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Plant Based Sources of Lipase, Their Application and Recent Advances in Immobilization Techniques

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Abstract- Lipase is a class of hydrolytic enzyme, which catalyses the breakdown of tri-glycerides and is used in different food processing, cosmetic, detergent and various other industries because of its multi-functional properties. In this review, the work has been mostly focussed on the studies that have been done on the lesser explored source of lipase, which are the plant based sources. The study conducted so far on various plants parts such as germinating and dormant seeds, leaves, fruits, as well as waste parts for the extraction of lipase has been analysed. The various application of such plant based lipases that have been so far reported has also been analysed and reported. This review also provides an insight into the recent advances in techniques that have taken place for the immobilisation of enzyme which could be used to make the enzymes more economical and feasible for industrial scale application.

Index Terms – biodiesel, bioremediation, detergent, fruit waste, immobilization, lipase, plant source,

1 INTRODUCTION

LIPASE (triacylglycerol acylhydrolases EC 3.1.1.3) is a water soluble enzyme which is involved in the breakdown of complex triglyceides into free fatty acid and glycerol over an oil–water interface (Reis et al., 2009) [1]. In addition, lipases are also efficient in catalysing the hydrolysis and transesterification of other esters and also the synthesis of esters ,hence exhibit enantioselective properties. (Houde et al., 2004) [2].showed the ability of lipases to perform chemical transformation (biotransformation) has make them increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries

It is a glycoprotein having two isoenzymes and is very non-specific. Molecular masses of known lipases range from less than 20 kDa as in the case of the small lid-less lipolytic enzymes lipase A from *Bacillus subtilis* and cutinase from *Fusarium solani pisi*, to about 60k Da for the larger fungal lipases (i.e. *Geothricum candidum* lipase). Lipases, being non-specific catalyse the breakdown of almost all triglycerides and hence is termed as regio-specific. (Mardani and Salehi, 2016) [3]reported that structurally, lipases have a $\alpha\beta$ hydrolase fold which is common to many hydrolytic enzymes of different origin and function. In $\alpha\beta$ hydrolase fold there is a central β sheet containing eight parallel β strands while the β2 strand runs anti-parallel to the other strands. B2- β8 strands are connected by six α helices. This $\alpha\beta$ hydrolase fold has an active site consisting of a catalytic triad which consists of a nucleophilic residue which can be either serine, cysteine or aspartic acid but almost always is a serine; a catalytic residue which can be aspartic acid or glutamic acid and a histidine residue. This catalytic triad is highly conserved with the histidine residue being the most conserved and the active site which is also known as the nucleophilic elbow contains the nucleophile as a pentapeptide in the form Sm-Nu-X-Sm where Sm represents small residues which most often is a glycine, X represents any residue and Nu is the nucleophilic residue. The active site forms a sharp γ turn which remains conserved while the nucleophile residue varies. Neves Petersen et al. (2001) [4], reported that lipases are negatively charged when they are at their most active state and at alkaline pH. They found that after the cleavage of ester bonds, the active site had a negative potential. The negative electrostatic potential was due to ionised free acid generated from the active site immediately after the cleavage of ester bonds. Rehm et al. (2010) [5], in their study analysed the activation of lipases which was based on opening of the lid domain when a hydrophobic solvent came in contact with the lipase. They analysed the lid opening feature in water as well as organic solvents and deciphered that the open lid conformation was stable but in water the lids closed when no contact was there between the hydrophobic solvent and the enzyme leading to the indication that lipase are activated only transiently in the presence of hydrophobic interface even if bound substrates are present.

