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A green alternative for oily wastewater treatment: lipase from *Acinetobacter haemolyticus* NS02-30

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ABSTRACT

Biological treatment of wastewater is negatively affected by high concentrations of oil and grease, which decrease the oxygen transfer rate in the aerobic process by forming a layer on the water surface, making removal of oil and grease from manufacturing wastewater essential. However, the most common currently used techniques are inadequate as well as costly, and chemical methods create environmental pollution. By contrast, fermentation can be used to produce extracellular microbial lipases relatively cheaply and in large quantities for industrial use. Biological methods that use lipase enzymes also represent an appropriate, "green" choice for removing oil and grease from wastewater. This study identified and characterized a novel lipase produced from bacteria isolated by screening soil and olive pomace samples for lipolytic activity. The bacteria was subsequently identified as *Acinetobacter haemolyticus*, and the lipase produced from this bacteria (LipAH02–30) was then purified and characterized. Findings are presented as means ± SD. Enzyme *K*m and V_{max} were 0.8 mM and 3.833 mmol/ml/min, respectively. Optimal temperature and pH for enzyme activity were identified as 40˚C and 9.0, with good stability exhibited at temperatures ranging between 10 and 30˚C and a pH range of 5.0–11.0. The effects of organic solvents, metal ions, surfactants, commercial detergents, enzyme inhibitors, oxidizing agents, protease and boric acid on LipAH02–30 activity were also examined. The enzyme was found to be highly stable at +4˚C, displaying 90% activity even after 60 d, and to be effective in hydrolyzing oils found in kitchen wastewater. The high stability demonstrated by LipAH02–30 over a wide range of temperatures and pHs, along with its ability to maintain its activity in the presence of various surfactants, oxidizing agents, proteases and commercial detergents, indicates it to be an effective solution for the treatment of lipid-rich wastewater.

Keywords: *Acinetobacter haemolyticus*; Lipase; Characterization; Stability; Wastewater treatment

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1. Introduction

The high levels of oil and grease found in domestic wastewater have a serious impact on the environment [1]. Moreover, when present in wastewater at high concentrations, lipids naturally form a layer on the water surface, decreasing the rate of oxygen transfer in the aerobic process and creating a major problem for biological treatment processes [2]. Current practices for oil and grease removal usually rely on physical and chemical pretreatment using processes such as dissolved air flotation and skimming to solve this problem [3]. However, not only are these commonly used processes very costly, they are unable to efficiently remove colloidal and emulsified particles [4]. The use of low-cost enzyme preparations represents a vital development in wastewater treatment [5] that has gained attention as a result of more stringent environmental regulations [6,7].

Lipases are hydrolases that under aqueous conditions act on the carboxyl ester bonds present in triacylglycerols to liberate fatty acids and glycerol. The long-chain triacylglycerols that form the natural substrates of lipases have very low solubility in water, and the hydrolytic reaction is catalyzed at the lipid–water interface [8]. In addition to hydrolysis, lipases are also involved in a wide range of conversion reactions that include esterification, interesterification, transesterification, alcoholysis, acidolysis, and aminolysis in nonaqueous media [9,10]. This versatility makes lipases the enzyme of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries [11].

Lipases are ubiquitous in nature and are produced by various plants, animals, and microorganisms. Lipases of microbial origin, mainly bacterial and fungal, represent the most widely used class of enzymes in biotechnological applications and organic chemistry. Because extracellular bacterial lipases are relatively easy to produce, they are of considerable commercial importance [8].

Due to their specific ability to hydrolyze oils and greases, lipases have been looked on as a very promising alternative for degrading lipid-rich industrial wastewaters [12], and a number of studies have been conducted in this regard [1,6,13]. *Acinetobacter* lipases have been the subject of several studies [14–16]; however, only one study in the literature has focused on an *Acinetobacter haemolyticus* lipase. In that study, the lipase produced from *A. hameolyticus* was isolated from healthy human skin [17]. The present study aimed to produce, purify, and characterize a lipase produced by *A. haemolyticus* isolated from a soil-olive pomace mixture and examine its efficacy in the hydrolysis of various edible and waste oils. To our knowledge, this is the first study to report on the purification and characterization of an *A. haemolyticus* lipase isolated from a soil-olive pomace mixture and the application of this enzyme in the hydrolysis of edible and waste oils.

