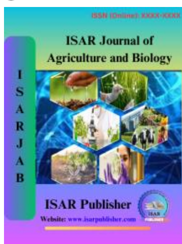
OPEN ACCESS



ISAR Journal of Agriculture and Biology
Vol-2, Iss-4 (Apr- 2024)
Abbriviate Title- ISAR J Agri Biol
ISSN *(Online)*- 2584-217X
https://isarpublisher.com/journal/isarjab
OPEN ACCESS

Lipases, Catalytic Cycle and Sources: A Review

Ezenwelu, Chijioke, O.[1], Okeke, Chisom M.[1], Udemezue, Onyeka, I.[2], Ngwu, Ogochukwu, R.[1], Ogana, Joy[1], Oparaji, Emeka, H.[3*]

[1]Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State

[2] Department of Applied Microbiology and Brewery, Nnamdi Azikiwe University, Awka, Anambra State.

[3] Department of Biochemistry, State University of Medical and Applied Sciences, Igbo-Eno, Enugu State.

***Corresponding Author**
Oparaji, Emeka, H.
Department of Biochemistry, State University of Medical and Applied Sciences, Igbo-Eno, Enugu State.

Article History
Received: 05.03.2024
Accepted: 25.03.2024
Published: 06.04.2024

Abstract: Enzymes are essential tools with a wide range of uses in the biotechnology sector. Lipases are widely distributed enzymes that have significant physiological importance and economic potential. At the oil-water interface, they catalyze the hydrolysis of triacylglycerols to liberate free fatty acids and glycerol. Lipases, in contrast to esterases, do not hydrolyze dissolved substrates in the main fluid; instead, they are activated only when adsorbed to an oil–water interface. It has been reported that microbes, plants, and animals all have lipases. The yield of lipases from plants are relatively low; sometimes it could be cheaper than other sources as it does not require fermentation techniques and unique in substrate specificity/ selectivity.

Keywords: Enzymes, Lipases, Properties, Sources.

Background of the Study

Lipases, (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are ubiquitous enzymes of considerable physiological significance large biased utility in the industrial sector. At the oil-water interface, they catalyze the hydrolysis of triacylglycerols to liberate free fatty acids and glycerol (Kiyouta *et al.,* 2011). (Abolemonaem *et al.,* 2011). Lipases, in contrast to esterases, do not hydrolyze dissolved substrates in the bulk fluid; instead, they are activated only when adsorbed to an oil–water interface (Martinelle *et al.,* 2015). Emulsified glycerine esters and long-chain fatty acids like triolein and tripalmitin will be divided by a genuine lipase. In aqueous solutions containing soluble substrates, lipases don't do anything. Esterases, on the other hand, exhibit typical Michaelis-Menten kinetics in solution. Lipases have a role in different phases of lipid metabolism in eukaryotes, such as the digestion, absorption, reconstitution, and metabolism of lipoproteins. Lipases are located in the tissues of plants' energy reserves (Balashev *et al*., 2011).

An essential protein chemistry technique for separating enzymes from their crude mixtures is the purification of lipases from their sources. According to Anosike (2002), lipase purification follows the fundamental stages of protein precipitation via common ion action. The common ion effect is the basis for the precipitation of extract using salts with varying concentrations. Salt redistribution in solution causes macromolecules to become salted at varying concentrations and salted at elevated concentrations. In order to remove the appended salts, the precipitated proteins are typically dialysed across gradient using a dialysis bag. As the purification process advances, it is seen that the specific activity of purified lipases—that is, lipase activity per milligram protein—increases several times. Subsequent purification stages entail exchanging ions via exchange and particle sized chromatography (Ejedegba *et al.,* 2011).

Numerous investigations have been conducted on the production of lipase by actinomyce strains, yeast, fungus, and bacteria (Khan et al., 2011). According to Khan et al. (2011), microbial lipases have several benefits over their comparable sources, including a high organism doubling time, ease of separation during downstream purifications, and a high biocatalyst yield. Aspergillus sp. strains, which belong to the Zygomycete class of filamentous fungi, are currently showing promise as abundant enzyme makers.

Lipase: Mechanism of Reactions.

The process of substrate hydrolysis commences when the carbon atom of the ester linkage carbonyl group is attacked by the oxygen atom of the catalytic serine. This results in the formation of a tetrahedral intermediate, which then forms hydrogen bonds with the nitrogen atoms in the oxyanion hole (Trodler and Pleiss, 2008). The negatively charged transition state that results from hydrolysis is stabilized as a result. The acyl-lipase complex is liberated along with an alcohol, and it is eventually hydrolyzed to release free fatty acid and regenerate the enzyme (Kuwahara *et al*., 2008). According to Trodler and Pleiss (2008), Petersen *et al*.'s mapping of the electrostatic surface of a number of lipases and esterases revealed that these enzymes' active sites are negatively charged within their ideal pH range of pH 6–10. As a result, following ester cleavage, the ionized carboxylic acid is released from the negatively charged carboxyl group's electrostatic repulsion against the active site's negative electrostatic potential, resulting in the so-called electrostatic catapult process (Peters *et al*., 2009).

***Corresponding Author:** **Oparaji, Emeka, H.**

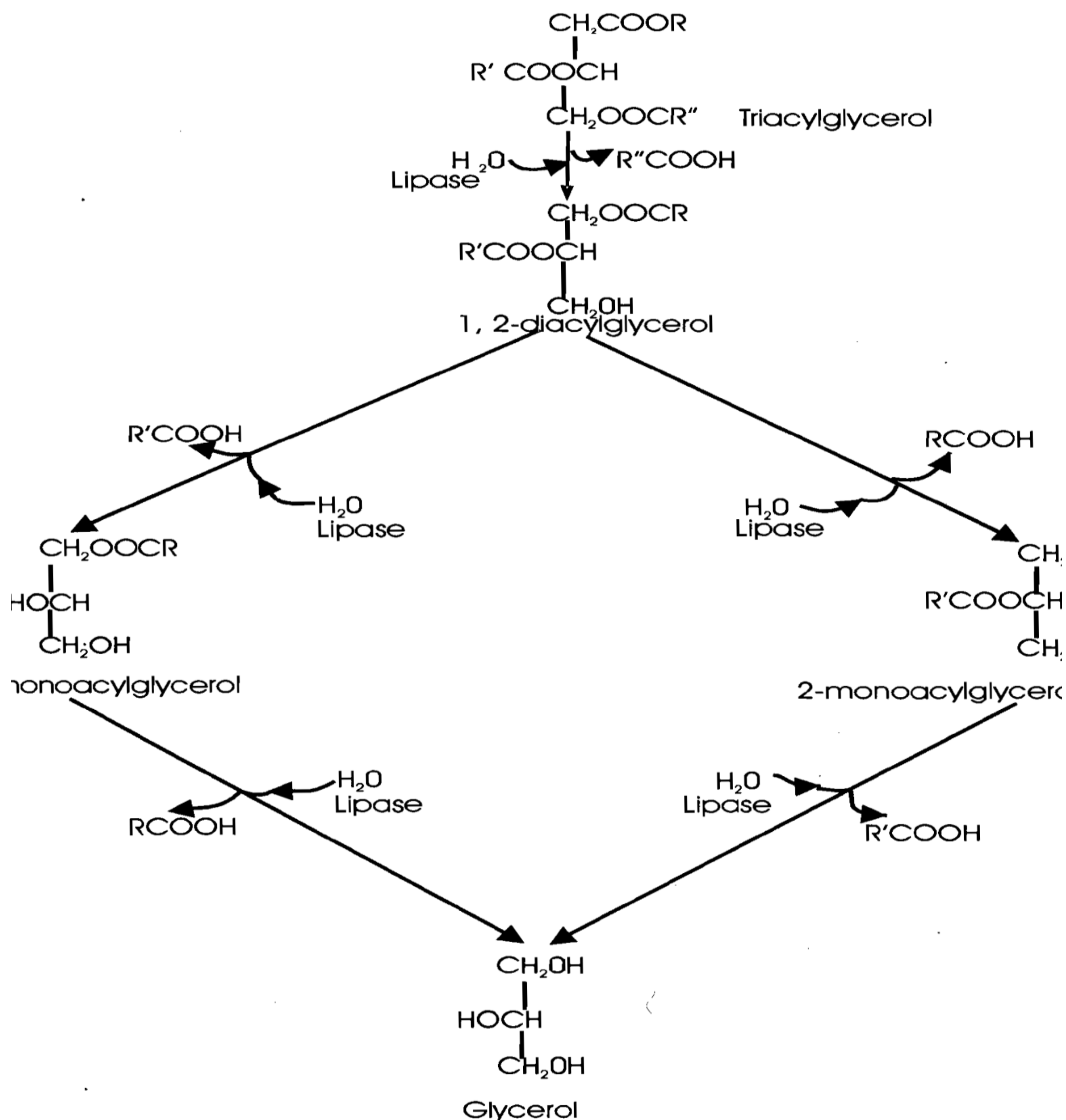


Fig. 1. Kinetics of lipase pathway (Source Aeger *et al.,* 2009).