Enzymes, as we are well aware of, is a quintessential part of all industries. Lipase is one such multifunctional enzyme which is used in varied industries starting from baking, brewing, detergent industries and so on and so forth. Lipases are enzymes that hydrolyse the triglycerols to glycerols and fatty acids. In the detergent industry, lipases are widely used because these group of biocatalyst is biodegradable, non-toxic, and can be produced from different sources such as microbial sources, plant sources, animal sources, etc. Detergent requires lipases as an additive in order to remove the oily stains from cloth for which alkaline lipases are mostly used. Many microorganisms ranging from *Staphyloccus sp.* Strain, *Pseudomonas fluoresens*, etc. are commercially harvested to produce lipases at industrial scale as they serve as excellent biocatalyst as they have many different active site motifs which helps them in catalysing a number of different reactions. However, sometimes the cost of production of microbial lipases can be unfeasible owing to various pre-treatment required, mostly in cases where the enzymes are intracellular.

The source of lipase can be broadly classified into three categories; animal, plant and microbial. Animal lipases can be further sub categorized as hepatic lipases, hormone-sensitive lipases, lipoprotein lipases, pancreatic lipases, etc based on their origin. Microbial lipases which are the most explored can be of various origins such as bacterial lipases which are categorized into eight family, they can also be of fungal origin which are widely used in industrial scale and also can be of yeast origin which is the most common terrestrial source. Plant lipases can also be of various origins like from the seed, fruit, peels, etc.

Many a times, enzymes lose their functionality or show reduced activity due to a number of factors that might influence their activity and for this reason researchers have developed the technique of enzyme immobilisation which is a process by which whereby the enzyme is attached to a solid surface which remains insoluble in the reaction mixture. Enzyme immobilization has been found to be an effective process by which the stability of the enzyme can be enhanced, which makes it suitable for a wide range of application; it also makes the enzyme reusable which makes the enzyme feasible from many industrial perspective.

2 LIPASES FROM PLANT SOURCES

Many plant tissues and parts which include seeds, leaves, fruits, peels, etc have shown the presence of lipase and among them the highest lipase concentration is seen in the seeds, especially the olegeneous seeds which have high amount of triacylglycerol. However other plant sources have also shown decent amount of lipase activity.

2.1 Lipase from Seeds

Eze and Ezema (2012) [6] isolated lipase from the endosperm of white melon seeds (*Cucumeropsis manni*) and purified them using 70% ammonium sulphate precipitation. Two lipase activity peak were obtained for the gel filtration and was designated as lipase A and lipaseB. Lipase A had a purification fold of 5.4 and an enzyme activity yield of 0.7 while lipase B had a purification fold of 11 and enzyme activity yield of 1.76. The optimum pH for lipsse A was 7.5 rendering it an alkaline lipase while the optimum pH for lipase B was 5.9 rendering it acidic lipase. The pH stability of the alkaline lipase was between 6.5 and 8.0 while that for the acidic lipase was between 3.5 and 6.0. The optimum temperature of both the lipases was around 37 °C but the enzyme could remain stable upto 42 °C. Ca and Zn ions increased the enzyme activity while Pb and Al ions showed inhibition of enzyme activity. The enzyme activity increased upto a substrate concentration of 0.06 mg/ml beyond which the activity declined. All these properties make the white melon seed lipase a potential source of industrial application.

(Gadge et al. 2012) [7]isolated lipases from germinating seeds of soybean, groundnut, pea and castor. The maximum lipase activity was shown by soybean and it was followed by castor seeds. The lowest enzyme activity was shown by groundnut and pea seeds. The optimum pH and temperature for the activity of lipase from these sources was found to be pH 8 and 30 °C respectively, beyond which the activity rapidly decreased. Low concentrations of Ca and Mg ions was seen to increase the enzyme activity while higher concentration and EDTA inhibited the enzyme activity. Characterisation of the biochemical properties of the germinating seed lipases in this study makes these sources a potential source of industrial enzyme application

