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A Review of Enzymatic Properties of Lipase in Plants, Animals and Microorganisms

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Abstract: Lipases are widely distributed in plants, animals, insects and microorganisms. In plants, lipases are mostly present in food reserve tissues of growing seedlings especially in those which contain large amounts of triacylglycerols. In animals, the lipases are found in pancreas, and on the surface of mucous cells of the gastric mucosa. In insects, these enzymes are found mostly in plasma, salivary glands, muscles and fat bodies. Lipases are versatile group of enzymes that not only hydrolyze the esters of long chain aliphatic acids from glycerol at oil/water interface but also involved in the transesterification reaction. Lipases have preference to hydrolyze triacylglycerol, diacylglycerol and monoacylglycerol to glycerol and free fatty acids. The pH ranges of plant lipase is between 4.0 to 9.0 and of those animals between 5.5 to 8.5 and of microorganisms it ranges some what higher than other two groups i.e, 6.0 to 10.0. The temperature ranges at which plant lipase remain active is between 20 to 38°C and of animals it lies between 37 to 60°C. The microbial lipase temperature is between 37 to 55°C. The plant and animal lipase activate in the presence of calcium and zinc, while they inhibit in the presence of EDTA, Triton X-100 and Tween 80. However, the microbial enzymes inhibit in the presence of $FeCl_3$, $ZnCl_2$ and $HgCl_2$. The molecular weight of lipase in plants ranges between 19000 to 270000. While animal lipase ranges 43000 to 300000. The microbial lipase molecular weight lies between 32000 to 97000.

Keywords: Enzyme, Lipase, Plant Lipase, Lipase Properties, Lipase activities

Introduction

Lipases are special kind of esterases belong to sub class 1 of hydrolytic enzymes class 3 and have been assigned sub-sub-class 3.1.1 due to their specificity for carboxylic acid ester bonds. They have thus been called carboxylic acid esterases and numbered as E.C.3.1.1 according to the classification recommended by enzyme commission of the International Union of Biochemists (Florkin and Stotz, 1965).

Glycerol-ester Hydrolases or triglycerides acyl Hydrolases (EC. 3.1.1.3) are generally known as lipases and may not have positional specificity for the primary ester bonds. Usually lipase hydrolyze triacylglycerols stepwise to diacylglycerols, monoacylglycerols, glycerol and free fatty acids. The most prominent member of the group is pancreatic lipase along with monoglyceride lipase and galactolipase (Brockerhoff and Jensen, 1974). The sequential breakdown of triacylglycerol by lipase to glycerol and free fatty acid is shown in Fig. 1. According to the reaction of lipase shown in Fig. 1,lipase do not attack the secondary ester bond of triacylglycerols and it has been proposed that 1 or 2-diacylglycerol intermediate isomerized to 1 or 3-isomer form and then primary esters are hydrolyzed (Ory, 1969). However, in subsequent studies (Noma and Borgstrom, 1971) demonstrated the sequence of reaction as under:

Triacylglycerol→1,2-diacylglycerol+FFA → 1-or 2 monoacylglycerol+FFA→ glycerol+FFA.

The presence of 1,3-diacylglycerol during the hydrolysis was examined by an acyl transfer reaction. Lipase catalyzes not only the hydrolysis of triacylglycerols but also synthesis of ester bonds by transesterification (Matsuo *et al.*, 1980).

1- Monoacylglycerol+Fatty acid → 1,3-diacylglycerol.

The primary ester group is attached preferentially with 1,2-or 1,3-diacylglycerols and are hydrolyzed at similar rates. The overall reaction is not specific for the position on the glycerol molecules (Noma and Borgstrom, 1971). The enzymatic reaction of lipolysis present in biological system is an example of heterogeneous catalysis. The lipases are perfectly soluble in water but the substrate are not and these reactions usually carried out by making emulsion of the substrate in order to provide a oil-water interfacial area to which the enzyme binds with substrate. The individual catalytic steps occur essentially at the lipid/water interfaces and this unique property is known as interfacial activation (Brockman, 1984). The interfacial activation is associated with conformational change in the lipase molecule (Entressangles and Desnuelle, 1974). Generally, glycerol trioleate (olive oil which contains 70% oleic acid) is used as substrate for the detection or determination of lipase activity and it is acceptable as universal and low cost substrate.

Lipases are widely distributed in plants, animals and microorganisms. In plants mostly lipases are present in food reserve tissues of growing seedlings and especially in those which contains large amount of triacylglycerols (Hill and Beevers, 1987). The storage triacylglycerols are localized in the organelles called lipid bodies (oleosomes, oil bodies, spherosomes) which are surrounded by a membrane (Jacks *et al.*, 1967; Ory *et al.*, 1968; Yatsu and Jacks, 1972). Lipase is the first enzyme in the gluconeogenic pathway, and is associated with the membrane of the lipid bodies where the intracellular lipid is localized (Lin *et al.*, 1983).

Lipase activity in plant seeds increases rapidly after germination of the seeds. In seeds containing oil (mainly triacylglycerols) as storage material, it is obvious that lipase action is essential in germination when reserve (insoluble) triacylglycerols are converted to soluble sugars which can be transported to the growing tissues to supply structural carbon and energy (Galliard, 1980).

