Review Paper:

Lipases with preferred thermo-tolerance in Food Industry

Govada Vayunandana Rao[1] and Kota Sobha[2*]
1. Department of Biotechnology, RVR and JC College of Engineering, Guntur 522 019, Andhra Pradesh, INDIA
2. Department of Chemical Engineering, RVR and JC College of Engineering, Guntur 522 019, Andhra Pradesh, INDIA
*sobhakota2005@gmail.com

Abstract

Lipases are defined as 'esterases' of carboxylic acid because of their explicitness for carboxylic acid ester bonds. In general, these enzymes can be of bacterial, fungal and plant (seeds) origin with catalytic property variations. Extensive use of lipases in industries is due to the exhibition of stereo, regio and substrate specificity and thermostability. This review provides a gist of lipase applications and from the synthesized gist it was evident that lipases are extensively used in dairy and flavour industries. Emphasis was laid on applications of thermostable lipases especially in food industry which provided novel strategies as a substitute for chemical flavours in food industry. Synthetic chemical flavours usually improve the food taste but they show certain devastating effects if they are not within acceptable daily intakes.

In order to beat the chemical flavour toxicity, an attempt is made here to tackle these issues with thermostable lipases produced flavours with evidence of some research in this direction. Finally, this review aims at providing the need to research more natural lipases and standardize their usage in food industry.

Keywords: Lipase, thermostability, food industry, catalysis, chemical flavours.

Introduction

An enzyme is a high molecular weight biological molecule made up of one or more polypeptide chains and functions as a biological catalyst, mediating several biochemical reactions with specific substrates. Enzymes are naturally present and can also be synthesized. Some naturally occurring and commonly encountered enzymes are Lactase, Amylase, Invertase, Cellulase, Proteases and Lipases[1]. Among all these enzymes, lipases have been broadly used in several industries such as detergents, food, dairy, flavouring and textiles before mid-1980[2] and also the global industrial enzyme market is expected to rise at a CAGR of 6.83% during 2019-2024. Food industry dominates the enzyme market with maximum share of 37%[3].

Lipases are otherwise called esterases of carboxylic acid because of their explicitness for carboxylic acid ester bonds. Lipases are numbered by Enzyme Commission as (3.1.1). Lipases catalyze a wide array of biological reactions such as lipolysis, esterification, transesterification and amidation[4]. Few commonly used industrial reactions using lipases are: Lipolysis is the breakdown of fats and oils into free fatty acids and glycerol, while esterification is a reaction procedure between an alcohol and an acid[5-7]. Transesterification defines the exchange of acid and ester groups: if the exchange happens between two esters, it is called inter-esterification and alcoholysis if displacement of alcohol from an ester by another alcohol is effected[8].

Classification of lipases: In general, lipases are segregated based on their specificity into 3 types:

1. Substrate specific: Lipases are specific towards a substrate for the desired product. For instance, biodiesel was produced by using *Pichia pastoris* expressed immobilized lipase from *R. oryzae* (1, 3-specific)[9,10].

2. **Regioselective:** These lipases are sub divided into 3 types:
A) Non-specific: Lipases which are not substrate specific, they catalyze hydrolysis of triacylglycerols into free fatty acids and glycerol into mono and diacylglycerols[11-14]. Structured lipids with high purity were obtained using canola oil in the presence of caprylic acid with lipase (*C. antarctica*) catalyzed acidolysis[15].

B) Specific: Lipases involve catalysis of triacylglycerols at C1 and C3 positions producing 2-monoacylglycerols and 1, 3 or 2-3 diacylglycerols[16]. For instance, fatty acids with long chains were synthesized in the presence of caprylic acid with immobilized lipase (1, 3-specific) catalyzed acidolysis of walnut oil[17].

C) Specific or selective type fatty acids: Lipases catalyze hydrolysis of long chain fatty acids having esters with double bonds on C-9[9]. For the synthesis of short chain esters, lipases with strongest specificities found in *Aspergillus niger* and *Candida lipolytica* were used[18].

3. Enantioselective: They extraordinarily hydrolyze from the prochiral predecessor. One of the racemate isomers over the other and these lipases can separate enantiomers in a racemic blend[16]. For instance, pharmaceutical products were produced by the lipase catalyzed transesterification of secondary alcohols[19].

Generally, lipases are omnipresent in nature[20] and can be of animal, microbial and vegetable origin with catalytic property variations[21]. Majority of the microbial lipases are extracellular and gotten from fungi, yeast and bacteria. On

further research, it has been disclosed that microbial lipases can likewise be acquired through fermentation of farming and dairy wastes[22,23].

Sources of Lipases

Bacterial sources: Bacterial lipases are extracellular or intracellular or membrane bound[24]. Bacteria are potential possibility for production and discover potential uses in biotechnology. Species of *Bacillus* such as *Bacillus pumilus, Bacillus subtilis, Bacillus coagulans* are common lipase producing bacteria[25]. For instance, by solid state fermentation for 28 hr at 70^0C, lipase L2 was extracted from *Bacillus* strain L2 from hot spring perak[26]. From the petroleum contaminated soil bacterium, lipase (Biopetro-4) was extracted after 120 hr of fermentation and the maximum lipase activity obtained was 1,675 U/mL[27].

Fungal Sources: Fungal lipases are most beneficial than bacterial sources due to low extraction methods and batch fermentation[28]. Of them, *Rhizopus sps., Aspergillus sps., Rhizomucor sps,* are the most prominent ones for lipase production[25]. These lipases can catalyze a wide range of reactions such as alcoholysis, hydrolysis, esterification and saponification[29,30]. For instance, *Streptomyces griseus* exhibited lipase activity of 51.9 U/mL[31]. Fifty-nine fungal strains producing lipase were isolated from Brazilian soils: among them *Colletotrichum gloeosporioides* was identified as a potential lipase strain producing 27, 700 U/L[32].

Plant Sources: Although bacteria and fungi are the most common sources for lipases, these are encountered with production of novel lipases known as 'seed lipases' with potential benefits[16]. In plants, lipases are located in tissues with better concentrations of triacylglycerols like the reserve tissues of developing seedlings[7]. Consortium of plant lipases is available from seed sources such as bean, sunflower, canola, Barbados nut, lupin, linseed, coconut, almond, black-cumin, oar barley, sesame and wheat[16]. The activity of lipases increases during germination due to the lipase catalyzed conversion of triacylglycerols to soluble sugars[33].

The plant lipases are active within the physical parameters viz. pH 4-9, temperature 20^0C - 38^0C with reported activation of these lipases in the presence of zinc and calcium and inhibition in the presence of Tween 80 and EDTA[34]. In the production of several pharmaceutical, detergent, food and cosmetic goods, lipase extracted from palm fruit mesocarp is discovered to be efficient[35]. Plant lipases seem to be effective due to their improved stability, low cost, easy acceptability and downstream processing[4].

Lipase Structure: Lipases generally weigh about 19-60 kDa[36] and the structure consists of α/β hydrolase fold and a catalytic triad. The general topology of canonical α/β hydrolase fold is based upon eight β folds (β1 to β8) with antiparallel β2 strand. The α-helices which are stuffed on the two sides of β sheets are linked to (β3 to β8) strands and the catalytic triad consists of amino acids such as Histidine, Serine, Aspartate or Glutamic acid[37]. The enzyme structures can be accessed from protein data bank.

Applications of Lipase in Food Industry: One of the most important reasons for extensive use of lipases was exhibition of stereo, regio and substrate specificity. Lipase catalyzes not only hydrolytic reactions but also synthetic reactions such as alcoholysis, acidolysis and esterification. They catalyze a consortium of enantio, regio and chemoselective biotransformations[20]. Lipases find promising applications in food industry for cheese ripening, vegetable and tea processing and also esterification reactions[38,39]. Lipases are also used in the synthesis of flavour and ripening compounds[40]. In dairy industry, lipases are mainly used to develop flavoured cheese, butter, sweets, milk and chocolates[41].

The major applications of lipases from microbial, fungi and plants are reviewed and the synthesized gist is given in table 1. From the tabulated information, it is evident that lipases are extensively used in dairy and flavour industries.

Synthetic chemical flavours Vs. Lipase produced flavours: Food additives are compounds which are added by food industries to food in order to enhance flavour, taste, texture, consistency, freshness and wholesomeness[65-69]. Flavour enhancers are the largest class of food additives as they usually improve the food taste or to give a specific taste. The chemical compounds responsible for flavours are alcohols, esters, ketones, aldehydes. These compounds are synthesized easily and replaced for natural flavours[70]. Food additives should be added in permissible limit concentrations and regulated quantities. They show some devastating effects on consumer if they are not within acceptable daily intakes (ADI's)[66].

The flavouring agents which are commonly used in food industries taken from literature are depicted in table 2. The flavouring agents which show toxic effects as reported by Occupational Safety and Health Administration (OSHA) and Food and Drug Administration (FDA) are provided in table 3.

As the tables 2 and 3 reflect the type of flavours produced with chemical additives and their toxic effects, an attempt is made here to tackle these issues by lipase produced flavours with the evidence of some research in this direction. The lipase catalyzed ester synthesis for flavours was studied by Garlapati and Banerjee.[92] Their studies reflect the flavours which are biologically synthesized with characteristics such as orange (octyl acetate) and pineapple (methyl butyrate) that serve as natural flavouring ingredients. This was achieved by transesterification reactions under solvent free conditions by immobilized *Rhizopus oryzae* NRRL 3562 lipase.

In general, chemical ester synthesis involves use of solvents for transesterification reactions which lead to

immunogenicity and toxicity reactions in consumers but this research stamps on it and says esters can also be synthesised without solvents which are steps for green technology. Brault and his research team[93] demonstrated short chain ester molecules producing isoamyl acetate (banana fragrance) with transesterification and esterification reaction in organic media by using LipIAF5-2 lipase expressed in *E.coli* recombinant system and the biocatalyst showed the highest activity at 40°C.

Ghazani and his team[94] synthesized cocoa butter equivalents which is a chocolate fat mimetic using inter-esterification reaction at 65°C in continuous bed reactor and batch system for 4 hours by using Lipozyme RM IM (Lipase) from *Rhizomucor miehei.* De Luca et al[95] stated that in general, the hydrolytic activities of lipases which have come from lactic acid bacteria, play an important role during ripening of cheese. By adding exogenous lipase (Thermophilic esterase EST2) from *Alyciclobacillus acidocaldarius*, cheese flavour intensified by reducing the ripening time and also by releasing short chain fatty acids. The mixed flavour of orange and strawberry can be achieved by using raw materials as solid waste from coconut oil industry.

Lipase from *Mucor miehei* was purified by ammonium sulphate precipitation and this crude lipase was immobilized on polyurethane foam and esterification reactions were carried out in the presence of citronellol and free fatty acid at 400°C at various reaction times. Finally, the orange and strawberry flavours indicate the esterification results[96]. The different natural flavours such as citronellyl acetate (lemon flavour), geranyl acetate (rose), isoamyl acetate (banana), can be produced by using immobilized lipase B from *C. antarctica*. The flavour synthesis can be obtained by lipase catalyzed esterification reaction under free solvent system for 72 hours at 200 RPM at 30°C[97].

The banana flavour producing compound isoamyl acetate was synthesized by lipase catalyzed hydrolytic reactions. The lipase (Type II esterase) was isolated from *B. licheniformis* S-86, which is tolerant towards organic solvents. The enzyme retained 100% activity at alkaline pH of 10-11 and showed moderate thermostability at 50°C for one hour (half-life)[98].