Sources of Lipase

In nature, lipases are found in large quantities and can have diverse catalytic properties. They can originate from bacteria, fungi, yeast, or animals, including the pancreatic, hepatic, and gastric regions (Thakur, 2012). The search for alternative sources of microbial lipases is encouraged by the fact that, despite their wide range, the industrial usage of these enzymes is still limited due to their expensive production costs (Haider et al., 2008). As biocatalysts, seed lipases have received a lot of attention lately. Due to some very intriguing characteristics like specificity, affordability, availability, and ease of purification, these enzymes can occasionally offer advantages over microbial and animal lipases. As such, they represent a fantastic alternative for possible commercial exploitation as industrial enzymes (Lin *et al*., 2008; Parques and Marcedo, 2006). Chemical and enzymatic catalysis are equally viable methods for transesterification, which modifies fats and oils. At present, alkaline metals serve as the reaction catalyst in the chemical process of industrial transesterification, which is carried out at elevated temperatures (Ribeiro *et al*., 2009).

Microbial lipases: Sources

There are lipases everywhere in nature, and microbial lipases are the most commonly utilized class of enzymes in biotechnological applications and organic chemistry. Sources of these microbial lipases include the following: Bacterial, fungi, yeast some algal growths etc.

Bacteria

Bacillus lipases are among the most widely used bacterial lipases, and they have intriguing qualities that could make them suitable candidates for biotechnological uses (Dustra et al., 2008). The most prevalent bacterial lipases include Bacillus subtilis, Bacillus pumilus, Bacillus licheniformis, Bacillus coagulans, Bacillus

stearothermophilus, and Bacillus alcalophilus. Furthermore, it has been observed that the following bacteria generate lipase: Pseudomonas sp., Pseudomonas aeruginosa, Burkholderia multivorans, Burkholderiacepacia, and Staphylococcus caseolyticus (Table 1). Ertugrulet al. (2007) isolated 17 bacterial strains that were capable of growing on OMW-based media, and they chose the strain that showed the most promise for producing lipase. The best lipase producer was found to be a strain of Bacillus sp. after screening on tributyrin agar medium. Following medium optimization, 168 U mL−1 of intracellular activity was detected Kiran *et al*. (2008) isolated 57 heterotrophic bacteria from the marine sponge *Dendrodorisnigra*, of which 37% produced a clear halo around the colonies on tributyrin agar plates for lipase production. Pseudomonas MSI057 in particular showed extensive clean zones surrounding the colonies. during that, this strain was chosen for additional research, and during optimization, a maximal lipase activity of 750 U mL−1 was discovered. A bacterium strain that was identified as Biopetro-4 was obtained by Carvalhoet al. (2008) from soil contaminated with petroleum. Following an analysis of many inducers for lipase activity, the highest value attained after 120 hours of fermentation was 1,675 U mL−1. After isolating a strain of B. stearothermophilus AB-1 from the air, Abada (2007) generated lipase, reaching a maximal lipase activity of 1,585 U mL−1 after 48 hours of fermentation. Takaç and Marul (2008) regularly subcultured samples in nutrient broth containing 1% (v/v) tributyrin in order to extract microbial cultures from soil that had been enriched. The procedure of isolation was carried out by samples serially diluted on tributyrin agar (TBA) plates. The goal of Bacillus sp. selection was to produce the largest opaque halo. For purification, active colonies were respread on TBA agar.

Shariff *et al*. (2007) obtained Bacillus sp. strain L2, a thermophilic bacterium, from a hot spring in Perak, Malaysia. After 28 hours of fermentation, an extracellular thermostable lipase activity was found using plate and broth assays at 70 °C. Because microorganisms require a high water activity (greater than 0.9), submerged fermentation systems are typically the preferred method for cultivating bacteria. For bacteria cultivated through solid state fermentation, there aren't many exceptions. However, production is typically high when the bacteria are well suited to this solid media. Mahanta *et al*. (2005) used a solvent-tolerant P. aeruginosaPseA strain to achieve a maximal lipase activity of 1,084 U gds−1. Using B. coagulans, Alkan *et al.* (2007) generated extracellular lipase, with a maximal lipase activity of 149 Ugds−1 after 24 h of fermentation. Fernandes *et al.* (2007) obtained a peak lipase catalysis of 108 U gds−1 after 72 h of fermentation by *B. cepacia*.

Fungi

The majority of lipase-producing fungi that are known to be economically significant are classified into the following genera: Rhizopus sp., Aspergillus sp., Penicillium sp., Geotrichum sp., Mucor sp., and Rhizomucor sp.

The strain, growth medium composition, cultivation circumstances, pH, temperature, and type of carbon and nitrogen sources all affect the amount of lipase that fungus produce (2004). The isolation and selection of novel strains is prompted by the industrial need for new sources of lipases with distinct catalytic properties. Different ecosystems, including industrial wastes, vegetable oil processing industries, dairy farms, and soil contaminated with oil and oilseeds, have been reported to harbor lipase-producing microbes (2001). In their 2010 study, Vishnupriya et al. discovered that Sterptomyces griseus produces lipase, with a maximal enzyme activity of 51.9U/ml. Using enrichment culture methods, Colen et al. (2006) identified 59 lipase-producing fungus strains from soil found in Brazilian savannas. Using an agar plate medium with olive oil emulsion and bile salts, fungi were isolated and grown for the primary screening experiment. The ratio of the colony radius to the lipolytic halo radius was used to choose twenty-one strains. Out of the eleven strains that were examined, the highest productive strain was found as Colletotrichumgloesporioides. Cihangir and Sarikaya (2004) identified a strain of *Aspergillus* sp. in another study. from soil samples from the different regions of Turkey and obtained an expressive activity of 17 U mL−1. Teng and Xu (2008) examined the synthesis of lipase by Rhizopuschinensis in SmF and found that, under ideal experimental settings, the maximal lipase activity was 14 U mL−1. The mutant strain of Rhizopus sp. was improved for lipase production by Bapiraju et al. (2005), and the optimal activity was 29 U mL−1. A maximal activity of 13 U mL−1 was obtained by Kaushik et al. (2006) during their study on the development of an extracellular lipase from Aspergilluscarneus. The generation of lipase by Penicillium verrucosum in SSF was studied by Kempka et al. (2008), and the optimal activity was approximately 40 U gram of dry substrate−1 (gds). In their 2008 study, Vargas et al. discovered that Penicillium simplicissimum produces lipase with an activity of 30 U gds−1. P. simplicissimum and P. verrucosum were separated from the babassu oil industry.

Because the techniques for measuring lipase activity differ, it is challenging to compare SmF and SSF quantitatively. Nonetheless, there may be interest in certain qualitative data that is provided in the literature. For instance, Kempkaet al. (2008) produced extracellular lipases utilizing Rhizopus homothallicus, exhibiting lipase activity of 1,500 U gds−1 and 50 U mL−1, respectively, by SSF and SmF. By cultivating Penicillium restrictiveum, Azeredo et al. (2007) obtained lipase activity of 17 U gds−1 and 12 U mL−1 for SSF and SmF, respectively. There have also been some recent publications detailing the usage of filamentous fungus's immobilized entire biomass.

Due to its ability to prevent biomass washout at high dilution rates, immobilization is useful. Furthermore, a high cell concentration in the reactor might be attained, and it is preferred that the biomass be separated from the medium (2000). According to Wolski et al. (2008), immobilized biomass from a recently isolated *Penicillium* sp. was used in submerged fermentation to enhance lipase synthesis through the application of response surface approach. The lipase activity that the authors achieved under optimal experimental conditions was approximately 21 U mL−1, which was greater than the activity that the identical microbe had acquired prior to immobilization. Yang and colleagues (2005) investigated the synthesis of lipase in repeated batches using immobilized Rhizopus arrhizus mycelium in submerged fermentation. After the process switched from batch to repeated-batch mode, the lipase productivity rose from 3 to 18 U mL−1 h−1. In their 2004 study, Ellaiah et al. produced lipase from the entire immobilized biomass of Aspergillus niger, and they observed comparable activity in both the free and immobilized biomass cultivations (4 U mL−1). The entire R. arrhizus cell was immobilized by Elitol and Ozer (2000), and despite multiple iterations of batch studies, the rate of lipase production remained consistent.