2. Materials and methods

2.1. Screening of lipolytic bacteria

Bacteria isolated from various oil-contaminated soils, olive pomace-soil mixtures and olive pomace samples were screened for lipolytic activity using tributyrin agar (TA) (Merck) [composition, g/L: peptone 5.0, beef extract 3.0, tributyrin 15 mL (v/v), agar-agar 15.0, pH 7.0 ± 0.2] and rhodamine-B agar [0.8% nutrient broth (NB), 0.4% NaCl, 2.5% olive oil, 1% agar, and 1% rhodamine B]. Isolates were streaked on TA and rhodamine-B agar plates and incubated at 30˚C for 72 h. Lipolytic activity in TA was evaluated according to the presence or absence of a hydrolysis zone around the bacterial colonies, with the presence of an inhibition zone considered to indicate positive lipolytic activity in this media. Lipolytic activity in rhodamine-B agar was evaluated by screening in a UV cabinet (Camag) at 254 nm, with the appearance of an orange fluorescent zone around the bacterial colonies considered to indicate lipolytic activity in this media.

2.2. Culture and growth conditions

The production of lipase by isolates was carried out in 100 ml NB inoculated with 2% inoculum of an overnight culture and incubated in 250-ml Erlenmeyer flasks for 24 h at 30˚C and 130 rpm. The culture medium was centrifuged in an Eppendorf centrifuge at 10,000 rpm and 4˚C for 10 min, and cell-free supernatant was used to identify lipase activity.

2.3. Lipase assay

Lipase activity was measured spectrophotometrically (Shimadzu spectrophotometer) at 410 nm using p-nitrophenyl palmitate (p-NPP) as a substrate as described by Winkler et al. [18], with some modifications [19,20]. The substrate and enzyme were incubated at 130 rpm and 30˚C for 30 min, and the absorbance was measured at 410 nm, with 1 unit of enzyme activity defined as the amount of enzyme required to liberate 1 μmol/min of p-nitrophenol under these conditions.

2.4. Identification of bacteria

The isolate showing the highest lipase activity was identified using conventional biochemical methods (gram staining, catalase, oxidase, and lactose fermentation tests) as well as the VITEK Gram-negative (GN) test system.

2.5. Detection the optimum time course

The time course of lipase production was studied for 52 h. The inoculum [2% (v/v)] was added to 500-ml Erlenmeyer flasks containing 250 ml NB and incubated at 130 rpm on an incubator shaker (Zhicheng) at 30˚C for 52 h. Samples were harvested at 4 h intervals. Bacterial growth was measured spectrophotometrically at 660 nm, and lipase activity was identified using a standard lipase assay.

2.6. Enzyme purification

The enzyme was precipitated from cell-free supernatant using ammonium sulfate (70% saturation) at pH 7.0. Precipitates were harvested by centrifugation at 10.000 rpm for 30 min, dissolved in a minimal amount of buffer (50 mM Tris–HCl, pH 9.0) and dialyzed in 50 mM Tris-HCl buffer (pH 9.0). The enzyme solution was then applied to a Sephacryl S-100 HR gel filtration column (AKTAprime™ plus) equilibrated with Tris-HCl buffer (50 mM, pH 9.0), and the elution fractions showing lipase activity were collected and subjected to ultrafiltration using a centrifugal membrane filter (Millipore 5.000 MWCO). The molecular mass of the lipase was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) performed with 8% (w/v) SDS polyacrylamide gels according to Laemmli [21].

2.7. Characterization of lipase

2.7.1. Determination of Km and V_{max}

The effect of substrate concentration (p-NPP, ranging from 0.05 to 2.0 mM) on reaction rate was assayed by adding 1 ml of enzyme solution to 9 ml substrate solution (pH 8.0) and incubating at 30˚C for 30 min. Absorbance was measured (λ = 410 nm), and Michaelis Menten constant (*Km*) and maximum velocity for the reaction (V_{max}) were calculated according to the Lineweaver–Burk graphic technique.

2.7.2. Effect of pH and temperature

The pH activity profile of the lipase was measured using p-NPP over a pH range of 4.0–10.6 with the following buffers (50 mM): phosphate-citrate (pH 4.0–6.0); Tris-HCl (pH 7.0–9.0); glycine-NaOH (pH 10.0–10.6). Due to the instability of p-NPP at pH above 9.0, lipase activity at pH above 9.0 was also measured titrimetrically using olive oil as the substrate. The reaction mixture was prepared by emulsifying 10% (w/v) olive oil in 10% (w/v) Triton X-100 in the above-mentioned buffers. A total of 1 mL of crude lipase was added to each 10-mL reaction mixture and incubated on an incubator shaker at 130 rpm and 30˚C for 30 min. The reaction was stopped by adding 1 mL of acetone/ethanol (1:1, v/v) solution. Next, 2–3 drops of phenolphthalein indicator were added to each reaction mixture, which was then titrated with 0.05 M NaOH solution. Lipase activity was calculated as micromoles of free fatty acids formed from olive oil per mL of crude lipase.