In biocatalysis, thermostability is an important property and occupies a prominent role which acts as a key for lipase selectivity for a particular application. Lipases which are extracted from thermo tolerant bacteria possess unique features such as chemical inactivation resistance and solvent tolerance[99,100]**.** Protein Data Bank (PDB) was used as search engine to extract thermo-stable lipases alone from plant, fungal, bacterial sources and the results are presented in table 4. We have found 12 bacterial lipases, 1 fungal lipase and null hit results for plant lipases.

Table 1
Summarization of some recent works on lipases in food industry

Source	Function/Purpose	Type of Product / Application	Industry
Immobilized microbial lipases	Hydrolysis or alcoholysis of fish oils	Production of PUFA's & omega 3 fatty acids	Oil[42]
Microbial	Transesterification of palm stearin and palm olein blends	Margarine formulation-trans free fat	Food dressing[43]
Microbial	Esterification	Enhance flavour in bakery products	Bakery[44]
Microbial	Interesterification of different edible oils	Cocoa butter equivalents	Natural oils[45]
Microbial	Egg yolk emusification	Dressings	Food dressing[46]
Microbial	Tras or interesterification	Flavour development and cheese ripening	Dairy[40]
Microbial	Transesterification of menhaden, borage & tuna oils	Polyunsaturated fatty acids (PUFAs)	Oil[6]
Microbial	Modification of fatty acid chain lengths	Enhance cheese flavours	Flavour[47]
Microbial (Acetinobacter sp.)	Mediated synthesis	Ester ethyl caprylate-fruity flowery fragnance/flavour (peach, apple, pineapple) enhancing compounds	Flavour & Fragnance[48]
Microbial (*Amycolatopsis mediterranei*)	Synthesis	Flavoured compound (ester isoamyl acetate), especially banana flavour	Flavour[49]

Microbial (*Burkholderia multivorans*)	Synthesis	Ethyl butyrate esters and food fragrances	Flavour & Fragrance[50]
Microbial (Highly region & fatty acid specific	Inter-esterification	New oils and fats	Oil[6]
Microbial *(Rhodotorulla mucilagenosa)*	Transesterification	Palm oil	Oil[51]
Microbial (*Streptomyces* sp.)	Transesterification	Vegetable oils with methanol	Oil[52]
Microbial (Synthetic resin bound truncated *C.antarctica* lipase	Transesterification	Corn and soybean oils with ethanol	Oil[53]
Microbial phospholipase (Lecitase Nova)	Hydrolysis of phopholipids	Degumming	Oil[54]
Microbial Phospholipases	Environmental friendly process	Phospholipids of vegetable oils (Degumming)	Oil[55]
Microbial Phospholipases	Degradation of wheat lipids	Traditional emulsifiers	Bakery[44]
Microbial phospholipases	Hydrolysis of phospholipids	Degumming	Oil[46]
Microbial sausage lipases	Measurement of long chain fatty acids	Fermentation steps	Dairy[56]
Fungal *(Aspergillus niger & oryzae)*	Lipolysis	Cheese manufacturing	Dairy[57]
Fungal (*Aspergillus oryzae)*	Processing	Improve shelf life of bakery products	Bakery[56]
Fungal (*Rizomucor miehei)*	Dehydration & fermentation	To increase the quality of black tea by reducing total lipid content	Tea processing[39]
Fungal *(Sn-1,3 Rhizomucor miehei*)	Acidolysis of tripalmitin, butterfat, palm oil	Human milk fat substitutes	Dairy[45]
Fungal *(Sn-1,3 Rhizomucor miehei*)	Acidolysis between lard and soybean fatty acids	Commercial product Betapol	Dairy[45]
Immobilized fungal lipase (P*enicillium* sp.*)*	Hydrolysis of sardine oils	Production of eicosapentanoic acid & docosahexanoic acid-(PUFA's)	Oil[42]
Plant (C.rugosa lipase)-CRL	Hydrolysis of glyceride fraction of salmon oil	Enrichment of omega 3 PUFA's	Oil[58]
Plant (Carica Papaya Lipase)-CPL	Low calorie long & short triacylglycerol 45synthesis	Infant formulae	Dairy[4]
Plant (Castor bean lipase)	Esterification of fatty acids & glycerols	Production of triacyl glycerols of interest	Dairy[59]
Plant (CPL self-immobilized in papaya latex)	Synthesis	Human milk fat substitute	Dairy[60]
Plant (CPL)	Interesterification of palm oils	Cocoa butter equivalent, chocolates	Food dressing[61]
Plant (*Nigella sativa)*	Transesterification & enrichment	Borage oil with gamma linoleic fatty acid	Oil[62]
Plant (Papaya lipase)	Transesterification of tripalmitin with fatty acids of rapeseed oil papaya latex	Human resembling milk fat	Dairy[63]
Plant (Papaya lipase)	Interesterification reactions of ethyl esters with tripalmitin	Structured triacylglycerols	Dairy[4]
Barley seed (*Hordeum vulgare L.)*,Maize seed (*Zea mays L.),*Linseed *(Linum usitatissimum),*Rapeseed (B*rassica napus L.)*	Esterification	Synthesis of low molecular weight esters	Flavour[64]
Castor bean seed (*Phaseolus vilgaris)*	Esterification	Synthesis of structured lipids	Edible and industrial[59]

Table 2
Some commonly used flavours in food products[71].

Chemical Agent	Type of Flavour	Product of Application
Isoamyl Acetate	Banana Pear	Confectionary, baked goods, chewing gums
Benzaldehyde	Almond	Chocolates, baked goods, fizzy drinks
Methyl Anthranilate	Concord grapes	Soft drinks, confectionaries
Limonene	Orange	Mask the bitter taste of alkaloids, food & beverages
Allyl Hexanoate	Pineapple	Confectionary and soft drinks
Ethyl Maltol	Aroma reminiscent of caramelised sugar	E-liquids
Vanillin	Sweet and pleasant aroma	Ice creams & chocolates

Table 3
Health hazards associated with some commonly used food additives

Flavouring Agent	Product of Application	Health Hazards
Diacetyl	Butter, cheese, caramel and butterscotch flavours	Obstructive lung disease and bronchiolitis obliterans[72-76]
2,3 Pentanedione	Reformulated liquid buttermilk flavouring	Injures upper respiratory tract and airway epithelium toxicity in rodents[77-80]
Acetoin	Microwave popcorn manufacturing plants and artificial butter flavouring	Irritation to respiratory tract, skin, mucus membranes and eyes[75,81,82]
Furfural	Butter flavourings	Tissue changes in small blood vessels and lungs in rodents[83,84]
Acetic acid	Microwave popcorn manufacturing plants	Skin, eyes and respiratory tract irritation[76]
Acetaldehyde	Microwave popcorn manufacturing plants	ENT irritation[76,83,85]
Benzophenone	Synthetic flavouring agent and adjuvants in foods	Renal tubular tumors (male rats) & hepatocellular tumors (male mice)[86,87]
Ethyl Acrylate	Synthetic flavouring agent and adjuvants in foods	Fore stomach tumors in rodents[86,87]
Methyl Eugenol	Synthetic flavouring agent and adjuvants in foods	Liver tumors and glandular stomach neuroendocrine neoplasms in rodents[86,88]
Myrcene	Synthetic flavouring agent and adjuvants in foods	Renal tubular tumors in F344/N rodents and hepatocellular tumors in B6C3F1 mice[86,89]
Pulegone	Synthetic flavouring agent and adjuvants in foods	Urinary bladder papilloma & carcinoma in F344/N female rodents and liver neoplasms in B6C3F1 male mice[86,90]
Pyridine	Synthetic flavouring agent and adjuvants in foods	Renal tumors in male rats F3344/N and liver tumors in B6C3F1 mice[86,91]

Mase et al[101] reported Lipase (39-A) from *Cryptococcus flavescens* used in cheese flavouring. The yeast strain (39-A) was isolated from the digestive juice of *N. ventricosa*, an endangered plant species. By ammonium sulphate precipitation and chromatographic techniques, lipase from strain 39-A was purified. The fermentation was carried out in a 3L fermenter tank containing 2L of medium and 40 ml of strain 39-A culture. The optimal conditions for the fermentation were reported to be temperature 25°C at 300 RPM. After 72 hours of cultivation, the cells were centrifuged and crude lipase solution was obtained and concentrated by filtration and ultrafiltration nearly with 100% recovery.

Finally, this lipase 39-A is demonstrated to have the ability to replace pre-gastric esterase in dairy industry[101]. As animals are the sources for pre-gastric esterase, their large scale use would cause a great devastation to animal diversity, husbandry and finally leading to ethical issues. Therefore, to intercept these issues, lipase strain 39-A from plant source (*Nepenthes ventricosa)* is definitely a powerful tool. Using rDNA technology, we can produce more number of lipases (with the characteristics of 39-A) which act as a future perspective and promising research in the field of biotechnology, bioreactor engineering and enzyme engineering.

Table 4
Thermo-stable lipases of high potential in food industry and their characteristics

Lipase	Source	Mol. Wt (Da)	Chains & Sequence Length	Residue Count	PDB ID
Thermophilic Carboxyl esterase EST-2	*Alicyclobacillus acidocaldarius*	34887.00	A & 310	310	1EVQ
D196N Mutant of Monoglyceride Lipase	*Bacillus* sp. (Strain H-257)	178104.83	A, B, C, D, E, F & 270	1620	4KE6
Monoacylglycerol Lipase	*Bacillus* sp. (Strain H-257)	55041.50	A, B & 250	500	4LHE
Monoacylglyceride Lipase	*Bacillus* sp. (Strain H-257) complex with PMSF	29850.99	A &270	270	3RLI
Monoacylglyceride Lipase	*Bacillus* sp. (Strain H-257) in complex with 1-myristoyl glycerol analogue	60024.38	A, B & 270	540	4KE7
D196N Mutant of Monoacylglyceride Lipase	*Bacillus* sp. (Strain H-257) in space group P212121	180193.91	A, B, C, D, E, F & 270	1620	4KEA
Monoacylglyceride Lipase	*Bacillus* sp. (Strain H-257) with 1-stearyol glycerol analogue	118927.38	A, B, C, D & 268	1072	4KE9
Monoacylglyceride Lipase	*Bacillus* sp. (Strain H-257) with monopalmitoyl glycerol analogue	119688.28	A,B,C, D & 270	1080	4KE8
Monoacylglycerol Lipses	*Bacillus* sp. (Strain H257)	29678.79	A & 270	270	3RM3
Thermostable Bacterial Lipase	*Geobacillus stearothermophilus*	8631.46	A, B & 388	776	1JI3
Thermoalkalophilic Lipase	*Geobacillus thermocatenulatus*	45034.00	A & 389	389	2W22
Fungal Lipase	*Thermomyces langinosus*	58684.97	A, B & 269	538	4EA6
Lipoate-Protein Lipase	*Thermoplasma acidophilium*	30549.19	A & 262	262	2ARS

Conclusion

Thermostable lipases find enormous applications in the arena of food industry due to their versatile nature. Nowadays, usage of synthetic chemical flavours in food is on the rise. To combat the ill effects of synthetic flavours, the usage of natural flavours produced by lipases in food industries should increase and thereby keep away the possible health hazards in consumers. Using lipases, a consortium of flavours is produced under solvent free conditions which is an emerging trend towards building blocks of green technology. Still the debate is continuing on sources of lipase viz. plant, animal, fungi, microbial for the best benefits to mankind.