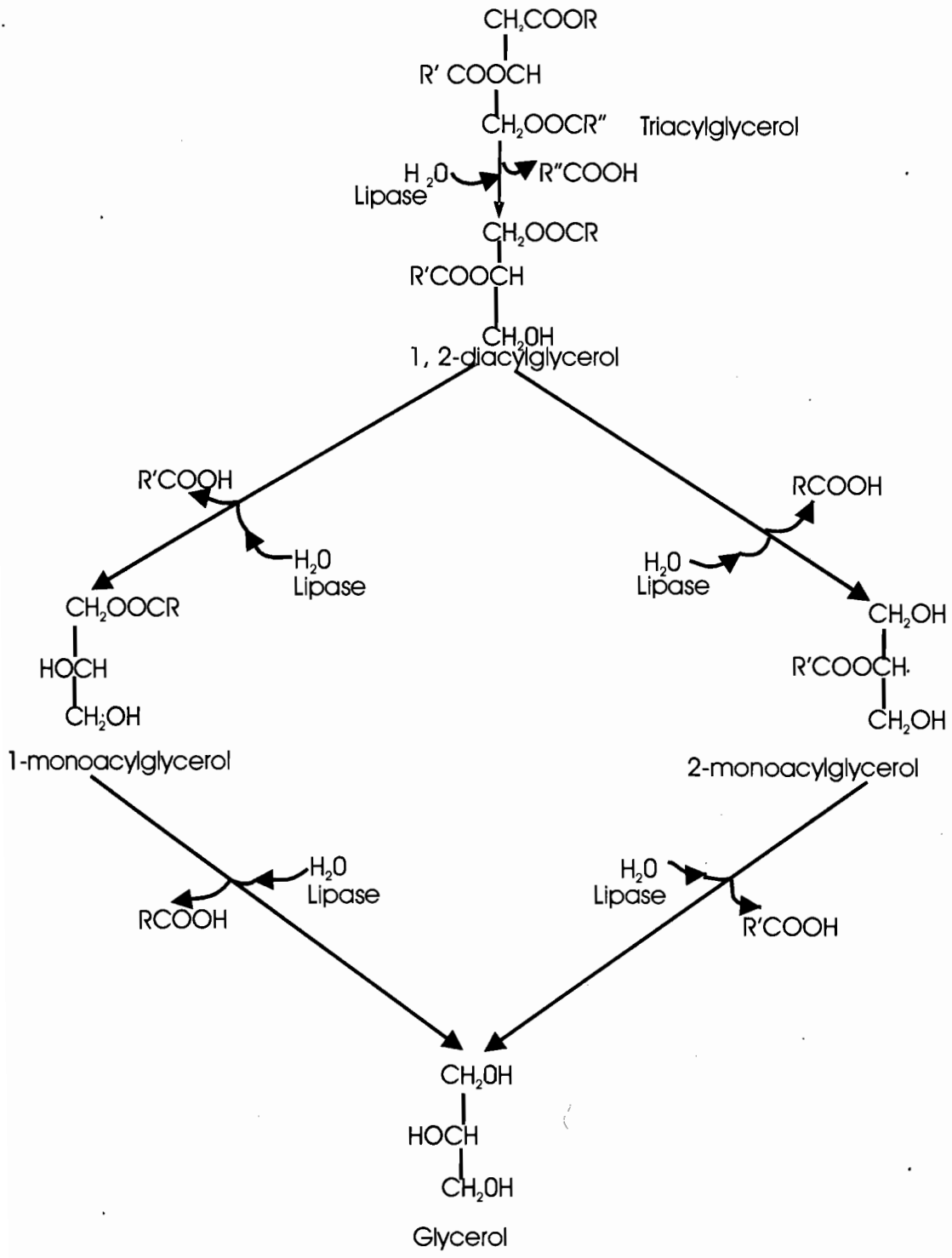

Fig.1: Reaction Mechanism of Lipase

Hill and Beevers (1987) suggested that lipase hydrolyzes triacylglycerol to glycerol and fatty acids which are converted to sugars and support the growth of young plants. Fatty acids released by lipases act as precursors for the phospholipid synthesis necessary to support proliferation of cellular endomembrane systems (Vakharia *et al.*, 1987), which may be their primary role in the germination process (Wilkinson *et al.*, 1984). The growing interest in this area arises from the potential applications of lipase. Among the enzymes so far investigated, lipase is one of the most advantageous because it is stable, inexpensive and widely used in the development of various applications. Lipases are produced from different sources such as plants, animals and microorganisms.

The amount of enzyme is not determined on the basis of its catalytic activity but it is estimated by measuring the decrease in substrate concentration or measuring the product concentration with the passage of time under the use of appropriate method of assay. Enzymologists have developed a number of assay methods in an attempt to throw some light on the complex enzymatic interactions involved in the breakdown of triacylglycerols and other lipids. For both detection and assay of lipases, it is essential that substrate be chosen and reaction conditions arranged so that the defination of a lipase be met i.e. hydrolysis of long-chain acylglycerols at an oil-water interface. Generally lipase is present in low amount in crude subcellular fractions, therefore the assay method should be sensitive and rapid. Mostly assay methods are based on the release of free fatty acids or labelled fatty acids from natural and radioactive substrates. Various methods have been developed to measure free fatty acids such as titration, colorimetric, fluorometric, thin layer chromatography and gas chromatography. An excellent general coverage of lipolytic enzymes, methods of assay and kinetics is given by Brockerhoff and Jensen (1974). A useful discussion of kinetics and assay of enzymes acting on water-insoluble lipid substrate is contained in their papers by (Gatt and Bartfai., 1977 a,b; Aizono *et al.*, 1973; Theimer and Roshinteck., 1978; Lin and Huang., 1984; Dahot and Memon., 1987; Handerson and Osborne., 1991; Ohinata *et al., 1997*).

Applications: Lipolytic enzymes are widely used in the manufacturing process throughout the world in varied and interesting applications. In recent years the growing demand of lipolytic enzymes has been increased due to its potential use in the various manufacturing process of industrial goods such as detergent industry, food industry and medicine, which inspired to search new sources for enzyme isolation. Lipases are incorporated with the products of pharmaceuticals, cosmetics, leather, detergents and perfumery. They are also used in the medical diagnostics and other organic synthetic materials (Boland *et al.*, 1991; Gandhi, 1997; Savendsen, 2000). In medical and therapeutic applications the manipulation of lipolytic activities will probably play a part in future methods for treating malfunctions of fat metabolism and thus control cardiovascular diseases. The pancreatic lipase is necessary for the absorption of fat. Hormone-sensitive lipase is used for the mobilization of fat from adipose tissue. The assay of serum lipase is a clinical tool of some importance in the diagnosis and to check the severity of pancreatitis. Microbial lipases are used in combination with other enzymes to help in the degradation of sewage. An acid lipase has been added to bread dough for the uniform production of monoglycerides which greatly improve the resistance of the bread to staling (Brockerhoff and Jensen, 1974). It is observed that uncontrolled lipolysis during milk processing can cause flavor defect and accumulation of free fatty acids in the extraction of cotton seed oil due to undesirable action of lipases requiring extra operation for their removal prior to marketing (Chapman, 1969). Godfrey and Reichelt (1983) also reported the use of lipase in a variety of industrial process such as removing of fat stains from fabrics, accelerating maturation in cheese and degrading fats in waste products.

Plant Lipases: Lipase activity has been identified in the various tissues of plants but relatively high concentration is found in seeds. Seeds are generally rich in triacylglycerols, which serve as compact source of energy for the newly emerging plant. During germination of the seed, the reserved triacylglycerols are disappeared, since the fatty acids can not be oxidized to provide energy until they are released from the triacylglycerol. Lipases are probably rate controlling during germination and the activity of the lipase is high during germination (Brockerhoff and Jensen, 1974).

Lipase Activity in Cereal Seeds: Cereal seeds contain significant amount of triacylglycerols normally concentrated in germ (embryo) and outer layers of the grain, although oats (5-9% oil) are unusual in having most of the oil in the endosperm. Because lipolytic activity in these seeds causes major problems in their commercial use, the responsible enzymes have received attention, particularly in wheat, rice and oats (Galliard, 1980).