The pH stability of the lipase was evaluated by preincubating aliquots of enzyme samples at 30˚C for 1 and 2 h with the respective pH buffers. Residual enzyme activity was measured using the standard p-NPP method and calculated against initial enzyme activity (100%).

The effect of temperature on lipase activity was evaluated by monitoring enzyme activity at temperatures ranging between 10 and 70˚C at pH 9.0 (optimum pH) using the standard p-NPP method. The optimum temperature was identified as 40˚C. Thus, subsequent experiments were performed at pH 9.0 (optimum pH) and 40˚C (optimum temperature).

The thermal stability of the lipase was evaluated by preincubating enzyme solutions at various temperatures ranging between 10 and 70˚C for 1 and 2 h. Residual enzyme activity was measured using the standard p-NPP method and calculated against initial enzyme activity (100%).

2.7.3. Effects of metal ions, boric acid, surfactants, oxidizing agents, detergents, enzyme inhibitors, protease, and organic solvents on lipase activity

The effects of various metal ions, boric acid, surfactants, oxidizing agents, detergents, enzyme inhibitors, protease (*Bacillus licheniformis* protease, ≥2.4 U/g, Sigma, P4860) and organic solvents on lipase activity were evaluated by preincubating the lipase with the respective compounds for 1 h at 30˚C. Residual enzyme activity was measured using the standard p-NPP method and calculated against activity of the enzyme without any additives (100%).

2.7.4. Storage stability of lipase

The storage stability of the lipase was evaluated by measuring its activity toward p-NPP for 60 d at various time intervals during storage at +4°C.

2.7.5. Hydrolysis of edible and waste oils

The ability of LipAH02–30 to hydrolyze edible oils was tested titrimetrically by measuring the amounts of fatty acids obtained from olive oil, sunflower oil, soybean oil, and corn oil treated with the lipase. Reaction cocktails were prepared with 10% (w/v) edible oil emulsified in 10% (w/v) Triton X-100 in a Tris-HCl buffer (50 mM, pH 9.0). For each edible oil, 1 ml lipase was added to 10 ml reaction cocktail and incubated in a shaker incubator at 40°C and 130 rpm for 30 min.

The ability of LipAH02–30 to hydrolyze kitchen waste oils was also tested titrimetrically as described above using various waste oils obtained from hotel kitchen grease traps. For each waste oil, 2 ml lipase was added to 20 ml reaction cocktail and incubated in a shaker incubator at 40°C and 130 rpm for 24 h. The pH of each reaction mixture was measured before the addition of the enzyme solution.

Reactions were stopped by adding 1 ml of acetone: ethanol solution (1:1), and 2–3 drops of phenolphthalein indicator were added to each reaction mixture. Fatty acid contents were determined by titration using a 0.05 M NaOH solution. Lipase activity was calculated as micromoles of free fatty acids formed from edible and waste oils per ml of crude lipase enzyme.

3. Results and discussion

In this study, 62 lipolytic bacteria were isolated from three soil samples, four olive pomace samples, and three soil-olive pomace mixtures using TA medium, and the lipolytic activities of these isolates were also examined using rhodamine-B agar medium.

Gram-staining results indicated 42 of the isolates to be Gram-negative. Quantitative lipase activities of the Gram-negative isolates were measured spectrophotometrically using p-NPP as a substrate, and 35 of the 42 isolates were found to have lipase activity (data not shown). Of these, the NS02–30 isolate obtained from a soil-olive pomace mixture had the highest lipase activity and was thus selected for further study. A VITEK GN test identified the NS02–30 isolate as *A. haemolyticus* with 98% certainty. Lipolytic *Acinetobacter* strains have been isolated from human skin; dairy and other food products; and diverse soil and water habitats, both pristine and highly polluted [22]. However, this is the first report on *A. haemolyticus* isolated from a soil-olive pomace mixture.

