Production, characterization and application of crude fungal lipase from *Aspergillus rubber* OP520917

Rania Abdel Razik, Mona S. Shafei

Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, 33 El Bohouth St. (from El Tahrir St.), Dokki, Giza, Egypt

Correspondence to Rania Abdel Razik, Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research institute, National Research Centre, 33 El Bohouth St. (from El Tahrir St.), Dokki, Giza, Egypt.
Tel:/Fax: 01227272695;
e-mail: Rania122bc@gmail.com

Received: 26 May 2023
Revised: 1 July 2023
Accepted: 14 July 2023
Published: 22 November 2023

Egyptian Pharmaceutical Journal 2023, 22:632–639

Background
The pancreatic and stomach juices both include the naturally occurring lipase enzyme. Its job is to digest lipids and fats, which helps to keep the gallbladder working properly. Long chain triglycerides are hydrolyzed by microbial lipases, E.C 3.1.1.3 because they are capable of producing changed molecules, the lipase enzymes that come from microorganisms are theoretically versatile and useful for a wide range of industrial applications.
Objective
This study sought to determine the amount of crude lipase produced by *A.rubber* OP520917 during submerged fermentation and its use in removing oil from fabrics and breaking down chicken fats.
Materials and methods
Aspergillus rubber OP520917 was tested for lipase production using submerged fermentation; lipase activity was estimated, characterized and tested for industrial applications. Influence of: the pH; incubation times; surfactants and organic solvents and their concentration on the production of lipase by the selected strain were evaluated.
Results and conclusion
Aspergillus rubber OP520917 was identified strain for lipase production. The biochemical characterizations of lipolytic activity of *A.rubber* were studied and documented that the best temperature was 37°Cat pH 4 after 4 days of incubation time. The medium used for enzyme production contains olive oil as a carbon source, Moreover, 30% of hexane was the best organic solvent for the strain. Where the relative activity increased to 200%. The lipase activities were maximal (210,145 U/ml) in the presence of surfactants tween80 and tween20 respectively at 1% concentration. Furthermore, Lipase activity was also tested for removal of oil stains from the fabrics and the degradation of natural chicken fats with crude enzyme as industrial applications. The results showed that more than 74% of fats degraded after 5 days of the incubation period.

Keywords:
Aspergillus rubber, destaining and degradation of animal fats application, lipase production

Egypt Pharmaceut J 22:632–639

1687-4315

Author's contributions: RA designed the study, RA and MS prepared the manuscript, RA did the practical part of this work.MS supervised the practical part of this work and reviewed the manuscript. RA and MS authors have reviewed and approved the final revision of the manuscript.

Introduction

Both the chemical and enzymatic catalysts are effective for industrial applications. However, there are several drawbacks in chemical catalysis such as undesirable by-product formation, high energy consumption and equipment corrosion [1] On the other hand, the enzymatic catalysis is preferable as it is specific, selective and thus preventing undesired modifications of substrate and the formation of the toxic byproduct in addition to the fact that it is less energy demanding [2,3].

The reaction is catalysed at the lipid-water interface by lipases, a subclass of esterase with long chains of triacylglycerol that are very poorly soluble in water [4,5] Due to their excellent stability in temperature, pH, and organic solvent extremes, lipases are particularly effective in catalysing processes in both aqueous and non-aqueous conditions [6]. According [5], lipases are known to have a hydrophobic lid that is essential for their interfacial activity.

The breakdown of oils and fats is catalyzed by lipases (glycerol ester hydrolyses, E.C 3.1.1.3). They also facilitate the formation of esters through Trans

esterification, thio esterification, and amylolysis [7] under micro-aqueous circumstances [8] Lipases are widely distributed in bacteria, yeasts, and fungi [9–11].

Studies on microbial lipases have expanded as a result of their practical industrial uses, such as fatty acid generation, fat hydrolysis, ester and peptide synthesis, racemic mixture resolution, and addition to detergents and food additives. Microbial lipases can be produced utilizing both submerged and solid state fermentation systems with a variety of carbon and nitrogen sources. The majority of literature publications confirmed that the solid state fermentation systems are superior techniques for the synthesis of microbial lipase.

The best sources of lipase are fungi, which are frequently employed in industrial applications, particularly in the food industry. *Aspergillus niger* is one of the most well-known lipase producers, and its enzyme is suitable for use in many developed applications [12]. According to various research, a wide range of species developed extracellular lipases. This throws light on the biochemical characterization of lipase activity and stability of *A.rubber* that can help in understanding the major aspects of its production as well as its potential in industrial applications.

Materials and methods

Chemicals

All media components were purchased from sigma Aldrich Chemical Company and were of analytical grade.