(Enujiugha et al. 2004) [8] Reported the presence of lipase in the dormant seeds of African oil seeds. In their study, they used the seeds of African oil seeds with different substrates such as palm oil, palm kernel oil, coconut oil and raphia oil. They found that the lipase activity was highest with palm kernel oil and coconut oil as substrates which have short chain fatty acids. They also found in their study that there is a linear relationship between the enzyme concentration and the lipolytic activity, highest activity shown in the range of. 0.5 and 0.75 gm and then showing steady rate of lipolysis up till the concentration reaches 4 gm, beyond which there was a drop in the rate of lipolysis but without any decrease in the enzyme activity. The optimum temperature for lipolysis was found to be 30 °C, above which the activity declined and the optimum pH was found to be a near neutral pH. Linear relationship between the reaction time and rate of lipolysis was found and the optimum was at 60 °C and it was found that inclusion of Ca ion increased the enzyme activity while NaCl, $HgCl_2$ and EDTA decreased the activity. The lipase thus obtained can be used for industrial purposes.

(Ejedegba et al. 2007)[9] isolated lipase from coconut seed (*Cocos nucifera* linn) endosperm and analysed the activity under different nutrient conditions such as nitrogen treatment, potassium treatments and Calcium treatment. They found that the enzyme activity was optimum at a pH range of 7.5-8.5 and enzyme had a stronger affinity for its native substrate, i.e coconut oil. They also reported that the enzyme activity was observed up till the substrate concentration reached 8mM; beyond which there was a decline and the optimum temperature was found to be 30 ºC except in case of nitrogen treatment, where the optimum temperature varied from 35-40 ºC.

(Tambun et al. 2020) [10]demonstrated in their study the generation of free fatty acids from avocado seeds by activating the lipase present in the avocado seeds. They found that the highest fatty acid content generated through the lipase enzyme was at 35 ºC, with water content of upto 40% and a reaction time of 12 hours.

(Kouteu et al., 2017) [11], extracted crude lipase powder from the seeds of Jatropha mahafalensis and Adansonia grandidieri and transesterification was carried out by analysing various parameters such as effect of alcohol polarity, glycerol and thermal water activity. For the alcohol analysis five linear primary alcohols namely ethanol, propanol, butanol, pentanol and hexanol were used. It was seen, irrespective of the alcohol, A. grandidieri crude lipase powder showed the highest yield and the alcohol polarity was inversely related to the FAAE. The increasing concentration of glycerol in the medium decreased the lipase activity and at 10% glycerol addition there was inhibition of the enzyme. A. grandidieri crude lipase powder was more sensitive to glycerol compared to J. mahafalensis. For optimum activity A. grandidieri seed lipase powder needed to be incubated at a thermal water activity of 0.33 or water content of 3.5% while that for J. mahafalensis was 10.7% which makes A. grandidueri crude lipase powder more effective. However, at a thermal water activity of 0.8 both the lipase were inactivated. A. grandidieri crude lipase powder because of its better properties was used further for the synthesis of fatty acid ethyl esters by stepwise addition pf ethanol. Of the various strategies applied, addition of 1/2 equivalent of ethanol after every 15 hours along with the addition of new crude lipase powder every 30 hours giving a yield of 91% and 96% after 96 hours. However, this strategy uses 25% of the crude lipase powder which may present as a disadvantage.

2.2 Lipase from Leaf Extract

(Kumari et al. 2019) [12],isolated lipase along with other enzyme was from the chloroplast of crude leave extract of lettuce and spinach through genetic engineering and assessed their efficacy in detergents. The chloroplast lipase was found to be exhibited an optimum temperature at around 70 ºC and worked well in a wide temperature range of 30-80 ºC while the commercial lipases showed an optimum activity at around 30 ºC and very little activity was seen in the temperature ranges of 70-80 ºC. Chloroplast lipase without protease inhibitors showed a higher activity by 30-25% compared to the commercial lipases with protease inhibitors.

