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Lipase Enzyme: a comprehensive review on production, optimization by using agro-industrial waste.

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Abstract:
Lipases are triacylglycerol hydrolases used in different types of industries such as in textile industry, detergent industry, in food processing, flavor development and their quality improvement, , medicinal field, cosmetics, bakery and confectionery, tea processing, biosensors, degreasing of leather, waste or sewage treatment plants, oil biodegradation, pulp and paper industry, in biodiesel production. The higher enzyme cost limits the production of lipases by considering this the present review focuses on the processes for optimizing the enzyme production though solid state fermentation by using agro-industrial waste like oil cakes. Oil cakes are waste produced after boiling process, and generally responsible for the lowering the production cost of lipase enzyme production.
Keywords: Lipases enzyme, Solid state fermentation, oil cakes, microbial lipases, optimization of lipases.

Introduction:

Lipases are (Triacylglecerol hydrolases EC 3.1.1.3) are extracellular hydrolytic enzymes perform cleavage of ester bonds in triacylgycerol and produces free fatty acids , di and monoglycerides, glycerol(Aguieiras et al., 2015)(A. C. D. Oliveira et al., 2014). These enzymes are also catalyze esterification's, breakdown of acids, amino acids, alcohols, and interesterifications reactions(Aguieiras et al., 2015). They are intracellular as well as extracellular and also highly specific and selective in nature (Villeneuve et al., 2000). They are found in animals, plants, and microorganisms like bacteria, fungus, actinomycetes. However the enzymes production from microorganisms gained a lot of attention because of their compelling characteristics and easy to production (Salihu et al., 2012). Among the bacteria the bacillus sp. Shows the highest enzyme producers and also stable in respect to temperature and P^H than other bacteria other genus of bacteria like pseudomonas, staphylococci are also found to be highest lipase producers. The fungal lipases are also widely used in industrial production. Aspergillus sp, rhizopus sp and rhizomucor were used mostly for the production of fungal lipases. The Candida sp. in yeast were highest producers of lipase and (Salihu et al., 2012)(BALTACI et al., 2019).

The use and presence of lipases was discovered a 300 years ago, their process nearly 90 years ago (Hasan et al., 2006). Nowadays enzyme used in fat and oleochemical industry, for production of biodegradable polymers, in textile industry, detergent industry, in food processing, flavor development and their quality improvement, resolution of racemic mixture, in medicinal field, cosmetics, bakery and confectionery, tea processing, biosensors, degreasing of leather, waste or sewage treatment plants, oil biodegradation, pulp and paper industry, in biodiesel production(Hasan et al., 2006).

Due to their gigantic use lipase enzyme production always attract the attention to produce it at as an industrial level. The high enzyme cost, the low rate of reaction and enzyme inhibitory substances in media are some the problems associated with the problems in enzyme production (Aguieiras et al., 2015). Therefore many of the scientists working hard to find the way to reduce the cost of enzyme production and to increase the purity of enzyme, also more stable enzyme at different range of P^H and temperature. The most production cost of enzyme comes from a utilization of costly substrate and hence needs to lower the cost of production of lipase enzyme. The agro-industrial wastes shows promising solutions for the production of low cost enzyme due to their easy availability and low cost (Budžaki et al., 2019). The agro-industrial waste are used for the various industrially potent molecule production (Sadh et al., 2018). Cereal grain waste contain high amount of hemicellulose used for the production of Xylo-oligosaccharides (Martins et al., 2022).

Solid state fermentation for lipase production:

The solid state fermentation gave promising results to enhance the product yields, to reduce the production cost by reducing the cost of downstream processes(De Almeida et al., 2016). Solid state fermentation (SSF) is the fermentation process in which water activity of substrate are near absence or minimum and the substrate itself act as a carbon and energy sources. The another type of fermentation is submerged fermentation (SmF) in which water activity remained higher, it uses molasses and broth medium for fermentation processes (Subramaniyam Ravichandran Stanford University 14 & 69, 2012).

Scientist nowadays mainly focusing to utilize the agro industrial waste to produce the different types of industrially important products by using solid state fermentation due to their low requirements of water and easy to handle. The agro industrial waste like cassava bagasse, sugar cane bagasse, oil cakes, apple pomace, coffee pulp, was mostly used in the recent era to produce value added products like antibiotics, amino acids, ethanol, single cell protein, secondary metabolites, organic acids, enzymes, mushrooms, biostimulants, biopesticides, in biorefining and bioplastic production, antioxidant, phenolic compound production as an antioxidants (Mirpoor et al., 2021)(Bhanja Dey et al., 2016) (Singhania et al., 2008)(Mattedi et al., 2023). The SSF has many advantages than the traditional methods like submerged fermentation (SmF) process for smooth and cost effective easy to utilize and variety of substrates, evading the processes like monitoring many parameters, higher product yield no foam generation, and easy to recover the end products and hence recent study attracts solid state fermentation to produce a novel and cost effective products(Webb, 2017). J A rodriguez etal showed that *Rhizopus homothallicus* (IRD13a) shows 60 fold increase in the production of lipase in solid state fermentation using sugarcane bagasse as a support, optimized by using nutrient content medium P^H, emulsifier, olive oil as inducer(Rodriguez et al., 2006).