Microorganisms

Aspergillus rubber OP520917 was kindely obtained from the culture collection at Institute of Pharmaceutical and Industrial Drugs chemistry of natural and microbial products department, National Research Centre (NRC) culture collection, Cairo, Egypt. The fungal strain was maintained on potato dextrose agar (PDA) and stored at 4°C.

Inoculum preparation

The fungal inoculum was prepared by scratching the spores of cultivated slants (4.3×10^6 spores/100 ml medium) into a sabouraud liquid medium.

Submerged fermentation (SmF)

Submerged fermentation was used for quantitative evaluation of *A.rubber* OP520917 where Erlenmeyer flasks (250 ml) containing 100 ml sabouraud broth medium which has the following composition (g/l): 40 g glucose; 10 g peptone [13]. The flasks were inoculated with 4.3×10^6 spores/100 ml of the tested microorganism and incubated at 30°C on a rotary shaker (160 rpm) for 4 days. At the end of the incubation period, the fermented medium filtrated using whatman No.1 was and the cell dry weight was measured and the lipase activity was assayed in the supernatant.

Lipase assay

Culture filtrate lipase(1 ml) was mixed with3-ml emulsion of olive oil in Arabic gum (10% w/v) and 2.5 ml of deionized water in 1-ml 0.1 M Tris-HCl buffer to assess lipase activity (pH 7.5). 10 ml of 99% ethanol solution was added to terminate the reaction after it had been incubated for 2 h at 37°C and 160 rpm. Thymolphthalein was used as an indicator as the mixture was then titrated against 0.05 N Na OH. Blank experiments were then carried out by boiling the enzyme. The amount of enzyme that releases 1 mole of free fatty acids per minute under test conditions is considered one unit of lipase activity [14].

Effect of incubation period on lipase production

On the productive medium, *A.rubber* was allowed to grow for various incubation times ranging from 24 to 120 h at 30 degrees Celsius on a rotary shaker (160 rpm). Enzyme activity was assessed after the extract was collected.

Characteristics for the lipase

The ideal conditions for lipase activity to be used in diverse applications were determined using the clear supernatant.

Effect of pH and temperature

Using citrate, phosphate, and glycine buffer, the impact of pH on lipase activity was evaluated at various pH values ranging from 3 to 10. A wide range of temperatures, from 25 to 50°C, were used to study how temperature affected lipase activity. The enzyme assay was conducted as previously described in order to establish the ideal pH and temperature

Effect of organic solvents and surfactants

This was determined by using different surfactants (tween 80, tween20) at different concentrations (0.5, 1, 1.5%v/v) and organic solvents either water soluble (ethanol, methanol and acetone) or water insoluble (hexane) were added to the enzyme solution and incubated at 37°C for 2 h. Activity of the enzyme was measured as previously mentioned.

Effect of enzyme concentration

To investigate the effect of the different enzyme concentration on the lipolytic activity, aliquots of crude enzyme (0.1–2.5 ml) were added to the reaction mixture which contained 3 ml olive oil. The lipase activity was determined and the relative activities were plotted against different concentration of the crude enzyme and relative activities.

Determination of lipase efficiency

The crude fungal lipase, were examined for their effectiveness in eliminating oil stains from fabrics and decomposing natural chicken fat.

Removing oil stains

Two drips of frying oil were used to stain a fabric that was 3 cm by 2 cm and 3 cm by 4 cm in size. The fabric pieces were placed on reagent bottles with four different treatments after being allowed to dry as follows: (a) Water (100 ml), (b) 1% (w/v) detergent in water (99 ml + 1 ml), (c) lipase in water (99 ml + 1 ml), and detergent and lipase in water (98 ml + 1 ml + 1 ml) are examples of the four different types of liquids. A cold-water treatment (25°C) and a hot water treatment (60°C) were performed in parallel for each treatment. For 30 min, all treatments were incubated and gently stirred. The fabric pieces were then taken out, dried, and checked for any signs of oil left over. Titrimetric assay was employed as previously mentioned to measure the quantity of free fatty acids produced with each treatment. Each sample's relative activity (%) was calculated and contrasted with the detergent-free control. the relative activity of control was defined as the enzyme activity without detergent incubated under similar conditions and was taken as 100%.

