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ORIGINAL PAPER

Some Biochemical Properties of Lipase from Bay Laurel (*Laurus nobilis* L.) Seeds

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Abstract Lipase was isolated from bay laurel (*Laurus nobilis* L.) seeds, some biochemical properties were determined. The bay laurel oil was used as the substrate in all experiments. The pH optimum was found to be 8.0 in the presence of this substrate. The temperature optimum was 50 °C. The specific activity of the lipase was found to be 296 U mg protein^{-1} in optimal conditions. The enzyme activity is quite stable in the range of pH 7.0–10. The enzyme was stable for 1 h at its optimum temperature, and retained about 68% of activity at 60 °C during this time. K_m and V_{max} values were determined as 0.975 g and 1.298 U mg protein^{-1}, respectively. Also, storage stability and metal effect on lipolytic activity were investigated. Enzyme activity was maintained for 9, 12, and 42 days at room temperature, 4 and −20 °C, respectively. Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, and Mg^{2+} lightly enhanced bay laurel lipase activity.

Keywords Bay laurel (*Laurus nobilis* L.) · Lipase · Defatting · Thermal stability · Metal ions

Introduction

Bay laurel (*Laurus nobilis* L.), known commonly as True Laurel, Sweet Bay, Grecian Laurel, or Roman Bay laurel, is an evergreen tree, and widely distributed in Mediterranean area and Europe. The leaves are dark-green and elliptical. In ancient times, the leaves were accepted as a symbol of fame, victory and peace, and heroes were decorated with a bay laurel wreath. The berries are black, aromatic and ovoid. Dried bay laurel leaves are mainly used as a spice in grills, soups and other foods. The volatile oils of the seeds and leaves are used widely in the beverage, perfume, food and soap industries. In folk medicine, the volatile oils from seeds and leaves are used externally for treatment of sprains, bruises and rheumatism [1], and as cream for hemorrhoids as an external application. Recent studies have been interested in the antioxidative activity and the antiepileptic effect [1], the gastroprotective effect [2], the antibacterial and the antifungal activities [3], the analgesic and the anti-inflammatory effects [4] of bay laurel seeds and leaves extracts.

Lipases are an important group of biocatalysts because of their ability to carry out the reactions both in aqueous and non-aqueous media. Lipases (triacylglycerol acylhydrolase, E.C. 3.1.1.3) catalyse the hydrolysis of triacylglycerol to release free fatty acids and glycerol. This hydrolytic reaction is reversible, and in the presence of decreased amounts of water, often in presence of the organic solvents, the enzymes are effective catalysts for various inter-esterification and trans-esterification reactions [5]. Their importance and application in stereospecific synthesis have increased in recent years. Therefore lipases have become industrially useful for the modification of fats and oils.

Because of their importance in the field of dairy science, detergents and oleochemistry, lipases have been purified and characterized from various animal, plant and microbial sources. Most of the works about lipases have focused on microbial lipases [6–8]. There has been a growing interest in plant lipases in recent years since they are cheap, very versatile and stabile in organic media. Lipases from plant families like *Euphorbiaceae* [9], *Caricaceae* [10–12], *Cucubitaceae* [13], and *Brassicaceae* [14] have been

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described as useful biocatalysts for different reactions such as hydrolysis, esterification and interesterification.

The extraction of protein becomes rather difficult due to the fatty material, when the enzyme is isolated from oil seeds. Therefore a defatting process should be applied as the first step during the isolation of the enzyme from the oil seeds [15]. In this study, the extraction of fatty material from bay laurel seeds was tested with ethanol, acetone, hexane, acetonitrile, diethylether and petroleum ether to determine the most appropriate solvent.

The bay laurel seeds are rich in triacylglycerols which have been reported to contain between 43 and 70% lipid by weight. Lauric acid (12:0) is the primary component of these triacylglycerols. The studies have shown that lipases isolated from some seeds have high substrate specificity for the fatty acids which are dominant in their seed. For example, castor bean lipase and oil palm fruit mesocarp lipase exhibit their highest activity toward triricinolein, and tricaprin, respectively [16]. Whereas we used bay laurel oil as the substrate in order to determine some properties of lipase-isolated bay laurel seeds in this study.

