Production, Purification and Characterization of Extra Cellular Lipase from *Serratia marcescens* and its Potential Activity for Hydrolysis of Edible Oils

Neihaya H. Zaki*, Sinan E. Saeed
Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad-Iraq.
E-mail:remaryamhameed@yahoo.com.

Abstract

Twelve isolates of *Serratia marcescen*s 32.4% were obtained out of 37 soil samples. One isolate (*Serratia marcescen*s N3) was selected according to high lipase production. Lipase of this isolate was produced in liquid medium, extracted and purified by two stages included: *CM*-cellulose and *DEAE*-cellulose column, the fold of purification for the enzyme eluted from DEAE column was 19.33 with 61.15% recovery. The optimum pH and temperature for enzyme activity was 8 at 35°c, but it lost most of its activity (62%) at pH 10 and (78%) at 65°c.

The effect of some metal ions and surfactant on lipase activity was also studied, since the presence of Ca+2 was more effective than that of Mg+2 in increasing lipase activity, and this indicate that lipase is a metalloenzyme. Surfactant like Tween 80 induced lipase activity (110%) followed by tween 20 (102%) while α –naphtholrin inhibited it to 21%.

The activity of *Serratia marcescens* N3 lipase to hydrolyse different kinds of edible oils was determined, maximum activity appeared with Gingily oil followed by olive oil, coconut, sesame, soybean, and sunflower were (122, 112, 104, 93, 92 and 82) U/ml respectively.

Keywords: *Serratia marcescen*s–Lipase- Enzyme Production – Purification - Characterization of enzyme - Edible oils.

Introduction

Lipases are class of enzymes which catalyze the hydrolysis of long-chain triglycerides. Microbial lipases are currently receiving much attention with the rapid development of enzyme technology [1]. Lipases are an important group of biocatalysts for biotechnology, and they find immense applications in food technology, dairy detergent, biomedical sciences, esters and amino acids derivatives, making of fine chemicals, agro-chemicals, bioremediation, and pharmaceutical industries [2].

Lipases are hydrolases, which act under aqueous conditions on the carboxy ester bonds present in triacylglycerols to liberate fatty acids and glycerol. The natural substrates of lipases are long-chain triacylglycerols which have very low solubility in water; and the reaction is catalyzed at the lipid-water interface [3]. Under micro-aqueous conditions, lipases posses the unique ability to carry out the reverse reaction, leading to esterification, alcoholysis, and acidolysis. Besides being lipolytic, lipases also posses esterolytic activity and thus have a very diverse substrate range, although they are highly specific as chemo-, regio- and enantioselectivity catalysts [4].

Lipases are produced from microbes and specifically bacterial lipases play a vital role in commercial ventures [2]. Some important lipase-producing bacterial genera include: *Bacillus, Pseudomonas, Burkholderia, Achromobacter, Alcaligenes, Arthrobacter, Staphylococcus and Chromobacterium* spp. Also *Serratia* spp has been studied for its ability to produce lipase [5]. Lipases are generally produced on lipidic carbon, such as oils, fatty acids, glycerol or tweens in the presence of an organic nitrogen source [6].

Bacterial lipases are mostly extracellular and are produced by submerged fermentation. The enzyme is most commonly purified by hydrophobic interaction chromatography, in addition to some modern approaches such as reverse-micellar and aqueous two-phase systems [7].

Microbial lipases form a versatile tool in biotechnology and in recent years they have become an important class of enzymes. Other important application of lipases are the uses of enzymes in supplements benefit in digestion of fats, conversion fats to energy, cleaning the

clogged veins and arteries and treatment the pancreatic insufficiency, cystic fibrosis, spastic colon, Crohn's disease and celiac disease [8]. With this usage, the exponential increase in the application of lipases in various fields in the past few years demands both qualitative and quantitative improvement. The quantitative enhancement requires strain improvement and medium optimization for over-production [9].

Bacterial lipases are mostly inducible enzymes, requiring some form oil, fatty acids, fatty acids alcohol for induction. Lipases can be enhanced by the presence of some compounds, i.e fatty acids, triglycerides or surfactants has often been to induce lipase activity[7]. Therefore, the current study was undertaken to optimize the lipase activity, which produced by *Serratia marcescence* on relatively low cost media.

Materials and Methods

Samples collection: Thirty-seven soil samples were collected from different locations in a farm in Baghdad city. Top soil was collected by spatula in to clean sterile plastic bags and stored at 4°C prior to use. The soil samples were air-dried (20°C) and passed through a sieve (mesh size, 2mm). Ten gram of sieved soil was suspended in 20 ml of basic salt medium (*BSM*) [10].

Isolation & characterization of *Serratia marcescens* :

One loopfull of suspension soil samples was plated on blood agar and MacConkeys agar, then incubated at 30°C for 18-24 h. Several biochemical tests were done to differentiate *Serratia marcescens* from the other species. These include the following tests: inability to ferment lactose, a negative indole test, a negative urease test,and ability to decarboxylate arginine, but not lysine [10]. In addition to these biochemical tests, *API 20E* identification was used to differentiate *Serratia marcescens* from the other types.