2.3 Lipase from Latex

(Abdelkafi et al. 2011) [13] isolated lipase from the crude powder of *Carica papaya* latex and the lipase activity was 2000±185 U/ g when tributyrene was used as substrate, 256±8 U/g when olive oil was substrate and 983±29U/g with trioctanoin as substrate. The *carica papaya* latex lipase was seen to be more effective on short chain fatty acids and the optimum pH range for the lipase activity was around pH 9, being only and no activity was seen below pH 6. The enzyme showed maximum activity at 50 ºC but the enzyme was not stable above 37 ºC, hence the thermostability of the enzyme was at 25 ºC. The enzyme activity was inhibited by addition of detergents above the critical micellar concentration but were resistant to NaTDC and caps similar to gastric lipase and *Yarrowia lipolytica* lipase. The enzyme could be stored at 20 ºC for only about 20 weeks after which it lost 50% of its activity but when stored at 4 ºC, it retained more than 90% of its activity for the same time period.

2.4 Lipase from Fruit Waste

(Okino-Delgado and Fleuri, 2014) [14] also isolated lipase from the waste parts of orange, i.e core, frit and peel . The maximum lipase activity was observed for the peel which was 57.55 U/g followed by core at 36.5U/g and frit at 22.95U/g. The optimum pH of the enzyme activity was at pH 6 for the core, pH 7 for peel and pH 9 for fruit. The optimum temperature for the enzyme activity was at 20 ºC and 70 ºC for core, 20-60 ºC for the peel and 20-70 ºC for the frit suggesting the presence of lipase isoforms. The presence of different salts increased the activity of the core and peel but no effect was seen on the fruit.

3 APPLICATION OF LIPASES FROM PLANT SOURCE

(Okino-Delgado et al., 2017) [15], extracted lipase from orange wastes, i.e; the core, peel and frit for the bioremediation of waste cooking oil. The orange waste lipase was purified using 60% ammonium sulphate and the enzyme activity was found to be 50.86U/g. Enzyme precipitated through acetone powder and microfiltration showed reduced enzyme activity, to the extend where the peel enzyme concentration was 0U/g when microfiltration was used. Orange waste lipase showed promising result in the bioremediation of waste cooking through transesterification reaction.

(Rasit and Chee Kuan, 2018) [16] utilized the lipase isolated from vegetal and fruit dregs by fermentation with molasses and water for pre-treatment of palm oil mill effluent Lipases along with other enzymes were isolated from the dregs and the maximum pH for the lipolytic activity was 7.The presence of lipase along with other enzymes like protease in the vegetable and fruit waste helped in reducing the total suspended solids as well as the chemical oxygen demand in the waste water. The presence of lipase also reduced the oil and grease of wastewater.

(Polizelli et al. 2013) [17] isolated lipase from the seeds of *Pachira acquatica* was and analysed its use as a detergent as well as its feasibility in poultry wastewater pre-treatment and fat hydrolysis. The optimum pH for the lipase activity was found to be in the range of 8-9 and a temperature of 40 ºC which makes it an ideal candidate as a detergent additive. The incubation time for the lipase was 90 minutes beyond which the enzyme activity declined. In the presence of Hydrogen Peroxide the enzyme activity decreased. Only 30% of enzyme activity was retained on addition of 1% hydrogen peroxide while 24% was retained in the presence of 3% hydrogen peroxide. The concentration of fatty acids in waste water prior to the treatment with lipase was approximately 0.23 mM which significantly increased to approximately 1.7 mM on treatment with the lipase for 60 minutes. After 72 hours of incubation, the fat particle degradation was 13% more compared to the control and an increase in the enzyme concentration to 30 mg/ml increased the percentage to 14.3%, however the activity being lower than that of pancreatic lipase. Hence, this lipase is more effective for vegetable oil compared to animal fats.

(Kumari et al., 2019) [11]isolated chloroplast lipase from the leaf extract was used to remove mustard oil stain from fabric and compared the efficacy to the commercial lipase.The efficacy was found to be comparable at 30C ºC but at 70 ºC, chloroplast lipase crude extract showed four times better clearance on the stained cloth. A high reflectance at 450 nm was observed for the de-stained cloth for the chloroplast lipase.