The study found the optimum time course of *A. haemolyticus* NS02–30 lipase production to be 28 h. After incubating the isolate for the optimum time, the culture supernatant was used in the purification stage. LipAH02–30, the lipase obtained from *A. haemolyticus* NS02–30, was partially purified with ammonium sulfate precipitation, dialysis and gel filtration column chromatography with Sephacryl S-100 HR. Approximately, 6.25-fold purification with 8.4% recovery was achieved.

The molecular mass of lipase obtained from *Acinetobacter* sp. has been reported to vary. For example, the *Acinetobacter calcoaceticus* LP009 lipase was reported to have a molecular weight of 23 kDa [23], whereas the lipase produced from *A. haemolyticus* TA 106 isolated from human skin was found to have a molecular mass of 60 kDa [24] and the *Acinetobacter* sp. O16 lipase was found to have a molecular mass of ≥200 kDa [25]. In the present study, the lipase purified from *A. haemolyticus* had a molecular mass of 282 kDa.

The *K*m and V_{max} values as a function of p-NPP concentration were determined to be 0.8 mM and 3.833 mmol/ml/min, respectively, and the optimum pH for *A. haemolyticus* lipase activity was 9.0. Previous studies have reported the optimum pH of *A. calcoaceticus* [26]. and *A. haemolyticus* TA 106 isolated from human skin [24] to be 9.0 as well.

A. haemolyticus lipase was found to retain more than 90% of its activity at pH 5.0–11.0 (Fig. 1), with maximum stability observed at a pH range of 8.0–10.0. Chen et al. [27] reported similar characteristics for an *Acinetobacter radioresistens* lipase, which was found to have an optimum pH of 10 and stability at a pH range of 6.0–10.0. A lipase isolated from *A. calcoaceticus* was found to be stable at an even broader range (pH 4.0–10.0) for 24 h [26].

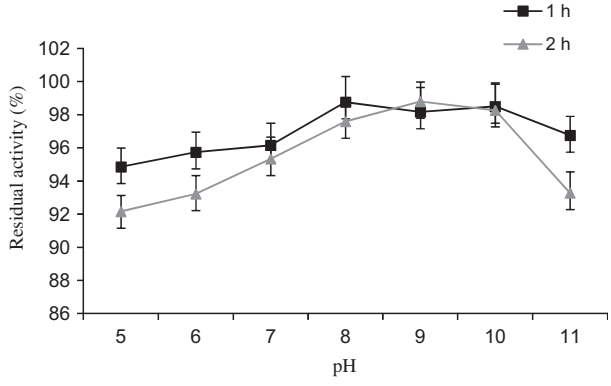

Fig. 1. Effects of pH on lipase activity. The maximum activity of the enzyme was taken as 100%.

This study found 40˚C to be the optimum temperature for lipase activity, which is the same result obtained for *A.calcoaceticus* lipase [26]. Residual activity of the lipase at 20 and 30˚C was above 90% (Fig. 2). In terms of temperature, maximum stability was observed at 10˚C after incubation for 2 h, whereas the enzyme was strongly inhibited at 70˚C. A previous study reported a lipase produced from *Acinetobacter johnsonii* retained 94.53% of its maximum activity at 20˚C, but was strongly inhibited at 70 and 80˚C [28], whereas the TA 106 lipase produced from *A. haemolyticus* found on human skin displayed good activity at 0, 30, and 37˚C [24].

The present study found the *A. haemolyticus* lipase activity increased with the addition of Mg^{2+}, Na^{+}, Fe^{3+}, H_3BO_3, Ni^{2+}, and Ca^{2+} (Table 1), but was strongly inhibited in the presence of Cu^{2+} and Zn^{2+}. According to the literature, the *A. johnsonii* lipase is also activated in the presence of Na^{+}, Ca^{2+}, and Mg^{2+} and inhibited by the presence of Cu^{2+} and Zn^{2+} [28], whereas the *A. calcoaceticus* lipase is highly enhanced in the presence of Ca^{2+}, Mg^{2+}, and K^{+} and partially inhibited by Cu^{2+}, Al^{3+}, Fe^{3+}, Ba^{2+}, and Zn^{2+} [26]. Activity of a lipase produced from *A. haemolyticus* TA 106 isolated from human skin was found to be significantly reduced by Ca^{2+}, Mg^{2+}, and Cu^{2+} [24].