Currently, extensive research is witnessed with fungi and bacteria while little work with animals and plants is evident from submissions in protein data bank. Hence, there is impending need for researchers to focus on plant lipases due their bioavailability in nature, improved stability, low cost, easy acceptability and downstream processing. These plant lipases would act as replacement for animal lipases which are extracted and used as pre-gastric esterases in food flavouring. Procurement of lipases from animal sources may warrant ethical issues and may trigger immunogenicity and toxicity reactions in human beings. Lipases can be engineered with the desired characteristics using rDNA technology protein engineering and plant bioreactors which act as promising tools in the area of biotechnology and bioreactor engineering. Finally, thermostability is the key deciding factor for the specific application of lipase. Lipases which exhibit more thermostability are the most potent.

References

1. Kiran S., Arshad Z., Noshen S., Kamal S., Gulzar T., Majeed M.S., Jannat M. and Rafique M.A., Microbial Lipases: Production and Applications, A Review, *Journal of Biochemistry, Biotechnology and Biomaterials*, **1(2)**, 7-20 **(2016)**

2. Gurung N., Ray S., Bose S. and Rai V., A Broader View: Microbial Enzymes and Their Relevance in Industries, Medicine and Beyond, *Bio Med Research International*, **2013**, 1-18 **(2013)**

3. Industrial Enzymes Market - Growth, Trends and Forecast (2019-2024), 230 **(2019)**

4. Gandhi N.N. and Mukherjee K.D., Reactivity of medium-chain substrates in the interesterification of tripalmitin catalyzed by papaya lipase, *Journal of the American Oil Chemists' Society*, **78(9)**, 965-968 **(2001)**

5. Florkin M. and Stotz E.H., Comprehensive biochemistry: enzyme nomenclature, 2nd ed, Elsevier Publishing Co., New York, **13**, 25-38 **(1965)**

6. Sharma R., Chisti Y. and Banerjee U.C., Production, purification, characterization and applications of lipases, *Biotechnology Advances*, **19(8)**, 627-662 **(2001)**

7. Svendsen A., Lipase protein engineering. Biochimica et Biophysica Acta (BBA), *Protein Structure and Molecular Enzymology*, **1543(2)**, 223-238 **(2000)**

8. Paques F.W. and Macedo G.A., Lipases de Látex Vegetais: Propriedades e Aplicações Industriais: A Review, *Química Nova*, **29(1)**, 93-99 **(2006)**

9. Ribeiro B.D., Castro A.M.D., Coelho M.A.Z. and Freire D.M.G., Production and use of lipases in bioenergy: A Review from the feedstocks to biodiesel production, *Enzyme Research*, **2011**, 1-16 **(2011)**

10. Clementz A.L., Del Peso G., Canet A., Yori J.C. and Valero F., Utilization of discard bovine bone as a support for immobilization of recombinant *Rhizopus oryzae* lipase expressed in *Pichia pastoris*, *Biotechnology Progress*, **32**, 1246-1253 **(2016)**

11. Sarmah N., Revathi D., Sheelu G., Yamuna Rani K., Sridhar S., Mehtab V. and Sumana C., Recent Advances on Sources and Industrial Applications of Lipases, *Biotechnology Progress*, **34(1)**, 5-28 **(2018)**

12. Villeneuve P., Plant Lipases and Their Applications in Oils and Fats Modification, *European Journal of Lipid Science and Technology*, **105(6)**, 308 **(2003)**

13. Macrae A.R. and Hammond R.C., Present and Future Applications of Lipases, *Biotechnology Genetics and Engineering Review*, **3(1)**, 193-218 **(1985)**

14. Sonnet P.E., Lipase Selectivities, *Journal of the American Oil Chemists' Society*, **65(6)**, 900 **(1988)**

15. Rivera I., Robles M., Mateos-Diaz J.C., Gutierrez-Ortega A. and Sandoval G., Functional expression, extracellular production, purification, structure modelling and biochemical characterization of *Carica papaya* lipase, *Process Biochemistry*, **56**, 109-116 **(2017)**

16. Barros M., Fleuri L. and Macedo G., Seed lipases: sources, applications and properties-A Review, *Brazilian Journal of Chemical Engineering*, **27(1)**, 15-29 **(2010)**

17. Todorova T., Guncheva M., Dimitrova R. and Momchilova S., Walnut oil–unexplored raw material for lipase-catalyzed synthesis of low-calorie structured lipids for clinical nutrition, *Journal of Food Biochemistry*, **39**, 603-611 **(2015)**

18. Song X., Qi X., Hao B. and Qu Y., Studies of substrate specificities of lipases from different sources, *European Journal of Lipid Science and Technology*, **110**, 1095-1101 **(2008)**

19. Borza P., Peter F. and Paul C., Improved enantioselectivity of *Candida antarctica* a lipase through sol-gel entrapment, *Chemical Bulletin*, **60**, 49-54 **(2015)**

20. Sharma S. and Kanwar S.S., Organic solvent tolerant lipases and applications, The *Scientific World Journal*, **2014**, 1-15 **(2014)**

21. Jachmanián I. and Mukherjee K.D., Esterification and Interesterification Reactions Catalyzed by Acetone Power from Geminating Rapeseed, *Journal of the American Oil Chemists' Society*, **73(11)**, 1527-1532 **(1996)**

22. Zubiolo C., Santos R.C.A., Figueiredo R.T., Soares C.M.F. and Santana L., Morphological and physicochemical aspects of microbial lipase obtained from novel agroindustrial waste encapsulated in a sol-gel matrix, *Journal of Thermal Analysis and Calorimetry*, **120(3)**, 1503-1509 **(2015)**

23. Marques T.A., Baldo C., Borsato D., Buzato J.B. and Celligoi M., Utilization of dairy effluent as alternative fermentation medium for microbial lipase production, *Romanian Biotechnology Letters*, **19(1)**, 9042-9050 **(2014)**

24. Vaquero M.E., Barriuso J., Martinez M.J. and Prieto A., Properties, structure and applications of microbial sterol esterases, *Applied Microbiology and Biotechnology*, **100(5)**, 2047-2061 **(2016)**

25. Thakur S., Lipases, its sources, Properties and Applications: A Review, *International Journal of Scientific and Engineering Research*, **3(7)**, 1344-1353 **(2012)**

26. Shariff F.M., Leow T.C., Murkhed A.D., Salleh A.B., Basri M. and Rahman R.N., Production of L2 lipase by *Bacillus* sp. Strain L2: Nutritional and physical factors, *Journal of Microbiology and Biotechnology*, **47(5)**, 406-412 **(2007)**

27. Carvalho N.B., Souza R.L., Castro H.F., Zanin G.M., Lima A.S. and Soares C.M.L., Sequential production of amylolytic and lipolytic enzymes of bacterial strain isolated from petroleum contaminated soil, *Applied Biochemistry and Biotechnology*, **150(1)**, 25-32 **(2008)**

28. Mehta A., Bodh U. and Gupta R., Fungal lipases: A review, *Journal of Biotech Research*, **8**, 58**-77 (2017)**

29. Ozturkoglu-Budak S., Wiebenga A., Bron P.A. and De Vries R.P., Protease and lipase activities of fungal and bacterial strains derived from an artisanal raw ewe's milk cheese, *International Journal of Food Microbiology*, **237**, 17–27 **(2016)**

30. Zhang L.Q., Zhang Y.D., Xu L., Li X.L., Yang X.C., Xu G.L., Wu X.X., Gao H.D., Du W.N., Zhang X.T. and Zhang X.Z., Lipases catalyzed synthesis of RDG diamide in aqueous water miscible organic solvents, *Enzyme and Microbial Technology*, **29**, 129-135 **(2001)**

31. Vishnupriya B., Sundaramoorti C., Kalaivani M. and Selvam K., Production of lipase from *Streptomyces grisesus* and evaluation of fermentation, *Bioprocess and Biosystems Engineering*, **31**, 119-125 **(2008)**

32. Cihangir N. and Sarikaya E., Investigation of lipase production by a new isolate of *Aspergillus* species, *World Journal of Microbiology and Biotechnology*, **20(2)**, 193-197 **(2004)**

33. Patil K.J., Chopda M.Z. and Mahajan R.T., Lipase biodiversity, *Indian Journal of Science and Technology*, **4(8)**, 971-982 **(2011)**

34. Mala V. and Umar D.M., Lipase activity of *Carissa carandas* fruit, *Science International-Lahore*, **7**, 161-164 **(1995)**

35. Suwanno S., Rakkan T., Yunu T., Paichid N., Kimtun P., Prasertsan P. and Sangkharak K., The production of biodiesel using residual oil from palm oil mill effluent and crude lipase from oil palm fruit as an alternative substrate and catalyst, *Fuel*, **195**, 82-87 **(2017)**

36. Jaeger K., Dijkstra B. and Reetz M., Bacterial biocatalysts: Molecular biology, three-dimensional structures and biotechnological applications of lipases, *Annual Reviews in Microbiology*, **53(1)**, 315-351 **(1999)**

37. Lotti A., Lipases: Molecular Structure and Functions, In Polaina J. and Andrew P.M., eds., Industrial Enzymes Structure, Function and Applications, Springer, 263-280 **(2007)**

38. Saxena R.K., Ghosh P.K., Gupta R., Davidson W.S., Bradoo S. and Gulati R., Microbial lipases: Potential biocatalysts for the future industry, *Current Science*, **77(1)**, 101-115 **(1999)**

39. Latha K. and Ramarethinam S., Studies on lipid acyl hydrolases during tea processing, *Annual Review of Plant Physiology*, **3**, 73-78 **(1999)**

40. Reetz M.T., Lipases as Practical Biocatalysts, *Current Opinion in Chemical Biology*, **6(2)**, 145-150 **(2002)**

41. Jaeger K. and Reetz M.T., Microbial lipases form versatile tools for biotechnology, *Trends in Biotechnology*, **16(9)**, 396-403 **(1998)**

42. Daniela F.M., Wilson G.M., Cesar R.F.T., Sonia M. and Benevides C.P., Fernandez-Lorente G., Guisan J.M. and Carmona E.C., Immobilization of Lipase from *Penicillium* sp Section Gracilenta (CBMAI 1583) on Different Hydrophobic supports: Modulation of Functional Properties, *Molecules*, **229(2)**, 339 **(2017)**

43. Sellami M., Ghamgui H., Frikha F., Gargouri Y. and Miled N., Enzymatic transesterification of palm stearin and olein blends to produce zero-trans margarine fat, *BMC Biotechnology*, **12(48)**, DOI: https://doi.org/10.1186/1472-6750-12-48 **(2012)**

44. Robert H., Lipases in baking, Lipinov. Romainville FR: Adebiotech **(2015)**

45. Bhaggan K., Specialty fats CBE and HMF substitute, Enzyme technology in oilseeds, oils and fats congress **(2016)**

46. Guerrand D., Lipases industrial applications: focus on food and agro industries, *OCL*, **24(4)**, 1-7 **(2017)**

47. Aravindan R., Anbumathi P. and Viruthagiri T., Lipase application in food industry, *Indian Journal of Biotechnology*, **6**, 141-158 **(2007)**