Lipase play a central role in the release of fatty acids from the reserve triacylglycerols of lipid bodies in the aleurone and scutellum of germinating cereals. Lipase are synthesized *de novo* on free ribosomes in the scutellum of germinating maize and are translocated to the membrane of the lipid bodies where they remain bound (Wang and Huang, 1987). Lipase synthesis in the aleurone is more complicated. Significant lipase activity is detected in isolated barley aleurone layers before gibberellic acid application, but preliminary imbibition of half grains may induce its synthesis (Fernandez and Staehelin, 1987).

Lipase activity in rice is concentrated in the outer layer of the grain and causes problems in the storage (Galliard, 1980). Aizono *et al.* (1973) have reported the enzymatic properties of rice bran lipase that maximum activity was observed at pH 7.5 and 8.0, but stable over the pH range from 4.0 to 9.0. The optimum temperature was 37°C and the enzyme was stable upto 40°C. The enzyme was activated by calcium ion (<0.01M) but inhibited with 0.1M. Lipase activity was also strongly inhibited by EDTA. It was more specific for glycerol than alkyl alcohol. Moreover, the enzyme is capable of hydrolyzing the olive oil, rice bran oil, and coconut oil, in addition to synthetic triacylglycerol and triolein. The hydrolysis rate was maximum at C_4 and decreases until C_{18}, among these triolein was hydrolyzed at the lowest rate. The enzyme catalyzes the hydrolysis of short chain ester bond faster than in longer ones. The hydrolysis of 2-oleo -1,3-distearin resulted in the formation of monoacylglycerol, 1,2-diacylglycerol; 1,3-diacylglycerol and fatty acids. Rice bran lipase preferentially splits fatty acid at the 1,3-position of substrate. Matsuda and

Hirayama (1979) further studied the enzymatic properties of a galactolipase from rice bran, that its molecular weight was about $4x10^4$ and the Km value 0.34mM for monogalactosyl diacylglycerol. The enzyme exhibited maximal activity at pH 7.5 in a Tris-HCl buffer and its optimum temperature was 35°C. It losts 95% of its activity at pH 7.5 at 50°C. The enzyme activity was inhibited by EDTA, Triton X-100, acetyltrimethylammonium chloride, NaCl and sodium dodecyl sulfate. Whereas, the activity increased in the presence of Ca^{2+} and sodium deoxycholate. Maximum hydrolytic activity was found with digalactosyldiacylglycerol. Monogalactosyldiacylglycerol and its lysolipids were hydrolyzed at about two-third of the rate of digalactosyldiacylglycerol. Cysteine, mercaptoethanol, and diisopropyl flourophosphate inactivated the purified galactolipase. It catalyzes the hydrolysis of the fatty acid ester at C_1 and 2-positions of the galactolipid. Funatsu *et al.* (1971) carried out the biochemical studies on rice bran lipase. The purification sequence showed a 480 fold increase in specific activity. The purified enzyme was elctrophoretically homogeneous by polyacrylamide disc gel electrophoresis and ultracentrifugation. The isoelectric point of the enzyme was found 8.56 by ampholine electrophoresis and the sedimentation coefficient was 2.97s with maximum and minimum absorption at 278 mμ (nm) and 250 mμ (nm) respectively. The molecular weight of lipase was estimated 40,000 and the value of extrapolation to zero time was 0.800 and 0.835. The optical rotatory dispersion (ORD) constant values of a_o and b_o at 239mμ (nm) were -164 and -123, respectively. Fujiki *et al.* (1978) also studied the chemical properties of rice bran lipase that the enzyme was comprised of disulfide linked sub units with molecular weight of 32000. Rice bran lipase hydrolyze long chain triacylglycerols and rice bran oil less readily than triacylglycerol of short chain (C_2 - C_4) fatty acids.

The lipolytic enzyme activity of buckwheat was 880 pmol/min/mg which was 7 times higher than wheat flour. The water activity above 0.28 did not depress the lipolytic enzyme activity during 1 month storage at 25°C. The activity was inhibited at 120°C within 7 sec. and increased free fatty acids.

The accumulation of free fatty acids in buckwheat during storage are mainly caused by lipase (Ohinata *et al.*, 1997). Tavener and Laidman (1972) have reported the induction of lipase activity in the germinating wheat grain. They found that the maximum lipase activity was upto 6-day of germination in the starchy endosperm. Lipase activity in the tissue of endosperm halves incubated with 1 mM hydroxylamine was optimum at 4th day. In endosperm halves, lipase activity can be induced in the starchy endosperm by glutamine and hydroxylamine. The activity in the bran of endosperm halves is induced by indol acetic acid in the presence of nitrogen sources. Both of these induction processes are inhibited by energy metabolism inhibitors, RNA and protein synthesis. The bran pre-incubated with cytokinin, hydroxylamine and indol acetic acid were able to induce lipase activity slowly, they further studied.

Oat contain a true lipase that hydrolyzes triolein and tributyrin. This is an alkaline lipase (optimum pH 7.4) first described by Martin and Peers (1953). They further reported that the lipase activity of oats was found mainly in the epidermal layers. It is important to know that it is essentially a property of the seeds, and does not arise from subepidermal fungal mycelium, which is known to occur in some specimens of grain. This lipase has been purified some 2000 fold on a dry matter basis compared with the activity in the original oat meal. The purified preparation has an optimum temperature of 37-38°C, and a Michaelis-Menten constant (Km) of 0.006M when tributyrin is the substrate. The purified lipase splits off one butyric acid radical only from tributyrin and does not hydrolyse the various mono- and di-butyrins at pH 7.4 and 37°C.

