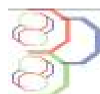

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Biochemical characterization of the lipase activities in dormant seeds from the ripe and unripe *Terminalia catappa* Linn (Myrtales: Combretaceae) fruits harvested in Côte d'Ivoire

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ABSTRACT
Objectives: The aim of this study was to check some biochemical properties of the lipase activities in dormant seeds from the ripe (LASRF) and unripe (LASUF) *Terminalia catappa* fruits harvested in Côte d'Ivoire.
Methodology and Results: Fresh fruits (ripe and unripe) of *Terminalia catappa* were carefully cracked and the seeds were removed, cleaned and washed off any adhering residue. The crude enzyme extracts isolated contained active lipases. LASRF was higher than LASUF. They showed lower affinity for medium with long C-chain esters than for short C-chain triglycerides especially coconut oil. The optimum conditions for lipolysis of LASRF and LASUF was found to be 45°C and 45 min incubation time, with a pH optimum near neutrality (pH 5). Optimum substrate concentration was 5 g in 2.5 ml of hexane. LASRF and LASUF were stable at temperatures between 30-37°C for 100 min. Calcium ion enhanced the enzymes responsible for LASRF and LASUF, while Mn^{2+}, Na^{+}, K^{+}, Fe^{3+}, Fe^{2+}, Zn^{2+} and EDTA strongly inhibited the same activities.
Conclusions and application of findings: The results of the present study showed that the oil of *Terminalia catappa* (tropical almond) seed would deteriorate with storage. The enzyme activities appear to be distinct from other known lipase activities in terms of substrate specificity, stability and chemical agent effect. The thermostable, lipid-hydrolyzing enzymes may be applied to treat lipid-rich industrial effluents, to produce inter-esterification substances in the food industry, or to synthesize useful chemical compounds. Stability of LASRF and LASUF in the presence of hexane makes it a good candidate for application in non-aqueous biocatalysts.
Keywords: Lipase activity, seed, *Terminalia catappa,* fruit.

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) and esterases (carboxyl ester hydrolases, EC 3.1.1.1) are hydrolytic enzymes that hydrolyse ester bonds. They are compounds of the glyoxysomal membrane found in most oil-rich species with a yet unclear metabolic role (Huang, 1987 & 1992). Lipases revealed limited substrate specificity and thus its physiological role in the overall breakdown of stored fat had yet to be proven in detail (Hoppe &Theimer, 1997). They are biotechnologically valuable enzymes, which specifically hydrolyze carboxyl esters of triglycerides into fatty acids, and are being used as animal feed supplements to increase bioavailability of n3 or n6 polyunsaturated fatty acids (Chakraborty & Raj, 2000a). Lipases also catalyse several industrially significant catalytic biotransformation reactions namely, esterification and trans-esterification (Huang & Akoh, 1994; Kosugi & Azuma, 1994; Akoh *et al*., 1996). The trans-esterification of vegetable oils in the presence of a short length alcohol, such as methanol or ethanol, lead to mixture of biodiesel (alkyl monoesters) and glycerol, as final product. This reaction may be conventionally catalyzed by chemical species that act as enzymes or Brönsted acids or bases (Suarez *et al*., 2007). Many vegetable oils, such as palm oil and soybean oil, can be employed in order to obtain biodiesel (dos Santos *et al*., 2008). The synthesis of flavour esters for the food industry, modification of triglycerides for the fat and oil industry, and resolution of racemic mixtures used by the synthesis of fine chemicals for the pharmaceutical industry can be performed with the lipases (Molinari *et al*., 1996). Each application requires unique properties with respect to specificity, stability, temperature and pH-dependence (Chakraborty & Raj, 2008b).

Most of the findings are available so far in the field of lipase research and development focus on microbial and animal lipases (Mukherjee & Kiewitt, 1996; Cambon *et al*., 2008). Plant lipases are interesting enzymes, however, that might combine the advantages of competitive prices and a large spectrum of specificities and stabilities (Villeneuve *et al*., 2007). During the last few decades, considerable attention has therefore focused on these enzymes (Lee *et al*., 2008). Lipase activity in dormant seeds of the African oil bean (*Pentaclethra macrophylla*) was more active on lauric oils (containing short-chain fatty acids), especially coconut oil. Lipolysis increased with enzyme concentration (non-linearly). Optimum temperature for the activity of the oil bean lipase was 30°C although substantial lipolysis was still evident at 80°C indicating the high thermostability of the enzyme (Enujiugha *et al*., 2004). Lipases belonging to plant families such as Euphorbiaceae (Giordani *et al*., 1991; Moulin *et al*., 1994), Asclepiadaceae (Giordani *et al*., 1991), Brassicaceae (Hills & Mukherjee, 1990) and Caricaceae (Giordani *et al*., 1991) have been described as providing useful biocatalysts for several applications (hydrolysis and synthesis). To use any lipase for hydrolysis, esterification, or any other application, it is essential to biochemically characterize the enzyme (Chakraborty & Raj, 2008b). The aim of this study was to check some biochemical properties of the lipase activities in dormant seeds from the ripe and unripe *Terminalia catappa* Linn (Myrtales: Combretaceae) fruits harvested in Côte d'Ivoire (West Africa). This was done in order to find new lipases for biotechnological applications and to follow the development of these enzymes, which are linked, to the progress of lipid accumulation in *Terminalia catappa* seed oil during storage.