48. Ahmed E.H., Raghavendra T. and Madamwar D., An alkaline lipase from organic solvent tolerant *Acinetobacter* sp. EH28: Application for ethyl caprylate synthesis, *Bioresource Technology*, **101**, 3628-3634 **(2010)**

49. Dheeman D.S., Henehan G.T.M. and Frias J.M., Purification and properties of *Amycolatopsis mediterranei* DSM 43304 lipase and its potential in flavor ester synthesis, *Bioresource Technology*, **102**, 3373-3379 **(2011)**

50. Dandavate V., Jinjala J., Keharia H. and Madamwar D., Production, partial purification and characterization of organic solvent tolerant lipase from *Burkholderia multivorans* V2 and its application for ester synthesis, *Bioresource Technology*, **100**, 3374-3381 **(2009)**

51. Srimhan P., Kongnum K., Taweerodjanakarn S. and Hongpattarakere T., Selection of lipase producing yeasts for methanol-tolerant biocatalyst as whole cell application for palm-oil transesterification, *Enzyme and Microbial Technology*, **48**, 293-298 **(2011)**

52. Mander P., Cho S.S., Simkhada J.R., Choi Y.H., Park D.J. and Yoo J.C., An organic solvent–tolerant lipase from *Streptomyces* sp. CS133 for enzymatic transesterification of vegetable oils in organic media, *Process Biochemistry*, **47**, 635-642 **(2012)**

53. Hughes S.R., Moser B.R., Robinson S., Cox E.J., Harmsen A.J., Friesen J.A., Bischoff K.M., Jones M.A., Pinkleman R., Bang S.S., Tasaki K., Doll K.M., Qureshi N., Liu S., Saha B.C., Jackson Jr J.S., Cotta M.A., Rich J.O. and Caimi P., Synthetic resin-bound truncated *Candida antarctica* lipase B for production of fatty acid alkyl esters by transesterification of corn and soybean oils with ethanol or butanol, *Journal of Biotechnology*, **159**, 69-77 **(2012)**

54. Kumar C.G., Malik R.K. and Tiwari M.P., Novel enzyme based detergent: An Indian perspective, *Current Sciences*, **75**, 1312-1318 **(1998)**

55. Clausen K., Enzymatic oil-degumming by a novel microbial phospholipase, *European Journal of Lipid Science & Technology*, **103**, 333-340 **(2001)**

56. Fariha H., Shah A.A. and Hameed A., Industrial Applications of Microbial Lipases, *Enzyme and Microbial Technology*, **39**, 235-251 **(2006)**

57. Custry F., Fernander N. and Shahani K.M., Effect of enzyme association on bread dough performance: A response surface study, *Food Science & Technology International*, **6**, 117-266 **(2000)**

58. Kahveci D. and Xu X., Repeated hydrolysis process is effective for enrichment of omega 3 poly unsaturated fatty acids in salmon oil by *Candida rugosa* lipase, *Food Chemistry*, **129(4)**, 1552-1558 **(2011)**

59. Tüter M., Castor Bean Lipase as a Biocatalyst in the Esterification of Fatty Acids to Glycerol, *Journal of the American Oil Chemist's Society*, **75(3)**, 417 **(1998)**

60. Tecelão C., Rivera I., Sandoval G. and Ferreira-Dia S., Carica papaya latex: A low cost biocatalyst for human milk fat substitutes production, *European Journal of Lipid Science and Technology*, **114(3)**, 266-276 **(2012)**

61. Pinyaphong P. and Phutrakul S., Synthesis of cocoa butter equivalent from palm oil by *Carica papaya* lipase-catalyzed interesterification, *Chiang Mai Journal of Science*, **36(3)**, 359-368 **(2009)**

62. Tuter M., Secundo F., Riva S., Aksoy H.A. and Ustun G., Partial Purification of *Nigella sativa* L-Seed Lipase and Its Application in Transesterification Reactions, *Journal of American Oils Chemists' Society*, **80(1)**, 43-48 **(2003)**

63. Mukherjee K.D. and Kiewitt I., Structured triacylglycerols resembling human milk fat by transesterification catalyzed by papaya (*Carica papaya*) latex, *Biotechnology Letters*, **20(6)**, 613-616 **(1998)**

64. Liaquat M. and Apenten R.K.O., Synthesis of Low Molecular Weight Flavor Esters Using Plant Seedling Lipases in Organic Media, *Journal of Food Science: Food Chemistry and Toxicology*, **65(2)**, 295 **(2000)**

65. Winter R.A., Consumer's Dictionary of Food Additives, Three River Press, New York, 112 **(1994)**

66. Kunkel E.M. and Barbara H.D., The Gale Group Inc., Macmillan Reference USA, New York, Gale Nutrition and Well-being A to Z **(2004)**

67. Daniel M., Reactions to Food Additives and Preservatives, Workman publishing Company, New Jersey, 117 **(2007)**

68. Marmion D.M., Handbook of U.S. Colorants: Foods, Drugs, Cosmetics and Medical Devices, USA Wiley, 3rd ed. **(1991)**

69. Branen A.L., Davidson P.M., Salminen S. and Thorngate III J.G., Food Additives, USA, CRC Press, 2nd ed. **(2002)**

70. Sunitha J. and Preethi R., FDST Food Additives, Acharya N. G. Ranga Agricultural University **(2000)**

71. https://www.chemoxy.com/about-2/knowledge-hub/the-science-behind-food-flavourings/ **(2019)**

72. FEMA, Respiratory Health and Safety in the Flavor Manufacturing Workplace, The Flavor and Extract Manufacturing Association of the United States, https://www.osha.gov /dts/shib/shib10142010.html, August **(2004)**

73. National Library of Medicine: Hazardous Substances Data Bank: Diacetyl, Retrieved November 7 **(2006)**

74. U.S. Department of Labor: Occupational Safety and Health Administration (OSHA), https://www.osha.gov/dts/shib/ shib10142010.html **(2019)**

75. Kanwal R., NIOSH, Report on Severe Fixed Obstructive Lung Disease in Workers at a Flavoring Manufacturing Plant, National Institute for Occupational Safety and Health (NIOSH) Health Hazard Evaluation Report #2006-0303-3043, April **(2007)**

76. Kanwal R., Kullman G., Piacitelli C., Boylstein R., Sahakian N., Martin S., Fedan K. and Kreiss K., Evaluation of flavorings-related lung disease risk at six microwave popcorn plants, *J. Occup. Environ. Med.*, **48(2)**, 149-157 **(2006)**

77. NIOSH, Interim Report, National Institute for Occupational Safety and Health (NIOSH) Health Hazard Evaluation Report, Interim #1, HETA 2008-0230, November 3 **(2008)**

78. NIOSH, Report on an Investigation of Buttermilk Flavouring Exposures and Respiratory Health at a Bakery Mix Production Facility, National Institute for Occupational Safety and Health (NIOSH) Health Hazard Evaluation Report, HETA 2008-0230-3096, November **(2009)**

79. Hubbs A.F., Moseley A.E., Goldsmith W.T., Jackson M.C., Kashon M.L., Battelli L.A., Schwegler-Berry D., Goravanahally M.P., Frazer D., Fedan J.S., Kreiss K. and Castranova V., Airway epithelial toxicity of the flavoring agent, 2, 3 pentanedione, 1506, The Toxicologist CD, *An official Journal of the Society of Toxicology*, **114(1)**, 317, https://www.cdc.gov/niosh/nioshtic-2/20036634.html **(2010)**

80. Morgan D.L., Kirby P.J., Price H.C., Bousquet R.W., Taylor G.J., Gage N. and Flake G.P., Inhalation toxicity of acetyl propionyl in rats and mice, 1492, The Toxicologist CD, *An Official Journal of the Society of Toxicology*, **114(S-1)**, 239 (**2010)**

81. NIOSH, Gilster-Mary Lee Corporation - Jasper, Missouri, National Institute for Occupational Safety and Health (NIOSH) Health Hazard Evaluation Report #2000-0401-2991, January **(2006)**

82. NEIHS, Chemical Information Review Document for Artificial Butter Flavoring and Constituents Diacetyl [CAS No. 431-03-8] and Acetoin [CAS No. 513-86-0] **(2007)**

83. Boylstein R., Piaciatelli C., Grote A., Kanwal R., Kullman G. and Kreiss K., Diacetyl emissions and airborne dust from butter flavorings used in microwave popcorn production, *Journal of Occupational and Environmental Hygiene*, **3(10)**, 530-535 **(2006)**

84. Mishra A., Dwivedi P.D., Verma A.S., Sinha M., Mishra J., Lal K., Pandya K.P. and Dutta K.K., Pathological and biochemical alterations induced by inhalation of furfural vapor in rat lung, *Bulletin of Environmental Contamination and Toxicology*, **47(5)**, 668-674 **(1991)**

85. Kreiss K., Gomaa A., Kullman G., Fedan K., Simoes E.J. and Enright P.L., Clinical bronchiolitis obliterans in employees at a microwave-popcorn plant, *New England Journal of Medicine*, **347(5)**, 330-338 **(2002)**

86. Flavor and Extract Manufacturers Association Transmittal Letter to Szabina Stice (FDA, CFSAN), April 27, https://www.federalregister.gov/documents/2018/10/09/2018-21807/food-additive-regulations-synthetic-flavoring-agents-and-adjuvants **(2018)**

87. FDA Memorandum from S. Thurmond, CFSAN Toxicology Team, Division of Petition Review, to J. Kidwell, Regulatory Group I, Division of Petition Review, June 21 **(2018)**

88. FDA Memorandum from J. Zang, CFSAN Toxicology Team, Division of Petition Review, to J. Kidwell, Regulatory Group I, Division of Petition Review, June 21 **(2018)**

89. FDA Memorandum from A. Khan, CFSAN Toxicology Team, Division of Petition Review, to J. Kidwell, Regulatory Group I, Division of Petition Review, June 21 **(2018)**

90. FDA Memorandum from N. Anyangwe, CFSAN Toxicology Team, Division of Petition Review, to J. Kidwell, Regulatory Group I, Division of Petition Review, June 21 **(2018)**

91. FDA Memorandum from T. Tyler, CFSAN Toxicology Team, Division of Petition Review, to J. Kidwell, Regulatory Group I, Division of Petition Review, June 27 **(2018)**

92. Garlapati V.K. and Banerjee R., Solvent-free synthesis of flavour esters through immobilized lipase mediated transesterification, *Enzyme Research*, **2013**, 1-6, http://dx.doi.org/10.1155/2013/367410 **(2013)**

93. Brault G., Shareck F., Hurtubise Y., Le´pine F. and Doucet N., Short-Chain Flavor Ester Synthesis in Organic Media by an E. coli Whole-Cell Biocatalyst Expressing a Newly Characterized Heterologous Lipase, *PLOS ONE*, **9(3)**, 1-13 **(2014)**

94. Ghazani S.M. and Marangoni A.G., Facile lipase-catalyzed synthesis of a chocolate fat mimetic, *Scientific Reports*, **8**, 15271 **(2018)**

95. De Luca V., Perotti M.C., Wolf I.V., Meinardi C.A. and Mandrich Luigi, The addition of the thermophilic esterase EST2 influences the fatty acids and volatile compound profiles of semi hard cheeses, *Food Science and Technology*, DOI: https://doi.org/10.1590/fst.06018 **(2018)**

96. Moentamaria D., Irfin Z. and Udjiana S., Immobilized Lipase Technology for Making Natural Flavor, *International Journal of Engineering Research and Development*, **11(12)**, 30-35 **(2015)**

97. Singh P., Saxena D.K. and Naik S.N., Synthesis of Food Flavors by Enzymatic Esterification Process, *International Journal of Science and Research*, **3(9)**, 2113-2116 **(2014)**

98. Torres S., Baigorí M.D., Swathy S.L., Pandey A. and Castro G.R., Enzymatic synthesis of banana flavour (isoamyl acetate) by *Bacillus licheniformis* S-86 esterase, *Food Research International*, **42**, 454-460 **(2009)**

99. Hasan F., Shah A.A. and Hameed A., Industrial applications of microbial lipases, *Enzyme and Microbial Technology*, **39(2)**, 235–251 **(2006)**

100. Yu S., Han W., Wang H., Zheng B. and Feng Y., A novel thermophilic lipase from *Fervidobacterium nodosum* Rt17-B1 representing a new subfamily of bacterial lipases, *Journal of Molecular Catalysis B: Enzymatic*, **66(1)**, 81–89 **(2010)**

101. Mase T., Asano K., Ikeda Y., Kato Y., Esaki H. and Isshiki S., Characterization and Application of Lipase 39-A from *Cryptococcus flavescens* for Cheese Flavoring, *Food science and Technology Research*, **19(1)**, 89- 95 **(2013)**.