Lipase activity of *Cajanus cajan* L. (matri) was found maximum upto 1 hr and then declined. The rate of reaction was increased upto 20% enzyme solution with 10% substrate concentration (Khan *et al.*, 1991; Dahot *et al.*, 1989). The maximum activity was found at pH 5.5 and at temperature of 30°C. The enzyme was activated in the presence of Ca^{2+} and Zn^{+2} but it was inhibited with EDTA, Mn^{2+}, Co^{2+} and mercaptoethanol (Khan *et al.*, 1991; Noomrio *et al.*, 1990; Dahot *et al.*, 1989). The enzyme was highly stable upto 40°C. The activity was thermostable and retains 3% activity at 90°C within 10 min. The lipase activity of *Cajanus cajan* was highly specific towards tristearin (Dahot *et al.*, 1989). Dialysed sample of *Cajanus cajan* seeds was purified on Sephadex G-100 column and it was separated into four fractions (I, II, III, IV) which were subjected to SDS-disc gel electrophoresis. Fraction II was found homogeneous and the molecular weight was estimated 19000 by SDS-polyacrylamide disc gel electrophoresis. The optimum temperature of *Cajanus cajan* seed lipase II was found 20°C and pH 5.0. The enzyme was found fairly stable upto 40°C but more than 20% activity remained active after 10 min incubation at 98°C (Noomrio *et al.*, 1990). Since the lipase is intimately linked to the lipid both physically and metabolically, Wang *et al.* (1984) suggested a difference in the genetic control of the gluconeogenic enzymes and a co-selection for high lipid content and high lipase activity through breeding. The lipase of the lipid bodies and the enzymes of the glyoxysomes are under separate genetic control. A noncoordinate expression of their activities occur in the high and low lipid maize lines. Selection for high lipid in maize lines apparently also selects for high lipase activity but not the subsequent gluconeogenic enzymes. The mechanism of coordinate selection for both high lipid and high lipase activity is unknown. There are about 50 genes for the expression of high lipid in illinosis high oil (Lin *et al.*, 1983) and they are only expressed only in kernel formation and not in seedling growth. Yet, lipase activity is absent in the forming and ungerminated kernel and appears only after germination (post germinative growth). It is unlikely that the genes for high lipid content are tightly linked to the lipase genes. It is possible that the high lipid genes and the lipase genes are both expressed in kernel formation but the later expression results in the production of pro-lipase protein or mRNA, which is processed to active lipase in post germinative growth. Alternatively, the lipases synthesized or degraded in post germinative growth in proportion to the availability of substrate and thus metabolic need.

Lipase Activity in Oil Seeds: During germination of oil seeds the utilization of the storage fats is initiated by the stepwise hydrolysis of the triacylglycerides to free fatty acids and glycerol (Stumpf, 1976). Huang and Moreau

(1978) studied lipolytic activity in a range of germinating oil seeds. With the exception of castor bean, none contained acid lipase activity, but all had an alkaline "lipase" activity in their storage tissues. During the germination of peanuts, the lipase activity was increased rapidly and it was highest at pH 9.0. The subcellular localization of alkaline lipase was studied by using sucrose gradient centrifugation of peanut cotyledon homogenate of 3-days old seedlings. About 60% of enzyme was found to be associated with glyoxysomes, 15% with the mitochondria, and 25% with a membrane fraction. The glyoxysomal lipase hydrolyzed only monopalmitin, where as the enzyme associated with the mitochondria and the membrane fraction hydrolyzed equal amount of tri; di- and monoacylglycerol. Theimer and Rosnitschek (1978) have studied the intracellular localization of lipase activity in *Brassica napus* L. (rape seed) cotyledons. The growth of the seedlings was observed in the light and in the dark. The higher lipase activity was found at 4th day of seedling growth when crude homogenates of the cotyledons produced about 5 µmol of fatty acids/min/pair of cotyledons with sunflower oil as a substrate at 37°C with pH 9.0. The maximum activity was observed at pH 8.5 with N-methylindoxyl-myristate as a substrate. Significant effect of light was not observed on the development of lipase activity from the cotyledons after fat utilization but it may be dependent on nitrogen nutrition of seedlings. Crude homogenates by sucrose density gradient centrifugation yielded two major and one minor visible protein bands in the gradients. About 90% of the lipase activity was associated with a microsomal membrane fraction by sucrose solution. One major protein band was recovered from the gradients at a density of 1.085 kg/l and lipase activity was maximum in this protein band. In a later study Rosnitschek and Theimer (1980) have studied some properties of a membrane bound triacylglycerol lipase of rape seed cotyledons. The apparent Km of the lipase reaction was 6.5 mmol/l with sunflower oil. About 25% of the total radioactivity was lost from the triacylglycerol within 60 min and 23.5% were recovered from the fractions representing 1,2- and 1,3-diacylglycerol, monoacylglycerol and free fatty acids. In addition, increasing amount of free glycerol accumulated in the text mixture, indicating that the lipolytic membranes of rape seed cotyledons are capable of splitting triacylglycerol into glycerol and free fatty acids. The rate of hydrolysis was highest with sunflower oil followed by linseed oil. In the presence of linoleic acid or oleic acid in sunflower oil, lipase activity was completely inhibited. Erucic acid, a major component of rape seed oil blocks the lipase activity. The microsomal lipase of rape seed cotyledons is also affected by the presence of inorganic ions and detergents in the assay mixture. Lipase activity was increased in the presence of 0-0.1 mol/l NaCl and above this concentration the system was obviously saturated with respect to the salt. Pre-incubation of the lipolytic membrane fraction in 0.15 mol/l NaCl solution increased lipase activity while sodium deoxycholate and $CaCl_2$ stimulated the activity; and EDTA, Triton X-100 and Tween-85 also inhibited the activity. The lipase activity was present in the soluble cell fraction and the oil body fraction of cotyledons of 4-day-old rape seed. Optimum activity was obtained with triolein emulsion as substrate at pH 9.0. Addition of deoxycholate to the triolein substrate emulsion improves the stabilization of the surface of the lipid globules and shifts pH optimum to pH 8.0. In contrast, rape seed oil emulsified in gum arabic was a poor substrate for the soluble lipase. The apparent activity of lipase was enhanced with the addition of deoxycholate to the emulsified rape seed oil and fatty acids. 1,2 or 2,3-diacylglycerols, and monoacylglycerols were produced at alkaline pH values. The autolysis of a crude oil body fraction demonstrating the lipolytic activity associated with these subcellular compartments by the liberation of free fatty acids from the native neutral lipids with maximum activity at pH 5.0 (Hoppe and Theimer, 1997a). Lipase prepared from 4-day old rape seed seedlings hydrolyzed the triacylglycerols of the native oil bodies. Native oil bodies purified from cotyledons of rape seed started to show autolysis between day 1 and 2 of germination, with optimum activity at pH 5.0. While optimum activity of the cytoplasmic (soluble) lipase was detected at pH 7.0 with native oil bodies as substrate. However, such lipase catalyzed degradation was found only with oil bodies isolated from seedlings at least 2 days old, but not with oil bodies obtained from dry seeds or 1-day old seedlings. The inhibition of the action of lipase is caused by the chaff unit membrane, surrounding such oil bodies. These data cast doubt on the role of the prominent oil body proteins (oleosins) in anchoring lipase. It is proposed that the interaction of lipase with the surface of the oil bodies relies on negatively charged constituents of the oil body coat, which are different from the oleosins. In early germination the activity of acylester acyl-hydrolase rises prior to lipase activity, it should be assumed due to the initial enzyme in storage oil mobilization in germinating rape seeds (Hoppe and Theimer, 1997 b).