MATERIALS AND METHODS

Materials: Ripe and unripe *Terminalia catappa* L. (Combretaceae) fruits were harvested from the trees at the University of Abobo-Adjamé (Abidjan, Côte d'Ivoire) in May 2009. The climate in this area is characterized by high humidity, precipitation up to 4,000 mm per annum and relatively high temperatures, averaging 27°C. Olive, maize, sunflower, soybean, palm and coconut oils were obtained from a local market of Abidjan (Côte d'Ivoire, West Africa). All the chemicals used in the experiments were of analytical grade and were products from Sigma Chemical Co. (St. Louis, MO).

Enzyme extraction: Crude enzyme extracts were prepared as reported previously with slight modifications (Abigor *et al.*, 2002). Fresh fruits (ripe and unripe) were carefully cracked and the seeds were removed, cleaned and washed of any adhering residue. 25 g of the seeds were ground with 30 ml of cold acetone using a Waring Blender. The acetone extract was filtered through cheese cloth and washed four times, with 20 ml each time, of cold acetone (4°C). The residue was air dried at room temperature (27°C) for 20 min. It was finely milled using a laboratory hammer mill and screened through a mesh of 0.5 mm. The meal obtained was stored in an airtight container at 4°C until required for assay.

Enzyme assay: Lipase activity was assayed using a modification of the titrimetric method of Khor *et al.* (1986). The assay mixture (standard assay procedure) contained 5 g of coconut oil, 2.5 ml of hexane to solubilize the oil, 5 ml of phosphate buffer 100 mM, pH 7.5 and 0.5 g of the crude extract. The mixture was incubated at 45°C for a period of 45 min with continuous stirring, using a magnetic stirrer. At the end of the incubation, 25 ml of acetone-ethanol (1:1 v/v) were added to stop the reaction and to extract the free fatty acids (FFAs).The FFAs in the mixture were then estimated by direct titration with 0.01 M NaOH using phenolphthalein as indicator. Lipase activity was expressed as the percent FFAs liberated after a 45 min incubation (Wetter *et al.*, 1957). Corrections were made for endogenous fatty acid production (assay mixture without substrate) and non-enzymatic fatty acid production (assay mixture without enzyme preparation) (Enujiugha *et al.*, 2004).

Temperature and pH optima: The effect of pH on lipase activity was determined by measuring the hydrolysis of coconut oil in a series of buffers at various pH values ranging from pH 3.5 to 8.5. The buffers used were acetate buffer (100 mM) from pH 3.5 to 5.5, phosphate buffer (100 mM) from pH 6.0 to 7.5 and Tris-HCl buffer from pH 8.0 and 8.5. The pH values of each buffer were determined at 25°C. Lipase activity was measured at 45°C under the standard test conditions. The effect of temperature on lipase activity was followed in 100 mM phosphate buffer pH 7.5 over a temperature range of 30 to 80°C using 5 g of coconut oil under the standard test conditions.

pH and temperature stabilities: The stability of lipase activity was followed over the pH range of 3.5 to 8.5 in 100 mM buffers. The buffers were the same as in the study of the pH and temperature optima (above). After 2 h of incubation at room temperature (27°C), aliquots were taken and immediately assayed for residual lipase activity. The thermal stability of the lipase activity was determined at 30, 37, 40, 45 and 50°C after exposure to each temperature for a period from 10 to 100 min. The enzyme was incubated in 100 mM phosphate buffer pH 7.5. Aliquots were drawn at intervals and immediately cooled in ice-cold water. Residual activities, determined in both cases at 45°C under the standard test conditions, are expressed as percentage activity of zero-time control of untreated enzyme.

Incubation time: To determine the optimum incubation time, the reaction mixture was incubated for varying durations (15-90 min) with crude extract at 45°C. Samples were withdrawn at regular intervals to determine the residual activity following the hydrolysis of coconut oil at 45°C. The lipase activity was assayed by standard assay procedure.

Type and concentration of substrate: Different substrates (olive, maize, sunflower, soybean, palm and coconut oils) were substitutively used at 5 g in the assay mixture, with subsequent incubation at 45°C for 45 min. Lipase activity was measured for each substrate substitution, as described in this paper. The dependence of rate of lipolysis on substrate concentration was determined by assaying lipase activity with varying concentrations (1, 2, 3, 4, 5 g) of substrate in 2.5 ml of hexane.