Degradation of natural chicken fats

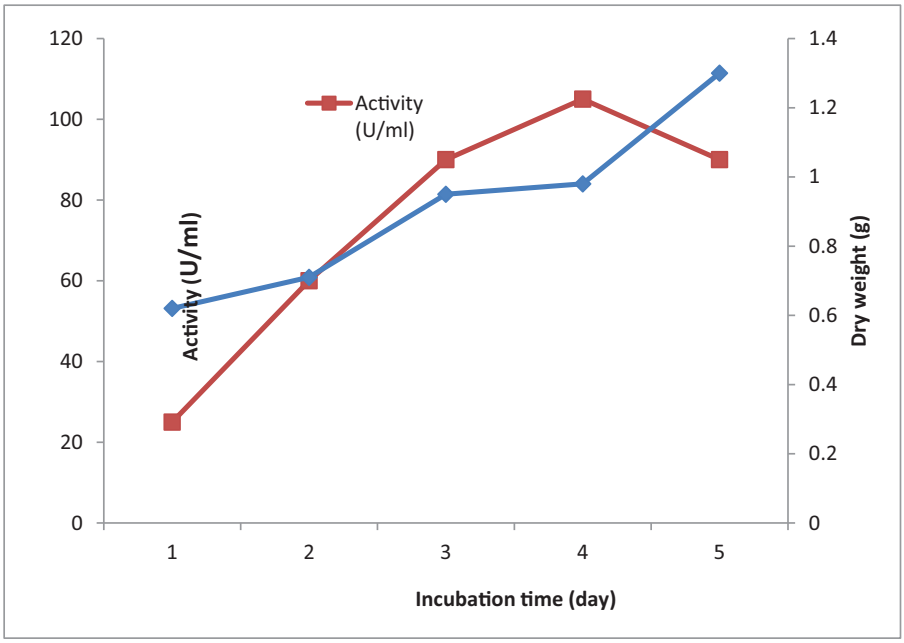
To assess the efficiency of the crude fungal lipase in breaking down natural fat, 2.5 g of chicken fat was autoclaved and placed in tubes before the crude enzyme were added. The tubes were then incubated for 1, 2, 3, 4, 5, and 6 days at their ideal temperature (50°C for *A.rubber*). Fat chunks were weighed following incubation, and the enzyme activity was assessed.

Results and discussions

Effect of incubation period on lipase activity

The incubation period plays an important role in the biosynthesis of lipase. In our study 4 days under submerged fermentation was found to be optimum incubation period for enzyme production by *A.rubber* OP520917 reaching its maximal values (105U/ml) at 0.98 g/l mycelial dry weight (Fig. 1). As the fermentation period increased the biomass increased up to 5 days where the enzyme activity started to decrease. Colla *et al.* [15] recorded 3 to 4 days as ideal incubation period to produce maximum lipolytic activity using *Aspergillus sp.* On the other Cesario *et al.* [16] and Kempaka *et al.* [17] adopted 6 and 7 days incubation period for maximal lipase

Figure 1



Effect of incubation period on lipase activity (U/ml) as indicated by the growth of *Aspergillus rubber* (dry weight g/flask).

activity. These diversities may be attributed to the wide varieties of fungal isolates as well as conditions and the media used [16].

Effect of pH on lipase activity

Lipase activity produced by *A.rubber* OP520917 was measured at various enzyme solutions ranging from pH 3 to 10. The lipase tends to be active under acidic to neutral (Fig. 2). Optimum lipase activity (155 U/ml) was found at pH 4.0 using citrate buffer. Nearly similar results were shown lipase produced from both *A.niger* and *P.simplicissimum* in pH 5 and pH 6 respectively [18,19]. In the same manner, *Aspergillus niger* J-1 [20] and *A.niger* NCIM1207 [21] had optimum activity under acidic conditions, pH 6 and 3 respectively. This is a result of the ionization state of the enzyme and, consequently, of the enzyme's reaction rate. Ionic interactions recognize the flexibility and confirmation of the enzyme and its active site at various pH settings. As a result, at various pH settings, the side groups' ionization state changes, interfering with these forces and denature the protein structure [22] As a result, every enzyme has an ideal pH range where they perform at their highest level.

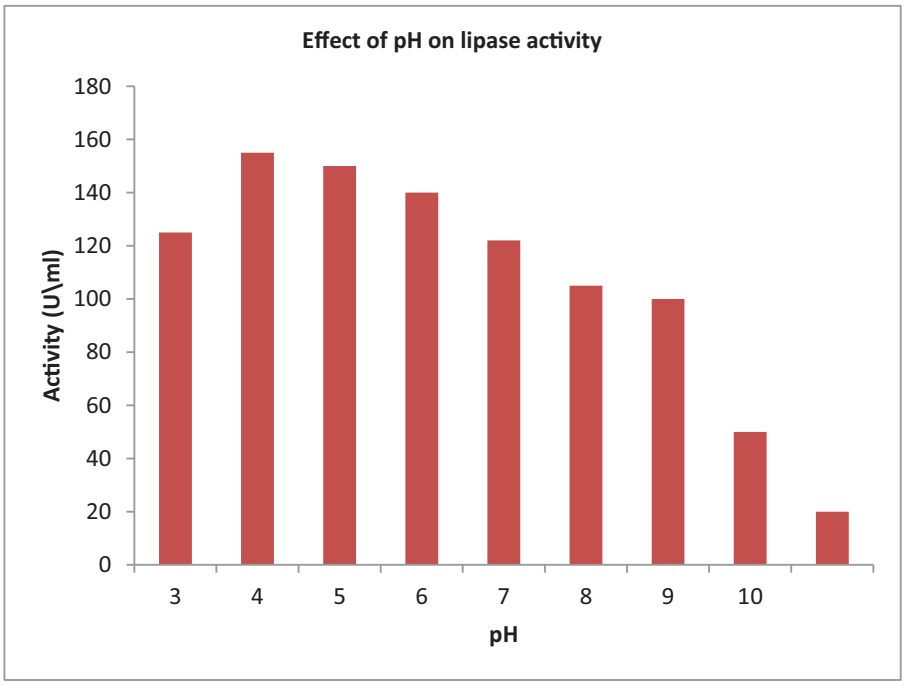
Effect of temperature on lipase activity