Yeast

According to Griebeler, *et al* (2009), *Candida rugosa, Candida tropicalis, Candida antarctica, Candida cylindracea, Candida parapsilopsis, Candida deformans, Candida curvata, Candida valida, Yarrowia lipolytica, Rhodotorula glutinis,* and *Rhodotorula pilimornae* are the primary terrestrial yeast species that have been shown to generate lipases. Cloning and overexpression of the lipase-encoding genes have been observed in Candida spp., Geotrichum spp., Trichosporon spp., and Y. lipolytica (2007). While C. rugosa and C. antarctica lipases have been widely applied in various industries, several new publications have reported lipase synthesis by other yeasts (Abada, 2018). In Delphi, India, areas used for petroleum and oil sludge, Kumar and Gupta (2008) discovered fifteen yeast species. The isolates were purified and checked for their lipolytic potential. Among these yeast strains, one strain was selected for further studies, based on the largest halo of lipolysis. On the basis of sequence homology, this strain was found to belong to *Rhodotorulamucilaginosa* genus and share 99% homology with the already existing database. Ciafardini *et al.* (2008) have discovered that freshly produced olive oil is contaminated by a rich micro-flora, capable of conditioning the physicochemical and organoleptic characteristics of the oil, through the production of enzymes. Several strains of yeast were identified as Saccharomyces cerevisiae, Candida wickerhamii, Williopsis californica, and Candida boidinii among the microorganisms that were isolated from this oil; of these, S. cerevisiae and W. californica shown good capacity to make lipase. It was shown that the lipase activity in W. californica was extracellular, whereas it was intracellular in S. cerevisiae. Olive oil mill wastewater (OMW), a dark-colored effluent produced by the three-phase olive oil extraction process, is the common name for it. D'Annibale et al. (2006) looked at the potential value of OMW as a growth medium for bacteria that produce extracellular lipase. C. cylindracea was the most promising strain out of the 12 examined. The most promising lipase producer among the yeasts described in the literature is Candida sp. Tan (2006) and he utilized the response surface methods to maximize the culture media for the strain Candida sp. 99-125 to produce lipase. After optimization, the authors reported the optimum lipase activity as 6,230 and 9,600 U mL−1 in shaken flasks and in a 5-L bioreactor, respectively. In a 30-L bioreactor, Tan *et al*. (2003) reached a maximum lipase activity of 8,300 U mL−1, thus showing that lipase activity values are highly influenced by the microorganism, substrates, and the operational conditions. In contrast to the high activities reached in the above-mentioned works, Rajendran *et al*. (2008) reported the optimum lipase activity of 3.8 U mL−1 by *C. rugosa*.

Table 1: Sources of Microbial Lipases (Thakur, 2012)

Micro organisms	Sources
Acinetobacterradioresistens	Bacterial
Pseudomonas sp.	Bacterial
Pseudomonas aeruginosa	Bacterial
Staphylococcus caseolyticus	Bacterial
Biopetro-4"	Bacterial
Bacillus subtilis	Bacterial
Burkholderiacepacia	Bacterial
Burkholderiamultivorans	Bacterial
Serratiarubidaea	Bacterial
Bacillus sp.	Bacterial
Bacillus stearothermophilus	Bacterial
Bacillus coagulans	Bacterial
Rhizopusarrhizus	Fungal
Rhizopuschinensis	Fungal
Aspergillus sp.	Fungal
Rhizopushomothallicus	Fungal
Penicilliumcitrinum	Fungal
Penicilliumrestrictum	Fungal
Penicilliumsimplicissimum	Fungal

Animal Lipase

Pancreative lipase can serve as a model and prototype for their digestive lipases, lipases of the plants and micro-organism and to a considerable extent, for the mobilizing lipases of tissue (Dutra *et al*., 2008). In addition, the enzyme has found extensive use as research tools in the field of lipid chemistry and biochemistry owing to the fact that specifically they hydrolyse the esters of primary alcohols. Pancreatic lipase was one of the earlier enzymes to be recognized by Claude Bernard in 1836. The purification of the lipase has usually been carried out from the dehydrated and defatted acetone powderof pig pancreas. The lipase was appeared in the first protein fraction that emerges from the column and having apparent molecular weight of 30kDa (Menoncin *et al*., 2008). Dietary fat affects health and diseases. The assimilation of dietary fats in the body requires that they can be digested by lipase. One lipase (pancreatic triglyceride lipase) is essential for the efficient digestion of dietary fats. Pancreatic triglycerol lipase is the arch type of the lipase gene family that includes two homologous of pancreatic triglyceride lipase, pancreatic lipase related proteins 1 and 2. Recently important advances have been made in delineating the mechanism of lipolysis.

Insect Lipase

Lipases in the eggs from the southern corn root worm inhibited DNP (10^{-5}) in the presence of the substrate (Bornscheuer et al 2012). The flight muscle of many insect seems to contain lipase especially active against diacylglycerols (Crabtree and Newholmes, 1972). Nandanan*et al*., 1973 have reported the properties and distribution of extra digestive lipase in the female pupa of *Trogodermagranurium* which hydrolysis triacylglycerol.

Seed Lipases

In studying the seed lipases, the physiological functions as well as the activity of the enzyme in agricultural products during storage must be clearly understood. In germinated oilseeds, mobilization of the stored fatty acid is essential to supply energy and carbon for embryonic growth. Lipolytic enzymes catalyze the first step of lipid mobilization, with the possibility of subsequently being controlled during and after the germination period (Quettier and Eastmond, 2009). Basically, oilseeds are composed of two fundamental parts: the husk or tegument and the kernel. The tegument or husk is the external layer of the seed covering the kernel, the latter being considered the main part of the seed. The kernel has two parts: the embryo or germ that will form the new plant when the seed germinates; and the albumin or endosperm, which stores the reserve nutrients that will feed the plant in the first stages of development. Grains generally contain proteins and, depending on the plant species, starch or triacylglycerols as energy reserve sources. In the mobilization of these three major nutrient reserves during germination, they are hydrolyzed specifically by proteases, amylases and lipases,respectively. Many researchers have investigated seed lipase at the height of its activity during grain germination (Parques and Macedo, 2006). From 20% to 50% of the dry weight of oilseeds is basically stored triacylglycerols.

During the germination period, the triacylglycerols stored in “oil bodies” or “oleosomes” are quickly used up in the production of energy for the synthesis of the sugars, amino acids (mainly asparagine, aspartate, glutamine and glutamate) and carbon chains required for embryonic growth (Quttier and Eastmond, 2009; Ejedegba *et al*., 2007; Borek, *et al.,* 2006).

Figure 2 shows the hydrolysis of seed triacylglycerol to free fatty acids and glycerol by the action of one or more lipases. The glycerol formed is phosphorylated and subjected to glycogenesis after its conversion to dihydroxyacetone phosphate (DHAP) (Quettier *et al.* 2008).

The free fatty acids are transported into the peroxisome, where they are activated into acytil-COA and initiate β-oxidation. The acetyl-COA produced by β-oxidation enters the glyoxylate cycle and subsequently takes part in glycogenesis to produce the sugar required by the embryo as an energy source during germination (Quttier and Eastmond, 2009)

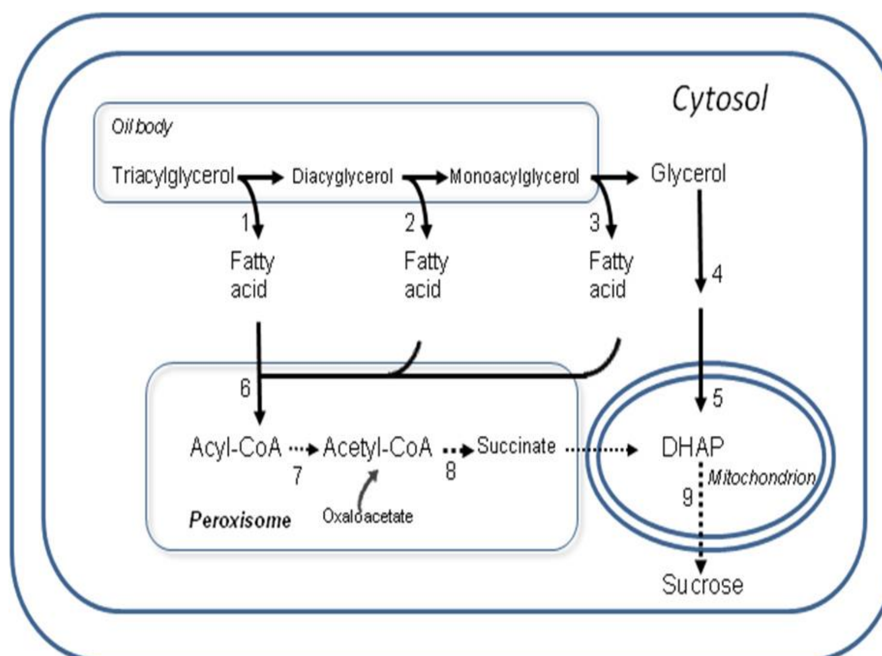


Figure 2:Triacylglycerols (TAG) stored in the lipid bodies are hydrolyzed to fatty acids (FA) and glycerol (Gly) by the sequential action of one or more lipases.