Enzyme concentration: Different quantities of the crude extract (0.25, 0.5, 0.75, 1 g of the acetone powder) were used in assay mixture with 5 g of coconut oil in 2.5 ml of hexane. Enzyme assay was carried out as described for lipase activity.

Chemical agents: The effects of NaCL, KCl, $MnCl_2$, $FeCl_3$, $ZnCl_2$, EDTA, DTNB and *p*CMB on the lipase activity of crude extract were investigated. One (1) ml of each of these solutions was added to separate assay mixtures and incubated for 45 min with continuous stirring. Final concentration of each chemical agent in the reaction mixture was 10 mM. Lipase activity was determined as described above. The percentage remaining activities were determined by comparison with the standard assay mixture with no metal ion added.

Statistical analysis: The Statistical Analysis System (SAS) (SAS, 1989) was used for the ANOVA; LSD means separation, single, Pearson and stepwise regression analyses. $P < 0.05$ was selected as the decision for significant differences.

RESULTS AND DISCUSSION

Lipolysis of different oils: The lipase activities in dormant seeds from the ripe (LASRF) and unripe (LASUF) *Terminalia catappa* fruits were tested toward olive, maize, sunflower, soybean, palm and coconut oils having different chain lengths **(Figure 1)**..

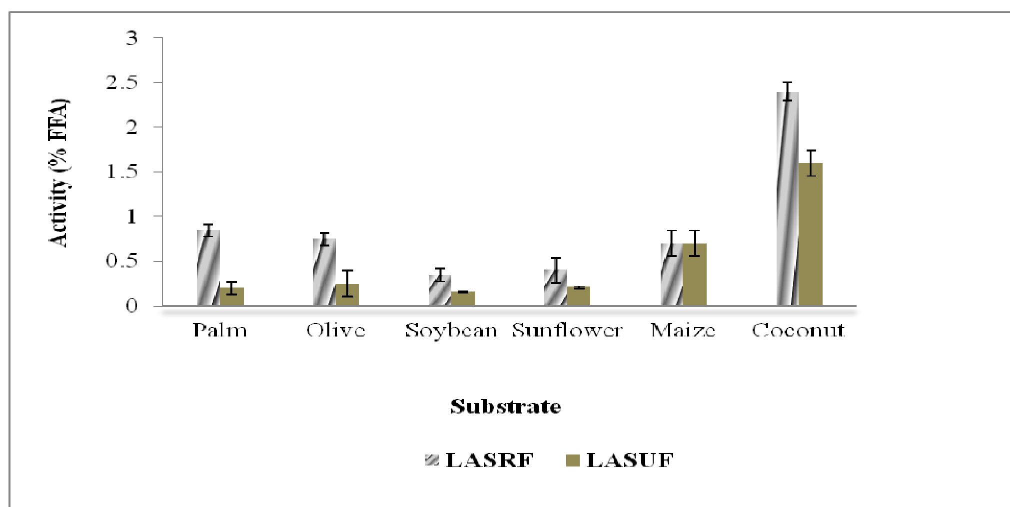

Figure 1: Lipolysis of different oils by the lipase activities of seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire. The reaction mixture contained 5 g substrate, 2.5 ml of hexane to solubilize the oil, 5 ml of phosphate buffer 100 mM pH 7.5 and 0.5 g of the crude extract. Assays were carried out at 45°C for 45 min. values are means of three independent assays ± S.E

In this study, the oils (substrates) were solubilized in hexane according to the method of Khor *et al.* (1986) to increase the interfacial area for the activity of the enzyme. It has been observed that the actual site of lipolysis is at the interface (Mukherjee, 1990). The enzymatic activity of a lipase is related to the interfacial area of the water-insoluble substrate. The highest activity for both crude extracts was obtained toward coconut oil, whereas very slight activity was obtained toward olive, maize, sunflower, soybean and palm oils. When olive, sunflower, soybean, coconut and palm oils were used, LASRF was higher than LASUF ($P<0.05$, Figure 1). This study confirms that the enzymes responsible for these activities in ungerminated (dormant) seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire are lipases. The common observation from past studies is that ungerminated (dormant) seeds have little or no lipase activity (Huang & Moreau, 1978). Most investigations on plant lipases have been carried out on oleaginous seeds in which activity is generally found to become prominent upon germination (Hassanien & Mukherjee, 1986). However, lipase activity has been observed in ungerminated seeds of castor bean (Ory *et al.*, 1962), *Jatropha curcas* (Abigor *et al.*, 2002) and the African bean *Pentaclethra. Macrophylla* (Enujiugha *et al.*, 2004). The fruit seeds need to be heated immediately after harvest to inactivate the lipase and to prevent an unacceptable level of free fatty acids in the oil.