Lipase activity was estimated between 25°C and 50°C and results are exposed in (Table 1). The extract showed activity in all temperatures tested. The lowest activity was at 25°C and continued to increase with temperature until it reached maximum at 37°C (155 U\ml) with relative activity 100%. Generally increasing the temperature increases the reactant molecules kinetic energy which in turn increases the effect of the shocks and the reactions rate. This phenomenon is observed at a particular temperature range when the enzyme's three-dimensional form is maintained. However, denaturation of the enzyme occurs at higher temperatures [23]. Our results agreed nearly with those obtained by Colla *et al.* [24] where the maximum lipase activity was at 37°C using *A.flavus*. Most of lipase activity produced by *Aspergilli* are at around 40°C as that obtained by Sundar and Kumaresapillai [25] using *A.niger* NCIM1207 as the maximum activity was at 40°C.Differently, Rashma and Shanmugam [26] stated that the optimum temperature was 27°C for maximum enzyme activity.

Table 1 Effect of temperature on the lipase activity

Temperature C°	Relative activity %	Activity (U/ml)
20	25.8	40
25	25.8	40
30	25.8	40
37*	100	155
40	41.9	65
45	38.6	60
50	38.6	60

Figure 2

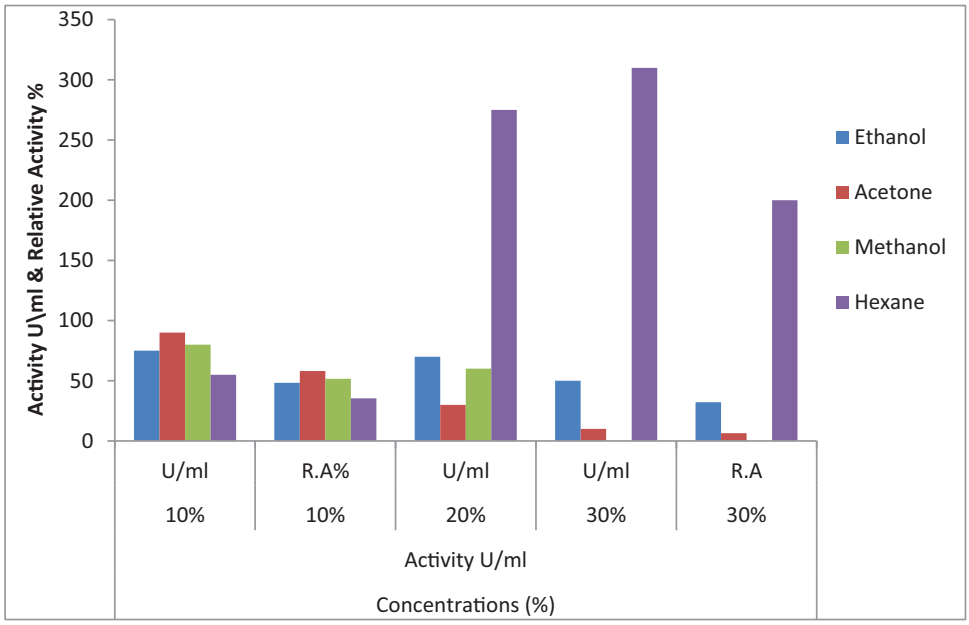


Effect of pH on lipase activity (U/ml).