(Mateos et al. 2020) [18] Isolated lipase from the latex of the fruit of *Araujia sericifera* and investigated the hydrolysis and transesterification of waste cooking oil The direct transesterification of waste cooking oil in excess of methanol took place at a temperature of 45 ºC for 24 hours of reaction. Free fatty acid was also released which indicated the hydrolysis reaction in the presence of the ASL lipase at 45 ºC for 48 hours and the highest lipase activity was reached with the addition of 5mg of lipase and 5ml of water. However, the reusability was significantly low rendering it unsuitable for reuse.

4 IMMOBILIZATION OF LIPASES

Many different materials have been used for immobilisation of enzymes through various techniques such as adsorption, covalent binding, cross-linking, entrapment, encapsulation, etc. For each of the methods of immobilization, different materials are being used ranging from the classical inorganic, organic, mineral , synthetic polymers, biopolymers or carbon-based material to the newer materials such as magnetic particles, nano-particles, mesosporous particles, ceramic materials, carbon nanotubes or electrospun materials or even biomaterials (Nur Mala Sari et al. 2020) [19] immobilized lipase on different biopolymers which include tapioca biopolymer starch, gelatin biopolymer and chitosan biopolymer by crosslinking with gluteraldehyde as well as sodium alginate biopolymer by entrapment. The highest activity of immobilized enzyme was seen in the case of tapioca starch biopolymer followed by gelatin. The lowest activity was reported for sodium alginate biopolymer. Enzymes immobilised by all the four techniques could be reused for about eight times with tapioca starch showing highest stability and reusability of 69.4%in the eighth round followed by sodium alginate with 65%, 48.2% in chitosan and lowest reusability of 15% in gelatin. The shelf life of the immobilised lipase declined rapidly after 30 days while the highest shelf life was observed fir the tapioca starch biopolymer followed by sodium alginate and chitosan. The lowest shelf life was observed for gelatin biopolymer. Hence, the study brings forth the advantage of cross linking technique for better immobilization.

(Bonine et al., 2014) [20], immobilized lipase from the seeds of *Pachira aquatica* tree and immobilized them using Calcium alginate as well as Alginate-PVA beads using the entrapment technique It was seen that using the calcium alginate entrapment technique, they found that best results were obtained when 3% alginate and 0.5mmol/l $CaCl_2$ as well as when the bead size is small. While, in case of alginate-PVA beads, the best percentage of Alginate was found to be 2% and the reusability was found to be six times with the last reaction showing an enzyme activity of last reaction. The optimum temperature for both free and immobilized enzyme was found to be 40 ºC for 90 minutes. Enzyme activity was found to be more stable at lower temperatures of 30-40 ºC which decreased after two hours while no enzyme activity was seen above 60 ºC. The immobilized enzyme showed a greater thermal stability and the alginate beads absorbed 3% more water than the Alginate-PVA beads which is important in order to reduce the interfacial denaturing character.

(Sun et al. 2015) [21] immobilized *Yarrowia lipolytica* lipase on WDA918 a macro-porous acrylic acid resin, LX-1000EP, which is an epoxy resin and LX-1000EA, an amino resin. WDA had more than 80% activity which was the highest. When enzyme concentration was increased around 4-6 mg/ml, the binding efficacy was around 60% for all the three resins. The

optimum temperature of the immobilized lipase was found to be 40 ºC which was 5 degree higher than the free enzyme but the enzyme was active at a temperature range of 20-70 ºC. The immobilized enzyme could retain 50% of their activity even at 70 ºC. The optimum pH of both the free and immobilized enzyme was at pH 9 but immobilized enzyme showed greater activity. The immobilized enzyme retained 80% of their activity even after 5 cycles. The maximum residual activity was retained by the amino resin, EA and the reusability was enhanced when treated with tetra butanol.

(Lau et al. 2014) [22],immobilized lipase on chitosan by incorporating them with sonicated graphite oxide The morohology of the beads without mixing with graphite oxide was studied at 2, 4 and 20 hours and the beads had appropriate morphology after 4 hours of incubation. These beads however were not stable and hence they were incorporated with graphite oxide which then showed a much rigid structure. The activity of the immobilized lipase with or without the graphite oxide is almost same at 3.23 and 3.48 units respectively indicating the graphite oxide's role in only mechanical stability but coupling with agents such as NHS and EDC enhanced the immobilized lipase activity. The immobilized lipase showed an activity of 64 U and showed 98 and 88% conversion with commercial enzymes like lipozyme and novozyme.