Effect of pH on lipase activity and stability: The effect of pH on lipase activity and pH stability was tested using coconut oil as substrate. As shown in figure 2, LASRF and LASUF have been found to be optimally active at pH 7.5.

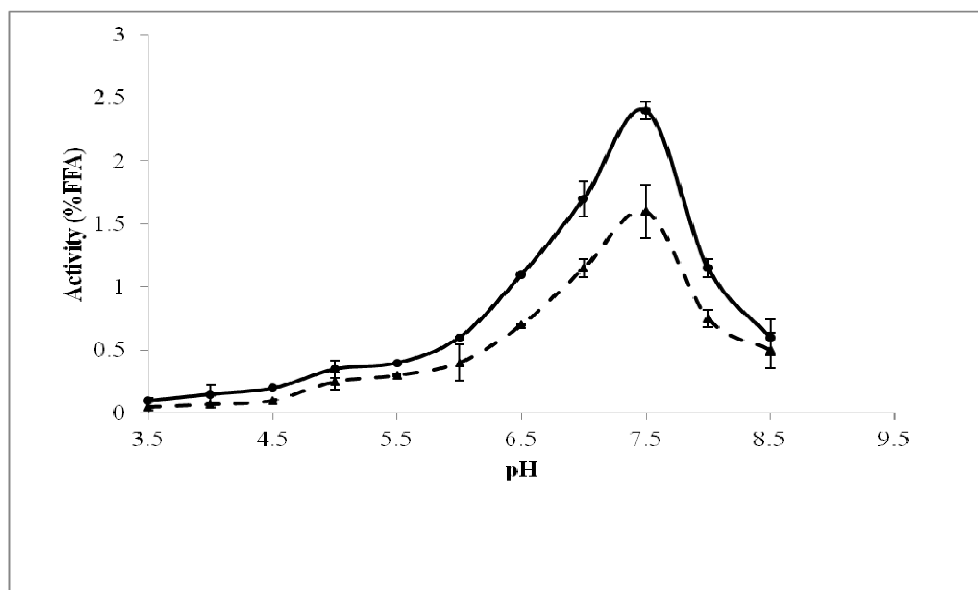

Figure 2: Effect of pH on the lipase activities of seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire. [●] lipase activity in seed from the ripe fruit, [▲] lipase activity in seed from the unripe fruit. The reaction mixture contained 5 g of coconut oil, 2.5 ml of hexane to solubilize the oil, 5 ml of buffer and 0.5 g of the crude extract. Assays were carried out at 45°C for 45 min. The effect of pH on lipase activity was in a series of buffers at various pH values ranging from pH 3.5 to 8.5; values are means of three independent assays ± S.E.

This value was similar to the optimum pH of lipase activities in the germinating seeds of *Jatropha curcas* (Abigor *et al.*, 2002). In general, there are no reports on microbial and plant lipases that are active at extremely acidic pH (<3.0). Exact mechanism behind pH sensitivity is also not clear. However, the stability studies of *Candida rugosa* lipase conducted by Hernaiz *et al.* (1994) focused mainly on the conformational changes in relation to the topology of lipase protein, caused by pH changes. That change might reduce or impose strain on the 'lid' overarching the active centre, thereby open or shut down the catalytic centre for substrate binding (Benjamin & Pandey, 1998). The enzymes responsible of LASRF and LASUF were quite stable between pH 6.0-8.0. The stability of these enzymes in acidic, neutral and basic pHs is important in terms of industrial applications. Similar results were reported earlier for esterases from *Geobacillus thermoleovorans* YN (Soliman *et al.*, 2007), *Mucor* sp. (Abbas *et al.*, 2002) and *Cucurbita pepo* cv "eskandrani" (Fahmy *et al*., 2007).

Effect of temperature on lipase activity and thermal stability: Lipase activity was tested at temperatures ranging from 30 to 80°C, using coconut oil as substrate. The reaction rate increased significantly ($P<0.05$, 2.5-fold and 3-fold respectively for LASRF and LASUF) when the temperature varied from 35°C to 45°C and reached its maximum value at 45°C. LASRF and LASUF strongly decreased above 50°C. The lipase activities in dormant seeds from the ripe and unripe fruits showed the same optimum temperature (45°C, Figure 3).