Effect of different concentrations of organic solvents on lipase activity

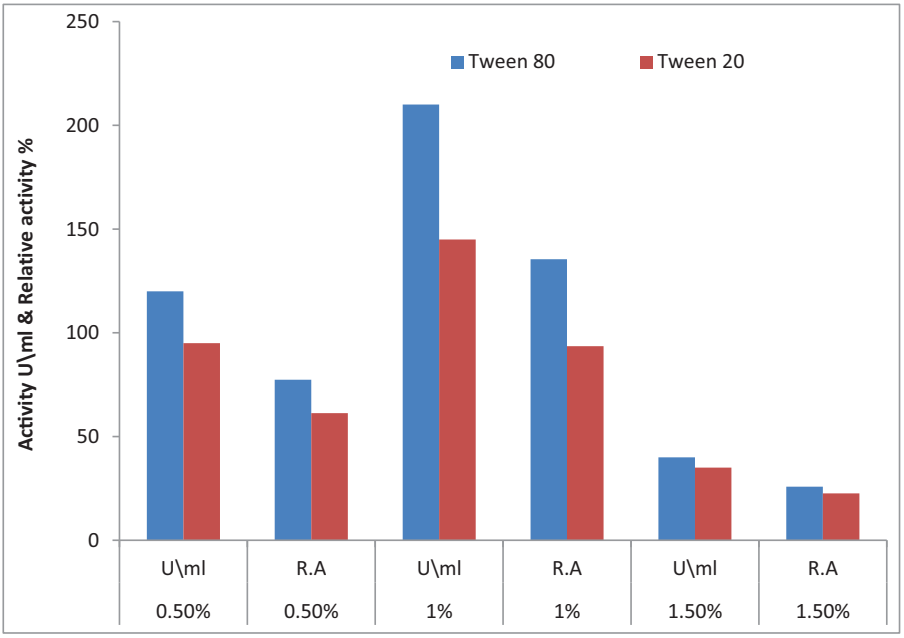
In biotechnological process, lipases are important in bioconversions in organic solvents. Which have influencing role on the activity of enzymes and their effectiveness. The organic solvents (methanol, ethanol, acetone& hexane) were used at two different concentrations (10, 30% v\v) to test the optimal activity for *A.rubber* OP520917. The effect of organic solvents on lipase activity was investigated as shown in (Fig. 3). The lipolytic activity of *A.rubber* OP520917 changed and showed different degrees of decline. However, *A.rubber* lipase showed good tolerance to hexane at 30% concentration and relative activity was 200% .While other organic solvents caused a decrease in the lipase activity. This may be attributed to the fact that hexane is a non-polar solvent with high log P 3.5. Klibanov [19] reported

Figure 3



Effect of different concentrations of organic solvents on *A.rubber* lipase activity (U/ml) and relative activity (%).

Figure 4



Effect of different concentrations of surfactants on lipase activity as indicated by U/ml and relative activity represented by (%).

previously that polar solvents with low log P 0.8 as in methanol reduced the enzyme activity while the non-polar solvents with high log P increased the enzyme activity. Similarly Gururaj *et al*. [27] reported that n-hexane increased lipase activity of *Acinobacter sp* AU07 by 1 fold therefore, during lipase-catalyzed organic synthesis, it is necessary to select a suitable reaction solvent [22].

Effect of surfactants

Surfactants in particular tween 80 and tween 20 have an effect on *A.rubber* OP520917 lipase activity. When Tween80 (1% v/v) was used, which is known to reduce the interfacial stress between the water and oil phases and improve the water-lipid interface area, lipase activity raise by 135.4% (Fig. 4). This raises the rate at which lipase-catalyzed reactions occur Shaoxin & Bingzhao [28]. This offers prospective benefits in the detergent industry. This in accordance with results obtained by Ben Bacha *et al*. [29], Das *et al*. [30] & Malekabadi *et al*. [31] who stated that tween 80 surfactants stimulated the enzymatic activity. Zheng *et al*. [32] and Sharma & Kanwar [33] stated that tween 20 SDS surfactants had inhibitory effect.

Our results showed that Tween 80 at 1% was found to be the most effective variable during lipase activity by *A.rubber*, because it served as a carbon source and an inducer. These results agreed with Salhiu *et al*. [34] who found that the presence of Tween 80 led to higher lipase production from *Penicillium citrinum*.

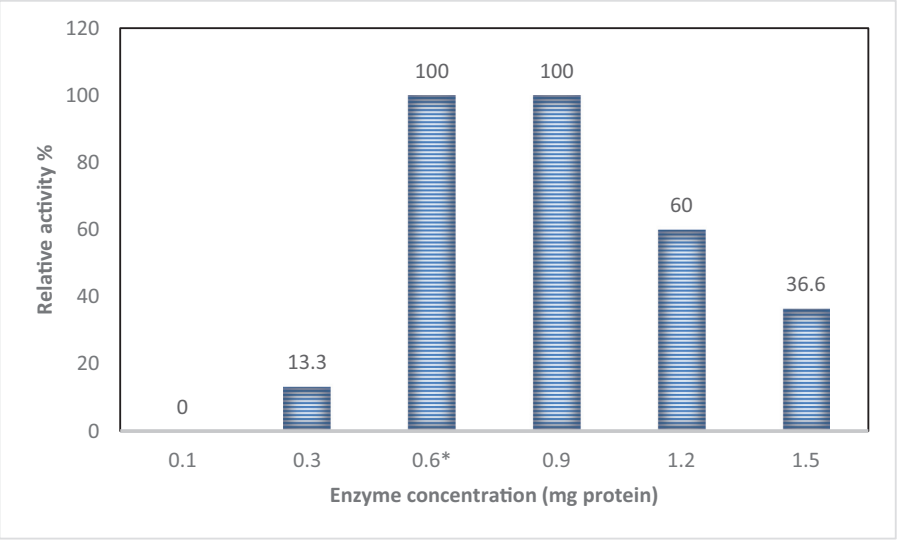
Effect of enzyme concentration *on A.rubber* lipase activity