(Received 25th July 2019, accepted 28th September 2019)

Bulletin of the National Research Centre

RESEARCH Open Access

Production and optimization of lipase using *Aspergillus niger* MTCC 872 by solid-state fermentation

Ashutosh Nema[1], Sai Haritha Patnala[1], Venkatesh Mandari[1], Sobha Kota[2] and Santhosh Kumar Devarai[1*]

Abstract

Background: Lipases are serine hydrolases that degrade triglycerides, an attribute that treasures wide applications in biodiesel production, detergent, chemical industries, etc. The most sought after the application is in the high quality and economical production of biodiesel under mild reaction conditions and simplified product separation. For the said application, fungal lipases are ideal catalysts that could effectively catalyze esterification and transesterification reactions with their specific ability to release fatty acids from 1, 3 positions of acylglycerols.

Results: In the present work, to facilitate bulk synthesis, lipase production using *Aspergillus niger* MTCC 872 was studied by solid-state fermentation (SSF). The chosen fungal strain was evaluated for lipase production using a mixture of agro-industrial substrates viz. rice husk, cottonseed cake, and red gram husk in various combinations at flask level. Tri-substrate mixture (rice husk, cottonseed cake, and red gram husk) combined in the ratio of 2:1:1 has shown the maximum lipase activity 28.19 U/gds at optimum cultivation conditions of temperature 40 °C, moisture content 75% (*v/w*), pH 6.0 and initial spore concentration of 5.4 million spores per mL. Further studies were performed for scale-up of lipase from flask level to lab scale using tray fermenter. Lipase activity was found to be 24.38 U/gds and 21.62 U/gds for 100 g and 1000 g substrate respectively.

Conclusion: This is the first report on the production of lipase from *Aspergillus niger* MTCC 872 using tri-substrate mixture of rice husk (RH), cottonseed cake (CSC), and red gram husk (RGH). Moreover, comparison between individual, binary, and tri-substrate mixture was carried out for which the highest lipase activity was observed for tri-substrate mixture. In addition, comparable results were found when scale-up was performed using tray fermenter. Thus, the current work signifies usage of agro-industrial residues as substrates for enzyme production by solid-state fermentation process as an effective alternative to submerged fermentation for industrial applications.

Keywords: Lipase, Solid-state fermentation (SSF), Agro-residues, Solid tri-substrate

Introduction

Over the recent years, lipases have emerged as one of the prominent biocatalysts and accounts nearly 10% of the enzyme market (Salihu et al. 2016). Fossil fuels are running out of reserves due to enormous usage in vehicular applications. Therefore, there is an imperative need to produce eco-friendly and clean energy alternatives like biodiesel in an economical and environmentally sustainable method. For this, lipases are extremely important as they hydrolyze acylglycerol into fatty acid and glycerol, and effect esterification and transesterification reactions (Stamenković et al. 2011; Christopher et al. 2014; Selvakumar and Sivashanmugam 2017). In general, lipases are triacylglycerol acyl hydrolases (EC 3.1.1.3), classified as serine hydrolases that catalyze triglycerides into diglycerides, monoglycerides, and fatty acids (Kanmani et al. 2015).

Lipases have proven potential for contributing to the multibillion-dollar market share in bio-industry and are being used in the processing of medicines (digestive enzymes), detergents (cleaning agent), food additives (flavor modifying enzymes), paper (control of pitch), cosmetics (exclusion of lipids), leather (elimination of fat

* Correspondence: devarai@iith.ac.in
[1]Industrial Bioprocess and Bioprospecting Laboratory, Department of Chemical Engineering, Indian Institute of Technology Hyderabad, Laboratory No: A-224, Kandi, Sangareddy District, Telangana 502285, India
Full list of author information is available at the end of the article

Springer Open
© The Author(s). 2019 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

from animal skin), wastewater (decomposition and oil removal), biodiesel production (transesterification reaction), etc. (Aravindan et al. 2007; Joseph et al. 2008; Andualema and Gessesse 2012; Selvakumar and Sivashanmugam 2017). Lipases are also used in the degradation of fatty wastes and biodegradation of polymers (Gombert et al. 1999; Sharma et al. 2001; Kanmani et al. 2015). Due to their distinctive huge catalytic potential, lipase is considered as one of the most crucial industrial enzymes (Saxena et al. 1999; Andualema and Gessesse 2012).

Lipases occur widely in plants, animals, and various microorganisms (Gilham and Lehner 2005; Show et al. 2015). In comparison to enzymes derived from animals and plants, enzymes originated from microbes are the better sources as they provide high stability and offer ease of cultivation. Microbial lipases which are used for commercial applications act as catalysts for a wide range of hydrolytic and synthetic reactions (Andualema and Gessesse 2012). Kynclova et al. (1995) screened various hydrolytic enzymes to meet the special demands. Among the screened microorganisms, fungal lipases turned out to be the best source for lipase as they are thermally stable with high turnover number and are presently receiving attention due to the easy recovery of extracellular enzymes (Kynclova et al. 1995; Mahadik et al. 2002; Salihu et al. 2013; Fleuri et al. 2014).

Industrially, lipases have been produced using submerged fermentation (SmF). Problems associated with this process include high cost of equipment, media, and higher probability of contamination. The traditional and emerging field of solid-state fermentation (SSF) has enabled the better production of a wide range of enzymes and metabolites which require less energy than SmF (Pandey 2003; Viniegra-González et al. 2003; Ashok et al. 2017). The credibility of solid-state fermentation (SSF) for production of enzyme has gained importance in developing countries (Singhania et al. 2009). SSF technology has been refined over the years and is currently the best method of obtaining fungal spores on the non-soluble matrix that acts as a natural habitat for filamentous fungi (Hölker et al. 2004; Colla et al. 2015). SSF also offers other advantages including resistance to contamination and ease of product extraction, and provides a better opportunity for the biosynthesis of low-volume-high-cost products. This is achieved by using low cost accessible agro-industrial residues as solid substrates, which provide both anchorage and nutrient source to microbial cells (Krishna 2005; Ashok et al. 2017). These agricultural residues are being generated in huge quantities in developing countries and problems associated with their disposal is also an important environmental concern. Thus, utilizing these residues as nutrient source for enzyme production will reduce overall production cost (Salihu et al. 2013). Hence, the selection of an appropriate substrate is an essential step during the production of enzymes in SSF. Various agro-industrial residues such as wheat bran, soybean cake, rice husk, gingelly oil cake, olive oil cake, sugar cane bagasse, babassu oil cake, and sheanut cake were studied (Salihu et al. 2013, 2016; Fleuri et al. 2014).

In the present study, we first explored the possibility of using a mixture of agro-industrial residues for the better production of Lipase using *Aspergillus niger* MTCC 872. In the next stage, physical parameters such as temperature, pH, moisture content, and initial spore concentration were optimized using one variable at a time method. The procedure adopted here for evaluating physical parameters was to evaluate the effect of individual factor and incorporate optimized level in the next factor optimization. In addition, optimized results were transferred to tray bioreactor at the laboratory scale and the efficacy of the strain for lipase production was evaluated.

Materials and methods

Microorganism

Aspergillus niger MTCC 872 was obtained from Microbial Type Culture Collection Centre and Gene Bank, Institute of Microbial Technology, Chandigarh, India. Obtained fungal strain was revived and grown on potato dextrose slant at 30 °C for 96 h and stored at 4 °C. Fungal spores were harvested with distilled water and obtained spore concentration was determined using Neubauer chamber.

Solid substrates and chemicals

Rice husk (RH), cottonseed cake (CSC), and red gram husks (RGH) were procured from the local market in Hyderabad, India. Other media components used in the experiment were obtained from Hi-media (Mumbai, India). All the chemicals were of analytical reagent grade.

Solid-state fermentation and extraction of the enzyme

Rice husk, cottonseed cake, and red gram husk were tested in various combinations (individual, combination of two and mixture of all the three substrates) by fixing the total weight to 5 g for lipase production. Five grams of substrate mixture was taken in a 250-mL Erlenmeyer flask and supplemented with 0.05 M potassium phosphate buffer (K_2HPO_4 and KH_2PO_4) to maintain an initial moisture content and pH. The flasks were sterilized by autoclaving at 121 °C (15 psi) for 15 min. After cooling, 4% (*v*/*w*) inoculum of the fungal strain *Aspergillus niger* MTCC 872 was added to the solid substrate. The flask was incubated at a temperature of 30 °C for 96 h (Edwinoliver et al. 2010; Dayanandan et al. 2012).

Solid substrate with fungal biomass was withdrawn at regular intervals under sterile conditions. In order to get a fully representative sample, the fermented mixture was

mixed thoroughly to get the uniformity. 0.5 g of moldy substrate was mixed with 10 mL of solution containing 1% (*w*/*v*) NaCl and 1% Triton X-100 (*w*/*v*). This mixture was ground using mortar and pestle. The resulting solution was then filtered using Whatman No. 1 filter paper. The residue left in the filter paper was dried at 70 °C for 24 h to obtain the dry solid weight. The filtrate was then centrifuged at 10,000 rpm for 10 min. The resultant supernatant was used as an enzyme source for Lipase assay represented in Fig. 1. All the analysis was done in triplicates. In order to evaluate optimized parameters, the effect of individual parameters was evaluated.

Scale-up to 1 kg capacity

The flask level studies were scaled up to 100 g of an optimized solid mixture containing rice husk, cottonseed cake, and red gram husk and were transferred to a 1-L Erlenmeyer flask, which provided sufficient surface area for the growth of microorganism on the solid substrates. The mixture was moistened with buffer and sterilized at 121 °C for 15 min. After cooling the substrates to room temperature, the inoculum was added and incubated for 96 h. Likewise, further scale-up in a tray bioreactor was performed using a steel tray of dimensions: length, 45.2 cm; breadth, 42.7 cm; and height, 2.5 cm. One kilogram of solid tri-substrate mixture was moistened (75% *v*/*w*) with buffer of pH6.0 and sterilized. Solid mixture was distributed on trays and once cooled, the inoculum was added and mixed inside laminar airflow chamber and kept for fermentation at 40 °C. Sampling was performed in tray bioreactor after complete manual mixing of all the substrate-biomass mixture. Samples were collected at every 24 h interval and analyzed for lipase activity (Doriya and Devarai 2018).