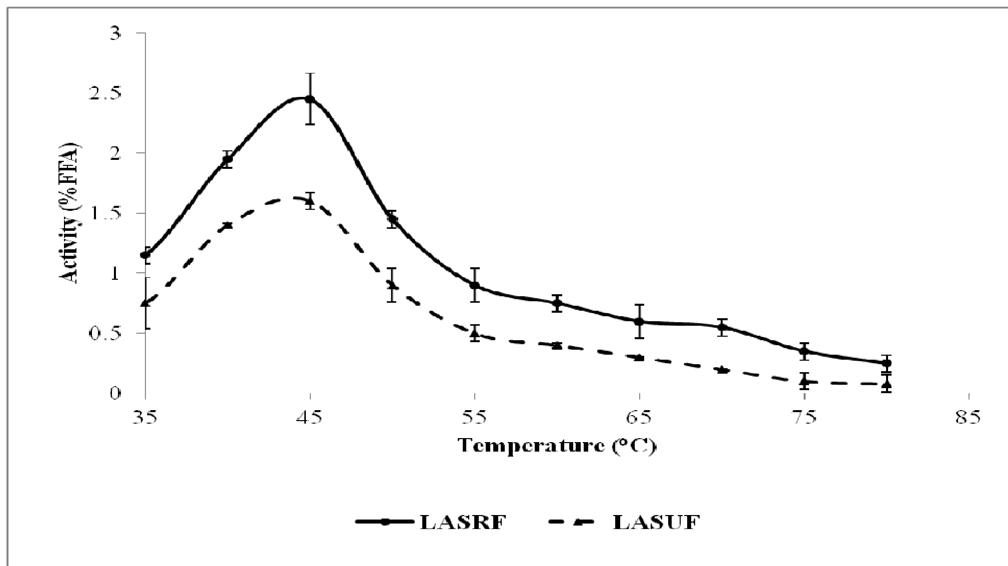

Figure 3: Effect of temperature on the lipase activities of seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire. [●] lipase activity in seed from the ripe fruit, [▲] lipase activity in seed from the unripe fruit. The reaction mixture contained 5 g of coconut oil, 2.5 ml of hexane to solubilize the oil, 5 ml of phosphate buffer 100 mM pH 7.5 and 0.5 g of the crude extract. The effect of temperature on lipase activity was followed over a temperature range of 30 to 80°C. Values are means of three independent assays ± S.E.

This value was similar to the optimum temperature lipase activities of juvenile *Cherax quadricarinatus* digestive gland (Lopez-Lopez *et al*., 2003) and lobster *Homarus americanus* (Biesot & Capuzzo, 1990). However, it differed from lipase activities in dormant seeds *Pentaclethra macrophylla* (Enujiugha *et al*., 2004) and palm *Elaeis guineensis* mesocarp (Abigor *et al*., 2002) which displayed a maximal activity around 30°C. LASRF and LASUF thermostabilities were also determined by measuring the residual activities after incubation of the crude enzymes at various temperatures. The enzymes responsible of LASRF or LASUF retained respectively 80% and 75% of their maximum activities after incubation for 30 min at 45°C (Figure 4).

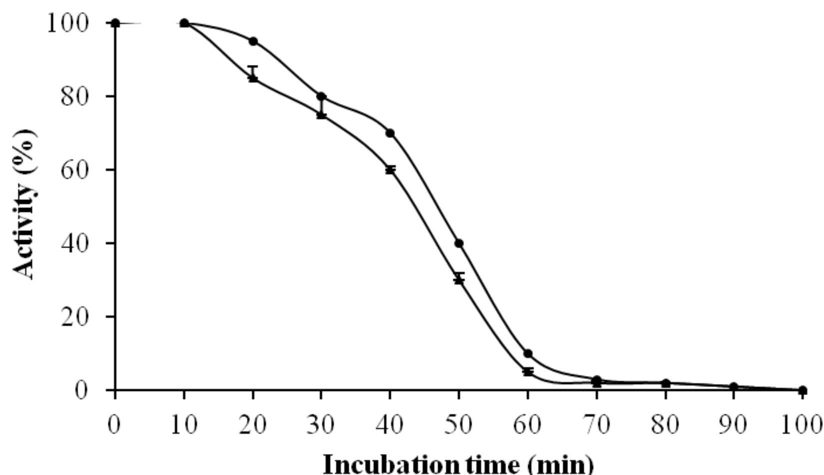

Figure 4: Thermal stability at 45°C of the lipase activities of seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire.

[●] lipase activity in seed from the ripe fruit, [▲] lipase activity in seed from the unripe fruit. The enzyme was incubated in 100 mM phosphate buffer pH 7.5 at 45°C. Aliquots were drawn at intervals and immediately cooled in ice-cold water. Residual activities were determined in both cases at 45°C under the standard test conditions. Values are means of three independent assays ± S.E.

When these enzymes were incubated at 50°C, they were completely inactivated after 20 min. LASRF and LASUF were most stable at temperatures between 30-37°C. Enzymes became inactive at temperatures above a critical level due to unfolding of the molecules (LapanJe, 1978). This process is usually reversible for most enzymes but prolonged heating results in irreversible loss of catalytic activity involving destruction of various covalent and noncovalent interactions (Ghosh & Nanda, 1993). Comparative studies of thermophilic and mesophilic enzymes have demonstrated that weak interactions such as hydrogen bonds (Macedo-Ribeiro *et al*., 1996), disulfide bonds (Hopfner *et al*., 1999), ion pairs (Vetriani *et al*., 1998), salt bridges (Criswell *et al*., 2003), hydrophobic interactions (Elcock, 1998), and compactness (Russell *et al*., 1997) are of importance for stability. However, no universal basis of stability has been recognized because the stability of different enzymes has different origins.