Our literature survey showed that no experimental data are available regarding the lipolytic activity of laurel seeds except for the California-laurel. Haas et al. purified partially lipase from California-laurel (*Umbellularia californica*) seeds, and its lipolytic activity was determined [17]. However, lipase isolation and characterization from bay laurel (*L. nobilis* L.) seeds has not yet been done, almost nothing is known about bay laurel seed lipase.

In the present study, lipase was isolated from bay laurel seeds which were defatted with ethanol, and some biochemical properties such as pH and temperature optima, pH and thermal stability, kinetic parameters, effect of metal ions and storage stability were investigated.

Materials and Methods

Plant Materials

Bay laurel (*L. nobilis* L.) seeds and laurel oil were purchased from the local market in Hatay, Turkey. The plant material was botanically authenticated at the Department of Biology, Faculty of Science and Art, Trakya University.

Reagents

Sodium deoxycholate and bovine serum albumin were purchased from Sigma, and the rest of solvents and chemicals from Merck. All chemicals were of analytical grade, and used without any purification.

Defatting of Seeds

After having their outer covering peeled off, the ungerminated seeds were ground in a blender. For defatting, 10 g of seeds were stirred with 50 mL of solvent for 40 min. This extraction procedure was repeated three times. At the end of every stirring period, the solvent phase was filtered, and fresh solvent was added to the seeds. All of the solvent phases were put together and evaporated under vacuum at 40 °C. This extraction procedure was carried out by using six different solvents which were ethanol, acetone, hexane, acetonitrile, diethylether and petroleum ether. The percentages of oil content of the extracts obtained were determined. The remaining precipitate, which was called seed powder, was used as the enzyme source, and stored at −20 °C until used.

Extraction of Bay Laurel Lipase

The seed powder defatted as mentioned above was used for lipase extraction. For this purpose, 10 g of seed powder obtained by using different solvents was stirred with phosphate buffer (100 mM, pH 7.0) at room temperature for 1 h, and then the suspension was centrifuged at 5,000×*g* at 4 °C for 15 min. The supernatant obtained was used as the crude lipase extract.

Protein Determination

Protein contents in the bay laurel seed lipase extracts were determined according to the Lowry method with bovine serum albumin as a standard [18].

Assay of Lipase Activity

Lipase activity was determined in bay laurel oil emulsions stabilized by emulsifying agents such as gum arabic and deoxycholate. Lipolytically released free fatty acids were determined by a pH-stat technique as described previously [6, 19]. This method yielded the highest and reproducible activity for the lipase [15]. Firstly bay laurel oil (3.5 g), and gum arabic 10% (w/v) were stirred with a magnetic mixer for 15 min, then Tris–HCl buffer (50 mM pH 8.0), sodium deoxycholate (40 mM) and $CaCl_2$ (0.01 N) were added to this substrate solution to obtain the substrate emulsion. Gum arabic was prepared in 50 mM Tris–HCl buffer, pH 8.0 as 10% (w/v). The enzymatic reaction was initiated by addition of 1 mL enzyme to the emulsion, and incubated at 37 °C at 200 rpm for 15 min in the shaker. The reaction was stopped by the addition of 10 mL

acetone–ethanol mixture (1:1), and the free fatty acids released were titrated with 0.1 N NaOH. One unit of lipase was defined as the number of micromoles of fatty acid hydrolyzed per minute under these conditions. Enzyme activity was expressed as enzyme unit per milligram of protein (U mg protein^{-1}) [6, 8].

Statistical Analysis

All analyses were run in triplicate. The results were presented as the means ± SEM. Statistical analyses were performed according to the GraphPad Prism 4 Demo (trial) program. Analyses of variance were performed using the ANOVA procedure. Significant differences ($P < 0.05$) between the means were determined using Dunnett's post hoc test.