Maintenance of *S.marcescens*: The culture of *S.marcescen*s was maintained at 4c and subcultured every two weeks. Nutrient agar medium was prepared and the pH of the medium was adjusted to 7.0, and was autoclaved at 121c for 15min. A loopful of bacterial culture was inoculated and incubated at 30c for 18 hr.[5].

Lipase screening of *S.marcescens* on media: *S.marcescen*s was activated in Brain-Heart infusion broth and incubated at 32°C for 24 hr., then 0.1ml of culture was streaked on to Rhan agar medium, which contained: (K_2HPO_4, 5g; $(NH_4)_2PO_4$, 5g; $CaCl_2.6H_2O$, 1g; $MgSO_4.7H_2O$, 1g; Tween 80, 5ml; $FeCl_2.6H_2O$, 0.001g; NaCl, 0.001g; Peptone, 2ml; agar, 20g and D.W., 1000ml at pH=7.2). The plates were incubated at 32°C for 4 days. Lipolytic activity was indicated by measuring the width of lipolytic zones (millimeters of areas of clearing around the colonies) [5,11].

Preparation of crude enzyme: *S.marcescens* was subcultured on brain heart infusion broth at 30c for 24hr. then 50ml of Rhan broth medium was inoculated by 5ml of bacterial growth (determined at O.D 1.2 / 600 nm) and incubated by shaking incubator (120 round/ min) for 48 hr at 30c. The growth was centrifuged at 5000 xg for 15 min at 4c. The lipase activity and protien concentration were determined in the supernatant solution (crude enzyme extract) [12].

Measurement of lipase activity: Activity of the crude lipase and chromatographic fractions were determined by the method of [12], except that the substrate was glycerol triciearate emulsion, the reaction solution composed of (2% gum arabic, 0.4M NaCl, and 5mM $CaCl_2$), blended for 2min at 50°C with the appropriate concentration of glycerol triciearate in a suitable buffer. A quantity (0.3ml) of the enzyme preparation was added to 2ml substrate solution in glass-stoppered test tubes that had been preincubated in a water bath at 37°C for 6min at pH 8. The mixture was held for 45 min in shaking incubator (120 cycle/min) at 35°C. The enzymatic reaction was terminated by adding 7.5 ml of the reagent (1000ml iospropane, 100ml heptan, 8ml of 1N H_2SO_4). After vigorous shaking for 2min and standing for 1 hr., the upper layer obtained was mixed with color reagent (1ml water, 5mg phenol red, 25mg Na-barbital, 200ml ethanol), and shaked for 30s. The absorbance was measured at the wavelength of 420nm. One lipase unit activity

was defined as μ moles of liberated free fatty acids/min at 37°C [11].

Measurement of protein concentration: The protein concentration was determined by the method of Lawry,*et.al* 1951[13] using Bovine Serum Albumin (*BSA*) (Sigma chemical *Co.*) for preparation of protein standard curve.

Purification procedures:

***CM*-Cellulose chromatography**: The crude enzyme was first applied to a column (22x3cm) of *CM*-Cellulose (Sigma *Co.*), which was pre-equilibrated with 10mM Potassium phosphate buffer pH 7.5. The lipase was allowed to bind to the gel for 2hr. at 4°C and was eluted with a linear gradient of 200ml Triton X-100 (0 to 1%). The flow rate was 1ml/1min., and fractions of 5ml were collected [11].

***DEAE*-Cellulose chromatography:** The lipase-rich fractions were pooled and applied to a column (16x2 cm) of *DEAE*-Cellulose (Sigma-chemical *co.*)**,** which was pre-equilibrated with 10mM potassium phosphate buffer at pH 6. The lipase was allowed to bind to the resin for 1hr at 4°C. The column was washed thoroughly with the same buffer to remove unabsorbed material, which included Triton X-100 and was eluted with a linear gradient of NaCl (0.05 to 1M) (300ml) in the same buffer at a flow rate of 1ml/1min. The eluate was collected in 5ml fractions [11].

Characterization of the purified lipase

Optimum pH: Glycerol triciearate emulsion were prepared in universal buffers covering the pH range 4 to 11 at 35C. The enzyme was diluted in the buffers and assayed separately in each substrate emulsion of the same pH [11].

pH stability: Portions of the purified lipase were held at 35C for 24hr in universal buffers for use at pH 4 to 11. The described assay was carried out for each lipase portion to obtain its stability [11].

Optimum temperature: Lipase activity of the enzyme was assayed at temperatures ranging from 5 to 65°C. The substrate emulsions were held at the respective temperature at pH 8 for 15 min before the addition of the enzyme [12].

Thermal stability: Portions of the purified lipase were held at temperatures ranging from 50 to 100°C for 15 min in tubes, then it was held in ice bath. The assay was carried out for each treatment to obtain lipase stability [11].

Effect of metal ions on lipase activity: The lipase was incubated with various metal ions (Fe^{+2}, Ni^{+}, Zn^{+2}, Mg^{+}, Ca^{+2} and Li^{+}) at concentrations of (1mM, 5mM, 10mM) in 0.025 mol/L of potassium phosphate buffer (pH 7.5) for 1hr. with constant shaking at 32°C, then the lipase activity was measured [14].