Our results showed that 1 ml containing 0.6 mg protein was the best enzyme concentration for lipase activity as shown in (Fig. 5) and these results agreed with Al-Haidari *et al*. [35] who stated that 1 ml of lipase concentration was the optimum concentration of enzyme that exerted the highest activity (2.31 U/ml) as more enzymes causes more colloid with substrate molecules. Cumulative enzyme concentration will speed up the chemical reaction rate, as long as there is substrate accessible for binding. Once all of the substrate is bound, the reaction will no longer speed up because there will be nothing for additional enzymes to bind.

Application of *A.rubber* lipase in removing oil stain

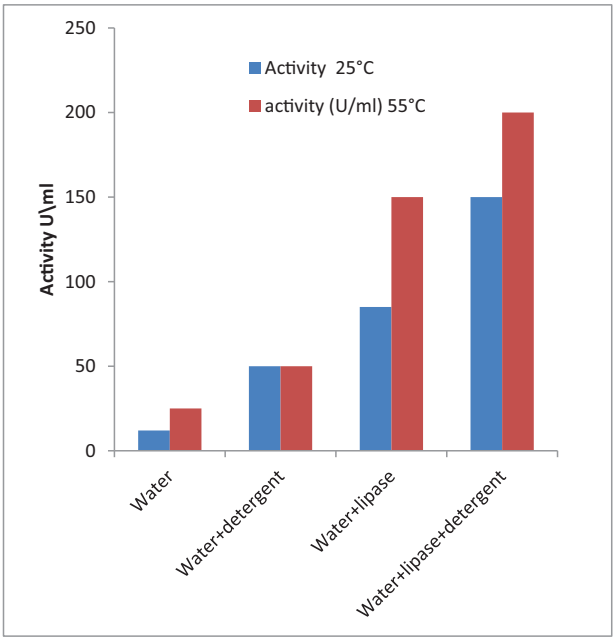
Oil stains on cotton garments were treated with hot and cold water four times each to test the effectiveness of *A.rubber* lipase. In comparison to low temperatures, lipase activity was found to be better at high temperatures (55°C) than low (25°C). When the cotton fabric was treated with the enzyme and the detergent, it was clear that the oil staining process had been successful. Moreover, adding crude lipase to cold or hot water boosted the enzyme activity in the absence of detergent by 1.7 and 3 folds, respectively. When crude lipase was added to cold or hot water in the absence of detergent, the enzyme activity rose by 1.7 and 3 times, respectively. The largest amount of oil distaining (200 U/ml) was achieved using hot water in the fourth treatment, which also included detergent, cold or hot water, and lipase. Our findings showed that oil stains may be eliminated even little in cold water when water, detergent, and enzyme were present. This suggests the potential for application and thus lowering energy use. Similar to this, Hemachander and Puvanakrishnan [36] showed that using detergent and *Rastonia pickettii* enzyme combined improved oil

Figure 5



Effect of different enzyme concentrations (weight/mg protein) on lipase relative activity (%).

Figure 6



Oil destaining efficiency of the lipase by using oil stains on cotton garments which were treated by a) water b) water+detergent, c) water + lipase, d) water+lipase+detergent.

removal by 24–27%. These results agreed with Prazeres and Cruz [37] using lipase from *Fusarium oxysporium* in removing oil stains while Das & Bhattacharya [30] using *Aspergillus tamarii* JGIF06. The effectiveness of removing oil stains in the presence of detergent and enzyme was the same in cold and hot water. This disagreed with our results in which the lipase activity in hot water was higher than in cold [30].

Degradation of chicken fats

It was used to break down chicken fats. After five days, it was shown that lipase was able to breakdown roughly 74% of the chicken fats (Fig. 6). Hence, lipase can address the problem of fat pollution in the environment in a safer and more affordable manner [21]. The removal of fats in the medical field as well as the lipolysis of fats in water sanitation and the prevention of water pollution can be accomplished using the enzyme from *A .rubber*, according to our findings [38] (Table 2).

Conclusion

In the present work, the extracellular enzyme was obtained from *A.rubber* OP520917 was characterized and showed several properties suitable for different applications. The lipase produced was stable to surfactants and organic solvents and thus suggesting its use for detergents and as biocatalysts for trans esterification reactions.