Properties of Bay Laurel Seed Lipase

pH Optimum and Stability

The pH optimum for the laurel seed lipase was determined at various pH from five to ten in the following buffers: 100 mM citrate buffer (pH 5–6), 100 mM Tris–HCl buffer (pH 7–8) and 100 mM carbonate buffer (pH 9–10). The pH stability was determined by measuring the residual lipase activity with the enzyme previously incubating in each buffer at 4 °C at 1 h. Lipase activity of each sample in each buffer was measured using the lipase assay described previously.

Temperature Optimum and Thermal Stability

Temperature optimum for the bay laurel seed lipase was determined by measuring the enzyme activity at various temperatures (30, 40, 45, 50, 55, 60 and 70 °C) using a circulation water bath. The lipase extract and substrate emulsion were incubated for 15 min at the different temperatures indicated above at the optimum pH (pH 8.0). Then lipase activity was determined as previously mentioned.

The thermal stability of the lipase was examined by measuring the residual activity after the enzyme mixture was incubated at various temperature (50, 60, 70 and 80 °C) for 15, 30, 45 and 60 min. Lipase activity of each sample was measured at the optimum conditions.

Enzyme Kinetics

Enzyme kinetics for the bay laurel lipase were studied by using bay laurel oil as a substrate, and the rate of the lipase reaction was measured at various substrate concentrations under optimum conditions (pH 8.0, 50 °C). The kinetic data were plotted between activities and substrate concentrations. The Michaelis–Menten constant (K_m) and maximum velocity (V_{max}) were determined from Lineweaver–Burk curve.

Effect of Metal Ions

To determine the effects of metal ions (Ca^{2+}, Mg^{2+}, Cu^{2+}, Co^{2+}, Fe^{2+}) on the bay laurel lipase activity, enzyme extract and relevant metal solutions with two concentrations (50 and 100 mM) were preincubated for 10 min at room temperature. The residual lipase activity was determined at optimum conditions after this mixture was added to substrate emulsion.

The Effect of Storage Time

The enzyme solutions were stored at room temperature, 4 and −20 °C. The lipase activity was determined at certain intervals at optimum conditions for each of them during this storage period.

Results and Discussion

Defatting of Seeds

Bay laurel seed is one of the oil seeds which contain considerable amount of fatty oil. Before the isolation of lipase from bay laurel seeds, the oil was removed by a defatting process. The defatting process was applied to bay laurel seeds after water contents were minimized by incubating at 37 °C for 4 days.

The defatting process was applied to remove the oil from bay laurel seeds at atmospheric pressure and room temperature. The extraction of fatty material from bay laurel seeds was tested by using different organic solvents. These solvents were hexane, petroleum ether, diethylether which solve lipids well, and ethanol, acetone, acetonitrile which can be usually used in protein and enzyme studies. After lipid extraction, the remaining precipitate, which was called seed powder, was used in order to isolate the enzyme. The oil contents of extracts, and the lipolytic activities of these powders dissolved in buffer were determined (Table 1). Ethanol was chosen as the solvent for defatting process according to the results as shown in Table 1. As seen in this table, the percentages of oil contents of obtained extracts had values close to each other. All the powders obtained with different solvents mentioned

Table 1 The percentages of oil contents of obtained extracts from bay laurel seeds, and relative lipolytic activities of obtained powders with different solvents

Solvent	The oil content[a] (%)	Relative activity[b] (%)
Ethanol	13.52 ± 0.231	100 ± 0.00
Diethylether	12.00 ± 0.404	66.22 ± 0.962
Hexane	11.30 ± 0.722	64.19 ± 1.153
Acetone	11.00 ± 0.404	60.15 ± 1.562
Petroleum ether	10.21 ± 0.462	49.45 ± 0.843
Acetonitrile	7.93 ± 0.346	44.75 ± 0.552

[a] Data are presented as mean ± standard error of mean (SEM)

[b] Relative activity was expressed of the percentages of the maximum activity detected (100%)

previously showed lipolytic activity. Because ethanol powder showed the highest lipolytic activity, it was chosen as the crude lipase source, and used in the following experiments. Whereas Prabhu et al. [15] found *n*-hexane to be the best solvent among the tested solvents such as petroleum ether, *n*-hexane, and *n*-heptane for the rice bran defatting.