Effect of surfactant on lipase activity: Effect of surfactant was detected by incorporating different surfactant namely (Tween 20, Tween 80, α-naphtholrin). The purified lipase was diluted with each surfactant at these concentrations (0.05, 0.1, 0.15, 0.2)w/v. After 1hr. incubation with constant shaking at 32°C, remaining activity was measured [15].

Hydrolysis of edible oils by the purified lipase: Substrate specificity of the lipase against triacylglycerols of vegetable oils (sunflower oil, soybean oil, sesame oil, coconut oil, gingily oil and olive oil) was determined by preparation of an emulsion by adding of the medium/substrate (3:1).

Activity % was expressed as the percentage of the maximum activity [15].

Results and Discussion

Isolation and characterization of *S. marcescens*: The results revealed that 12(32.4%) isolates of *S. marcescen*s were obtained out of 37 soil samples. Sundaramoorthy, et.al.2009 [16] showed that 20 samples of *S. marcescen*s isolated from soil and only 5 of them were produced red pigment and lipase as virulence factor. Other researches refered that *S. marcescen*s isolated from soils and it produced many virulence factors like lipase, chondroitinase, prodigiosin and proteases [17, 18, 19 and 20].

Lipase screening of *S.marcescens* on medium: The width of hydrolysis areas were measured to compare the lipase production of *S. marcescens* isolates, the lipase production of *S. marcescens* appeared after 18hr, and the widest area observed after 36 hr (Fig.(1)).

From this figure it can seen that (N_3) isolate produced lipase enzyme (16mm) at higher level than the other soil isolates. Also from (Table (1)) the highest lipolytic activity of lipase was 0.73 U/ml from (N_3) isolate when compare it with other soil isolates, thus, this isolate was chosen for purification step.

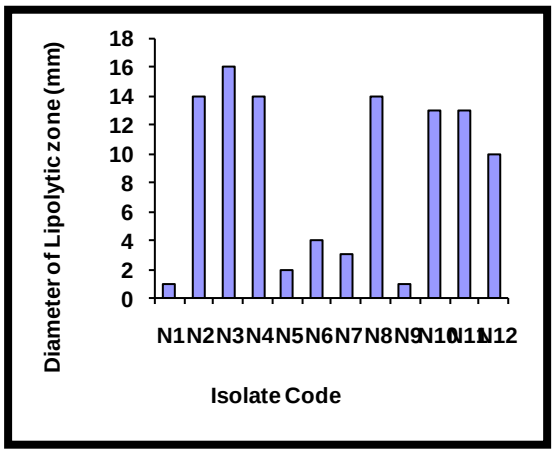

Fig.(1) Diameters of lipolytic zones of lysis for all Serratia marcescens isolates.

Table (1)
Comparasion between inhibition zones and lipase activity for all S. marcescens isolates.

Isolate code	*Inhibition zone (mm)*	*Lipolytic activity (U/ml)*
N1	1	0.25
N2	14	0.51
N3	16	0.73
N4	14	0.51
N5	2	0.27
N6	4	0.31
N7	3	0.29
N8	14	0.51
N9	1	0.25
N10	13	0.49
N11	13	0.49
N12	10	0.42

Singh and Banerjee, 2007 [21] exhibited that *Pseudomonas aeruginosa* produce lipase after 24hr. Also Haider and Pakshirajan, 2007[22] exhibited that soil microorganisms produced higher lipase at 24 and 72hr., while Abdou, 2003 [11] showed that psychrophilic strain of *S. marcescen*s produced lipase after 6hr only.

Purification of lipase: A representative purification profile is summarized in (Table (2)), and from (Fig.(2)), two peaks of protein appeared in the eluted fractions with one peak of lipase activity located in the first peak of protein. On the CM-cellulose column, the highest lipase activity was eluted in fractions (17 to 28), specific activity was 4.46-fold, and about 89% of its activity was recovered.

Active fractions were applied to the second step of purification (DEAE-cellulose), the highest lipase activity was between (0.05 and 1M) NaCl in fractions (34 to 41), specific activity was increased (19.33-fold) in this step with (61.15%) recovery. From (Fig.(3)), two peaks of protein appeared in the eluted fractions with one peak of lipase activity located in the first peak of protein.

Extracellular lipase produced by psychrophilic *S. marcescen*s and purified by ion exchange and gel filtration chromatography with recovery equal to 45% and 65% respectively [22,23], while Immanuel, et.al.2008 [5] showed that lipase activity from *Serratia rubidaea* increased 8-fold after partial purification by ether alcohol extraction. Matsumae, 1994 and Gao, et.al.2004 [24,25] showed that lipase activity from *S. marcescen*s Sr41 and *S. marcescen*s Euc101 enhanced 1.7 and 9.5-fold respectively.