Table 2 Degradation of chicken fats by *A.rubber* lipase

Time	Weight of fats/g	Activity U/ml
Control	**2.5**	**155**
1st day	2.13	120
2nd day	1.82	80
3rd day	1.4	65
4th day	0.9	60
5th day	0.65	60

Acknowledgements

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Chowdhury A, Chakraborty R, Mitra D, Biswas D. Optimization of the production parameters of octyl ester biolubricant using Taguchi's design method and physico-chemical characterization of the product. Ind Crops Prod 2014; 52:783–789.

2 Cavalcanti ED, Aguieiras EC, da Silva PR, Duarte JG, Cipolatti EP, Fernandez-Lafuente R, Freire DM. Improved production of biolubricants from soybean oil and different polyols via esterification reaction catalyzed by immobilized lipase from *Candida rugosa*. Fuel 2018; 215:705–713.

3 Fernandes KV, Papadaki A, da Silva JAC, Fernandez-Lafuente R, Koutinas AA, Freire DMG. Enzymatic esterification of palm fatty-acid distillate for the production of polyol esters with biolubricant properties. Ind Crops Prod 2018; 116:90–96.

4 Aulakh SS, Prakash R. Optimization of medium and process parameters for the production of lipase from an oil-tolerant *Aspergillus sp*. (RBD-01). J Basic Microbiol 2010; 50:37–42.10.

5 Khan FI, Lan D, Durrani R, Huan W, Zhao Z, Wang Y. The lid domain in lipases: Structural and functional determinant of enzymatic properties. Front Bioeng Biotechnol 2017; 5:16.

6 Tan JS, Abbasiliasi S, Ariff ABNgHS, Bakar MHA, Chow YH. Extractive purification of recombinant thermostable lipase from fermentation broth of Escherichia coli using an aqueous polyethylene glycol impregnated resin system. Biotech 2018; 8:1–7.3

7 De Oliveira UM, Lima de Matos LJ, De Souza MCM, Pinheiro BB, dos Santos J, Gonçalves LR. Effect of the presence of surfactants and immobilization conditions on catalysts' properties of *Rhizomucor miehei* lipase onto chitosan. Appl Biochem Biotechnol 2018; 184:1263–1285.

8 Palla CA, Pacheco C, Carrín ME. Production of structured lipids by acidolysis with immobilized *Rhizomucor miehei* lipases: selection of suitable reaction conditions. J Mol Catal B: Enzym 2012; 76:106–115.

9 Jaeger KE, Reetz MT. Microbial lipases form versatile tools for biotechnology. Trends Biotechnol 1998; 16:396–403.

10 Jaeger KE, Eggert T. Lipases for biotechnology. Curr Opin Biotechnol 2002; 13:390–397.

11 Villalba M, Verdasco-Martín CM, Dos Santos JC, Fernandez-Lafuente R, Otero C. Operational stabilities of different chemical derivatives of Novozym 435 in an alcoholysis reaction. Enzyme Microb Technol 2016; 90:35–44.

12 El Menoufy HA, Gomaa SK, Haroun AA, Farag AN, Shafei MS, Shetaia YM, Abd El Aal RA. Comparative studies of free and immobilized partially purified lipase from *Aspergillus niger* NRRL-599 produced from solid-state fermentation using gelatin-coated titanium nanoparticles and its application in textile industry. Egypt Pharm J 2022; 21:143.

13 Abd El Aal RA, Shetaia YM, Shafei MS, Gomaa SK, El Menoufy HA, El-Refai HA. Optimization of parameters for lipase production by *Aspergillus niger* NRRL-599 using response surface methodology. Egypt Pharm J 2019; 18:165.

14 Nadia N, Nehad ZA, Elsayed E, Essam MA, Hanan MA. Optimization of lipase synthesis by *Mucor racemosus*-Production in a triple impeller bioreactor. Malaysian J Microbiol 2010; 6:7–15.

15 Colla LM, Primaz AL, Benedetti S, Loss RA, Lima MD, Reinehr CO, Costa JAV. Surface response methodology for the optimization of lipase

production under submerged fermentation by filamentous fungi. Braz J Microbiol 2016; 47:461–467.

16 Cesário LM, Pires GP, Pereira RFS, Fantuzzi E, da Silva Xavier A, Cassini STA, de Oliveira JP. Optimization of lipase production using fungal isolates from oily residues. BMC Biotechnol 2021; 21:1–13.

17 Kempka AP, Lipke NL, da Luz Fontoura Pinheiro T, Menoncin S, Treichel H, Freire DM, De Oliveira D. Response surface method to optimize the production and characterization of lipase from *Penicillium verrucosum* in solid-state fermentation. Bioprocess Biosyst Eng 2008; 31:119–125.