The specific activity of the bay laurel lipase was found as 296 U mg protein^{-1} in optimal conditions (pH 8.0, 50 °C). The lipolytic activity of latex of babaco fruit was 1.01 IU mg^{-1} by using tributyrin as substrate [12]. The lipolytic activity of latex of *Euphorbia characias* was determined as 141 and 79 IU g^{-1} by using sunflower TAG and linseed TAG as substrate, respectively [20]. However Palocci et al. [9] reported that lipolytic activity of latex of *Euphorbia characias* was 785 and 1183 IU mL^{-1} by using sunflower seed oil and linseed oil as substrate, respectively. Lipase activity of California laurel seeds was determined as 0.79 μkat g^{-1} dry weight using trilaurin as substrate [17].

pH Optimum and Stability

The pH is one of the important parameters capable of altering enzymatic activities in aqueous solution. Relative activity as a function of pH is depicted in Fig. 1. Relative activity was determined as a percentage of the observed highest lipase activity in the relevant experiment, which was taken as 100%. As seen Fig. 1, the optimum pH of bay laurel lipase was 8.0 for bay laurel oil as substrate. This value is similar to that of lipases in oil palm fruit mesocarp, California-Bay laurel seeds, and rice bran, which were 9.0, 8.5 and 7.0–7.5, respectively [15, 17, 21]. The optimum pHs for different plant lipases have been generally reported in the basic pH values (7.0–9.0), and this value have been changed depending on enzyme source and substrate.

Figure 2 shows the residual percentage activity of the lipase from bay laurel seeds for various pH values, between

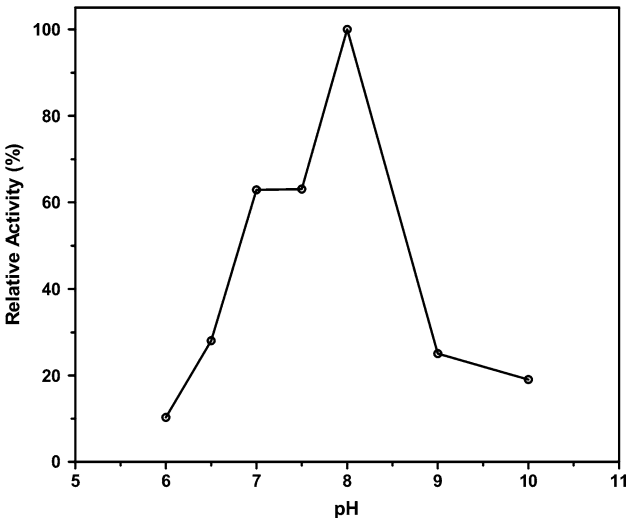

Fig. 1 Effect of pH on bay laurel lipase activity. Activities were assayed using 100 mM buffer solutions of different pH [citrate buffer (pH 5–6), Tris–HCl buffer (pH 7–8) and carbonate buffer (pH 9–10)]. Lipase activity of each sample in each buffer was measured using the lipase assay described in the "Materials and Methods" section. The highest activity obtained was taken to be 100% for calculating the relative activities

5.0 and 10.0. The pH stability profile for the bay laurel lipase has shown that the lipase activity was fully retained at its pH optimum value and around the basic pH values, but the enzyme was less stable at lower pHs.