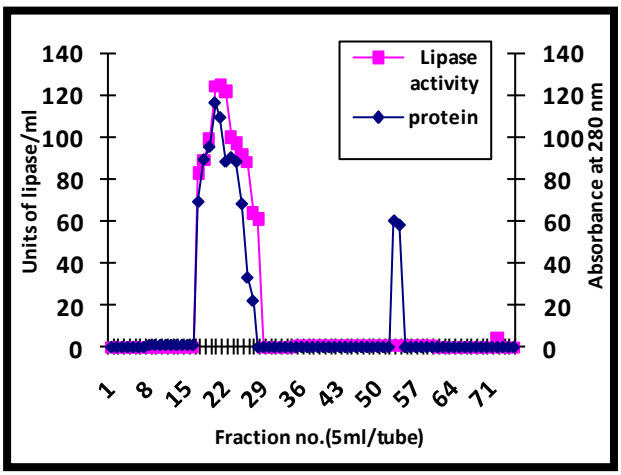

Fig.(2) CMC-cellulase chromatography of Serratia marcescens lipase. Column (22x3cm), 10mM K-PO4.

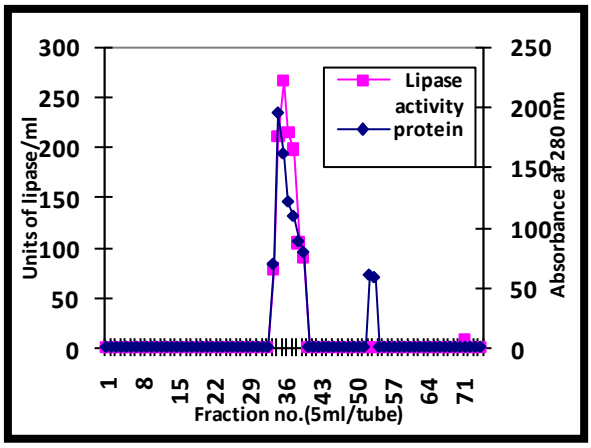

Fig.(3) DEAE-cellulase chromatography of Serratia marcescens lipase. Column (16x2cm), 10mM Potassium phosphate buffer.

Table(2)
Purification steps of lipase from S. marcescens.

Purification step	*Protein conc mg/ml*	*Lipo-lytic activity U/ml*	*Specific activity U/mg protein*	*Purity index (fold)*	*Total activity (U)*	*Recovery (%)*	*Size (ml)*
The crude enzyme	52	69	1.32	1	310.86	100%	67
CM-cellulose	21	124	5.90	4.46	275.2	88.52	15
DEAE-cellulose	17.1	434	25.52	19.33	190.1	61.15	10

Characterization of the purified lipase:

Effect of pH on lipase activity: The lipase activity was evaluated at different pH values at 32°C (Fig.(4)). From this figure the enzyme reached to maximum activity (100U/ml) at pH 8 and about *95U/ml* at *pH* 7. Purified lipase from *S. marcescens* exhibited maximum activity at pH 8 [5, 24], while Gao,et.al.2004 [25] showed that optimum pH for lipase activity from *S. marcescens* was 6.5, also Bachkatova and Severina,1980 [26] showed optimum pH was 6.3 from other strain of *S. marcescens*.

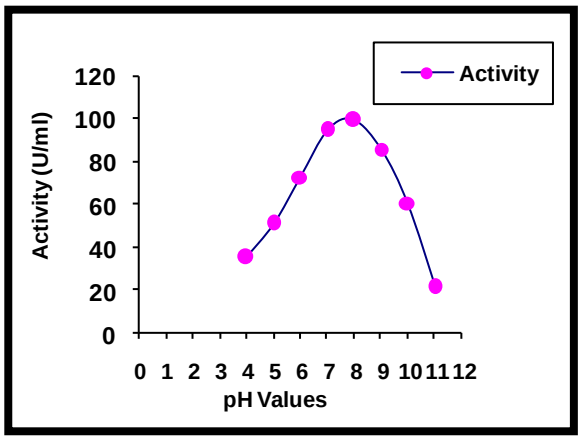

Fig.(4) Effect of pH values on N3 lipase activity.

Effect of pH on lipase stability:

Remining activity of lipase enzyme reached to 22 % and 20% at pH 4 and 11, respectively (Fig.(5)).

The purified lipase showed maximum stability at pH 8, but under pH 6 lipase lost about 30U/ml of its activity after holding it at 32°C. Abdou, 2003[11] showed that lipase stability of psychrophilic *Serratia. marcescens* was between 8-9, while Makhzoum, *et.al.*1995 [27] showed lipase of other strain of *S. marcescens* was stable in pH between 6-9, while lipase from *S. grimisii* was stable over the range of 7-9 [28].

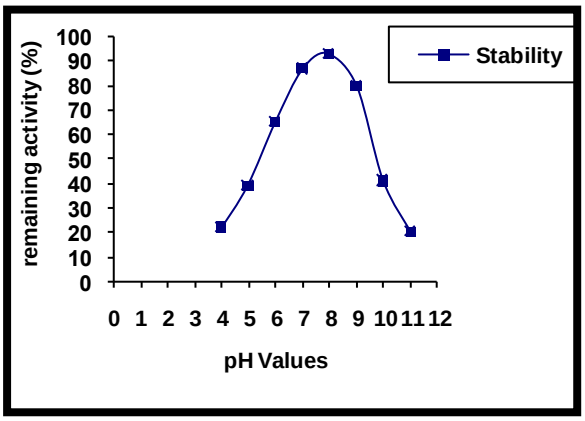

Fig.(5) Effect of pH values on N3 lipase stability.