Oil cakes as a substrate for lipase production:

There are different products are used by using agroindustrial waste among them oil cakes (by product after extraction of oil from different oil seeds) used for enzyme production like lipase, phytase and glutaminase, alpha amylase glucoamylase, proteases production (Ramachandran et al., 2007). They are also used in the mushroom production and responsible for their higher yield. Recently they are used for production of bioplastics and in biorefineries (Mirpoor et al., 2021). They are also responsible for the increase in the solubility of rice straw wherever used as substrate(Ramachandran et al., 2007). Oil cakes have higher content of fatty acids, dry matter along with crude protein, crude fiber, ash, calcium, phosphorous. The oil cakes are differ in amino acid content and some are deficient for one or two amino acid therefore it is necessary to utilize the oil cakes as per need (Ramachandran et al., 2007)(Mirpoor et al., 2021).

In world the crops like soybean, rapeseed, cotton, sunflower, groundnut, sesame, black cumin, coconut, palm kernel, canola, Olive grown as oil crops. The cultivation of soybean is the mostly used as crops in the world than other and having a high source of proteins and essential amino acids and they are also high source of phenolic compound including flavones(Mirpoor et al., 2021). But other than soybean other cotton is also used as oil crops as well as in for fabric and other many more. Cotton seed oil cakes also containing high amount of proteins fatty acids and it used as animal feed and fertilizer applications.

It has similar amount of amino acids as soy meal products(He et al., 2015). Other oil cakes vary in their composition as described in table. The major industrial uses of oil cakes were enzyme, organic acid, antibiotics, vitamins, enzyme immobilization, and mushroom production (Ramachandran et al., 2007) (Budžaki et al., 2019).

Optimization of lipase Production:

Lipase production by using oil cakes as substrate has been studied far long ago, and scientists nowadays working hard to optimize the process and lipase production. The media supplements will increase the lipase production by 58% than in the tray reactors and addition of carbon source also can be useful to increase the production of lipase (do Nascimento et al., 2021). The mixing of two substrate palm kernel oil cake and sesame oil cake increases the production of lipase from 127 to 460 U/g (F. Oliveira et al., 2017). The optimum production conditions for production of lipase by using pseudomonas sp. shows that at P^H 5.9, moisture 33% and at optimum temperature 28^0C the lipase production increased by 0.7 % and shows that the agro-industrial waste play a significant role in the lipase production by solid state fermentation (Faisal et al., 2014).

By using different parameters like supplementation of carbon and nitrogen source such as sucrose, glucose, lactose, peptone, yeast extract and ammonium sulphate at increasing concentration upto 1 to 5 % showed that at 3% concentration of sucrose highest lipase activity was observed. The 5% yeast extract shows higher biomass production, but not a lipase production at P^H 6 and at temperature 37^0C (Bharathi et al., 2019). On the basis of colony characters the species of pseudomonas, bacillus, Escherichia coli, klebsiella Sp. are found to be most producers of lipase at 37 ^{0}C in the presence of 3% sucrose and 5% yeast extract (Bharathi et al., 2019).

There is broad range of microbes need environment based on their growth and cultural characteristics. On tributyrin broth containing yeast extract, NaCl, peptone, and 1% olive oil at 36^0C, maximum production of lipase were found at 48 hours incubation time, with supplementation of carbon and nitrogen sources i.e. palm oil as carbon source and peptone as nitrogen source. Along with scientist studies P^H temperature and agitation speed and found that maximum enzyme production were at 36^0C, P^H 7 and at 160 rpm (Priyanka et al., 2021). By using a central composite design (CCD), an experimental design of mathematical model showed that glucose concentration should be lower until soy and yeast extract have optimum values and hence the oil content in soy flour acts as an inducers for lipase production (Yordanova et al., 2014)(Rigo et al., 2010). The complementary production of lipase with protease in a single media gaining the industrially significant views (Sangeetha et al., 2010).