Effect of incubation time: A linear logarithmic relationship was observed between reaction time and rate of lipolysis (LASRF or LASUF) at shorter incubation periods (10 to 40 min). After 45 min of incubation, the curve declined **(Figure 5)**.

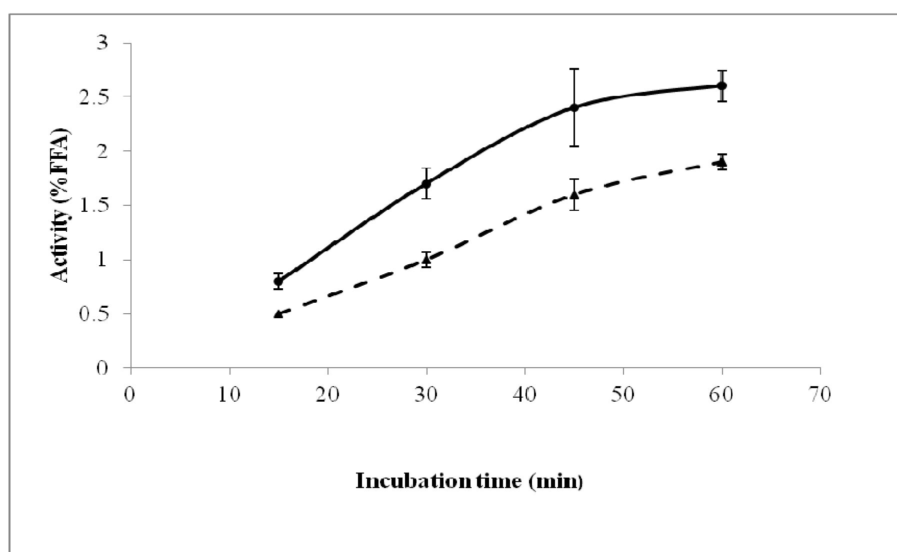

Figure 5: Effect of incubation time on the lipase activities of seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire. [●] lipase activity in seed from the ripe fruit, [▲] lipase activity in seed from the unripe fruit. The reaction mixture contained 5 g of coconut oil, 2.5 ml of hexane to solubilize the oil, 5 ml of phosphate buffer 100 mM pH 7.5 and 0.5 g of the crude extract. The reaction mixture was incubated for varying durations (15-90 min) with crude extract at 45°C. Values are means of three independent assays ± S.E.

Abigor *et al*. (1985) observed a linear relationship at shorter incubation periods for oil palm lipase. Some other researchers, working with lipase from different sources, also found that the rate of hydrolysis or lipolysis is linear on a logarithmic scale (Khor *et al*., 1986). With peanut alkaline lipase, a linear rate of hydrolysis was exhibited for over 60 min (Sanders & Pattee, 1975). This trend could be attributed to zero order reaction, indicating that the substrate is not limiting and the products of hydrolysis exhibit no inhibitory action.

Effect of coconut oil amount: A linear logarithmic relationship between coconut oil amount and rate of lipolysis was observed at amount up to 4 g. At higher amount (> 4g), there was a drop in the rate of lipolysis with an apparent decline in the LASRF or LASUF of the enzyme. At this stage, increasing the amount of the substrate would result in inhibition of the enzymes responsible for LASRF and LASUF (Figure 6).

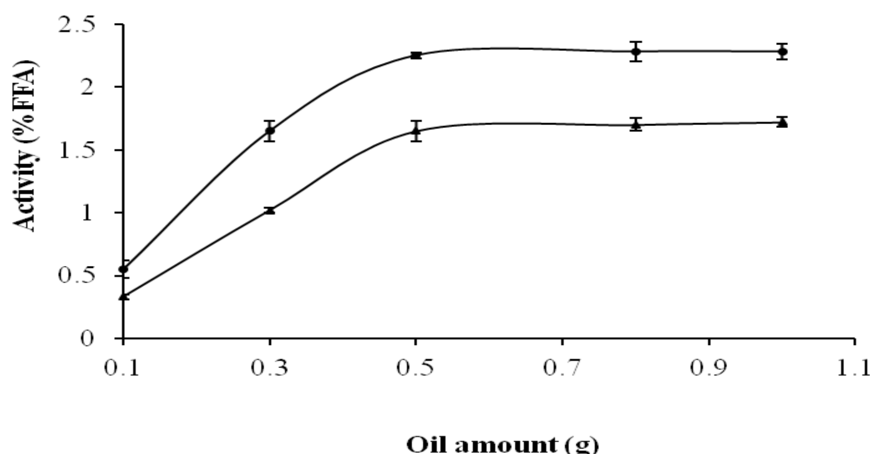

Figure 6: Effect of coconut oil amount on the lipase activities of seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire. [●] lipase activity in seed from the ripe fruit, [▲] lipase activity in seed from the unripe fruit. The reaction mixture contained 5 g of coconut oil, 2.5 ml of hexane to solubilize the oil, 5 ml of phosphate buffer 100 mM pH 7.5 and 0.5 g of the crude extract. The reaction mixture was incubated for 45 min at 45°C. Values are means of three independent assays ± S.E.