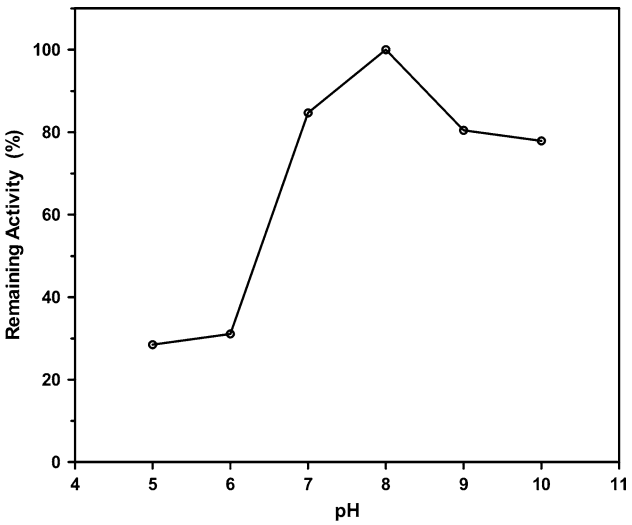

Fig. 2 Effect of pH on stability of bay laurel lipase. The pH stability was determined by measuring the residual lipase activity with the enzyme previously incubating in each buffer [100 mM citrate buffer (pH 5–6), 100 mM Tris–HCl buffer (pH 7–8) and 100 mM carbonate buffer (pH 9–10)] at 4 °C at 1 h. Lipase activity of each sample in each buffer was measured as described previously. Remaining activities were calculated as a percentage of maximum activity obtained

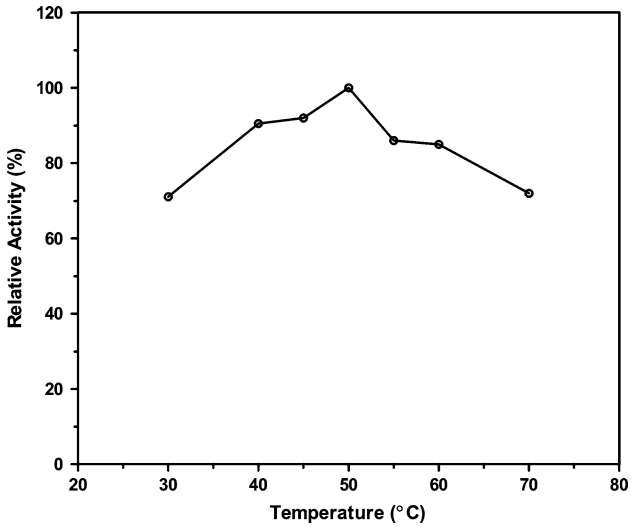

Fig. 3 Effect of temperature on bay laurel lipase activity. Activity of bay laurel lipase was studied by incubating the reaction mixture at different temperatures (30, 40, 45, 50, 55, 60, and 70 °C). The highest activity obtained was taken as 100% for calculating the relative activities

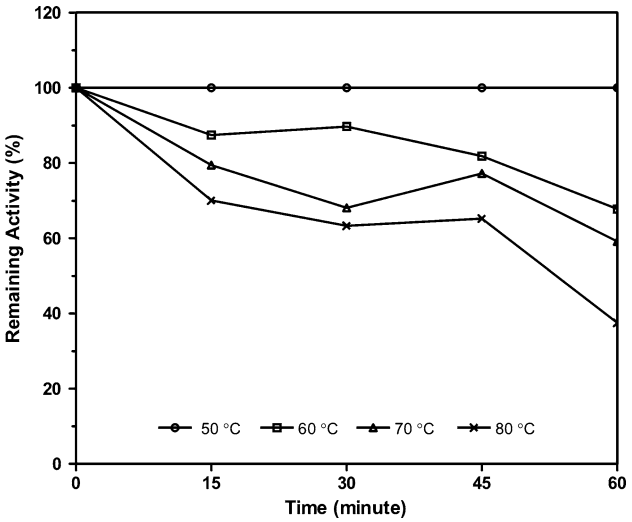

Fig. 4 Effect of temperature on stability of bay laurel lipase. *Open circles*, 50 °C; *open square*, 60 °C; *open triangle*, 70 °C; *cross*, 80 °C. Enzyme solutions were incubated at the different temperatures for 15, 30, 45, and 60 min, and remaining activities were determined under optimum assay conditions

Temperature Optimum and Thermal Stability

The activity of bay laurel lipase was measured at different temperatures (30–70 °C). As presented Fig. 3, the optimum temperature for maximum activity of the bay laurel lipase was determined to be 50 °C using bay laurel oil as the substrate. A similar temperature optimum was reported as 45 °C for *Euphorbia characias* latex lipase using tributyrin as the substrate [20]. Dhiuque-Mayer et al. [12] also carried out lipolysis reactions catalysed by *Carica papaya* latex lipase at 50 °C. However, Ngando Ebongue et al. [21] observed that the optimum temperature was 35 °C for oil palm seed (*Elaeis guineensis* Jacq) mesocarp lipase using olive oil as the substrate.