Microbial lipases:

The most microbial lipases are bacterial, fungal and yeast based. The bacterial lipases are more thermo tolerant especially from the bacillus species.(Javed et al., 2018). The bacterial genera producing industrial based lipases are bacillus, Pseudomonas botryococcus and Burkholderia and they are highly stable low energy requirements, highly specific, lower production and processing time, low cost producing, and grow on easily available substrates (Vishnoi et al., 2020). The fungal species that produces lipases are Aspergillus Sp., Penicillium Sp., Rhizopus sp., Fusarium sp., Geotrichum sp., Trichoderma sp., and Mucor sp.(Kumar et al., 2023).

The advantages of using fungal lipases are high efficacy under mild conditions, easier to practice, capable of decrease contamination, accessibility to dicerse fungal sources, enhancement of catalytic power through genetic engineering (Kumar et al., 2023). The major sources of yeast based lipases are Yarrrowia sp., Candida sp.,.(Kumar et al., 2023). The disadvantage among the use of fungal based lipases are their high sensitivity towards carbon, nitrogen temperature, metal ions, surfactants and moisture content (Villeneuve et al., 2000), (Hasan et al., 2006).

The table shows the microorganism's produces lipase enzyme and the scientist trying to optimize the process of enzyme production. There are different parameters used for the production of lipase like moisture content, optimization of P^H, carbon source, nitrogen source, inoculum size, and temperature in solid state fermentation.

Table 1 Microbes producing Lipase enzyme and there parameters to optimize enzyme production

Process optimization							Lipase activity
Lipase producers	Substrate for lipase production	P^H	Temperature (in ^{0}C)	Carbon source & (Moisture)	Nitrogen source	Inducer	
Penicillin (Di Luccio et al., 2004)	Soy Cake	7	27	7.6 Wt %	-	Olive oil 10%	
Bacillus Amyloliqufician s (Mazhar et al., 2023).	Soy cake, wheat bran, oil wastes, mlasses	7	40	Carbon source (Sucrose + Fructose) = 1% & 60 %	Nitrogen Source: Peptone + Yeast Extract+ NH_4NO_3 (all 1%)	Olive oil	Soy cake 41 u/ ml
Rhizopus Arrhizus (Dobrev et al., 2018).	Corn flour, wheat bran, wheat flour, sunflower cake	7	30	Glucose + sucrose+ starch & 66%	Peptoe + tryptone	Olive oil	1021 U/g
Aspergillus ibericus MUM	palm kernel oil	7	30	1% glucose &	NH4Cl ranged	Olive oil	460 U/g

(F. Oliveira et al., 2017)	cake (PKOC)			60%	from 0% to 5%		
Aspergillus niger,(Putri et al., 2020)	Jatropha seed cake + rice bran	7	30	2% maltose + 1.5% glucose	2% peptone	Olive oil	176 U/g
Rhizopus oryzae (Vaseghi et al., 2013)	Sugarcane bagasse	8	45	80% moisture	1% peptone	Olice oil	225 U/gds
Aspergillus niger (ZULKIFLI & RASIT, 2022), (A. Paithankar Rewatkar, 2014)	Copra waste Groundnut ,oil cake	7 + 7.5	35 + 30	60% moisture +	-	-	170 U/ml + 7.89 mg/L
Pseudomonas aeruginosa PseA (Mahanta et al., 2008)	Jatropha seed cake	7	35	50%	$NaNO_3$	-	625 U/gm
Bacillus licheniformis (Annamalai et al., 2011)	Peanut oil cakes	9	55	-	-	-	730 U/ml
Burkholderia contaminans LTEB (Galeano et al., 2017)	Sugarcane bagasse	7	50	60%	-	Soybean oil + Palm oil	192 U/ml

Conclusion and discussion:

The demand to produce the lipases at lower cost, stability in the temperature, P^H and salt concentrations, easy for processing, ecofriendly and easy to manipulate under genetic conditions. The bacterial lipases looks more stable for industrially production than fungal based because of their stability in the environment in harsh conditions. Although fungal and bacterial species maintained their importance in lipase production bacterial lipases shows promising results in solid state fermentation which is easy to handle. Because of some drawbacks of heat production in the production system solid state fermentation limits their use, but scientists trying hard to overcome this limitation, and looking forward to produce the lipases by using solid state fermentation.

The major cost of enzyme production comes from the utilization of raw substrates hence the use of agro- industrial waste based lipase enzymes shows gaining interest, because of they are cheap and easy avail. The experiments suggest that the some parameters like temperature, P^H, Salt concentration, inducer sources, carbon, and nitrogen source can enhance the production of lipases in the medium. Hence form the review we conclude that production of lipases from bacterial sources as their high stability and easy to handle can be a promising source of lipase production in solid state fermentation by optimizing their process for production.

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