The free fatty acid level of palm oil is a major determinant of quality and commercial value. It is therefore important to know the reasons why fatty acids appear, and lipase of various kinds have been implicated. According to Hartley (1967) oil palm mesocarp contains a very active endogenous lipase. Desassis (1957) suggests that as much as 40% of the triglyceride present in mesocarp could split in 15 min. Purified fungal lipases splits fatty acids from triacylglycerides (Tombs and Blake, 1982) at the rate of the order of 2000 µmol min^{-1} mg^{-1}. If the specific activity of the enzyme in mesocarp was similar to that of fungal lipases, the remarkably high rate of fatty acid production would have needed about 1% lipase in the mesocarp tissue. The mesocarp contains only about 1% protein and it seems unlikely that the whole of this would be a lipase. Most fruit contain only a few percent fatty acids and the level is variable. This has been attributed to variable activation of the supposed endogenous lipase by for example, bruising of the fruit (Coursey, 1963). The origin of the idea that oil palm mesocarp contains a powerful endogenous lipase is attributed to Desassis (1957) and Fickendey (1910). This is hardly conclusive evidence for an endogenous lipase, since heating to 100°C would eliminate lipases from any source, as well as minimising the microbial population. It is generally accepted that fungi can indeed bring about lipolysis of oil palm mesocarp (Hartley, 1967; Coursey, 1963; Fickendey, 1910). Handerson and Osborne (1991) have detected lipase activity in ripening and mature fruit of the oil palm extract. The lipase activity was inactivated *in vitro* above 0°C but *in vivo* the enzyme activity

increases as ripining proceeds. The highest activity was present in the ripest fruit coincident with the abcission of the fruit base from the pedicel. Activity rose from background values in the unripe fruit to just detectable in fruit that were first synthesizing carotene and lipid (Ca 125-130 days after synthesis).

Lipase activity of this aqueous layer was always lower than that of the equivalent of frozen mesocarp powder. The enzyme activity of both aqueous extracts and mesocarp powder decreased after 30 min incubation with the increase of assay temperature from 18 to 35°C and this was assumed to be associated with a greater instability of the lipid micelles at these temperatures. Tween 20, Tween 80 or Triton X-100 in the extraction medium and cocktail increases stability of enzyme activity. Tween 20 was found to be superior for the retaining activity. To improve extractibility and stability of the enzyme by increasing ionic strength were unsuccessful. At 1.0M NaCl, enzyme activity was reduced by 30% in the lipid layer and by 50% by 200mM in the aqueous layer. Addition of Ca^{2+}, papain or PMSF also failed to enhance or prolong the lipase activity in mesocarp powder or in lipid or aqueous layer of the homogenates. Detection of enzyme activity from mesocarp powder [^{14}C] oleat hydrolysis product increase with time. The reaction is linear for at least 20 min at 18°C and the rate of reaction increases with increasing concentration of the glycerol tri [1-^{14}C] oleat substrate in the medium. Maximum product released per 30 min assay occurred at 6mg from mesocarp powder but inhibition was not observed with increasing sample weight. The lipase activity of ripe fruit is sensitive to chilling, which inactivate enzyme activity at 8°C without restoration of activity at 25°C. However, enzyme activity is stable at least 8 days in fruit held continuously at 20°C or for short period (30 min) at 45°C. The addition of lipid present in the tissue of lipase inactivated ripe fruit neither enhances activity from unripe mesocarp or reduces the activity from ripe mesocarp. An active lipase enzyme is indeed absent from unripe fruit. Oo and Stumpf (1983) have reported the presence of lipase activity in the *Elaeis guineensis* (palm oil) seedlings. An active lipase was found to be present in shoot extracts during germination and reached highest after 18 to 21 days. The optimum pH for lipase activity was found to be 6.2 and enzyme was more active with monopalmitin in comparison to other substrates.

Afolabi *et al.* (1991) have analyzed eight seed oils from *Vernonia galamensis, Ximenia kaffra,* castor, corn, soybean, palm kernel, sunflower and olive as a substrate for the action of lipase (acetone powder) followed by methylation using diazomethane in ethyl ether. The *V. galamensis* lipase (acetone powder) is probably nonspecific in the hydrolysis of triacylglycerols. The reaction products were analyzed by gas chromatography and mass spectrometry and the presence of different fatty acids, confirm the lipolytic hydrolysis of the lipase (acetone powder). The lipolytic hydrolysis of triacylglycerol probably does not result in isomerization because the resulting fatty acids are unaffected during hydrolysis. The fatty acids obtained are representative of the fatty acid spectrum of the parent oil.

In the scutella of corn, lipase activity is present in the lipid bodies of germinated but not ungerminated seeds. It has an optimal activity at neutral pH values, and is highly specific for the native corn triacylglycerols. Lin *et al.* (1983) have reported that the lipase activity in lipid bodies increased during seedling growth, parallel to the increase of catalase activity. At 6th day, the lipase activity releases 47 nmol fatty acid /min/scutellum at 34°C. The lipid bodies were isolated from ungerminated seeds and the maximal autolytic activity was found at pH 7.5. About 50% of the lipase activity was recovered in the lipid bodies, and the rest was distributed among other subcellular fractions. The optimum activity was found with trilinolein, or N-methylindoxylmyristate at pH 7.5. Lipase activity was slightly enhanced in the presence of 0.05 M NaCl and was reduced by $CaCl_2$, EDTA and phosphate. The enzyme activity was completely lost by Triton-X and SDS. The lipase was found active on trilinolein and triolein but it was totally inactive on tristearin, tripalmitin, trierucin, triarachidin and tribehenin. The corn lipase was purified by DE-52 ion exchange chromatography and sucrose density gradient centrifugation from isolated lipid bodies. Only one protein band was detected by SDS PAG electrophoresis. The molecular weight was 65,000 by electrophoresis but 270,000 by sucrose density gradient centrifugation. The lipase was purified about 272 fold. Total proteins are associated with the membrane of the isolated lipid bodies but the lipase contained a substantial amount of neutral amino acids (Lin and Huang, 1984).

Maeshima and Beevers (1985) have purified glyoxysomal lipase from castor bean glyoxysomal membrane by PEG fractionation, CM-cellulose and hydroxylapatite chromatography. Only one band was visible after SDS-polyacrylamide gel electrophoresis and the molecular weight was estimated to be 62,000. The reaction was stimulated by Na^+ and K^+ salts. Deoxycholate, octylglucoside and Triton-X 100 inhibited the reaction. Triricinolein (the endogenous lipid) was hydrolyzed by the purified enzyme. However, when intact glyoxysomes in 0.5M sucrose were incubated in trypsin the lipase activity was rapidly inhibited, but the isocitrate was scarcely affected. An antibody preparation raised in a rabbit against the purified enzyme, inhibited the purified enzyme in glyoxysomal membranes. Ory *et al.* (1962) reported that the lipase activity was completely inhibited by mercuric ion and PCMB, but the inhibition was reserved by cysteine and EDTA. Ory (1969); Huang and Moreau (1978); Muto and Beevers (1974) studied the dry seeds of castor bean, and reported that little or no activity of lipase was observed but highest activity was found at pH 9.0 during the seed germination. Castor bean contains two type of lipase activities such as acidic and alkaline in nature. In dry castor bean, an acidic active lipase was present but it was disappeared completely after 6th day of germination. In contrast alkaline lipase was absent in the dry seeds but its activity increased markedly after 6 days of germination. Muto and Beevers (1974) observed that total lipid of the castor bean endosperm remains unchanged until the beginning of day 3, and completely disappeared at day 7. Simple lipid comprised essentially all of the lipid until day 5. The enzyme in the fat layer shows optimal activity at pH 5.0. The Km values of acid and alkaline lipases for N-methylindoxylmyristate were 1.67 and 0.23 mM, respectively.