A property of *Acinetobacter* lipases is the positive effect of Ca^{2+} on enzyme stabilization and activity [22]. This is most probably a function of the Ca^{2+}-binding pocket [14,29], which enables correct configuration of the active site [30]. Many lipases are inhibited in the presence of Zn^{2+} [31–34] and Cu^{2+} [32,34]. The effects of metals as well as various enzyme inhibitors have been studied with respect to the suitability of lipases for industrial applications [22]. The inhibitory nature of transition metals is thought to be a function of the interaction of metal ions with the charged side-chain groups of surface amino acids, which helps to conform and stabilize the enzyme [35].

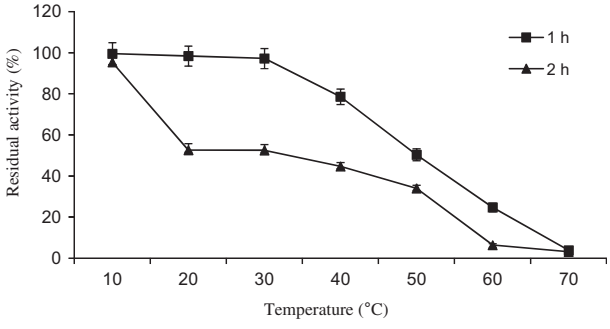

Fig. 2. Effects of temperature on lipase activity. The maximum activity of the enzyme was taken as 100%.

Table 1
Effects of metal ions, boric acid, surfactants, oxidizing agents, and enzyme inhibitors on LipAH02–30 activity

	Residual activity (%)
Control	100
Metal ions (5 mM)	
Mg^{2+}	172.41
Cd^{2+}	71.63
Ca^{2+}	114.57
Cu^{2+}	27.88
Na^{2+}	124.03
Mn^{2+}	97.11
Co^{2+}	70.67
Ni^{2+}	114.9
Zn^{2+}	43.26
Fe^{3+}	122.69
Boric acid (5 mM)	
H_3BO_3	117.3
Surfactants (1%)	
Tween 20	87.3
Tween 40	111.37
Tween 60	49.16
Tween 80	52.38
Triton X-100	80.42
Saponin	51.32
Oxidizing agents (0.1%)	
Sodium hypochlorite	84.77
H_2O_2	76.58
Sodium perborate	95.31
Oxidizing agents (1%)	
Sodium hypochlorite	0.00
H_2O_2	0.00
Sodium perborate	36.23
Enzyme inhibitors (0.1%)	
EDTA	42.01
PMSF	84.37
Iodoacetic acid	21.42
β-mercaptoethanol	102.05
SDS	5.28

The present study's findings regarding enzyme activity and stability in the presence of surfactants is also given in Table 1. LipAH02–30 was highly active in the presence of Tween 40 and remained stable in the presence of Tween 20 and Triton X-100; however, Tween 60, 80, and saponin strongly inhibited enzyme activity. While some lipases are also inhibited in the presence of Triton X-100 [36,37], many others are stimulated [34,38]. The *A. johnsonii* lipase LP28 has been shown to be stable in the presence of Triton X-100,

Tween 20, Tween 80, saponin, sodium cholate and sodium taurocholate [28]. In contrast, *A. haemolyticus* lipase TA 106 isolated from human skin is completely inhibited in the presence of Triton- X-100, Tween 20, and Tween 80 [24].

The present study also examined the response of LipAH02–30 to a number of known enzyme inhibitors (Table 1). LipAH02–30 activity was found to be strongly affected by EDTA, which has been similarly shown to inhibit the activity of the *A. haemolyticus* lipase TA 106 isolated from human skin [24]. Other studies have reported some enzymes to be inhibited by EDTA [32,34,39] and some to remain stable in the presence of EDTA [31,36,38]. The enzymes inhibited by EDTA may be metalloenzymes [40].

LipAH02–30 was also observed to exhibit a reduction in activity (by 15.63%) in the presence of PMSF [41]. While many lipases are inhibited in the presence of PMSF [31,39,42], some do remain stable [38].

Both iodoacetic acid and SDS significantly inhibited LipAH02–30 activity. In contrast, the *A. haemolyticus* lipase TA 106 isolated from human skin was activated in the presence of SDS [24].

LipAH02–30 was shown to be stable in the presence of β-mercaptoethanol. Previous studies [43,44]. have shown incubation of enzymes in the presence of reducing agents such as β-mercaptoethanol and dithiothreitol (DTT) to result in activity independent of intact disulfide linkages.