Oil Seed Lipases

Genuine lipases are those that hydrolyze fatty acids bonded to their respective triacylglycerols. In vegetables, they are present in oleaginous seeds (oilseeds) and other cereal seeds. During the germination period of oilseeds, the lipid reserve is rapidly used up in the production of energy for embryonic growth. During this period, lipolytic activity is very high and, depending on the plant species, the lipase may be located in the membrane of the lipid bodies or in other cellular compartments (Borgston and Borckman, 2004). The following are the most widely studied oilseed grains with respect to lipase extraction and characterization: beans (Enujiugha *et al*., 2004); sunflower seeds (Sagiroglu and Arabaci, 2005); linseed (Sammour, 2005) and cotton seeds (Gupta and Kumar, 2008), although lipases from other oilseed sources are currently being investigated. Some of these studies and the characteristics of the oilseed lipases, such as those from beans, sunflower seeds, canola, Barbados nut, lupin, linseed, coconut, French peanut, almond, laurel and black cumin, are discussed in other researches.

Bean Lipases

Bean lipases are well known and characterized. They are generally more active at pH levels close to neutrality, with an optimum temperature of 30ºC and specificity for short and medium chain fatty acids. Enujiugha*et al.* (2004) studied the lipolytic activity of Africa bean seeds (*Pentaclethra macrophyllaBenth*) and the enzyme showed greater activity with oils containing short chain fatty acids, especially coconut oil. The optimum temperature of the lipase was 30ºC, but at 80ºC it still showed lipolytic activity, indicating that it is a heat stable enzyme. The optimum pH was close to neutrality. The authors also observed the effects of different salts on enzymatic activity and found that the presence of Ca^{2+} salts enhanced the activity at 64%, whereas sodium chloride and mercury chloride inhibited the activity at 36% and 28.55%, respectively. The addition of EDTA caused inhibition of 28% of the activity. French bean lipase was studied by Kermash and Van de Voort (1986), who investigated the effects of pH, calcium concentration and the addition of emulsifying agents. The results showed that the enzyme presented greater activity at pH 7.0 and that the addition of calcium had an inhibitory effect, whereas the addition of the emulsifier Tween-20 resulted in a four-fold increase in enzyme activity.

The specificity of the French bean lipase was compared to that of pancreatic lipase using as substrates triacetin, olive oil and French bean oil. The raw French bean lipase showed greater activity than pancreatic lipase in all these substrates, the highest activity being detected with triacetin. Castor beans contain a lipase with some peculiar characteristics. The enzyme showed optimum activity at a pH close to 4.5 and was inactivated at pH values above 6.0 at a temperature of 30ºC. This acid lipase exhibited specificity for short and medium chain fatty acids and also for non-saturated ones. In addition, it showed some regioselectivity for fatty acids at the positions sn-1 and sn-2 (Benjamin and Pandey, 2017). Thakur *et al*. (2012) studied castor bean lipase as a biocatalyst in esterification reactions. The author evaluated parameters such as incubation time, pH, enzyme concentration and the glycerol to fatty acid molar ratio. The best reaction conditions were found to be 4 hours at pH 4.0 with 20% of enzyme extract and a 5:1 glycerol to fatty acid molar ratio. According to the author, this enzyme shows good prospects for industrial use.

Black-Cumin Lipases (*Nigella sativa*)

Thakur (2012) studied some applications of the seed lipase from black-cumin, a plant from Southeast Asia, with respect to its use in oleochemical reactions. The esterification of oleic acid with ethanol was evaluated without removal of the water from the medium. Greater ester production was observed at 45°C using a proportion of 1:1.5methanol:oleicacid and 50% of pressed seed in relation to the total weight of the reagents. The esterification of glycerol with oleic acid was also studied without removal of the water, using different substrate proportions. The highest conversion of oleic acid was obtained with a glycerol to oleic acid molar ratio of 4:1 at 45°C, giving a product containing 31.2% of monolein, 37.3% of diolein, 15.7% of triolein and 15.8% of oleic acid. Zhao *et al*. (2008) evaluated the adsorption of the Nigella sativa lipase onto Celite at different pH values. They observed that adsorption increased from 14.4 to 24.3mg.g-1 as the pH was reduced from 8.0 to 5.0, and that pH 6 was the optimum pH value for activity. Thus, the authors established a pH value of 6.0 as the best for adsorption, and, under this condition, the best for activity as well. The Nigella sativa seed lipase was also studied by Thakur (2012). The seed lipases were partiallypurified and then applied in transesterification andesterification reactions in the presence of differentorganic solvents. The experiments showed that the performance of the vegetable lipase was similar to that of microbial lipases, demonstrating its great potential for industrial applications.

Sunflower Seed Lipases (*Heliantu sannuus L.*)

The reduction in stored lipids during the germination of sunflower seeds was studied by establishing a correlation with the seed proteins (Benjamin and Pandey, 2012). The results showed that seeds growing in the sunlight showed greater lipolytic activity and a concomitant expressive increase in proteins with molecular weights of 40-50 kDa. Sagiroglu and Arabaci (2005) studied the physical-chemical properties of purified sunflower seed lipase (*Heliantu sannuus L*). The researchers observed that the monomeric lipase usually presented a molecular weight of 22 kDa and a pI of 8.0, and showed behavior similar to that of a typical lipase, with a Km of 1.33 mM and Vmax of 555 U/mg. It showed a preference for triacylglycerols with mono-unsaturated fatty acids, a high temperature of 50ºC and a high pH value of 7.5. Enzyme stability was also evaluated during grain storage, and it was shown that the lipolytic activity was preserved in a temperature range from 35 to 50ºC with an alkaline pH value for a period of about four months.

Canola Lipases (*Brassica napus L.*)

Sana *et al*. (2004) evaluated the physical and chemical properties of germinated canola seed lipase (Brassica napus L.) purified by chromatography in a column packed with Sephadex G-50, DEAE and CM-cellulose. Using triolein as the substrate, the highest activity was found at pH 7 with a temperature of 37°C. In the presence of Ca^{2+} and Bi^{3+} ions, the lipolytic activity was found to increase by 165% and 124%, respectively. Fe^{3+}, Fe^{2+}, Zn^{2+}, Hg^{2+} and Cu^{2+} ions inhibited the lipolytic activity and Al^{3+} and Pb^{2+} ions had no influence on the hydrolysis activity. Lin *et al*. (1986) studied the activity of canola lipase in different substrates. The enzyme showed high activity when acting on trierucic, tripalmitate and 4-methyl- umbeliferyloleate. Liaquat and Apenten (2000) studied the formation of low molecular weight esters in an organic medium composed of hexane, using enzymes precipitated from

different vegetable seeds by ammonium sulphate. Direct esterification of acetic, butyric and caproic acid by ethanol, butanol, isobutanol or (Z)-3-hexen-1-ol was also carried out. Of the grain lipases studied (wheat, linseed, barley and canola), canola seed lipase showed the highest degree of flavor formation, producing (Z)-3-hexen-1- yl butyrate and (Z)-3-hexen-1-yl caproate with an efficiency of about 96%.

Lupin Lipases (*Lupinusluteus* L.)