Lipase assay

Lipase activity was determined using the modified titrimetric method (Arzoglou et al. 1994). The reaction mixture containing 5 mL of olive oil emulsion substrate, 20 mL of 0.1 M potassium phosphate buffer, and 1 mL of enzyme extract was mixed and incubated in a rotary shaker at a temperature of 30 °C at 130 rpm for 30 min. This reaction mixture was then quenched using 15 mL acetone-ethanol (1,1) mixture. The amount of fatty acids released was titrated against 0.05 N NaOH until the solution turned into pink color. Blank assays were conducted adding the enzyme just before titration. One unit (U) of lipase activity is defined as the amount of enzyme which produces 1 μmol of fatty acids per minute under the assay conditions. In general, enzyme activity can be expressed in terms of units per milliliter or units per gram dry substrate.

$$\text{Lipase activity } (U/L) = \frac{A * C}{V} * 10^6$$

Where A = mL of NaOH consumed per minute; U = μmoles of fatty acids released per minute.

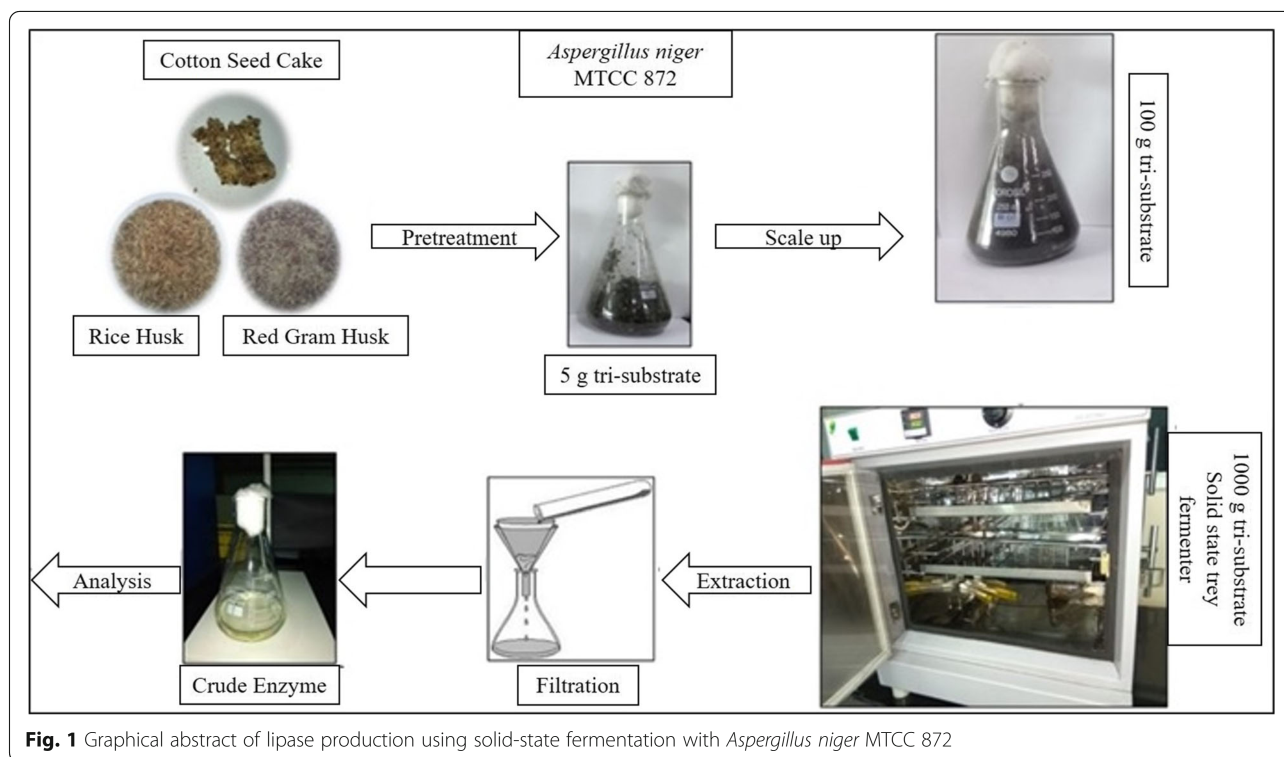

Fig. 1 Graphical abstract of lipase production using solid-state fermentation with *Aspergillus niger* MTCC 872

C = concentration of NaOH in mmoles/L; V = enzyme sample volume in μL; 10^6 converts sample volume to liters.

U/mL = μmoles of fatty acids released per minute per mL of crude enzyme; U/gds = μmoles of fatty acids released per minute per gram of dry solid substrate from which enzyme has been extracted.

Results

Effect of solid substrate

In the current work, the effect of three different agricultural residues, rice husk, cottonseed cake, and red gram husk, were evaluated using solid-state fermentation. Effect of individual solid substrate on lipase activity is represented in Fig. 2a. Highest lipase activity of 7.17 U/gds was observed for RGH after 24 h, and the lowest activity of 4.2 U/gds was observed using RH. Effect of binary mixture with equal ratio is shown in Fig. 2b. Maximum lipase activity for RGH:RH binary mixture was observed as 9.89 U/gds after 72 h of fermentation. SSF process was also studied using ternary substrate mixture (RH:CSC:RGH) by varying proportions of the components. SSF was carried out at 30 °C with an initial moisture content of 60% for 72 h. As represented in Fig. 2c, lipase activity ranged from 6.26 U/gds to 12.93 U/gds using ternary mixture in different ratios for the production of lipase using *Aspergillus niger* MTCC 872. Maximum lipase activity of 12.93 U/gds was obtained for the substrate ratio 2:1:1 (RH:CSC:RGH) and minimum lipase activity of 6.26 U/gds for the substrate ratio 1:1:2 after 24 h of fermentation.

Effect of temperature

Effect of temperature was studied using fermentation with optimized substrate concentration by incubating at different temperatures ranging from 30 °C to 45 °C. Optimal lipase activity of 13.07 U/gds was found using mixed substrate 2:1:1 at 40 °C at 48 h as shown in Fig. 3a.

Effect of pH

SSF was carried out using previously optimized tri-substrate mixture with composition 2:1:1 (RH:CSC:RGH) at 40 °C and moisture content at this stage was maintained at 60%. During the pre-treatment, pH of tri-substrate mixture is varied from 5.5 to 8 with an increment of 0.5. Influence of pH was investigated by measuring lipase activity as shown in Fig. 3b. Maximum lipase activity of 25.12 U/gds was observed at pH 6. On further increasing in the pH, decline in lipase activity, i.e., at pH 7.5 lipase activity of 7.14 U/gds was observed.

Effect of moisture content

To evaluate the impact of moisture content on lipase production, experiments were conducted using the aforementioned optimum conditions for tri-substrate

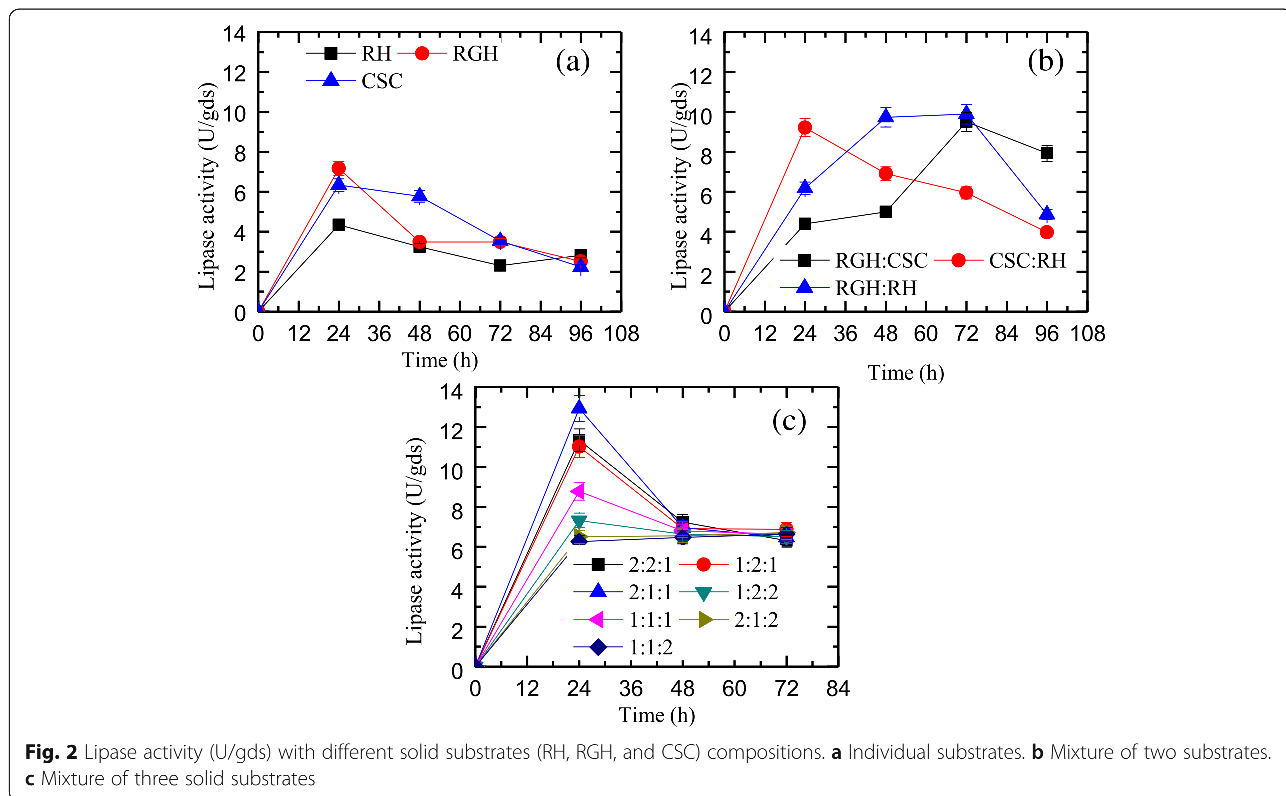

Fig. 2 Lipase activity (U/gds) with different solid substrates (RH, RGH, and CSC) compositions. **a** Individual substrates. **b** Mixture of two substrates. **c** Mixture of three solid substrates