The stability of LipAH02–30 to oxidizing agents was also checked in the presence of sodium hypochlorite, H_2O_2, and sodium perborate (Table 1). After incubation for 1 h at 30˚C in the presence of 0.1% sodium hypochlorite, H_2O_2, and sodium perborate, respectively, 84.77, 76.58, and 95.31% of residual lipase activity was observed, whereas incubation with 1% sodium perborate resulted in 36.23% residual activity, and incubation with 1% concentrations of sodium hypochlorite and H_2O_2 inhibited all enzyme activity. In contrast, the *A. haemolyticus* lipase TA 106 isolated from human skin was stable in the presence of 1% H_2O_2 [24]. In another study, *A. johnsonii* LP28 lipase retained 60.87, 78.25, and 83.88% of its activity after exposure to 1% solutions of hydrogen peroxide, sodium hypochlorite, and sodium perborate, respectively [28].

Lipases are generally inhibited in the presence of commercial detergents at high concentrations. In the present study, after preincubation for 1 h at 30˚C in the presence of Tursil and Bingo at low concentrations, LipAH02–30 retained 77.6 and 73.21%, respectively, of its initial activity (Fig. 3). The *A. johnsonii* lipase has been shown to exhibit very little loss in activity after exposure to various commercial detergents [28], and the *A. haemolyticus* lipase TA 106 isolated from human skin has demonstrated enhanced activity in the presence of various commercial detergents [24]. *Staphylococcus aureus* SXL and SSL lipases

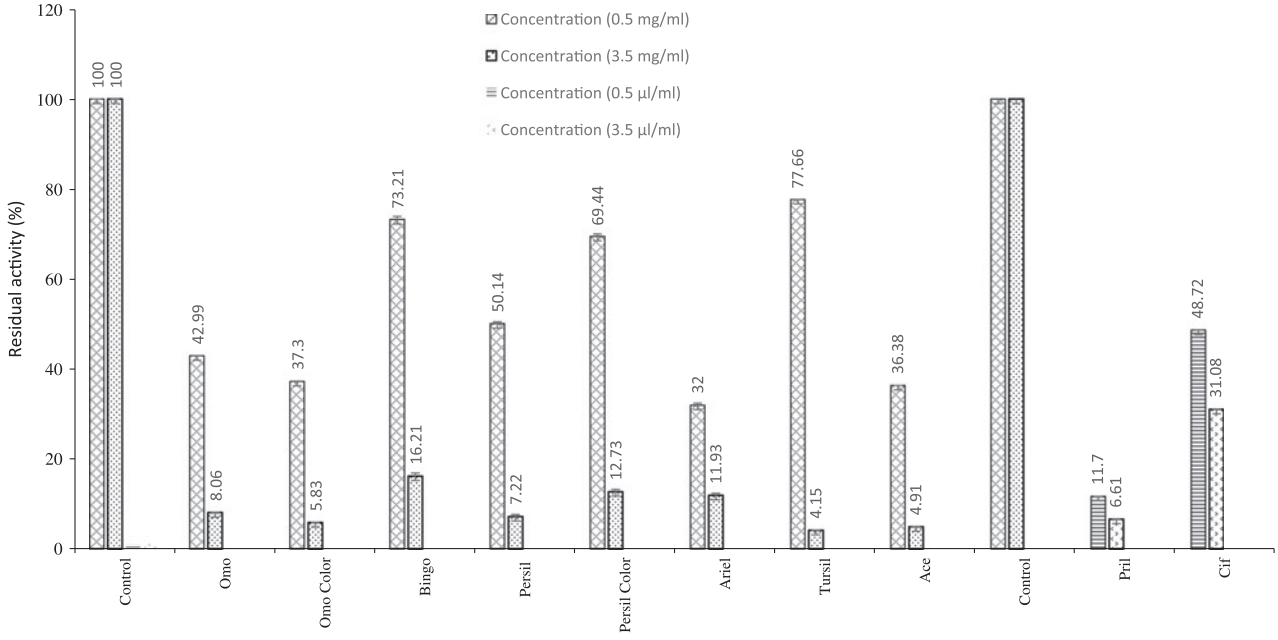

Fig. 3. Effect of commercial detergents on lipase activity.