Borek *et al.* (2006) observed the lipolytic activity of seed lipases from lupin (Lupinusluteus L.) cultivated in vitro in a medium with and without sugars. They observed increasing lipolytic activity up to a maximum of 96h of incubation under both conditions. The authors also showed that seedscultivated in a medium without sugar produced greater lipolytic activity than seeds cultivated in a medium with sugars. The lipids stored in the seed are for use as energy reserves and also for carbohydrate synthesis. Thus, seeds cultivated in a medium without sugars require lipids to obtain carbohydrates, which, in turn, require lipases to release the lipids. Kim and Hou (2006) studied the physical and chemicalproperties of lipases from non-germinated lupin seeds. The results showed that the lipase presented optimum activity at a pH of about 5.0 and temperature of 45°C. The lipolytic activity increased in the presence of potassium (10mM), calcium (1mM) and magnesium (1mM) ions and the enzyme showed greater specificity for fatty acids at positions 1 and 2 of the lupin seed oil triacylglycerols, and was more active with saturated than unsaturated fatty acids.

Oat Lipases (*Avenafatua*)

Oat lipase, partially purified by ion change chromatography, was studied by Mohamed *et al*. (2000). Four isoenzymes, EI, EII, EII and EIV, were separated by chromatography. The enzymes EII and EIII presented Km values of 0.52 and 0.38 mM, respectively; optimum activity at pH 9.0 and half the maximum activity at pH values of 8.5, 10 and 10.5; and optimum activity at 75°C and 65°C, respectively. The enzymes were heat stable, EII retaining 39% and EIII 23% of their activity at 90oC. The enzymes were stimulated in the presence of Ba2+ and Ca2+ ions, but inhibited by Mn2+ and Zn2+ ions.Resistance to high temperatures and activity atalkaline pH values are important properties for possible industrial applications.

Barley Lipases (*Hordeumvulgare*)

Kubicka et al. (2000) identified lipase in germinated barley grains. The lipolytic activity was measured using glycerol tributyrate as substrate at pH 8.0. The authors found maximum activity two days after seed germination, followed by a decrease in activity, concomitantly with a decrease in stored lipids.

Sesame Lipases (*Sesamumindicum L.*)

The lipase of germinated sesame seed was studied by Wanasundara*et al.* (2001). The authors evaluated the lipolytic activity for 10 days after germination and found maximum activity four days after germination. The enzyme showed an affinity for short chain fatty acids at a temperature of 38ºC.

Barbados Nut Lipases (*Jatrophacurcas L.*)

Seed lipases from Jatrophacurcas L., also known as Barbados nut, were studied by Abigor*et al*. (2002). The lipase was partially purified using a combination of ammonia sulphate precipitation and ultrafiltration. The enzyme showed high activity in triolein at a high pH of 7.5 and temperature of 37°C. The authors observed that the addition of Fe2+(15mM) inhibited 90% of the enzymatic activity, whereas the addition of Ca2+ increased it by 130% and that of Mg2+ by 30%. Staubmann*et al*. (1999) also studied Barbados nut seeds and found two esterases (JEA and JEB) and a lipase (JL). They only observed lipolytic activity in the grain during the germination period, reaching a maximum 4 days after germination. All three enzymes (precipitated by ethanol and purified by gel filtration) presented optimum activity at an alkaline pH of about 8.0. Using p-nitrophenyl butyrate as the substrate, JEA showed a value for Km of 0.02 mM and Vmax of 0.26 µmol. mg-1. min-1, and under the same conditions, JEB showed a value for Km of 0.07 mM and Vmax of 0.24 µmol. mg-1. min-1. Both esterases hydrolyzed tributyrin, nitrophenyl esters up to a chain length of C4 and naphthylesters up to a chain length of C6. As for JL. It hydrolyzed short and long chain triacylglycerols and gave above 80% triacylglycerol conversion in transesterification reactions.

Almond Lipase (*Amygdaluscommunis*)

Almond seed lipases (Yesiloglu and Baskurt, 2008) presented optimum activity at a pH value of about 8.5. The enzyme, semi purified with ammonium sulfate, showed similar behavior to the other lipases with Km and Vmax values of, respectively, 25 mM and 113.63 µmol.min-1.mg-1 using tributyrin as the substrate. It showed good hydrolysis activity with all the substrates studied (olive oil, corn, soybean, sunflower seed, almond, tributyrin and triolein). With respect to temperature, the lipase was stable between 20°C and 90°C, and stood out for having an optimum activity at 65°C in soybean oil. Its activity in soybean oil was increased by the addition of Ca^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+} and Ba^{2+} salts, but was strongly inhibited by Mg^{2+}, Cu^{2+} and Ni^{2+} salts. The enzyme retained its activity after six months storage at -20°C.

Wheat Lipases (*Triticumae stivum*)

Wheat germ lipase, originally described by Thakur (2012), is commercially available. Some authors have classified wheat lipase as an esterase, but Korneeva*et al.* (2008), Jing *et al.* (2003) and Kapranchikov*et al.* (2004) carried out their studies using triolein as the substrate, and showed good activity. Rose and Pike (2006) determined the lipolytic activities of both wheat and wheat bran. Lipase activity in wheat bran ranged from 2.17 to 9.42 U/g, and in whole kernel wheat from 1.05 to 3.54 U/g. Optimal olive oil and water concentrations were 0.4 to 0.8 mL and 0.15 to 0.20 mL per g of defatted sample, respectively. Optimal incubation temperature was 40°C, and incubation times of up to 8h were linear. The authors observed that the lipolytic activity was highly related to the pool of free fatty acids in the stored wheat. Purified wheat lipase (143 ±2 kDa) showed optimum activity at alkaline pH values close to 8.0 and at 37°C. The enzyme maintained 20% of its activity after incubation at temperatures from 60 to 90°C for an hour at pH 8.0 (Kapranchikov*et al.,* 2004). Similar results were reported by Jing *et al.* (2003) in their study using micro emulsions with triolein as the substrate. They found a Km of 76±4 µmol/L and a Vmax of 59±2 µmol/mL.h. The active enzyme site was studied by Korneeva*et al.* (2008) using the Dixon method. The lipase was inactivated by diethylpyrocarbonate and dicyclohexylcarbodiimide, suggesting that the active enzyme site contained an aspartame, carboxylic or glutamic acid group and a histidine imidazole group. Other tests demonstrated that apparently the OH group of serine had an important function in the catalytic action of the enzyme.

Corn Lipases (*Zea mays* L.)

Lipolytic activity in corn grains was only observed two days after seed germination, and started to decrease along with the decrease in total stored lipids. The peak in lipolytic activity was about 5-6 days after germination. This enzyme shows much greater activity with triolein than with mono and diolein. Liu *et al.* (2008) observed that corn seed lipase showed higher activity with triacylglycerols that contained linoleic and oleic acids. Liaquat and Apenten (2009) studied the synthesis of esters with different molecular weights by seed lipases precipitated with ammonium sulfate, in a medium containing organic solvents. Corn lipase showed better activity with short chain fatty acids in the following order: acetic (2C) > butyric (4C) >caproic acids (6C), in an organic medium using isopentanol and 72 h of reaction time. Zhong*et. al.* (2007) studied recombinant dog lipase expressed in transgenic corn seed. Of the different surfactants evaluated, the enzyme showed greater stability in Tween 80 at a concentration of 0.01% (w/w) and pH 5.5. This study showed the possibility of genetically modifying the seeds, aiming at changing or increasing the lipolytic activity.

Sorghum Lipase (*Sorghum bicolor L*eaves)

Burukutu is an alcoholic drink produced with germinated sorghum seed, dried in the sun without removing the germ, ground and suspended in water for simultaneous saccharification and fermentation (Kun, 1997). Uvere and Orji (2002) evaluated the lipolytic activity during sorghum malting and fermentation (red and white varieties) for the production of Burukutu. The 5-day malt was fermented for 48h to produce Burukutu, and the lipolytic activity was observed during this period. The pH of the fermentation medium decreased from 5.33 to 3.88 and from 5.35 to 3.85, for the red and white seed malts, respectively. During fermentation, the red malt enzyme showed greater activity in a shorter time than the white malt enzyme. Sorghum was also studied by some researchers as an alternative to barley malt replacement. Boareto*et al* (2007)detected lipolytic activity in non-germinated sorghum seeds during grain malting and mashing. The authors observed a slight decrease in enzyme activity during steeping for 24 hours, but IT increased several fold in the course of germination. Between 24 and 60% of the lipolyticactivity was retained after cooking at 48°C, but no activity was found after mashing at 65°C. About 68% of the lipase activity of 72 h old malt was detected in the plumule, while 29% and 3% were in the endosperm and radical, respectively. No study was carried out to characterize the physical-chemical properties of the lipase present in sorghum grains; only the presence of lipolytic activity was determined. Since sorghum grains are being used to obtain products of great value, studies should be carried out to characterize the enzyme and its possible inhibitors in order to obtain products with better sensory quality.

