction the rate of reaction decreases by 11% probably due to removal of Ca^{2+} from the system by chelation [19, 20]. The reasons for the activation and inactivation of enzymatic activity by the substances examined are not clear. However, since the lipase action is suggested to occur at oil-water interface, any parameter which changes this interface, such as surfactants like emulsifying agents and detergents are likely to change the rate of reaction. Also any substance e.g. $HgCl_2$ which can modify the enzyme protein is likely to modify the rate of reaction.

The effect of pH was determined as a optimum pH of 5.0 was found as shown in Fig. 4. The optimum temperature of action was found to be 40° as shown in

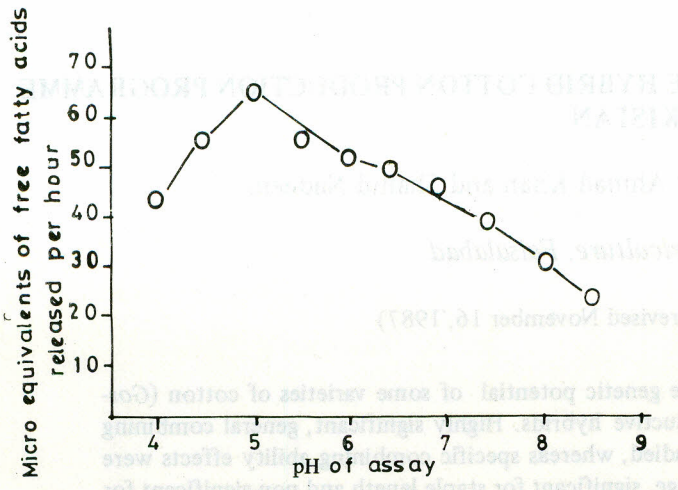

Fig. 4. Effect of pH on Olive oil emulsion by *Moringa oleifera* enzyme preparation.

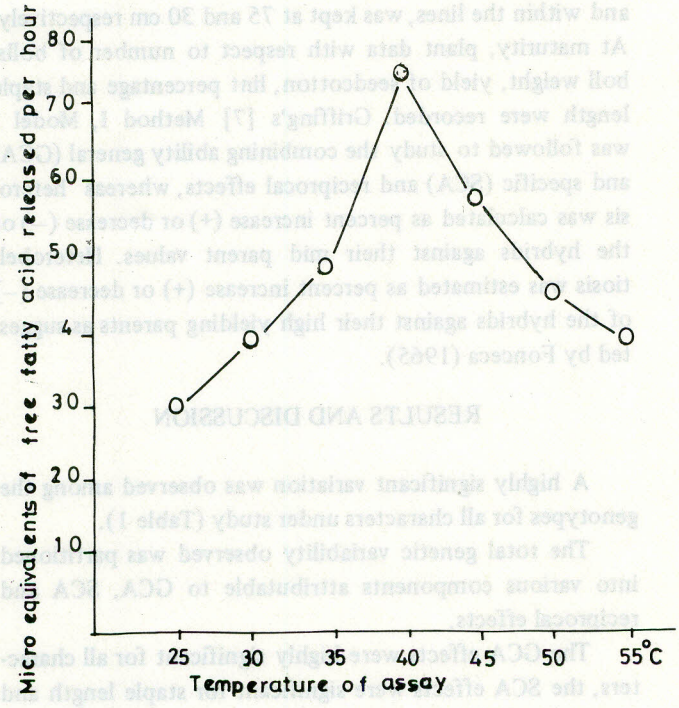

Fig. 5. Effect of temperature on Olive oil emulsion by *Moringa oleifera* enzyme preparation.

Fig. 5. Both of these parameters differed from those found in the phospholipase B activity previously reported [4]. This observations however, is not an adequates criterion for the presence of two different enzymes, since the literature reports that different substrates are likely to have different optimal pH and temperature values [17-21].

Further work on purification leading to the homogeneity of the protein could provide more conclusive results. Work in this direction is in progress.

REFERENCES

1. J.L. Meers, *Chem. in Britain,* **12**, 115 (1976).
2. E.L. Ruban, *Nauka* (Moscow), 195 (1977).
3. D. Chapman, "Introduction of Lipids", McGraw Hill Publishers (1969).
4. A.R. Memon and M. Umar Dahot, *J. Chem. Soc. Pakistan,* **7**, 7 (1985).
5. A.G. Gornall, C.J. Bardwill and M.M. David, *J. Biol. Chem.* **177**, 751 (1969).
6. J.J. Gallai-Hatchard and R.H.S. Thompson, *Biochem. Biophys. Acta.* **98**, 128 (1965).
7. G.H. De Hass, L. Sarda and J. Roger, *Biochem. Biophys. Acta.* **106**, 638 (1965).
8. A.J. Slotboom, G.H. De Haas, P.P.M. Bonsen and L.L.M. Van Deenen, *Chem. Phys. Lipids,* **4**, 15 (1970).
9. D.J. Hanahan, *J. Biol. Chem.,* **195**, 199 (1952).
10. P.V. Subbaiah and J. Ganguly, *Biochem. J.,* 118, 233 (1970).
11. Daniel M. Raybin, Leroy and A. Kernberg *Biochem.,* **11**, 1754 (1972).
12. J. Shiloah, G. Klibansky, A. Devries and A. Berger, *J. Lipid. Res.,* **14**, 267 (1973).
13. S. Gatt, *Biochem. Biophys. Acta.,* **159**, 304 (1968).
14. G.H. De Haas, P.P.M. Bonsen, W.A. Pieterson and L.L.M. Van Deenen, *Biochem. Biophys. Acta.,* **239**, 252 (1971).
15. H. Vanden Bosch, A.J. Aarsman, J.G.N. Jong and L.L.M. Van Deenen, *Biochem. Biophys. Acta.,* **348**, 197 (1974).
16. G.W. De Goede, J. Samallo and G.L. Scherpho, *Biochem. Biophys. Acta.,* **424**, 195 (1976).
17. F. William Shipe Jr., *Arch. Biochem.* **30**, 165 (1951).
18. A.K. Balls, M.B. Matlack and I.W. Tudeer, *J. Biol. Chem.,* **122**, 125 (1937).
19. G.H. De Haas, N.M. Postema, W. Nieuwenhuizen and L.L.M. Van Deenen, *Biochem. Biophys. Acta.,* **159**, 103 (1968).
20. J.F. Uthe and W.L. Magee, *Can J. Biochem.,* **49**, 776 (1971).
21. P.J. Fodor and A. Chari, *Enzymologia,* **13**, 258 (1949). (1949).
22. M.W. Akhter, J.D.E. Patterson and J.A. Blain, Pakistan J. Biochem., *7*, 81 (1974).
23. Y. Ota and K. Yamada, Agr. Biol. Chem., *30*, 351 (1966).