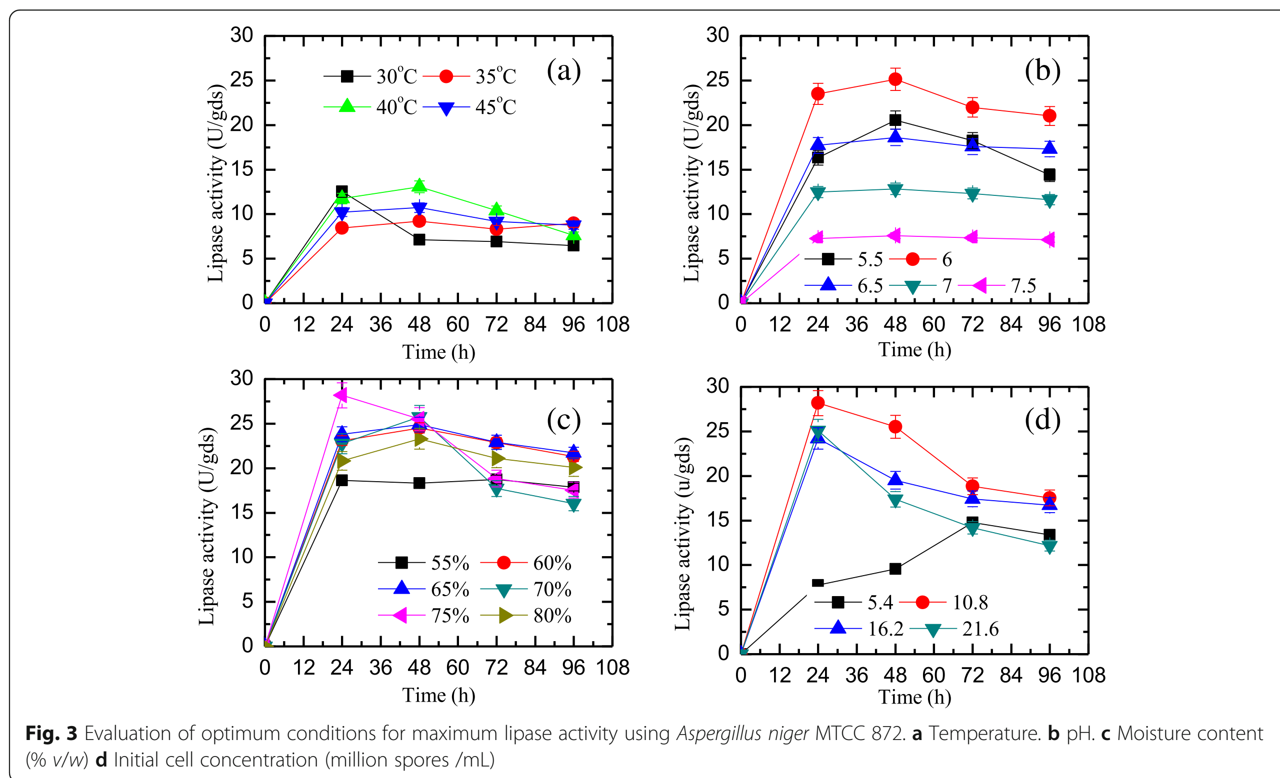

Fig. 3 Evaluation of optimum conditions for maximum lipase activity using *Aspergillus niger* MTCC 872. **a** Temperature. **b** pH. **c** Moisture content (% *v/w*) **d** Initial cell concentration (million spores /mL)

mixture. Lipase activity was obtained by varying moisture content from 55% to 80% (*v*/*w*) using phosphate buffer solution (pH 6) prior to fermentation as represented in Fig. 3c. Maximum lipase activity of 28.19 U/gds was observed at 75% moisture content after 24 h fermentation. Increase in lipase activity was observed with increase in moisture content from 55 to 75% (*v*/*w*). On further increment, decrease in lipase activity was observed.

Effect of spore concentration

To study the effect of initial cell concentration, experiments were conducted with aforementioned optimized parameters. Lipase activity was determined for solid tri-substrate inoculated with 4% (*v*/*w*) of *Aspergillus niger* MTCC 872 suspension consisting of 5.4, 10.8, 16.2, and 21.6 million spores per mL and shown in Fig. 3d. Highest lipase activity was 28.19 U/gds observed at cell concentration of 10.8 million spores per mL after 24 h.

Scale-up using tray bioreactor

Experiments were carried out in a tray bioreactor under the condition in which 100 g and 1000 g of optimized substrate mixture was fermented using previously optimized cultivation conditions. After 48 h of fermentation, the lipase activity was found to be 24.38 U/gds and 21.62 U/gds for 100 g and 1000 g substrate respectively as shown in Fig. 4. Lipase activity using different solid substrate at optimized conditions from *Aspergillus* species are shown in Table 1.

Discussion

SSF involves the growth and metabolism of the microorganisms on the moist solid substrate without any free-flowing water. Three different agricultural by-products rice husk, cottonseed cake, and red gram husk were evaluated for lipase activity with individual, binary mixture in equal

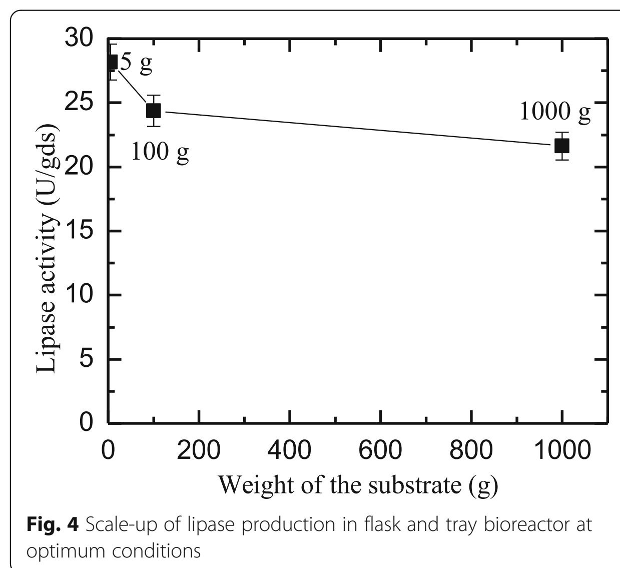

Fig. 4 Scale-up of lipase production in flask and tray bioreactor at optimum conditions

Table 1 Comparison of various works on lipase production

S. no.	Microorganism	Substrate	Optimum conditions	Lipase activity (U/gds)	Reference
1.	*Aspergillus niger* (O-4)	Wheat bran, rice husk	35 °C, pH– 6	42.82 ± 1.65	(Colla et al. 2015)
2.	*Aspergillus niger*	Shea butter cake	30 °C, 168 h, pH– 7.0	3.35	(Salihu et al. 2013)
3.	*Aspergillus niger*	Rice bran	30.3 °C, pH– 6.87	121.53	(Hosseinpour et al. 2012)
4.	*Aspergillus niger* MTCC 2594	Wheat bran, Coconut oil cake and Wheat rawa	30 °C, 72 h, pH– 7.0, MC 60%	745.7 ± 11	(Edwinoliver et al. 2010)
5.	*Aspergillus niger* MTCC 2594	Gingelly oil cake	30 °C,120 h, pH– 7.0, MC 60%	58.6	(Damaso et al. 2008)
6.	*Aspergillus niger* J-1	Olive oil and Glucose	30 °C, 168 h, pH– 7.0	9.14	(Falony et al. 2006)
7.	*Aspergillus niger* NCIM 1207	Wheat bran	45 °C, pH– 2.5	630	(Mahadik et al. 2002)
8.	*Aspergillus niger* MTCC 872	Rice husk, cotton seed cake and red gram husk	40 °C, pH– 6, MC 70%	27.2 (5 g) 24.38 (100 g) 21.62 (1000 g)	Current work

ratios and tri-substrate mixture in different ratios represented in Fig. 2a, b, and c, respectively. Nutrient sources (primarily carbon and nitrogen) and surface area are the two major factors that support fungal growth on the solid substrate. Highest lipase activity was observed for RGH and nearly similar activity for CSC after 24 h, whereas RH has shown lowest activity. Figure 2b represents binary mixture of substrates with equal ratio and maximum lipase activity was observed for RGH:RH after 72 h of fermentation. At the same time, comparable activity was observed for the mixture of RGH and CSC. Mixture of RGH:RH shows growth in the activity till 48 h and then retaining its activity till 72 h with a small increment. In case of RGH:CSC, rapid growth in activity was observed from 48 h to 72 h. Mixture of CSC:RH has shown notable growth for 24 h, thereafter gradual decline in the activity was observed. Binary mixtures taken in equal ratios have shown higher activity when compared with individual substrate. Similar results were observed when SSF was conducted with different combinations of rice bran, sugar cane bagasse, and wheat bran. Maximum lipase activity with mixed substrate of sugarcane bagasse and wheat bran was observed whereas for individual substrate the activity was found to be lower (Babu and Rao 2007). In the next stage of present work, SSF process was studied using ternary substrate mixture in the ratio of RH:CSC:RGH. Fermentation was carried out at 30 °C with an initial moisture content of 60% for 72 h. Figure 2c shows lipase activity for ternary mixture taken in different ratios using *Aspergillus niger* MTCC 872, representing the significance of selected solid substrates and their mixture composition. Maximum lipase activity was obtained for the substrate ratio 2:1:1 (RH:CSC:RGH) after 24 h of fermentation. With 2:2:1 and 1:2:1 tri-substrate mixtures, lipase activity was found to be similar. Lowest lipase activity was observed with tri-substrate composition of 1:1:2. Of largely, it was observed that the variation in the ratio of substrate mixture would influence the lipase activity. The above

results suggest that RGH and CSC are the primary nutrient source whereas RH plays a key role in providing sufficient surface area to promote fungal growth. To date, several studies were conducted to optimize fermentation conditions for lipase production using individual substrate, but very few have evaluated effect of different combinations of substrate during.

In the present work, ternary mixture has given maximum activity when compared to binary and individual substrate. This observation was in line with study conducted by Edwinoliver et al. (2010), wherein mixture of different substrates improved the growth and enzyme production, as it is difficult to acquire all essential nutrients from single substrate (Edwinoliver et al. 2010). In another study, high glutaminase activity was observed using a mixed substrate than single substrate (Sathish et al. 2008). Further physical parameters were optimized for this mixture composition and translated to a tray bioreactor for scale-up studies. Figure 3a represents the effect of temperature on the lipase activity. Optimized substrate concentration was incubated at different temperatures ranging from 30 °C to 45 °C with an increment of 5 °C. At 30 °C, lipase activity increased for 24 h thereafter sudden drop in the activity was observed. Enzyme activity increased with increase in temperature till 40 °C. Further increase in temperature led to decrease in biosynthetic activity. Similar results were observed where *Aspergillus niger* strain was optimally active in the temperature range of 40 °C to 60 °C during lipase production (Falony et al. 2006). Optimal lipase activity was found using mixed substrate 2:1:1 at 40 °C and 48 h as shown in Fig. 3a. This is in line with the study conducted by Kamini et al. (1998) where *Aspergillus niger* strains have been reported to be active between 40 °C