Table 2: Most studied seed lipases and their main features and biochemical properties (Barros *et al*., 2010).

Lipase Source	Optimum pH	Optimum Temperature	Activator	Inhibitor	Substrate	Specificity Position	Application
Africa bean seed *(Pentacleta macrophyllaBenth)*	7.0	30°C	Ca2+	EDTA	Coconut oi	-	Hydrolysis
French bean seed (*Phaseolus vulgaris*)	7.0	35°C	Tween-20	Ca2+	Olive oil, triacetin, oil french bean	-	Hydrolysis
Castor bean seed (*Phaseolusvulgaris*)	4.5	30°C	Ca2+	p-Chloromercu ribenzoic acid	p-nitrophenyl butyrate	sn-1 sn-2	Esterification
Almond seed (*Amygdalus communis L.*) -	8.5	65°C	Ca2+, Fe2+, Mn2+, Co2+ and Ba2+	Mg2+, Cu2+ and Ni2+	Soybean oil	-	Hydrolysis
Rice seed (Oryza sativa)	11.0	80°C	-	-	Olive oil	sn-2	Hydrolysis
French peanut (*Panchiraaquatica Bombacaceae*)	8.0	40°C	Ca2+	Mg2+ Hg2+, Mn2+, Zn2+ and Al3+	p-nitrophenyl acetate	-	Hydrolysis
Wheat seed (*TriticumaestivumL.*)*- - -	8.0	37°C	-	-	Triolein	-	Hydrolysis, Esterification

A summary of the most widely studied seed lipases, their main physical and chemical featuresand their applications are shown in Table 3.

Specificity of Seed Lipases

With some exceptions, oilseed lipases are generally more active with triacylglycerols containing short chain fatty acids. Commonly used substrates include commercially produced plant oils with unknown purity and non-specific polluting agents, triacylglycerols with short chain fatty acids such as acetic and butyric acids, saturated and non-saturated acylglycerols and fluorescent substrates with molecular structures composed of monoesters (Enujiagha *et al*., 2004). According to Thakur (2012), seed lipases show selectivity for the dominant fatty acids in the seed. For example, castor bean lipases show selectivity for triricinolein; palm tree lipase for tricaproin or trilaurein; elm lipase for tricaproin and Vermonia sp. lipase for trivernolein. Other seed lipases can quickly hydrolyze a great variety of fatty acids, such as canola and pinus seed lipases. Canola seed lipase usually presents activity with most lipids, but discriminates fatty acids with cis-4 or cis-6 double-bonds.

Table 3: Hydrolysis of several triacylglycerols by lipases from various seed sources

Triacylglycerols Pinus	**Castor**	**Bean**	**Corn**	**Rapeseed**	**Rapeseed (erucid acid increased)**	**Elm**	**Mustard**	**Palm**	**Pinus**
TricaproinC10:0	43	27	81	-	-	100	-	100	74
Trilaurein C12:0	60	00	31	-	-	4	-	60	78
Trimyristin C14:0	26	00	92	-	-	3	-	15	89
Tripalmitin C16:0	46	00	27	51	39	0	39	35	85
Tristearin C18:0	62	00	36	89	40	0	40	-	103
TrioleinC18:1	55	38	44	138	96	4	96	-	96
Trilinolein C18:2	57	100	89	116	89	6	89	-	100
Tricinolenin C18:3	100	0	83	-	-	0	-	-	114
Tribehenin C22:0	-	0	16	-	-	0	-	-	83
Trierucin C22:1	36	45	100	100	100	0	100	-	-
Triarachidin	-	-	-	-	-	-	-	-	60

Table 1 shows that the seed lipases evaluated had greater activity for the triacylglycerols containing the fatty acids found in higher percentages in the nutrient reserve tissue of that seed. Such affinity is of great physiological significance for the seed (Quettier and Eastmond, 2009). Corn lipase, for instance, presented greater activity with the triacylglycerols containing oleic and linolenic acids, which are the main constituents of corn oil. Similarly, elm lipase showed a high level of specificity and activity for tricaprin, and less than 10% of activity with the other triacylglycerols studied. This specificity level is even greater than that of palm seed lipase for caproic acid, which represents a greater percentage of the storage triacylglycerols of this seed (Boareto*et al*., 2007). With synthetic substrates, lipases are found to present the same pattern they present with natural substrates (Table 2). Lipases from corn, canola and elm seeds were more active with 4-methylumbelliferyl oleate, whereas the lipase from pinus seed was more active with 4-methyl-umbelliferyl laurate, showing no connection with fatty acid chain size, since it presented high laurate and oleate activities and low stearate activity.

Table 4: Hydrolysis of synthetic substrates (acyl 4-methyl-umbelliphenyl) by lipases from various sources.

Substrate (4-methyl umbelliferyl)	**Corn**	**Canola**	**Elm**	**Pinus**
Laureate	32	35	169	100
Palmitate	12	4	29	28
Estearin	5	44	53	6
Oleate	100	100	100	81
Elaidic	-	-	-	51

Conclusion

Lipases are significant biocatalyst with numerous biotechnological applications. Search for high catalytic lipases have lead to the production and engineering of the catalyst from numerous sources. With afore enlisted sources of the enzyme, more has to be done on area of recombinant engineering for efficient production of high spread catalytic cycle lipase for future generational biotechnological application.

Funding Information

This work was solely funded by **DR. Ezenwelu, C. and his research laboratory**

Ethics

Authors declared no ethical issues that may arise after the publication of this manuscript.