The results showed that lipolysis of 5 g of coconut oil could be optimally achieved at a 0.50 g enzyme amount.

Effect of crude extract amount: A linear logarithmic relationship was observed between crude extract amount and rate of lipolysis (LASRF or LASUF) at shorter enzyme amounts (0.1 to 0.5g). After 0.5 g of enzyme amount, the curve declined (Figure 7).

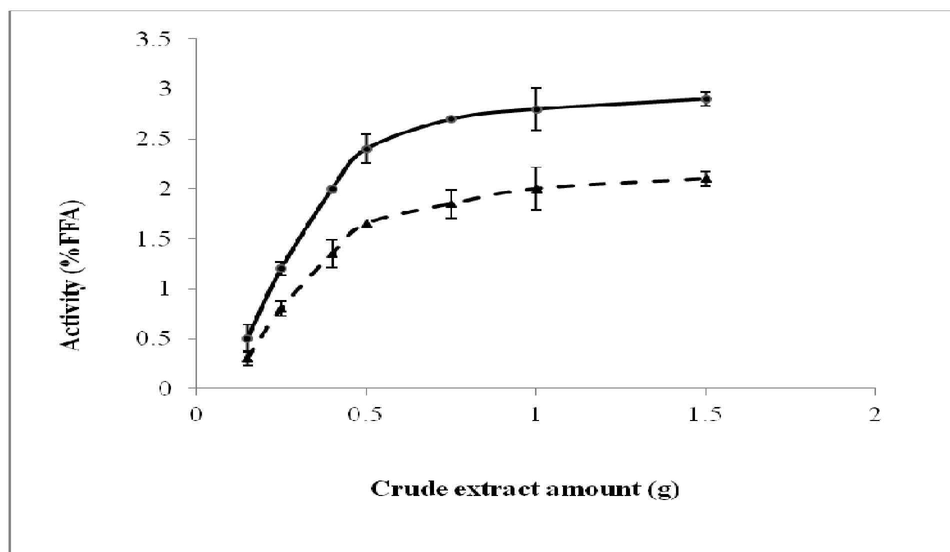

Figure 7: Effect of crude extract amount on the lipase activities of seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire. [●] lipase activity in seed from the ripe fruit, [▲] lipase activity in seed from the unripe fruit The reaction mixture contained 5 g of coconut oil, 2.5 ml of hexane to solubilize the oil, 5 ml of phosphate buffer 100 mM pH 7.5 and the crude extract. The dependence of rate of lipolysis on crude extract amount was determined by assaying lipase activity with varying amount (0.2, 0.3, 0.4, 0.5, 1.0, 1.5 g). The reaction mixture was incubated for 45 min at 45°C. Values are means of three independent assays ± S.E.

The results agree with the findings of Hassanien and Mukherjee (1986) and Sanders & Pattee (1975) that the relationship is linear and passes through the origin for peanut, rape, mustard and lupine lipase preparations. However, they differed to the observations of Enujiugha *et al*. (2004) that the relationship is non-linear but incremental. These results mean that between 0.1 and 0.50 g enzyme amount, a very high rate of lipolysis was observed. Beyond this, activity increased steadily, but rate of lipolysis was reduced. The results showed that lipolysis of 5 g of coconut oil could be optimally achieved at a 0.50 g enzyme amount.

Effect of some chemical agents on lipase activity: LASRF and LASUF were strongly inhibited by ethylene diamine tetraacetic acid (EDTA) **(Figure 8)**, suggesting that the lipases are metalloenzymes. EDTA inhibition of the enzyme activity could be attributed to its chelating ability.