Effect of temperature on lipase activity: (Fig.(6)) showed that optimum temperature for the enzyme was 35°C, and about 90% of its maximum activity occurred at 30°C and 40°C. Abdou, 2003 [11] exhibited optimum temperature of lipase activity from *S. marcescens* at 37°C, while Immanuel, et.al.

2008 [5] showed optimum temperature of lipase activity from *S. rubidaea* between (25-35°C). In contrast Bachkatova and Severina, 1980 [26] showed that highest lipase activity was from *S. marcescens* 345 at 45°C

Effect of temperature on lipase stability:

The lipase purified from *S. marcescens* showed some stability at 60°C (Fig.(7)), but the least residual activity was at 85°C, and it was completely inactivated at 90°C. Abdou, 2003 [11] exhibited that lipase activity from *S. marcescens* is not susceptible to low heat inactivation and is not as thermostable as other bacterial lipases.

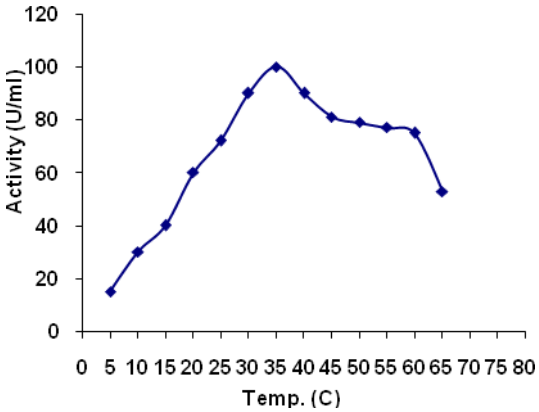

Fig.(6) Effect of temperature on lipase activity.

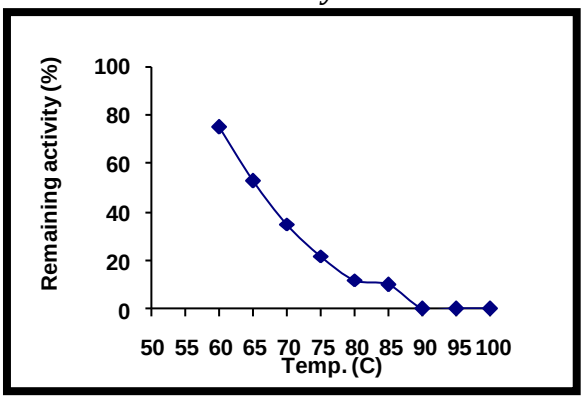

Fig.(7) Effect of temperature on lipase stability.

Effect of metal ions on lipase activity: *S. marcescens* lipase was treated with monovalent and bivalent cationic metals. Some of these metals showed activation effect, others had inhibition effect. 10mM of Ca^{+2}, Mg^{+2} and Li^{+} increased lipase activity to 126%, 103% and 76% respectively. Others 10mM of Zn, Fe and Ni^{+} inhibited lipase activity to 36%, 26% and 11% respectively (Fig.(8)).

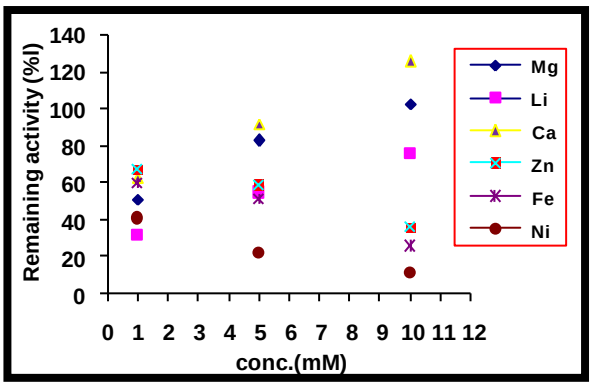

Fig.(8) Effect of metallic ions on lipase activity.

The presence of Ca^{+2} was shown to be more effective than that of Mg^{+2} in increasing lipase activity, thus, the lipase seems to be dependent on Ca^{+2} on its activity and in this way, for further enzyme purification, $CaCl_2$ (10mM) might be included in the culture medium [29]. Pogori,et.al.2008[15] showed that Zn^{+2} and Cu^{+} ions depressed the lipase activity but Mg^{+2} and Ca^{+2} were found to stimulate the lipase activity of *Rhizopus chinensis*, while Yu,et.al.2007[30] exhibited that Mn^{+2} and Ba^{+2} stimulate lipase activity of *Yarrowia lipolytica*.

Effect of surfactants on lipase activity: The influence of surfactants on lipase activity of *S. marcescens* was studied, lipase induced by Tween 80 (110% at concentration 0.2% w/v) followed by Tween 20 (102%), while α-naphtholrin inhibited the lipase activity at concentration 0.2% w/v to about 21% (Fig.(9)). Similarly, lipase activity of *S. marcescens* exhibited that Tween 80 activated the enzyme, while α-naphtholrin inhibited it [24,26].