18 Gutarra ML, Godoy MG, Maugeri F, Rodrigues MI, Freire DM, Castilho LR. Production of an acidic and thermostable lipase of the mesophilic fungus *Penicillium simplicissimum* by solid-state fermentation. Bioresour Technol 2009; 100:5249–5254.

19 Klibanov AM. Improving enzymes by using them in organic solvents. Nature 2001; 409:241–246.

20 Falony G, Armas JC, Mendoza JCD, Hernández JLM. Production of extracellular lipase from *Aspergillus niger* by solid-state fermentation. Food Technol Biotechnol 2006; 44:235–240.

21 Mahadik ND, Puntambekar US, Bastawde KB, Khire JM, Gokhale DV. Production of acidic lipase by *Aspergillus niger* in solid state fermentation. Process Biochem 2002; 38:715–721.

22 Adlerceutz P. Immobilization & applications of lipase in organic media. Chem Soc Rev 2013; 42:6406–6436.

23 Alabdalall AH, Al-Anazi NA, Aldakheel LA, Amer FH, Aldakheel FA, Ababutain IM, Al-Khaldi EM. Application and characterization of crude fungal lipases used to degrade fat and oil wastes. Sci Rep 2021; 11:1–10.

24 Colla LM, Ficanha AM, Rizzardi J, Bertolin TE, Reinehr CO, Costa JAV. Production and characterization of lipases by two new isolates of *Aspergillus* through solid-state and submerged fermentation. Bio Med Res Int 2015; 12:1-9.

25 Sundar WA, Kumaresapillai N. Isolation, purification and medium optimization of lipase enzyme producing strains of *A.niger* isolated from several natural sources. Int J Pharm Pharmac Sci 2013; 5:321–324.

26 Rashma CH, Shanmugam P. Isolation and characterization of the lipase from *Aspergillus Brasiliensis*. Int J Biotechnol Bioeng Res 2013; 4:481–486.

27 Gururaj P, Ramalingam S, Devi GN, Gautam P. Process optimization for production and purification of a thermostable, organic solvent tolerant lipase from *Acinetobacter sp*. AU07. Braz J Microbiol 2016; 47:647–657.

28 Shaoxin C, Lili Q, Bingzhao S. Purification and properties of enantio selective lipase from a newly isolated *Bacillus cereus* C71. Process Biochem 2007; 42:988–994.

29 Ben Bacha A, Moubayed NM, Al-Assaf A. An organic solvent-stable lipase from a newly isolated Staphylococcus aureus ALA1 strain with potential for use as an industrial biocatalyst. Biotechnology and applied biochemistry 2016; 63:378–390.

30 Das A, Shivakumar S, Bhattacharya S. Purification and characterization of a surfactant-compatible lipase from *Aspergillus tamarii* JGIF06 exhibiting energy-efficient removal of oil stains from polycotton fabric. Biotech 2016; 6:131–139. 3

31 Malekabadi S, Badoei-Dalfard A, Karami Z. Biochemical characterization of a novel cold-active, halophilic and organic solvent-tolerant lipase from *B. licheni formis* KM12 with potential application for biodiesel production. Int J Biol Macromol 2018; 109:389–398.

32 Zheng X, Chu X, Zhang W, Wu N, Fan Y. A novel cold-adapted lipase from *Acinetobacter sp*. XMZ-26: gene cloning and characterization. Appl Microbiol Biotechnol 2011; 90:971–980.

33 Sharma S, Kanwar SS. Purification and bio-chemical characterization of a solvent-tolerant and highly thermostable lipase of *Bacillus licheniformis* strain S CD11501. Proc Natl Acad Sci India Sect B: Biol Sci 2017; 87:411–419.

34 Salihu A, Alam MZ, AbdulKarim MI, Salleh HM. Lipase production: an insight in the utilization of renewable agricultural residues. Resour Conserv Recycl 2012; 58:36–44.

35 Al-Haidari AMD, Alsaadawi IS, Khudhair SH. determination the optimum conditions of the activity and stability of lipase extracted from sunflower germinated seeds. Iraqi J Sci 2021; 62:431–440.

36 Hemachander C, Puvanakrishnan R. Lipase from *Ralstonia pickettii* as an additive in laundry detergent formulations. Process Biochem 2000; 35:809–814.

37 Prazeres JND, Cruz JAB, Pastore GM. Characterization of alkaline lipase from *Fusarium oxysporum* and the effect of different surfactants and detergents on the enzyme activity. Braz J Microbiol 2006; 37:505–509.

38 Uppada SR, Akula M, Bhattacharya A, Dutta JR. Immobilized lipase from *Lactobacillus plantarum* in meat degradation and synthesis of flavor esters. J Genet Eng Biotechnol 2017; 15:331–334.