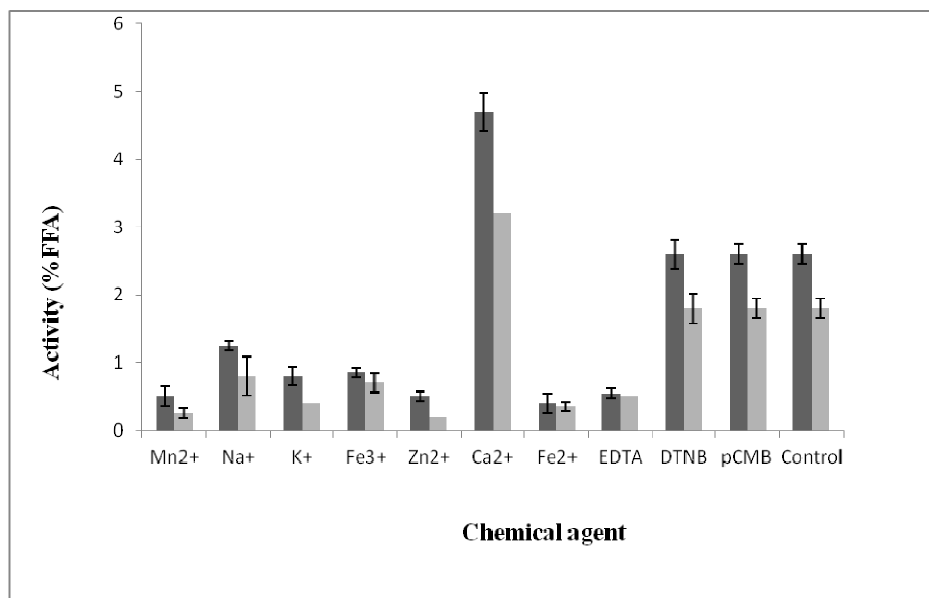

Figure 8: Effect of some chemical agents on the lipase activities of seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire. One (1) ml of each chemical agent solution was added to separate assay mixtures and incubated for 45 min with continuous stirring. Final concentration of each chemical agent in the reaction mixture was 10 mM. Lipase activity was determined as described above. The percentage remaining activities were determined by comparison with the standard assay mixture with no metal ion added. Values are means of three independent assays ± S.E.

It will naturally perform what is known as the "chelation process" of the system and thereby disrupt the formation of the enzyme substrate complex. This invariably affects the formation of the end product (Enujiugha *et al*., 2004). The inhibitory effect of EDTA on lipases is well-documented (Dring & Fox, 1983; Bozoglu *et al*., 1984; Roussis *et al*., 1988; Baral and Fox, 1997). This result indicated that the lipase also possesses a triad of three amino acids at its catalytic site just like many other lipases (Chakraborty & Raj, 2008b). The chemical agents *para*-chloromercuribenzoic acid (*p*CMB) and 5,5'-dithio-2,2'-dinitro-dibenzoic acid (DTNB) had no significant effect on LASRF and LASUF (Figure 8) indicating the absence of sulfhydryl groups in the enzyme molecules. This is in contrast to the findings of Hassanien and Mukherjee (1986) and Sanders and Pattee (1975). Calcium ion was found to enhance LASRF and LASUF while Mn^{2+}, Na^{+}, K^{+}, Fe^{3+}, Fe^{2+} and Zn^{2+} ions strongly inhibited the same activities (Figure 8). All inhibitions occurred to different degrees but not completely. Similar results were obtained with the esterolytic enzyme of *Anoxybacillus gonensis* A4 (Faiz *et al*., 2007) and most Pseudomonas lipases (Stead, 1986; Fox *et al*., 1989). Abigor *et al*. (2002) and Haas *et al*. (1992) also observed an enhancement of lipase activity by calcium ion. These enzymes require the presence of Ca^{2+} ion, for the maintenance of their stable and active structures. This ion is bound strongly to specific binding sites on the surfaces of the enzyme molecules. The binding sites of lipase are usually constructed from negatively charged carboxylate side-chain groups of aspartyl and glutamyl residues, brought together by folding of the polypeptide chain (Gray, 1995). The polypeptide chain is 'cross-linked' by the metal ion bridge and the enzyme-calcium ion complex

should, therefore, be more rigid and hence more stable (Gray, 1995). In conclusion, dormant seeds from the ripe and unripe *Terminalia catappa* fruits harvested in Côte d'Ivoire contained active lipases (called respectively LASRF and LASRF). LASRF was higher than LASUF ($P<0.05$). They showed lower affinity for medium to long C-chain length esters than for short C-chain triglycerides especially coconut oil ($P<0.05$). These results showed that the oil of *Terminalia catappa* (tropical almond) seed would deteriorate with storage. The enzymes responsible of LASRF and LASUF are metallo-, mesophilic, neutral lipases and were stable at 37°C for 1001 min. They were activated by calcium ion and strongly inhibited by Mn^{2+}, Na^{+}, K^{+}, Fe^{3+}, Fe^{2+} and Zn^{2+}. These lipases have several advantageous features for industrial applications (the production of infant formula, the making of cosmetics, the biological detergents and cleaning). The thermostable, lipid-hydrolyzing enzymes may be applied to treat lipid-rich industrial effluents, to produce inter-esterification substances in the food industry, or to synthesize useful chemical compounds (octyl laurate, biodiesel). Stability of LASRF and LASUF in the presence of hexane makes it a good candidate for application in non-aqueous biocatalysts.

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