The knowledge about the thermal stability of an enzyme is useful for exploring the potential applications of the enzyme. The thermostability of the lipase from bay laurel seeds was studied at 50, 60, 70 and 80 °C. The results are presented in the form of the residual percentage activity at Fig. 4. Bay laurel lipase was stable at 50 °C for 1 h. The enzyme retained about 68% of its activity at 60 °C whereas it retained 59% at 70 °C, for 1 h of incubation. However, the enzyme lost about 60% of its activity at 80 °C for the same incubation period. Based on our results, it can be considered that bay laurel lipase is a relatively thermostable enzyme. Thus, bay laurel lipase can be used in hydrolysis processes of triglycerides which require the high temperatures. The half-life values ($t_{1/2}$) of bay laurel lipase at 60, 70, and 80 °C were 108.9, 78.5, and 42.5 min, respectively. The midpoint of thermal inactivation (T_m), where the activity is diminished by 50%, is calculated from the plot of percent residual activity versus temperature. For this purpose, bay laurel lipase samples were incubated for 60 min at various temperatures between 50 and 80 °C, and remaining activities were assayed. T_m was found as 74.2 °C (data not shown). These high $t_{1/2}$ and T_m values also revealed that bay laurel lipase is enzyme which has high thermal stability.

Enzyme Kinetics

Lineweaver–Burk plots showed linear relationships and kinetic parameters were calculated for bay laurel lipase. The plot was drawn for the kinetic analysis of the reaction rate in the range of 0.8–3.5 g concentrations of bay laurel oil as substrate (Fig. 5). K_m and V_{max} values were determined 0.975 g and 1.298 U mg protein^{-1}, respectively. K_{cat}/K_m ratio as catalytic efficiency was calculated as 0.774 s^{-1} g^{-1}.

Effect of Metal Ions

Cofactors are not required for the expression of lipase activity, and divalent cations, such as Ca^{2+}, generally stimulate the activity. It has been postulated that this is based on the formulation of calcium salts of long-chain fatty acids [22]. To determine the effect of metal ions, lipase activity was examined with different metal solutions in the standard reaction solution in the absence of Ca^{2+} ions. The obtained activity results are given in Table 2.

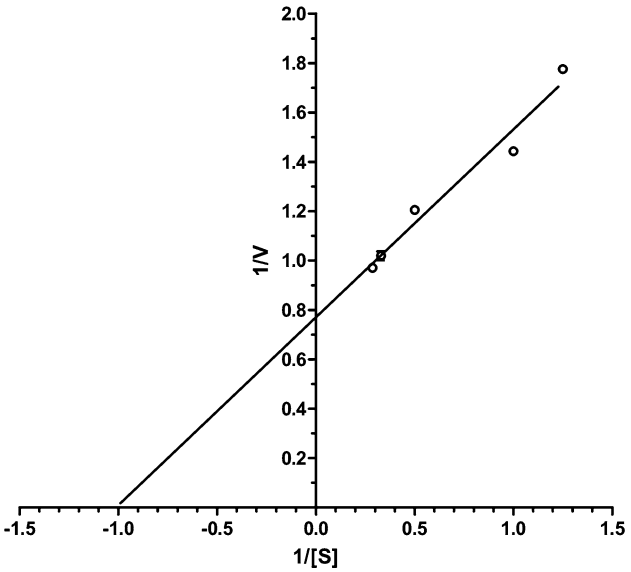

Fig. 5 Effect of substrate concentration on bay laurel lipase activity. Bay laurel oil at the different concentrations (0.8–3.5 g) in 50 mM Tris–HCl (pH 8.0) was used as substrate. The rate of the lipase reaction was measured under optimum conditions (pH 8.0, 50 °C). The Lineweaver–Burk curve was plotted between activities and substrate concentrations