Teissere *et al.* (1995) purified the fatty acyl-ester hydrolase from post germinated sunflower seeds. Fatty acyl ester hydrolase was not detectable in dry seeds of sunflower using various p-nitrophenyl caprylate or

emulsified sunflower oil as substrate. After imbibition of seeds enzyme activity slowly developed in cotyledon and was maximal after 5th day. The triacylglycerol level was began to decrease after one day and showed a continuous decrease through out the post germinating period. One main activity peak was eluted upon performing Macro-preparative anion chromatography and one band was visible under SDS-PAGE analysis. The molecular mass was 44 kDa and purification factor was close to 615 with a yield of 6.7% and was found homogenous by SDS-PAG electrophoresis. The total number of amino acids was 459 for a molecular mass of 45 kDa. The glycine and serine contents were high. The maximum absorbance of the protein was at 277.5nm at pH 8.0. The purified acyl-ester hydrolase was very stable at 30°C and maximum activity was obtained at pH 8.0 using p-nitrophenyl caprylate as substrate. o-phenanthroline, Mg^{+2}, Ca^{2+}, or Mn^{2+} had no effect on the enzyme activity but EDTA had only a very slight inhibitory effect at a concentration of 10mM. The DTT, DTNB and PMSF efficiently inhibited lipase activity but the activity was enhanced with 1% Triton X-100. The enzyme was capable to hydrolyze various p-nitrophenyl esters and highest rate was obtained with p-nitrophenyl caproate.

Five bacterial lipolytic enzymes thought to constitute a different group from the classical lipases and esterases. The occurrence of fatty acyl-ester hydrolase activity in post-germinated sunflower is due to a *de novo* synthesis of the protein (Beisson, 1997). Albasi *et al.* (1997) have investigated the influence of the enzyme and the substrate concentrations (sunflower oil). The interfacial area was modified by changing the volume fraction of oil, while keeping other variables constant. A phase inversion is indicated, which influenced the reaction kinetics by the possible formation of enzyme multilayers, due to stearic hindrance.

Lipophilization of soy proteins isolates, containing high amount of lysine residues was achieved by biocatalysis. Reactions have been carried out at 60°C, when the acylation was carried out by lipozyme without solvent. A level of 60, 33 and 42% of lysine residues were covalently attached by capric, lauric and oleic groups, respectively. The lipophilization was achieved by *Rhizopus arrhizus* lipase in the presence of tertiobutanol, a level of 50% of lysine residues were acylated by a mixture of palmitic and stearic acids (Roussel *et al.*, 1997).

Lipase Activity in Other Plants: Dahot and Memon (1987) have reported some properties of *Moringa olifera* (sohanjro) seed lipase. The optimum pH of crude enzyme extract was found 5.0 and temperature at 40°C. The rate of enzyme reaction was increased in the presence of $MnCl_2$ and $CaCl_2$ while it was inhibited with $HgCl_2$. Lipase activity was completely lost at 80°C within 10 min.

Akhtar and Kausar (1978) have reported the lipase activity in the resting and germinating states of *Cucumis melo* (garma seeds). The enzyme exhibited two optimum pH i.e 4.0 and 6.0. The activity at pH 6.0 was found to be stable as compared to the pH 4.0. In 24 hr 60% activity was lost at pH 6.0 and 70% at pH 4.0. The lipase activity was increased alongwith increase in germination period. The lipase activity decreased from tip to the base of primary root. Maximum activity was observed in the apical portion while the optimum temperature for the lipase activity was found to be 40°C. The enzyme was stable upto 40°C. At higher temperature the activity was rapidly lost. The enzyme was activated by low concentration of calcium and sodium ions. The garma seed lipase showed specificity towards ricinoleic acid glycerides of castor oil. *Cucumis melo* seed lipase showed greater activity in ethyl methyl ketone as compared to di-isopropyl ether and n-heptane.

Kausar and Akhtar (1979) have reported the isolation and characterization of *Hibiscus cannabinus* (kenaf) seed lipase. The maximum extraction of the enzyme was achieved at pH 7.0 with 39% recovery but in the presence of 0.5 M NaCl the recovery was 57%. The enzyme was most active at pH 5.0. The enzyme was found to be stable around pH 4.0 to 6.0 at 40°C. The optimum temperature for lipase activity was found to be 37°C. The enzyme activity was completely lost at 80°C within 1 hr. EDTA completely inhibited the enzyme activity while $MgCl_2$ and $CaCl_2$ significantly activated the enzyme activity. Where as, NaCl and ascorbic acid have no significant effect on the lipase activity. The maximum activity was observed against tributyrin.

Carica papaya latex is the principal source of the protease papain but this also contains lipase, which catalyzes the lipolysis of triacylglycerols. Latex lipase has a selectivity for short-chain acyl groups as well as a 1,3-diacylglycerol. These selectivities can be used in the synthesis of structured triacylglycerols. The latex lipase-catalyzed reactions specifically used in the synthesis of low-calorie triacylglycerol analogs (Foglia and Villeneuve, 1997).

Pahoja *et al.* (2001) have reported the characteristic properties of lipase from *Caesalpinia bonducella L.* seeds. The maximum activity was found at pH 7.0 and at 30°C and the pH stability was observed in between 6 to 7.5. Lipase activity was fairly stable upto 60°C and retaining 90% activity. Where as the activity was completely lost at 90°C within 10 min. *Caesalpinia bonducella L.* seeds was found specific towards coconut oil and the activity was slightly increased in the presence of calcium but the enzyme activity was decreased by the addition of sodium deoxycholate, Tween 80 and Triton X-100. Mala and Dahot (1995) also studied the lipase activity of *Carissa carandas* fruit and reported that the optimum pH of crude enzyme extract was found 5.0 and temperature at 30°C. The lipase activity was found heat labile and was completely lost at 80°C within 15 min. The rate of enzyme reaction was increased in the presence of calcium but decreased by the addition of EDTA, PMSCL and o-phenanthroline.