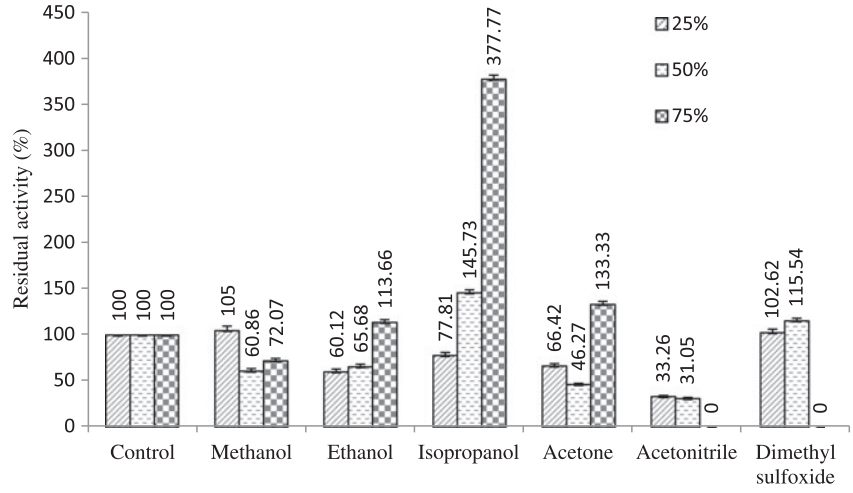

Fig. 4. Effects of water-soluble organic solvents on lipase activity.

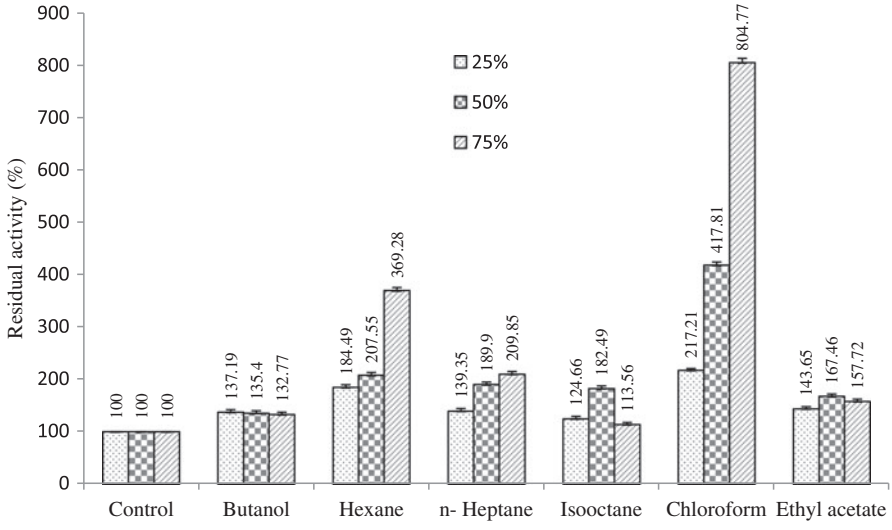

Fig. 5. Effects of water-insoluble organic solvents on lipase activity.

retained 38 and 25% of their activity in the presence of Ariel powder laundry detergent [45], whereas *Fusarium solani* N4–2, a low-temperature-resistant, alkaline lipase, retained >75% of its activity in the presence of commercial powder laundry detergents such as Omo and Ariel [46].

The effects of various organic solvents on LipAH 02–30 activity are presented in Figs. 4 and 5. In general, the enzyme was highly active in the presence of water-insoluble organic solvents. Similarly, the activity of *A. haemolyticus* TA 106 lipase isolated from human skin has been shown to be enhanced by organic solvents like n-butanol and isopropanol [24]. Because of the considerable desirability for industrial applications, the stability of enzymes toward organic solvents has recently been emphasized [20,32,38]. Enzymes used in organic synthesis in nonaqueous systems are required to show stability in the presence of organic solvents [22]. Given that numerous lipolytic strains have been isolated from petroleum-polluted environments [47,48], *Acinetobacter* lipases appear to be ideally suited for such purposes.

The effects of various concentrations of commercial protease (*B. licheniformis* protease, ≥2.4 U/g) on LipAH02–30 activity are presented in Fig. 6. Although the enzyme was generally inhibited in the presence of