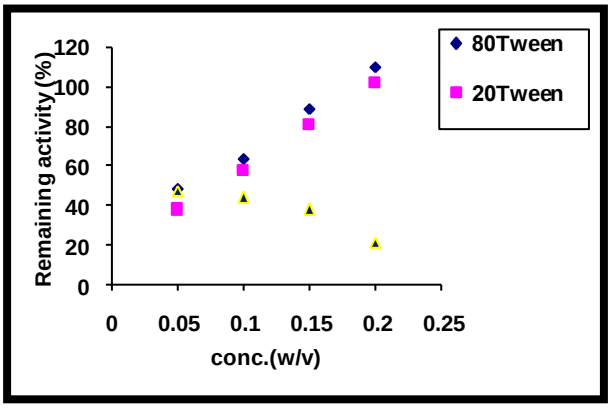

Fig.(9) Effect of Surfactants on lipase activity.

Hydrolysis of edible oils by the purified lipase: One of the most important properties of lipases is the substrate specificity towards triacylglycerols [15]. The *S. marcescen*s lipase efficiently hydrolyzed a variety of vegetable oils (sunflower, soybean, sesame, coconut, gingily and olive oil) at pH 7 and 35°C (Fig.(10)).

All the tested triglycerides were found to induce the lipase activity with different levels of enzyme activity. Among the oils tested sunflower oil was showed (82U/ml) of lipase activity. All others resulted in activities of above 90 U/ml, (Gingily, 122; olive oil, 112; coconut, 104; then sesame oil 93, and soybean 92) U/ml of lipase activity.

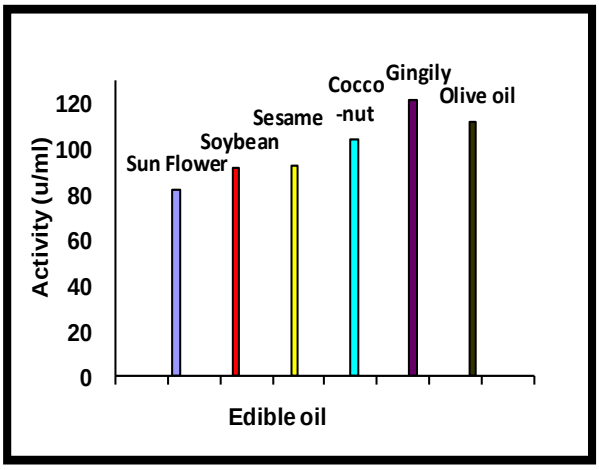

Fig.(10) Lipase activity toward different kinds of edible oils.

Gingily oil was found to be suitable for maximizing the lipase activity (122 U/ml) from *S. marcescence*, this is because triglycerides are important substrates for lipase production and enzyme activity and they act as inducer for it [15]). The lipase was found to have abroad substrate specificity, which has potential in the biocatalysis industry and has an economic potential application in the oleochemical industry [15].

This study is in agreement with the previous work of Matsumae and Shibatani, 1994 (24) which reported that olive oil induced the hydrolysis activity of lipase at pH 8 and 45°C. Also Machanan,et.al.,2007[31] showed that *S. marcescen*s can hydrolyzes green oil (palmorosa oil) and induced lipase activity of the enzyme at (3:3 v/v) substrate / media, while Immanuel,et.al.2008(5) found that gingili oil (15ml/L) is suitable substrate for lipase activity from *S. rubideae*.

(Volpato,et.al.2007) [32] showed that olive oil (3g/L) and soybean oil (2.5g/L) induced the lipase activity of *Staphyllococcus caseolyticus*, while Liu,et.al. 2008[33] found that the purified lipase from *Aureobasidium pullulans* has the highest hydrolytic activity towards peanut oil. Sharon, et.al.1998 [34] reported that castor oil induced lipase activity from *P. aeruginosa*, While sunflower oil and olive oil induced extracellular lipase by *Yarrowia lipolytica* [35], while many vegetable oils induced the lipase activity of *Candida rugosa* [36].

Lipase activity from *Rhizopus oryzae* induced by olive oil and coconut oil [37], while lipase activity of *Rhizopus delemar* induced by sunflower oil [38], also [15] found that vegetable oils have inducing effect on lipase activity by *Rhizopus chinensis*, and soybean oil enhanced lipase activity among other oils. EL-Bondkly and Keer, 2007 [39] exhibited that mutant of the fungus *Penecillium roquefortii* induced lipase activity when using olive oil and inhibited lipase when used cotton seed oil [40].

References

[1] Hasan, F.; Shah, A.A.; and Hameed, A.. Industrial application of microbial lipases. Enzyme and Microbial Technology; 39(2), 235-251; 2006.

[2] Gupta, R.; Gupta, N.; and Rathi, p. Bacterial lipases: an overview of production, purification and biochemical properties. Appl. Microbiol. Biotechnol.; 64: 763–781, 2004.

[3] Snellman, E.A; sullivan, ER.; and colwell, R.R.. Purification and properties of the extracellular lipase, Lip.A, of *Acinetobacter* sp.*RAG*-1- Eur. J.Biochem., 269, 5771-5779, 2002.