and 55 °C. Effect of pH is essential during SSF as change in pH could impact microbial growth and lipase activity. The influence of pH was investigated by varying the pH of potassium phosphate buffer ranging from 5.5 to 8. The pH affects stability of enzymes by changing the electrostatic interactions of their protein structure, causing changes in the amino acids ionization status, which defines the secondary and tertiary structures of protein and therefore its activity and stability (Eerappa et al. 2008). Optimum pH was determined by measuring lipase activity under fermentation conditions of 40 °C and moisture content 60% using optimized substrate mixture represented in Fig. 3b. Initially, lipase activity increased with increase in the pH and maximum lipase activity was observed at pH 6. On further increasing the pH, decrement in the lipase activity was observed. Another study reported that *Aspergillus niger* possesses good pH stability (pH 4–0–10.0) during lipase production (Kamini et al. 1998). Higher pH ranging from 7.5 to 8.0 did not show significant effect on lipase production by *Aspergillus niger* MTCC 872. Optimum moisture content and water activity are extremely important for growth of microbes during solid-state fermentation. Numerous fungi grow in low-moisture environment and hence they are quite adaptable to SSF technique when compared to bacteria. In the current work, lipase production was affected by moisture content variation as shown in Fig. 3c. Maximum lipase activity was observed at 75% moisture content after 24 h fermentation. Most of the reports suggest that maximum lipase activity is obtained at moisture content ranging from 60 to 80%. Also, higher moisture content would lower the substrate porosity thereby affecting fungal growth. At moisture level of 55 to 75%, lipase activity increased. Low moisture level would result in minimal growth due to a reduction in nutrient diffusion and low substrate swelling (Baysal et al. 2003). In contrast, 50% of moisture was optimal for protease and lipase production from *Pseudomonas aeruginosa* using Jatropha seed cake as a substrate (Mahanta et al. 2008). In another study, maximum lipase production from *Aspergillus niger* was observed at 71% moisture content using wheat bran as substrate (Mahadik et al. 2002). Hence, initial moisture content during the SSF technique would influence the substrate medium thereby affecting the microbial growth and substrate decomposition. Maximum lipase activity was obtained with optimized parameters for substrate ratio, temperature, pH, and moisture content of 2:1:1 (RH:CSC:RGH), 40 °C, pH 6, and 75% respectively. Further experiments were conducted to study the effect of initial cell concentration shown in Fig. 3d. At lower cell concentration of 5.4 million spores per mL, slow growth was observed since most of the nutrients were utilized for increasing cell concentration. Highest lipase activity was observed at cell concentration of 10.8 million spores per mL after 24 h. On further increasing the initial cell concentration decrease in lipase activity was observed, this might be due to rapid depletion of nutrient sources in the media to sustain the overall cell concentration. Scale-up of lipase production using 100 g and 1000 g substrate mixture resulted in 89.33% and 79.48% of the enzyme activity at flask level. Similar results were obtained, when scale-up was carried out for mixture of wheat bran and gingelly oil cake for which lipase activity of 95% and 84% of flask level for 100 g and 1000 g respectively was observed (Mala et al. 2007). In another study using wheat bran, coconut oil cake, and wheat rawa, lipase activity for 100 g and 3 × 1000 g was found to be 96% and 83% of the flask level lipase activity using *Aspergillus niger* MTCC 2594 (Edwinoliver et al. 2010).

Conclusion

Based on the current study, it can be concluded that *Aspergillus niger* MTCC 872 is an acidic lipase producer using substrate mixture composed of rice husk, cotton seed cake, and red gram husk which is easily available across the Indian markets. From the results, it can be perceived that CSC and RGH are the key nutrient component and RH provides sufficient surface area for fungal growth. The optimum conditions for substrate ratio, temperature, moisture content, and pH are found to be 2:1:1, 40 °C, 75% and 6.0 respectively. They also play a crucial role in lipase production. Lipase activity in tray bioreactor was found to be slightly lower, in comparison to SSF conducted in flasks. In conclusion, *Aspergillus niger* MTCC 872 strain and the solid substrates used in the present work are promising sources of lipase production.

Abbreviations

CSC: Cotton seed cake; RGH: Red gram husk; RH: Rice husk; SSF: Solid-state fermentation

Acknowledgements

The authors sincerely thank the Director of Indian Institute of Technology Hyderabad for his continued encouragement and support. We are also indebted to Kruthi Doriya for helping in manuscript and technical support. Devarai Santhosh Kumar thanks Science and Engineering Research Board-Department of Science and Technology-ECR/2017/000241. Sobha Kota acknowledges the UGC minor research project grant (grant no. 3026).

Funding

Science and Engineering Research Board-Department of Science and Technology-ECR/2017/000241.

Availability of data and materials

All the data generated and analyzed during the study are included in the article.

Authors' contributions

AN, VM, and SHP performed the experiments, and SK supported during the manuscript preparation. SKD guided AN, VM, and SHP during the experimentation and manuscript preparation. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors have read and approved to submit it to Bulletin of the National Research Centre. There is no conflict of interest of any author in relation to the submission.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
[1]Industrial Bioprocess and Bioprospecting Laboratory, Department of Chemical Engineering, Indian Institute of Technology Hyderabad, Laboratory No: A-224, Kandi, Sangareddy District, Telangana 502285, India. [2]RVR and JC College of Engineering, Guntur, Andhra Pradesh 522 019, India.

Received: 4 February 2019 Accepted: 8 May 2019
Published online: 24 May 2019

References
Andualema B, Gessesse A (2012) Microbial lipases and their industrial applications: review. Biotechnology. 11:100–118
Aravindan R, Anbumathi P, Viruthagiri T (2007) Lipase applications in food industry. Indian J Biotechnol 6:141–158
Arzoglou P, Goudoula C, Tsantili P, Lessinger J, Ferard G, Mignot V, Samyn N (1994) Transferability of lipase titrimetric assays: deductions from an interlaboratory study. Clin Chem Lab Med 32:773–778
Ashok A, Doriya K, Rao DRM, Kumar DS (2017) Design of solid state bioreactor for industrial applications: an overview to conventional bioreactors. Biocatal Agric Biotechnol 9:11–18
Babu IS, Rao GH (2007) Lipase production by Yarrowia lipolytica NCIM 3589 in solid state fermentation using mixed substrate. Res J Microbiol 2:469–474.
Baysal Z, Uyar F, Aytekin C (2003) Solid state fermentation for production of α-amylase by a thermotolerant *Bacillus subtilis* from hot-spring water. Process Biochem 38:1665–1668
Christopher LP, Hemanathan K, Zambare VP (2014) Enzymatic biodiesel: challenges and opportunities. Appl Energy 119:497–520
Colla LM, Ficanha AM, Rizzardi J, Bertolin TE, Reinehr CO, Costa JAV (2015) Production and characterization of lipases by two new isolates of *Aspergillus* through solid-state and submerged fermentation. Biomed Res Int 2015:1–9
Damaso MCT, Passianoto MA, De Freitas SC, Freire DMG, Logo RCA, Couri S (2008) Utilization of agroindustrial residues for lipase production by solid-state fermentation. Brazilian J Microbiol 39:676–681
Dayanandan A, Ravi SHV, Shanmugavel A, Gnanamani A, Rajakumar GS (2012) Solid state bioprocessing for scale up of *Aspergillus tamarii* MTCC5152 lipase and its degreasing effect on cow hide. Indian J Sci Technol 5:2978–2983
Doriya K, Devarai SK (2018) Solid state fermentation of mixed substrate for L-asparaginase production using tray and in-house designed rotary bioreactor. Biochem Eng J 138:188–196
Edwinoliver NG, Thirunavukarasu K, Naidu RB, Gouthaman MK, Nakajima K, kamini NR (2010) Scale up of a novel tri-substrate fermentation for enhanced production of *Aspergillus niger* lipase for tallow hydrolysis. Bioresour Technol 101:6791–6796
Eerappa R, Acharya P, Ahmad S, Sankaranaryanan R, Rao NM (2008) Structural basis for the remarkable stability of Bacillus subtilis lipase (Lip A) at low pH. Biochim Biophys Acta 1784:302–311
Falony G, Armas JC, Mendoza JCD, Hernández JLM (2006) Production of extracellular lipase from *Aspergillus niger* by solid-state fermentation. Food Technol Biotechnol 44:235–240
Fleuri LF, de Oliveira MC, de Lara Campos Arcuri M, Capoville BN, Pereira MS, Okino Deglado CH, Novelli PK (2014) Production of fungal lipases using wheat bran and soybean bran and incorporation of sugarcane bagasse as a co-substrate in solid-state fermentation. Food Sci Biotechnol 23:1199–1205
Gilham D, Lehner R (2005) Techniques to measure lipase and esterase activity in vitro. Methods. 36:139–147
Gombert AK, Pinto AL, Castilho LR, Freire DMG (1999) Lipase production by Penicillium restrictum in solid-state fermentation using babassu oil cake as substrate. Process Biochem 35:85–90
Hölker U, Höfer M, Lenz J (2004) Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Appl Microbiol Biotechnol 64:175–186
Hosseinpour MN, Najafpour GD, Younesi H, Khorrami M, Vaseghi Z (2012) Lipase production in solid state fermentation using Aspergillus Niger: response surface methodology. Int J Eng Trans B Appl 25:151–159
Joseph B, Ramteke PW, Thomas G (2008) Cold active microbial lipases: some hot issues and recent developments. Biotechnol Adv 26:457–470
Kamini NR, Mala JGS, Puvanakrishnan R (1998) Lipase production from Aspergillus Niger by solid-state fermentation using gingelly oil cake. Process Biochem 33:505–511
Kanmani P, Aravind J, Kumaresan K (2015) An insight into microbial lipases and their environmental facet. Int J Environ Sci Technol 12:1147–1162
Krishna C (2005) Solid-state fermentation systems - an overview. Crit Rev Biotechnol 25:1–30
Kynclova E, Hartig A, Schalkhammer T (1995) Oligonucleotide labelled lipase as a new sensitive hybridization probe and its use in bio-assays and biosensors. J Mol Recognit 8:139–145
Mahadik ND, Puntambekar US, Bastawde KB, Khaire JM, Gokhale DV (2002) Production of acidic lipase by *Aspergillus niger* in solid state fermentation. Process Biochem 38:715–721
Mahanta N, Gupta A, Khare SK (2008) Production of protease and lipase by solvent tolerant *Pseudomonas aeruginosa* PseA in solid-state fermentation using Jatropha curcas seed cake as substrate. Bioresour Technol 99:1729–1735
Mala JGS, Edwinoliver NG, Kamini NR, Puvanakrishnan R (2007) Mixed substrate solid state fermentation for production and extraction of lipase from *Aspergillus niger* MTCC 2594. J Gen Appl Microbiol 53:247–253
Pandey A (2003) Solid-state fermentation. Biochem Eng J 13:81–84
Salihu A, Bala M, Alam MZ (2016) Lipase production by *Aspergillus niger* using sheanut cake: an optimization study. J Taibah Univ Sci 10:850–859
Salihu A, Bala M, Bala SM (2013) Application of Plackett-Burman experimental design for lipase production by *Aspergillus niger* using shea butter cake. ISRN Biotechnol 2013:1–5
Sathish T, Lakshmi GS, Rao CS, Brahmaiah P, Prakasham RS (2008) Mixture design as first step for improved glutaminase production in solid-state fermentation by isolated *Bacillus* sp. RSP-GLU. Lett Appl Microbiol 47:256–262
Saxena RK, Ghosh PK, Gupta R, Davidson WS, Bradoo S, Gulati R (1999) Microbial lipases: potential biocatalysts for the future industry. Curr Sci 77:110–115
Selvakumar P, Sivashanmugam P (2017) Optimization of lipase production from organic solid waste by anaerobic digestion and its application in biodiesel production. Fuel Process Technol 165:1–8
Sharma R, Chisti Y, Chand U (2001) Production, purification, characterization and applications of lipases. Biotechnol Adv 19:627–662
Show PL, Ling TC, Lan JCW, Tey BT, Ramanan RN, Yong ST, Ooi CW (2015) Review of microbial lipase purification using aqueous two-phase systems. Curr Org Chem 19:19–29
Singhania RR, Patel AK, Soccol CR, Pandey A (2009) Recent advances in solid state fermentation. Biochem Eng J 44:13–18
Stamenković OS, Veličković AV, Veljković VB (2011) The production of biodiesel from vegetable oils by ethanolysis: current state and perspectives. Fuel. 90:3141–3155
Viniegra-González G, Favela-Torres E, Aguilar CN, Sergio de Jesus RG, Diaz-Godinez G, Christopher A (2003) Advantages of fungal enzyme production in solid state over liquid fermentation systems. Biochem Eng J 13:157–167