Animal Lipases: Pancreatic lipase can serve as a model and prototype for their digestive lipases, lipase of the plants and microorganisms and to a considerable extent, for the mobilizing lipases of tissues. In addition, the enzyme has found extensive use as a research tool in the field of lipid chemistry and biochemistry owing to the fact that specifically hydrolyzes the esters of primary alcohols. This property has been much exploited in the analysis and synthesis of fats and other glycerides (Litchfield, 1972). Pancreatic lipase was one of the earlier enzymes to be recognized by Claude Bernard in 1856. The purification of lipase has usually been carried out from the dehydrated and defatted acetone powder of pig pancreas. The lipase was appeared in the first protein fraction that emerges from the column and having apparent molecular weight of 300,000 (Downey and Andrews, 1965). Dietry fats affect health and

disease. The assimilation of dietary fats into the body requires that they can be digested by lipases. One lipase, (pancreatic triglyceride lipase) is essential for the efficient digestion of dietary fats. Pancreatic triglyceride lipase is the arch type of the lipase gene family that includes two homologues of pancreatic triglyceride lipase, pancreatic lipase-related proteins 1 and 2. Recently, important advances have been made in delineating the mechanism of lipolysis. The cDNA sequences encoding pancreatic triglyceride lipase and the related proteins have been described. The tertiary structure of human pancreatic triglyceride lipase has been determined alone and in a complex with colipase, a pancreatic protein required for lipase activity in the duodenum. This structural information has allowed the rational design of site specific mutants of pancreatic triglyceride lipase. Together with the structural information these mutants have greatly advanced our understanding of the molecular details governing lipolysis (Lowe, 1997). Porcine pancreas lipase was immobilized on polyacrylamide beads possessing carboxylic functional groups and this was activated by a water soluble carbodimide. The maximum activity was obtained at pH 8.9 and the apparent optimum temperature for the immobilized enzyme was about 7°C, higher than that for the soluble enzyme. The immobilization stabilized the enzyme against heat and urea treatment. Cross linking of the immobilized enzyme with glutaraldehyde or 3,5-difluoronitro-benzene also improved the thermal stability (Bagi *et al.,* 1997). The pure dromedary pancreatic lipase (DrPL) is a monomer and has a molecular mass of about 45kDa. This enzyme hydrolyzes more efficiently the shorter chains than the long chains of triacylglycerols. The lipase activity was maximum at pH range 8.0. Dromedary pancreatic lipase is inactivated at 60°C and is not stable at pH less than 5. The natural detergents (NaTDC), synthetic detergent (Triton X-100) or protein (bovin serum albumin) act as potent inhibitors for DrPL activity. The addition of colipase in the hydrolysis system is necessary when lipase is inhibited by synthetic detergent or protein. The addition of colipase and NaTDC is also required to restore the DrPL activity (Mejdoub *et al.,* 1997).

The strong enzymatic activity was localized on the surface of mucous cells of the gastric mucosa in animals at postpartal day 1 after ingestion of milk. Activity of gastric lipase persisted as long as animals were nursed. No gastric lipase could be demonstrated in weaned and adult cats. Lingual lipase was not found at any developmental stage. It may be suggested that in the new born cat, lipase of the gastric mucosa is responsible for milk fat lipolysis (Knospe and Plendl, 1997). The activity of pregastric lipase and primary pregastric lipase against tributyrylglycerol has been determined over a range of pH and temperatures. Optimum pH was ranged from 5.6 to 6.5 for goats and from pH 5.5 to 6.2 for kids. The maximum temperature ranged from 43 to 60°C for kid lipase, which extended slightly below pH 5.5. The enzyme was also used as catalysts for the hydrolysis of mono acid triacylglycerols (C-4:0 to C-12:0) at temperature 40°C and pH 6.5 and it was found maximum against tributyrylglycerol. Anhydrous milk fat was hydrolyzed by the commercial extracts of pregastric lipase of goats and kids, and the resulting profiles for free fatty acids were very similar to one another (Lai *et al.,* 1997). The enzyme found in the gastric juice of the animal is capable to hydrolyze the primary ester bonds of triglycerides with optimum pH 7.0. Molecular weight of enzymatic protein determined by the gel filtration was 43000. The specific activity of the gastric juice lipase at pH 7.5 and 37°C was 1.2 unit. Similar to porcine pancreatic lipase, the enzyme hydrolyzes tributyrin twicely than triolein. According to their behaviour on gel filtration and ion exchange chromatography, tributyrinase and lipase are identical. The enzyme is insensitive to DFP and to surface denaturation by the emulsified substrate but it can be protected by bile salt and albumin (Brockerhoff, 1971).

Insect Lipases: The lipases in the eggs from the southeren corn root worm were inhibited by DNP (10^{-5}M) in the presence of the substrate (Krysan and Guss, 1971). The flight muscles of many insects seem to contain lipases especially active against diacylglycerols (Crabtree and Newsholme, 1972). Nandanan *et al.* (1973) have reported the properties and distribution of extra digestive lipase in the female pupa of *Trogoderma granarium* which hydrolyses triacylglycerols. The homogenate of alimentary canal and the rest of the body tissues were assayed for lipase activity separately. Lipase activity was not detected in the alimentary canal while the rest of the tissues contained lipase activity. The enzyme activity in the rest of the body tissues decreased slightly when the alimentary canal was added to it. The rate of hydrolysis was increased almost linear with time upto 60 min. The optimum pH for lipase activity was 7.6. However, a second peak seemed to appear in the acid range of pH 5.0 and the activity was considerably reduced from pH 8.6 onwards. The optimum temperature for the enzyme activity was 37°C and the rate of hydrolysis increased steadily with increase in the enzyme concentration. The Km value was determined 3.10×10^{-4}M. The heavy metal ions like Hg^{2+} did not inhibit the enzyme activity. Calcium did not either activate or inhibit the activity. Sodium taurocholate and deoxycholate has no effect on the enzyme activity. The lipase activity in the pharate adult was almost equal to that of the pupa. Subsequently, the enzyme activity declined with age in the adult and the 8-day old adult show very little activity. Price (1975) have reported the presence of the lipase activity in tissues of third-instar larvae of the blowfly and *Calliphora erythrocephala*. Plasma from 6-day calliphora larvae was incubated at 30°C for various periods with the lipase substrate and the rate of reaction was increased with increase in the time period upto 4 hrs. In tissues from 7-day larvae lipase activity was measured upto pH 3.0 to 8.0. In salivary glands maximum lipase activity was found at pH 6.0, while in fat body and muscle the maximum at pH 7 to 8, and in plasma at 6 to 8. Lipase activity in tissue from third-instar calliphora larvae of various ages and the maximum activity in salivary glands was found in 6-day larvae. In fat body, a slight fall in activity over the period from the 5 to 6 day larvae was observed but the activity was increased in 7-day larvae. In muscle, the optimal activity was observed in 7-day larvae. In plasma, lipase activity was increased in 5 to 6-day larvae and then declined. Lipase from fat body was isolated from 4-day old (feeding stage) calliphora larvae. Lipase was released rapidly during the first 30 min of incubation and the rate of reaction then decreased considerably. Salivary glands from 6-day larvae released the greatest amount of lipase but the rate of enzyme

release subsequently decreasing in the glands from 7-day larvae. In 7-day larvae contains lipase activity in plasma 56.3%, salivary glands 16%, fat body 11.3% and muscles 9.6%.