[4] Jager, K-E; E; Eggert, T.; Eipper, A.; and Reetz, M. T.. Directed evolution and the creation of enantioselective biocatalysts. Appl. Microbiol. Biotechnol. 55, 519-530, 2001.

[5] Immanuel, G.; Jwbadhas, A.; and palaresam, A. Investigation of lipase production by milk isolate, *Serratia rubidaea*. Food Technol. Biotechnol. 46:(1). 60 -65, 2008.

[6] Jaeger, K.E.; and Eggert, T. Lipases for biotechnology. Curr. opin. Biotehnol., 13 : 390-397, 2002.

[7] Saxena, R.K; Sheoran, A; and Davidsan, S. Purification strategies for microbial lipases. J. Microbial Methods. 52: 1-18, 2003.

[8] Alquati, C., Gioia, L.D.; and Lotti, M. The cold–active lipase of *Pseudomonas fragi*. Eur. J. Biochem., 269: 3321 – 28, 2002.

[9] Lima, V; Mitchell, D; and Fontana, J.D. Effect of nitrogen and carbon sources on lipase production by *Penicillium aurantiogriseum*. Food Technol. Biotechnol., 41 : 105 -110, 2003.

[10] Holt, J.G. Bergey's Manual of Determinative Bacteriology. Williams-Wilkins Co, Baltimore, U.S.A., pp 787, 1994.

[11] Abdon, A.M. Purification and partial characterization of Psychrotrophic *Serratia marcescens* lipase. J.Dairy Sci. 86 : 127 – 132, 2003.

[12] Fox, P. F.; and Stepaniak, L. Isolation & some properties of extracellular heat–stable lipases from *Pseudomonas fluorescens* strain *AFT*-36. Dairy Res., 50: 77–89, 1983.

[13] Lowry, O.H; Rosebrongh, N.; Farr, A.; and Rand all, R. Protein measurement with the folin phenol reagent. J. Biol. chem.,. 193: 265 – 275, 1951.

[14] Muslim, S. Purification and partial charactarization of extracellular lipase from *Pseudomonas fluorescens*, 2006.

[15] Pogori, N.; cheikyoussef, A.; and Wang, D. Production and biochemical characterization of an extra cellular lipase from *Rhizopus chinensis* CCTCC M201021. Biotechnology, 7:710 – 717, 2008.

[16] Sundaramoorthy, N.; Yogesh, p.; and Dhandapani, R. Production of prodigiosin from *Serratia marcescens* isolated from soil. Indian J. of Science and Technology, 2: (10). 32 – 34, 2009.

[17] Venil, C.; Sangeetha, N.; and Lakshmana-perunalsamy, P. Statistical optimization of medium components for the production of lipase by *Serratia marcescens* SBOB. The Inter. J. of Microbiology. 7: (1), 2009.

[18] Haba, E.; Bresco, O.;and Manresa, A. Isolation of Lipase- Secreting bacteria by deploying used frying oil as selective substrate. Enzyme and Microbial Technology, 26(1) :40-44, 2000.

[19] Ke, T.; Zhangfu, L.; Hong, J.; and Shigu;, L. Isolation of *S. marcescen*s as a chondroitinase–producing bacterium and purification of a novel chondroitinase Ac. Biotechnol. lett. 27(7) :489-93, 2005.

[20] Giri, V.; Anandkumar, N.; and Pennathnr, G. A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescence* isolated from Soil. J. B.M.C Microbiol. 4(11), 2004.

[21] Singh, S.; and Banerjee, U.C. Purification and characterization of trans- 3- (4-methoxy Phenyl) glycidic acid methyl ester hydrolyzing lipase from *Pseudomonas aeruginosa*. Vol. 42(7): 1063-1068, 2007.

[22] Haider, A.; and Pakshirajan, K. Screening & optimization of media constituents for enhancing lipolytic activity by a soil microorganism using statistically designed experiment. Vol. 141(2-3): 337-390, 2007.

[23] Zhao, L. L.; chen, X. X; and Xu, J-H. Strain improvement of *Serratia marcescen*s Ecu1010 and medium cost reduction for economic production of lipase. World J. of Microbiology & Biotechnology. 26(3): 537-543, 2009.

[24] Matsumae, H.; and Shibatani, T. Purification and characterization of the lipase from *Serratia marcescen*s *Sr* 418000 responsible for a symmetric hydrolysis of 3-Phenylglycidic acid esters. J. of Fermentation and Bioengineering. 77 (2): 152-158, 1994.

[25] Gao, L.; Xu, J.; and Lin, Z-Z. Optimization of *Serratia marcescen*s lipase Production for enantioseletive hydrolysis of 3-Phenylglycidic acid ester. J. of Industrial Microbiology and Biotechnology. 31 (11) : 525–530, 2004.

[26] Bachkatova, N. A.; and Severina, L. O. Isolation and charaterization of intracellular lipase from *Serratia marcescen*s. 345. Prik. Biokhim Mikobiol. 16 (3): 315 – 26, 1980.