(Dumri and Hung Anh 2014) [23] synthesised a complex consisting of Ag nanoparticle and, polydopamine and used it for immobilization of lipase (LPA complex) which then was used for biodiesel production Ag Nanoparticle which is known to have antimicrobial property, hence protecting the lipase at the same time providing stability and controlled morphology was coated with a layer of polydopamine which occurs as a result of self-polymerization of dopamine on noble metals. This complex was used to immobilize lipase in the ratio of polydopamine:lipase in 1:1 without the need of any coupling agent such making the immobilization much more economical. The immobilization yield was higher compared to other covalent linking immobilization techniques and because of the self-polymerization property of dopamine, immobilization requires only 4 hours. The optimum temperature for the immobilized lipase was 40 ºC and retained about 95% of its activity even after 6 hours while the native enzyme could only retain 40% of their activity after 6 hours. For biodiesel production, the immobilized lipase produced 95% biodiesel at 40 ºC whereas the lyophilized lipase could only produce 86%. The immobilized could be used for upto 7 cycles with a decrease of only 27% activity by the seventh cycle which makes this technique industrially feasible as bio-complex could be recovered easily after use by centrifugation.

(Zou et al., 2020) [24] used silica clay modified with aminopropyltriethoysilane and lipase was immobilized by activating the complex with gluteraldehyde With lipase concentration of 10 mg/ml at pH 7.5 and 30 ºC temperature for 60 minutes the optimum gluteraldehyde concentration was 1% beyond which the activity of the immobilised lipase complex decreased. However, with increase in the gluteraldehyde concentration to 3%, the immobilization efficiency increased from 70-78%. Hence to strike a balance between activity and efficiency 2% gluteraldehyde was used for immobilization of lipase to produce biodiesel. The highest activity of lipase was achieved at 20 mg/ml and a temperature of 50 ºC beyond which the activity decreased. When the lipase concentration was increased from 0.2- 0.6 mg/ml, the conversion yield of biodiesel increased from 70.6%-85.0%, however beyond 0.6 mg/ml, the yield decreased. The yield increased when the temperature was around 45 ºC beyond which it decreased and the optimum contact time was 30 hours. The immobilized enzyme retained more than 67% of the activity even after 5 cycles which render them highly reusable and industrially feasible as silica clay is a cheap material and easily available.

(Kumar and Melo 2016) [25] used jackfruit's exocarp part of the seed pouch as a membrane for immobilization of urease enzyme. The Km of the immobilized enzyme was 13.8 mM compared to 39.24 mM of the free enzyme. The decrease in the Km indicated an increase in enzyme activity of the immobilized enzyme. The pH of the immobilized enzyme was found to be 7 while the optimum temperature range was found to be 35-40C. The membrane was reusable for up to 38 times with 2.5 mM and 10 mM urea concentration. The membrane was stable for up to 46 days with 10 mM urea concentration and retained up to 85% of the initial enzyme activity which proves jackfruit membrane a good biocompatible membrane for enzyme immobilization.

4 CONCLUSION:

Lipases are an important contributor in many industries and hence are required in large quantities. Most industrial lipases are of microbial origin, however acquiring lipases from microorganism is most often a cumbersome task. Plant based lipases are not a widely explored genre but has huge potential. The most used source of lipase from plants are the seeds, however other parts such as peels, frits, etc which usually form a source of waste can be also used as a source of lipase. The utilization of such products for the extraction of industrially important enzyme lipase, would not only provide an alternate source but also curb problems related to agro-industrial waste and thus help in waste management which in turn, makes such sources much more economical. The work related to such plant based sources has picked velocity only in the recent years and it still at a native stage which makes it an interesting area of study.

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