REFERENCES

1. Alonso, F. O. M., Oliveira, E. B. L., Dellamora-Ortiz, G. M., & Pereira-Meirelles, F. V. (2005). Improvement of lipase production at different stirring speeds and oxygen levels. *Brazilian Journal of Chemical Engineering*, *22*, 9-18.
2. Amaral, I. P., Carneiro-da-Cunha, M. G., Carvalho Jr, L. B., & Bezerra, R. S. (2006). Fish trypsin immobilized on ferromagnetic Dacron. *Process Biochemistry*, *41*(5), 1213-1216.
3. Bilal, M., Jing, Z., Zhao, Y., & Iqbal, H. M. (2019). Immobilization of fungal laccase on glutaraldehyde cross-linked chitosan beads and its bio-catalytic potential to degrade bisphenol A. *Biocatalysis and agricultural biotechnology*, *19*, 101174.
4. Anosike, E. (2001). Basic enzymology, UNIPORT press, Portharcourt, Nigeria. 1-120.
5. de Queiroz Antonino, R. S. C. M., Lia Fook, B. R. P., de Oliveira Lima, V. A., de Farias Rached, R. Í., Lima, E. P. N., da Silva Lima, R. J., ... & Lia Fook, M. V. (2017). Preparation and characterization of chitosan obtained from shells of shrimp (Litopenaeus vannamei Boone). *Marine drugs*, *15*(5), 141.
6. Sivasamy, A., Cheah, K. Y., Fornasiero, P., Kemausuor, F., Zinoviev, S., & Miertus, S. (2009). Catalytic applications in the production of biodiesel from vegetable oils. *ChemSusChem: Chemistry & Sustainability Energy & Materials*, *2*(4), 278-300.
7. Abdul Aziz, H. (2007). *Reactive Extraction Of Sugars From Oil Palm Empty Fruit Bunch Hydrolysate Using Naphthalene-2-Boronic Acid* (Doctoral dissertation, Universiti Sains Malaysia).
8. Bambase, M. E., Nakamura, N., Tanaka, J., & Matsumura, M. (2007). Kinetics of hydroxide-catalyzed methanolysis of crude sunflower oil for the production of fuel-grade methyl esters. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, *82*(3), 273-280.
9. Benjamin, S., & Pandey, A. (1997). Enhancement of lipase production during repeated batch culture using immobilised Candida rugosa. *Process Biochemistry*, *32*(5), 437-440.
10. Berrios, M., Siles, J., Martin, M. A., & Martin, A. (2007). A kinetic study of the esterification of free fatty acids (FFA) in sunflower oil. *Fuel*, *86*(15), 2383-2388.
11. Bilal, M., & Iqbal, H. M. (2019). Chemical, physical, and biological coordination: An interplay between materials and enzymes as potential platforms for immobilization. *Coordination Chemistry Reviews*, *388*, 1-23.
12. Biró, E., Németh, Á. S., Sisak, C., Feczkó, T., & Gyenis, J. (2008). Preparation of chitosan particles suitable for enzyme immobilization. *Journal of biochemical and biophysical methods*, *70*(6), 1240-1246.
13. Boareto, Á. J., De Souza Jr, M. B., Valero, F., & Valdman, B. (2007). A hybrid neural model (HNM) for the on-line monitoring of lipase production by Candida rugosa. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, *82*(3), 319-327.
14. Bobu, E., Nicu, R., Lupei, M., Ciolacu, F. L., & Desbrieres, J. (2011). Synthesis and characterization of n-alkyl chitosan for papermaking applications. *Cellulose Chemistry and Technology*, *45*(9), 619.
15. Borek, S., Ratajczak, W., & Ratajczak, L. (2006). Ultrastructural and enzymatic research on the role of sucrose in mobilization of storage lipids in germinating yellow lupine seeds. *Plant science*, *170*(3), 441-452.
16. Borgston, B. and Brockman, H. (2004). Lipases. Amsterdam, *Elsevier*. 312-322
17. Bornscheuer, U. T., Bessler, C., Srinivas, R., & Krishna, S. H. (2002). Optimizing lipases and related enzymes for efficient application. *TRENDS in Biotechnology*, *20*(10), 433-437.
18. Bornscheuer, U. T. (2002). Microbial carboxyl esterases: classification, properties and application in biocatalysis. *FEMS microbiology reviews*, *26*(1), 73-81.
19. Bueno, A. V., Velasquez, J. A., & Milanez, L. F. (2009). Effect of soybean oil ethyl ester/diesel fuel blends on engine efficiency. *International Journal of Vehicle Design*, *50*(1-4), 229-247.
20. Burkert, J. F. D. M., Maldonado, R. R., Filho, F. M., & Rodrigues, M. I. (2005). Comparison of lipase production by Geotrichum candidum in stirring and airlift fermenters. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, *80*(1), 61-67.
21. Cammarota, M. C., & Freire, D. M. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource technology*, *97*(17), 2195-2210.
22. Carpio, C., Gonzalez, P., Ruales, J., & Batista-Viera, F. (2000). Bone-bound enzymes for food industry application. *Food chemistry*, *68*(4), 403-409.
23. Cardona, C. A., Rincón, L. E., & Jaramillo, J. J. (2011, May). Integral analysis of feedstocks and technologies for biodiesel production in tropical and subtropical countries. In *Proceedings of the World Renewable Energy Congress—Sweden, Linköping, Sweden* (pp. 8-13).
24. Carvalho, N. B., de Souza, R. L., de Castro, H. F., Zanin, G. M., Lima, Á. S., & Soares, C. M. (2008). Sequential production of amylolytic and lipolytic enzymes by bacterium strain isolated from petroleum contaminated soil. *Applied biochemistry and biotechnology*, *150*, 25-32.
25. Cavalcanti, E. D. A. C., Gutarra, M. L. E., Freire, D. M. G., Castilho, L. D. R., & Sant'Anna Júnior, G. L. (2005). Lipase production by solid-state fermentation in fixed-bed bioreactors. *Brazilian Archives of Biology and Technology*, *48*, 79-84.
26. Chang, K. L. B., Tai, M. C., & Cheng, F. H. (2001). Kinetics and products of the degradation of chitosan by hydrogen peroxide. *Journal of Agricultural and Food Chemistry*, *49*(10), 4845-4851.
27. Chen, W., Chen, H., Xia, Y., Zhao, J., Tian, F., & Zhang, H. (2008). Production, purification, and characterization of a potential thermostable galactosidase for milk lactose hydrolysis from Bacillus stearothermophilus. *Journal of Dairy Science*, *91*(5), 1751-1758.

28. Chen, J., Leng, J., Yang, X., Liao, L., Liu, L., & Xiao, A. (2017). Enhanced performance of magnetic graphene oxide-immobilized laccase and its application for the decolorization of dyes. *Molecules*, *22*(2), 221.
29. Chikere, C. B., & Ekwuabu, C. B. (2014). Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria. *African Journal of Environmental Science and Technology*, *8*(6), 401-406.
30. Ciafardini, G., Zullo, B. A., & Iride, A. (2006). Lipase production by yeasts from extra virgin olive oil. *Food microbiology*, *23*(1), 60-67.
31. Cunha, A. G., Fernández-Lorente, G., Bevilaqua, J. V., Destain, J., Paiva, L. M., Freire, D. M., ... & Guisán, J. M. (2008). Immobilization of Yarrowia lipolytica lipase—a comparison of stability of physical adsorption and covalent attachment techniques. In *Biotechnology for Fuels and Chemicals: Proceedings of the Twenty-Ninth Symposium on Biotechnology for Fuels and Chemicals Held April 29–May 2, 2007, in Denver, Colorado* (pp. 169-176). Humana Press.
32. D'Annibale, A., Sermanni, G. G., Federici, F., & Petruccioli, M. (2006). Olive-mill wastewaters: a promising substrate for microbial lipase production. *Bioresource technology*, *97*(15), 1828-1833.
33. Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology research international*, *2011*.
34. Diaz, J. M., Rodríguez, J. A., Roussos, S., Cordova, J., Abousalham, A., Carriere, F., & Baratti, J. (2006). Lipase from the thermotolerant fungus Rhizopus homothallicus is more thermostable when produced using solid state fermentation than liquid fermentation procedures. *Enzyme and Microbial Technology*, *39*(5), 1042-1050.
35. Domszy, J. G., & Roberts, G. A. (1985). Evaluation of infrared spectroscopic techniques for analysing chitosan. *Die Makromolekulare Chemie: Macromolecular Chemistry and Physics*, *186*(8), 1671-1677.
36. Dutra, J. C., da Terzi, S. C., Bevilaqua, J. V., Damaso, M. C., Couri, S., Langone, M. A., & Senna, L. F. (2008). Lipase production in solid-state fermentation monitoring biomass growth of Aspergillus niger using digital image processing. In *Biotechnology for Fuels and Chemicals: Proceedings of the Twenty-Ninth Symposium on Biotechnology for Fuels and Chemicals Held April 29–May 2, 2007, in Denver, Colorado* (pp. 431-443). Humana Press.
37. Eckaenfelder, G. (2009). Industrial waste water quality. Proceedings of the Water Environment Federation **5**:1-4.
38. Ejedegba, B. O., Onyeneke, E. C., & Oviasogie, P. O. (2007). Characteristics of lipase isolated from coconut (Cocos nucifera linn) seed under different nutrient treatments. *African Journal of Biotechnology*, *6*(6).
39. El-Shora, H., Ibrahim, M. and Elmekabaty, M. (2017). Immobilization and thermostability of lipase from *Jatropha seed. Microbiology Research Journal, International,* 21(2): 1-11.
40. Enujiugha, V. N., Thani, F. A., Sanni, T. M., & Abigor, R. D. (2004). Lipase activity in dormant seeds of the African oil bean (Pentaclethra macrophylla Benth). *Food Chemistry*, *88*(3), 405-410.
41. Environmental Protection Agency (EPA) (2008). Ambient water quality criteria for polynuclear aromatic hydrocarbons. U.S. Environ. Protection Agency Repository, 440/5-80-069.pp 193.
42. Ertuğrul, S., Dönmez, G., & Takaç, S. (2007). Isolation of lipase producing Bacillus sp. from olive mill wastewater and improving its enzyme activity. *Journal of hazardous materials*, *149*(3), 720-724.
43. Falony, G., Armas, J. C., Mendoza, J. C. D., & Hernández, J. L. M. (2006). Production of Extracellular Lipase from Aspergillus niger by Solid-State Fermentation. *Food Technology & Biotechnology*, *44*(2).
44. Fickers, P., Ongena, M. A. R. C., Destain, J., Weekers, F., & Thonart, P. (2006). Production and down-stream processing of an extracellular lipase from the yeast Yarrowia lipolytica. *Enzyme and microbial technology*, *38*(6), 756-759.
45. Fomekong, A., Messi, J., Kekeunou, S., Tchuenguem-Fohouo, F. N., & Tamesse, J. L. (2008). Entomofauna of Cucumeropsis mannii Naudin, its impact on plant yield and some aspects of the biology of Dacus bivitattus (Diptera: Tephritidae). *African Journal of Agricultural Research*, *3*(5), 363-370.
46. Freire, G. and Castilho, F. (2008). Lipases emBiocatálise. In: Bon et al. (org). Enzimasembiotecnologia: Produção, Aplicação e Mercado. Rio de Janeiro, Interciência.
47. Fukuda, H., Kondo, A., & Noda, H. (2001). Biodiesel fuel production by transesterification of oils. *Journal of bioscience and bioengineering*, *92*(5), 405-416.
48. Gordillo, M. A., Montesinos, J. L., Casas, C., Valero, F., Lafuente, J., & Sola, C. (1998). Improving lipase production from Candida rugosa by a biochemical engineering approach. *Chemistry and Physics of Lipids*, *93*(1-2), 131-142.
49. Griebeler, N., Polloni, A. E., Remonatto, D., Arbter, F., Vardanega, R., Cechet, J. L., ... & Ninow, J. L. (2011). Isolation and screening of lipase-producing fungi with hydrolytic activity. *Food and Bioprocess Technology*, *4*, 578-586.
50. Guan, G., Sakurai, N., & Kusakabe, K. (2009). Synthesis of biodiesel from sunflower oil at room temperature in the presence of various cosolvents. *Chemical Engineering Journal*, *146*(2), 302-306.
51. Hadiyanto, M. C., Soetrisnanto, D., & Christwardhana, M. (2013). Phytoremediations of palm oil mill effluent (POME) by using aquatic plants and microalgae for biomass production. *Journal of Environmental Science and Technology*, *6*(2), 79-90.
52. Haider, M. A., Pakshirajan, K., Singh, A., & Chaudhry, S. (2008). Artificial neural network-genetic algorithm approach to optimize media constituents for enhancing lipase production by a soil microorganism. *Applied biochemistry and biotechnology*, *144*, 225-235.
53. Hansen, S. (2007). Feasibility study of performing an life cycle assessment on crude palm oil production in Malaysia (9 pp). *The International Journal of Life Cycle Assessment*, *12*, 50-58.
54. He, Y. Q., & Tan, T. W. (2006). Use of response surface methodology to optimize culture medium for production of lipase with Candida sp. 99-125. *Journal of Molecular Catalysis B: Enzymatic*, *43*(1-4), 9-14.