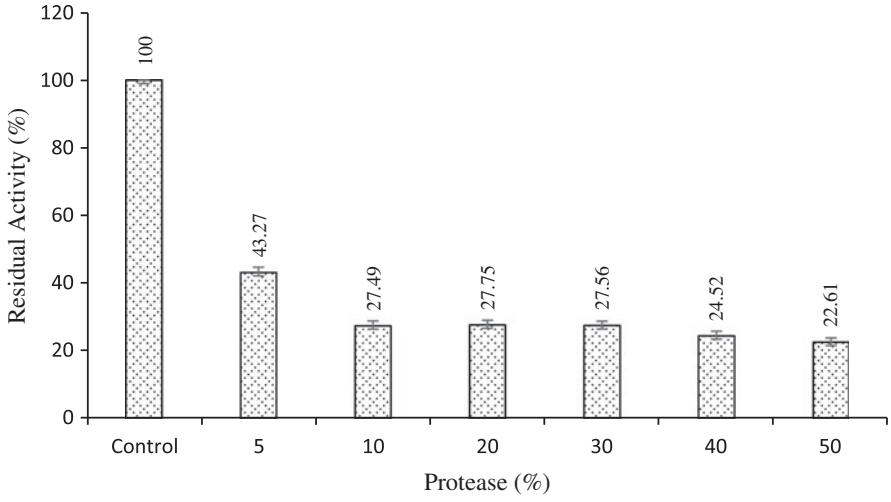

Fig. 6. Effect of protease (*B. licheniformis* protease, ≥2.4 U/g) on lipase activity.

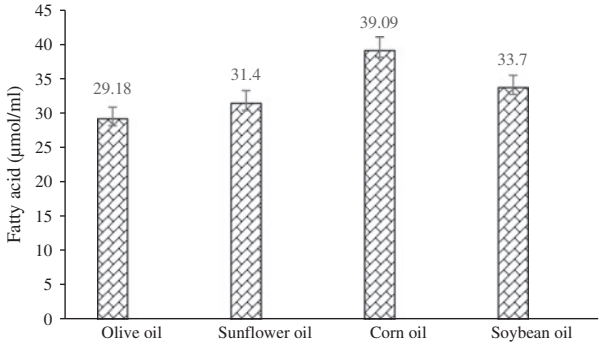

Fig. 7. Hydrolysis of edible oils.

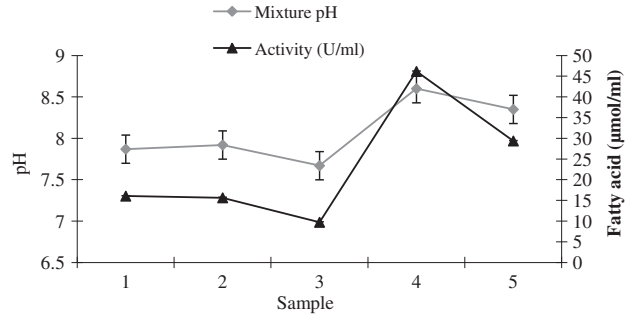

Fig. 8. Hydrolysis ability of waste oils and pH of the reaction mixture.

protease, it managed to retain 43.27% of its initial activity after exposure to commercial protease at a low (5%) concentration. The *A. johnsonii* lipase was reported to retain 76% of its activity in the presence of an alkaline protease (10,000 U/mL) [28].

In terms of storage stability, LipAH02–30 was found to be highly stable at +4˚C, displaying 90% activity even after 60 d of storage.

Fig. 7 illustrates the activity of LipAH02–30 in hydrolyzing various edible oils. As the figure shows, LipAH02–30 was able to successfully hydrolyze all the edible oils tested in this study, with the highest hydrolytic activity demonstrated towards corn oil. These findings suggest LipAH02–30 to have high potential for use in the hydrolysis of various triglycerides.

Fig. 8 illustrates the activity of LipAH02–30 in hydrolyzing various waste oils. LipAH02–30 was able to successfully hydrolyze all the waste oils tested in this study. Moreover, the hydrolytic activity of the lipase was shown to be related to the initial pH of the reaction mixture, with the greatest hydrolytic activity observed towards the waste oil with the highest initial pH and the least hydrolytic activity observed towards the waste oil with the lowest initial pH. A previous study examining the hydrolytic activity of a lipase produced from *Aureobasidium pullulans* HN2.3 found this enzyme to be capable of hydrolyzing various edible oils, with the greatest hydrolytic activity observed towards peanut oil [31].

In sum, this study, which is the first to report on the purification, characterization and potential application of a lipase produced by *A. haemolyticus* isolated from a soil-olive pomace mixture, found the lipase (LipAH02–30) to have a number of industrially important characteristics, such as high activity and stability at alkaline pHs and resistance to some metal ions, surfactants, enzyme inhibitors, bleaches, detergents, and organic solvents. The ability of LipAH02–30 to

hydrolyze various edible and waste oils makes it suited to applications in the field of lipid degradation, especially the removal of triglycerides from oily wastewater.

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