Table 2 The effect of some metal ions on bay laurel lipase activity

Compound	Concentrations (mM)	Specific activity[a] (U mg protein^{-1})
None	–	267.90 ± 1.185
$CaCl_2$	50	276.06 ± 0.751[b]
$CaCl_2$	100	275.50 ± 0.513[b]
$MgCl_2$	50	262.13 ± 0.731[b]
$MgCl_2$	100	262.00 ± 0.819[b]
$CoCl_2$	50	261.93 ± 1.501[b]
$CoCl_2$	100	283.96 ± 0.769[b]
$CuCl_2$	50	289.13 ± 1.565[b]
$CuCl_2$	100	316.13 ± 0.841[b]
$FeCl_2$	50	281.00 ± 1.629[b]
$FeCl_2$	100	300.13 ± 1.671[b]

[a] Data are presented as mean ± standard error of mean (SEM)

[b] Significantly different when compared to group none (as control) ($P < 0.05$)

Some surprising results were observed. First of all, Ca^{2+}, which is known as activator for lipases, slightly enhanced bay laurel lipase activity. Secondly, bay laurel lipase activity was enhanced by, Cu^{2+}, Fe^{2+}, Co^{2+}, and Mg^{2+} ($P < 0.05$). The effect of metal ions on plant lipases activity has not been studied sufficiently whereas there are several studies on microbial lipases. For example, Ca^{2+}, Mg^{2+}, Co^{2+}, and Na^{+} enhanced lipase activity whereas Cu^{2+}, Ba^{2+}, and Fe^{2+} strongly reduced lipase activity for extracellular lipase from *Mucor hiemalis f. hiemalis* [6]. Also Mg^{2+} and Ca^{2+} stimulated lipase activity, but Co^{2+}, Cu^{2+}, Fe^{2+}, and Ni^{2+} ions caused inhibition for regiospecific lipase from *Aspergillus terreus* [7].

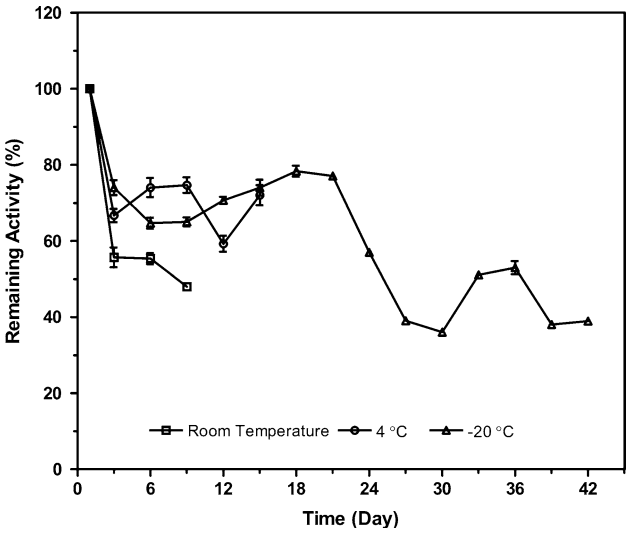

Fig. 6 Effect of storage time on stability of bay laurel lipase. The activities of enzyme solutions stored at different temperatures (room temperature, 4 and −20 °C) were measured at certain intervals at optimum assay conditions

The Effect of Storage Stability

The storage stability of an enzyme is one of the most important parameters for scheduling its application in a particular reaction. The storage stability of bay laurel lipase was investigated at room temperature, 4 and −20 °C. Bay laurel lipase lost about 48% of its activity after 9 days at room temperature. The observed lipase activity at 4 °C was about 61% on the 12th day. After this period, lipase activity was measured a few more times at 3-day intervals during storage and the activities were not found to be meaningful. It may be because of increasing microbial contamination in the enzyme solution. The activities of bay laurel lipase extracts stored at −20 °C were measured for 42 days, and decreased about 77% after 21 days (Fig. 6).