55. Kamimura, E. S., Mendieta, O., Sato, H. H., Pastore, G., & Maugeri, F. (1999). Production of lipase from Geotrichum sp and adsorption studies on affinity resin. *Brazilian Journal of Chemical Engineering*, *16*, 103-112.
56. Kapranchikov, V. S., Zherebtsov, N. A., & Popova, T. N. (2004). Purification and characterization of lipase from wheat (Triticum aestivum L.) germ. *Applied Biochemistry and Microbiology*, *40*, 84-88.
57. Kasaai, M. R. (2009). Various methods for determination of the degree of N-acetylation of chitin and chitosan: a review. *Journal of agricultural and food chemistry*, *57*(5), 1667-1676.
58. Kaushal, J., Singh, G., & Arya, S. K. (2018). Immobilization of catalase onto chitosan and chitosan–bentonite complex: a comparative study. *Biotechnology Reports*, *18*, e00258.
59. Kempka, A. P., Lipke, N. L., da Luz Fontoura Pinheiro, T., Menoncin, S., Treichel, H., Freire, D. M., ... & de Oliveira, D. (2008). Response surface method to optimize the production and characterization of lipase from Penicillium verrucosum in solid-state fermentation. *Bioprocess and Biosystems Engineering*, *31*, 119-125.
60. Kermasha, S., Van de Voort, F. R., & Metche, M. (1986). Caracterization of french bean (Phaseolus vulgaris) seed lipase. *Canadian Institute of Food Science and Technology Journal*, *19*(1), 23-27.
61. Khan, M. Y., Dahot, M. U., & Noomrio, M. H. (1991). Investigation of lipase activity from Cajanus cajan L. seed. *Pak. J. Sci. Ind. Res*, *34*, 384-386.
62. Kim, K. W., Min, B. J., Kim, Y. T., Kimmel, R. M., Cooksey, K., & Park, S. I. (2011). Antimicrobial activity against foodborne pathogens of chitosan biopolymer films of different molecular weights. *LWT-Food Science and Technology*, *44*(2), 565-569.
63. Kim, B. S., & Hou, C. T. (2006). Production of lipase by high cell density fed-batch culture of Candida cylindracea. *Bioprocess and biosystems engineering*, *29*, 59-64.
64. Kiran, G. S., Shanmughapriya, S., Jayalakshmi, J., Selvin, J., Gandhimathi, R., Sivaramakrishnan, S., ... & Natarajaseenivasan, K. (2008). Optimization of extracellular psychrophilic alkaline lipase produced by marine Pseudomonas sp.(MSI057). *Bioprocess and Biosystems Engineering*, *31*, 483-492.
65. Kiyota, H., Higashi, E., Koike, T., & Oritani, T. (2001). Lipase-catalyzed preparation of both enantiomers of methyl jasmonate. *Tetrahedron: Asymmetry*, *12*(7), 1035-1038.
66. Koopmans, C., Iannelli, M., Kerep, P., Klink, M., Schmitz, S., Sinnwell, S., & Ritter, H. (2006). Microwave-assisted polymer chemistry: Heck-reaction, transesterification, Baeyer–Villiger oxidation, oxazoline polymerization, acrylamides, and porous materials. *Tetrahedron*, *62*(19), 4709-4714.
67. Korma, S., Niazi, S., Alahamad, I., Ammar, A. and Alyousef, H. (2018). Production, classification, properties and application of different chitosan. *International Journal of Research in Agricultural Science.*

.

68. Korneeva, O. S., Popova, T. N., Kapranchikov, V. S., & Motina, E. A. (2008). Identification of catalytically active groups of wheat (Triticum aestivum) germ lipase. *Applied Biochemistry and Microbiology*, *44*, 349-355.
69. Krajewska, B. (2004). Application of chitin-and chitosan-based materials for enzyme immobilizations: a review. *Enzyme and microbial technology*, *35*(2-3), 126-139.
70. Kuwahara, K., Angkawidjaja, C., Matsumura, H., Koga, Y., Takano, K., & Kanaya, S. (2008). Importance of the Ca2+-binding sites in the N-catalytic domain of a family I. 3 lipase for activity and stability. *Protein Engineering, Design & Selection*, *21*(12), 737-744.
71. Ladero, M., Ruiz, G., Pessela, B. C. C., Vian, A., Santos, A., & Garcia-Ochoa, F. (2006). Thermal and pH inactivation of an immobilized thermostable β-galactosidase from Thermus sp. strain T2: Comparison to the free enzyme. *Biochemical Engineering Journal*, *31*(1), 14-24.
72. Lazarova, V., & Manem, J. (2000). Innovative biofilm treatment technologies for water and wastewater treatment. *ChemInform*, *31*(32), no-no.
73. Liese, A., Seelbach, K., & Wandrey, C. (Eds.). (2006). *Industrial biotransformations*. John Wiley & Sons.
74. Lin, E. S., Wang, C. C., & Sung, S. C. (2006). Cultivating conditions influence lipase production by the edible Basidiomycete Antrodia cinnamomea in submerged culture. *Enzyme and Microbial Technology*, *39*(1), 98-102.
75. Lineweaver, H., & Burk, D. (1934). The determination of enzyme dissociation constants. *Journal of the American chemical society*, *56*(3), 658-666.
76. Liu, Z., Chi, Z., Wang, L., & Li, J. (2008). Production, purification and characterization of an extracellular lipase from Aureobasidium pullulans HN2. 3 with potential application for the hydrolysis of edible oils. *Biochemical Engineering Journal*, *40*(3), 445-451.
77. Lotrakul, P., & Dharmsthiti, S. (1997). Lipase production by Aeromonas sobria LP004 in a medium containing whey and soybean meal.
78. Carrillo-López, A., Cruz-Hernández, A., Cárabez-Trejo, A., Guevara-Lara, F., & Paredes-López, O. (2002). Hydrolytic activity and ultrastructural changes in fruit skins from two prickly pear (Opuntia sp.) varieties during storage. *Journal of Agricultural and Food Chemistry*, *50*(6), 1681-1685.
79. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1), 265-275.
80. Luo, Y., & Wang, Q. (2013). Recent advances of chitosan and its derivatives for novel applications in food science. *Journal of Food Processing & Beverages*, *1*(1), 1-13.
81. Mahanta, N., Gupta, A., & Khare, S. K. (2008). Production of protease and lipase by solvent tolerant Pseudomonas aeruginosa PseA in solid-state fermentation using Jatropha curcas seed cake as substrate. *Bioresource technology*, *99*(6), 1729-1735.