[27] Makhzoum, A.; Knapp, J.; and Oulusu, R. Factors affecting growth and extracellular lipase production by *Pseudomonas fluorescens* 2D. Food Microbiol. 12 : 277–299, 1995.

[28] Abdou, A.M.. Studies on some Gram - negative Proteolytic and lipolytic

microorganisms in milk and milk products. Ph.D Thesis., Zagazig Univ. (Benha branch), kaliobyia, Egypt, 1997.

[29] Secades, p.; and Guijarro, J. A. Purification and characterization of extracellular lipase from the fish pathogen *Yersinia rucheri* and effect of culture conditions on production. Appl. Environ. Microbiol. 62 (9) : 3969–3975, 1999.

[30] Yu, M., Shaowei, Q; and Tan, T. Purification and characterization of the extracellular lipase *Lip* 2 from *Yarrowia lipolytica*. J. Bacteriol., 138: 663–670, 2007.

[31] Mchanan. S.; Mathan, N,; and palanisuomy,. Biodegradation of palmarosa oil (green oil) by *Serratia marcescen*s. Int. J. Environ. Sci. Tech., 4(2): 279–283, 2007.

[32] Volpato, G.;Rodrigues, C.; and HecR, J.X.. Production of organic solvent tolerant lipase by *Staphylococcus caseolyticus* Ex17 using raw glycerol as substrate. J. of Chemical Technology and Biotechnology. 83(6) : 821 -828, 2007.

[33] Liu, Z.; chi, Z.; wang,L.; and Li, J. Production, Purification, and characterization of an extracellular lipase from *Aureobasidium pullulans* HN2.3 with potential application for the hydrolysis of edible oils. Biochemical Engineering Journal. 40 (3): 445-451, 2008.

[34] Sharon, S.; Yamarido, T., and Kato,.y. Purification and characterization of a Lipase from *Pseudomonas aeruginosa* KK A -5 and its role in castor oil hydrolysis. J. Ind. Micrbiol. Biotechnol. 20: 304–307, 1998.

[35] Domanguez, A.; Deive, M.; and Lengo, M. Effect of lipids and surfactants on extracellular lipase production by *Yarrowia lipolytica*. J. Chem. Technol. Biotechnol., 78:1166-1170, 2003.

[36] Lakshmi, B., Abraham, B.; and Pennathur G. Effect of vegetable oils in the secretion of lipase from *Candida rugosa* (*DSM* 2031), Lett. Appl. Microbiol. 29: 66–70, 1999.

[37] Shukla, P; Gupta, K., and Kango, P. Production of lipase by hyper- lipolytic *Rhizopus oryzae KG*-10 on low – Value oil emulsions. Research J. of Microbiology. 2(9): 671 -677, 2007.

[38] Acikel, U; Ersan, M.; and Acikel, Y. S. Optimization of critical medium components using response surface methodology for lipase production of *Rhizopus delemar*. Food and Bioproducts Processing. 88 (1) : 31 – 39, 2009.

[39] EL-Bondkly, A. and Keer, A. A. UV and *EMS*-induced mutations affecting synthesis of alkaloids and lipase in *Penicillium roquifortii*. Arab J. Biotech. 10(2): 241-248, 2007.

[40] Kodrigues, C.; Batista, E.; and Meirelies, A. Deacidification of vegetable oils by solvent extraction. Recent Patents on Engineering. (1): 95-102, 2007.

الخلاصة

تم الحصول على 12 عزلة بنسبة 32.4% تعود لبكتريا *Serratia marcescens* من مجموع 37 عينة تربة. تم اختيار العزلة (N3) التي انتجت انزيم اللايبيز بكمية عالية لأستخلاص وتنقية ذلك الانزيم بمرحلتين هما: التنقية خلال عمودي CM-cellulose و DEAE-cellulose وكان عدد مرات التنقية 19.33 وبحصيلة نهائية 61.15%. تم توصيف انزيم اللايبيز المنقى ووجد بأن الرقم الهيدروجيني الامثل لفعالية الانزيم هو 8 ودرجة الحرارة المثلى هي 35°م, ولكنه فقد 62 % من فعاليته الاصلية عند الرقم الهيدروجيني 10 وكذلك فقد 78% منها عند درجة حرارة 65°م.

فضلا عن ذلك تم دراسة تأثير بعض العناصر المعدنية وعوامل الشد السطحي على فعالية اللايبيز ووجد بأن بعض العناصر المعدنية مثل الكالسيوم كان اكثر تأثيرا من المغنيسيوم في زيادة فعاليته ، وتؤكد هذه النتيجة ان اللايبيز هو انزيم معدني. كما وجد ان Tween 80 قد حفز فعالية الانزيم عند 110 % يتبعه Tween 20 102%، بينما α-naphtholrin قد ثبط الانزيم الى 21 %.

وقد اظهرت النتائج ايضا تأثير بعض الزيوت الصالحة للأكل على فعالية اللايبيز لبكتريا *Serratia marcescens* ومن ضمنها (زيت الزنجبيل 122، زيت الزيتون 112، زيت جوز الهند 104، ثم زيت السمسم 93 وزيت فول الصويا 92 يليها زيت دوار الشمس فقط 82) U/ml.