References

1. Simic M, Kundakovic T, Kovacevic N (2003) Preliminary assay on the antioxidative activity of *Laurus nobilis* extracts. Fitoterapia 74:613–616
2. Afifi FU, Khalil E, Tamimi SO, Disi A (1997) Evaluation of the gastroprotective effect of *Laurus nobilis* seeds on ethanol induced gastric ulcer in rats. J Ethnopharmacol 58:9–14
3. Erturk O (2006) Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Biologia 61(3):275–278

4. Sayyah M, Saroukhani G, Peirovi A, Kamalinejad M (2003) Analgesic and anti-inflammatory activity of the leaf essential oil of *Laurus nobilis* Linn. Phytother Res 17(7):733–736
5. Gupta R, Rathi P, Gupta N, Bradoo S (2003) Lipase assays for conventional and molecular screening: an overview. Biotechnol Appl Biochem 37:63–71
6. Hiol A, Jonzo MD, Druet D, Comeau L (1999) Production, purification and characterization of an extracellular lipase from *Mucor hiemalis f. hiemalis*. Enzyme Microb Technol 25:80–87
7. Yadav RP, Saxena RK, Gupta R, Davidson WS (1998) Purification and characterization of a regiospecific lipase from *Aspergillus terreus*. Biotechnol Appl Biochem 28:243–249
8. Kermasha S, Safari M, Bisakowski B (1998) Characterization of purified lipase fractions from *Rhizopus niveus*. J Agric Food Chem 46(10):4451–4456
9. Palocci C, Soro S, Cernia E, Fiorillo F, Belsito CMA, Monacelli B, Monache GD, Pasqua G (2003) Lipolytic isoenzymes from *Euphorbia* Latex. Plant Sci 165:577–582
10. Gandhi NN, Mukherjee KD (2001) Reactivity of medium-chain substrates in the interesterification of tripalmitin catalyzed by papaya lipase. J Am Oil Chem Soc 78(9):965–968
11. Foglia TA, Villeneuve P (1997) *Carica papaya* latex-catalyzed synthesis of structured triacylglycerols. J Am Oil Chem Soc 74(11):1447–1450
12. Dhuique-Mayer C, Caro Y, Pina M, Ruales J, Dornier M, Graille J (2001) Biocatalytic Properties of lipase in crude latex from babaco fruit (*Carica pentagona*). Biotechnol Lett 23:1021–1024
13. Eze SO, Chilaka FC, Alumanah EO (2005) Lipase activity in some *Cucubitaceae* species. Bio-research 3(1):66–71
14. Hills MJ, Kiewitt I, Mukherjee KD (1990) Lipase from *Brassica napus* L. discriminates against *cis*-4 and *cis*-6 unsaturated fatty acids and secondary and tertiary alcohols. Biochim Biophys Acta 1042(2):237–240
15. Prabhu AV, Tambe SP, Gandhi NN, Sawant SB, Joshi JB (1999) Rice bran lipase: extraction, activity and stability. Biotechnol Progr 15(6):1083–1089
16. Hellyer SA, Chandler IC, Bosley JA (1999) Can the fatty acid selectivity of plant lipases be predicted from the composition of the seed triglyceride. Biochim Biophys Acta 1440:215–224
17. Haas MJ, Cichowicz DJ, Dierov JK (2001) Lipolytic activity of California-laurel (*Umbellularia californica*) seeds. J Am Oil Chem Soc 78(10):1067–1071
18. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the phenol reagent. J Biol Chem 193:265–275
19. Hoppe A, Theimer RR (1996) Titrimetric test for lipase activity using stabilized triolein emulsions. Phytochemistry 42(4):973–978
20. Caro Y, Villeneuve P, Pina M, Reynes M, Graille J (2000) Lipase activity and fatty acid typoselectivities of plant extracts in hydrolysis and interesterification. J Am Oil Chem Soc 77(4):349–354
21. Ngando-Ebongue GF, Dhouib R, Carriere F, Amvam-Zollo PH, Arondel V (2006) Assaying lipase activity from oil palm fruit (*Elaeis guineensis* Jacq.) mesocarp. Plant Physiol Biochem 44:611–617
22. Indian Academy of Science (1999) Potential biocatalysts for the future industry. http://www.ias.ac.in/currsci/jul10/articles18.htm. Accessed July 1999