Microbial Lipases: The microbial lipases are resulted from investigation of food spoilage, especially of dairy products. The short chain free fatty acids released are directly responsible for flavour defects, while the long chain fatty acids could presumably be converted more readily to carbonyl and other volatile compounds as free acids. In contrast, free fatty acids in some dairy products particularly in cheese, contribute to desirable flavour. The triacylglycerol content of many microorganisms, particularly the true bacteria is low or even non existent. The role of endocellular lipase in the cellular economy is puzzling (Oleary, 1967).

Geotrichum candidum is a septate mold growing as a white pad on the surfaces of cheese and sour cream (Foster *et al.*, 1957) and was found potent for extracellular lipase. The enzyme was purified by ammonium sulfate fractionation and DEAE-Sephadex A-50 chromatography. The highest activity was observed within the pH range of 7.5 to 9.0 and the optimum temperature was observed at 45°C (Macedo *et al.*, 1997).

The physiological response of the *Penicillium restrictum* fungus towards cell growth and enzyme production upon variable carbon and nitrogen nutrition showed that higher C/N ratio favoured cell growth but not enzyme production. Low extracellular lipase activities were observed using glucose as carbon source suggesting glucose regulation. Final lipase accumulation of 13,000 U/L was obtained, by optimized specific air flow rate (Qa) of 0.5 vvm and an impeller speed (N) of 200 rpm. Agitation showed to be an important parameter to ensure nutrient availability in a growth medium having olive oil as carbon source (Freire *et al.*, 1997).

Azzabi *et al.* (1981) have reported the lipase activity in various fungi, grown on rape seed oil. Four of the five species grew rapidly at 37°C i.e >2.5 mm day^{-1}. *M. pusillus, A. corymbifera*, and *S. thermophile* were found strongly lipolytic but *A. fumigatus* was moderately lipolytic, which was slower growing species. Only *A. versicolor* was not lipolytic. These species were grown on the medium with and without salt and it was observed that the salt generally increased the rate of growth but had little or no effect on the degree of lipolysis. Three fungal species were grown on liquid media over 6 days. *P. hordei* grew faster but *A. versicolor* grew slower than *A. candidus*. The regression of free fatty acids on growth was examined and statistically significant correlation between the weight of mycelium and the amount of free fatty acids released was found for *P. hordei* and *A. candidus*.

Sugiura *et al.* (1977) have studied the purification, crystallization and properties of triacylglycerol lipase from *Pseudomonas fluorescence*. Enzyme was purified by ammonium sulfate precipitation, Sephadex G-75 and DEAE- cellulose chromatography with 521 fold and yield was 13.7%. The purified enzyme was homogenous on disc gel electrophoresis and its molecular weight was found to be 32000 by gel filtration on Sephadex G-75. After 24 hrs *Pseudomonas fluorescence* lipase was crystallized on the wall of glass tube. The specific activity was 4200 units/mg. The optimum pH was 7.0 but 80% or above of the maximum activity was observed in between pH 5 to 9. The effect of pH on the stability of enzyme was examined at 37°C for 60 min. The enzyme retained more than 80% of the original activity at pH range of 5-11. The lipase was stable below 40°C, but inactivated at 70°C.

The optimum temperature for hydrolysis was observed at 50-55°C. The enzyme was partially inhibited by $FeCl_3$ and N-bromosuccinimide while 30% inhibition was observed with $ZnCl_2$ and $HgCl_2$. Iodine completely inactivated the enzyme however, the inhibitory effect of iodine on the lipase disappeared in the presence of substrate. The enzyme hydrolyzed considerably all triacylglycerols used and had a higher activity upon tricaproylglycerol (11600 µmol/min/mg protein) and tripalmitoylglycerol (9630 µmol/min/mg protein).

Lipase from *Candida cylindracea* was used to catalyze the enantioselective esterification in isooctane to resolve naproxen. The conversion was 33.5% over 11 days. The enantiomer excess of product was about 100% at this conversion when octanol acted as the other substrate (Cui *et al.*, 1997). Inorganic nitrogen sources were not as effective as peptone for the production of lipase by newly isolated strain of *candida lipolytica*, when oleic acid or triacylglycerides were used as carbon source. Repression by glucose and stimulation by oleic acid and long chain triacylglcerols (triolein and olive oil) were observed. High extracellular lipase activity was only observed at late stationary phase, where as intracellular lipase levels were constant and almost undetectable during the cultivation period. The produced enzyme was attached to the cell wall, mainly at the beginning of cultivation. The crude lipase showed optimum activity at pH 8.0-10.0 and at temperature 55°C. Moreover, this preparation maintained its full activity for at least 370 days at 5°C (Pereirameirelles, 1997).

Lipase was purified from *Aeromonas sobria* LP004 (isolated from raw milk) to 10.29 fold in a homogeneous state by ultrafiltration and column chromatography on Phenyl Sepharose. The molecular weight of the lipase determined by SDS-PAGE was 97000. The maximum activity was obtained at pH 6.0 and at temperature 45°C. Enzyme was found stable under alkaline conditions (pH 6.5-10.0) at temperatures lower than 40°C. This lipase could be classified as a 1,3- position specific enzyme and its catalytic activity was calcium dependent. PMSF (a serine enzyme inhibitor) and 2-mercaptoethanol (a reducing agent) did not affect the enzyme activity (Lotrakul and Dharmsthiti, 1997).

Conclusion

Lipase enzyme are widely used in various manufacturing process of industrial goods and food materials, such as detergent, medicine, cosmetics, leather, perfumery and some food items. The lipase can be found in wide vatiety of plants, animals, microorganism and insects. Lipase can be measured and purified by titration, colorimetric, fluorometric, thin layer chromatography, gas chromatography, ammonium sulfate precipitation, Sephadex G-75 and DEAE-cellulose chromatography. They may be acidic, neutral and alkaline in nature. Lipase acitivity has been identified in the various tissues of the plants but relatively higher concentration is found in seeds. About 60% of enzymes are found to be associated with glyoxysomes, 15% with the mitochondria

and 25% with a membrane fraction of some oil seeds.

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