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Immobilization of apricot pectinesterase (*Prunus armeniaca* L.) on porous glass beads and its characterization

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ABSTRACT

Pectinesterase isolated from Malatya apricot pulp was covalently immobilized onto glutaraldehyde-containing amino group functionalized porous glass beads surface by chemical immobilization at pH 8.0. The amount of covalently bound apricot PE was found 1.721 mg/g glass support. The properties of immobilized enzyme were investigated and compared to those of free enzyme. The effect of various parameters such as pH, temperature, activation energy, heat and storage stability on immobilized enzyme were investigated. Optimum pH and temperature were determined to be 8.0 and 50 °C, respectively. The immobilized PE exhibited better thermostability than the free one. Kinetic parameters of the immobilized enzyme (K_m and V_{max} values) were also evaluated. The K_m was 0.71 mM and the V_{max} was 0.64 μmol min^{-1} mg^{-1}. No drastic change was observed in the K_m and V_{max} values. The patterns of heat stability indicated that the immobilization process tends to stabilize the enzyme. Thermal and storage stability experiments were also carried out. It was observed that the immobilized enzyme had longer storage stability and retained 50% of its initial activity during 30 days.

1. Introduction

Enzymes are biological catalysts that are highly effective and very specific under ambient conditions; therefore enzymatic processes have great industrial applications. General expectations from the commercially used enzymes are efficient use of reactants, maximizing catalytic velocity and enhancement of the operational lifetime [1]. To improve their economic feasibility in food, pharmaceutical, medical, industrial and technological processes, soluble enzymes are usually immobilized onto a solid support. Immobilization of the enzymes onto solid support is currently a very active area of research because of their wide range of applications. There are several advantages over the use of soluble enzyme preparations including easier separation of reaction products from the incubation mixture, the ability to recover and reuse enzyme, stabilization of tertiary structure of enzyme and increased enzyme stability and operational lifetime [2,3]. Thus the recovery and purification of the final products from enzymes become more reliable, simple and efficient [4]. During immobilization, many factors such as immobilization method, the size, shape and composition of the carrier material and the specific conditions can affect the activity of an immobilized enzyme. The success of an immobilized enzyme for practical applications depends strongly on the properties of the carriers employed. The carrier material must be insoluble in water, should have a high capacity to bind enzyme, should be mechanically stable and must not have deleterious effect on the enzyme action. Various modified supports for covalent immobilization such as polymers, silica and glass, have been widely investigated. Hydrophilic biopolymers based on natural polysaccharides such as dextran and cellulose, synthetic organic polymers such as polyacrylamide and polystyrene can be given as the most widely studied polymeric supports. Silica gel, aluminum oxide, apatite and glass supports are also preferred inorganic support materials. Organic polymeric carriers are the most widely studied materials because of the presence of rich functional groups, which provide essential interactions with the enzymes. However, the organic supports suffer a number of problems such as poor stability towards microbial attacks and organic solvents and disposal issues. In contrast, inorganic materials such as silica, alumina, and layered double hydroxides are known to be thermally and mechanically stable, non-toxic, and highly resistant against microbial attacks and organic solvents [5].

Inorganic carriers employed in biotechnology are usually modified bifunctional organosilanes: in particular 3-aminopropyltriethoxysilane. Such modified carriers, especially porous glasses

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having controlled pore dimensions are widely applied in biotechnology, predominantly for enzyme immobilization [6]. The hydroxyl groups on the surface of silica and glass provide desired functionality that can react with hydrolysable groups of organosilane and cyanogens bromide coupling agents. Silanization technique is used to change the physical and chemical properties of solid hydrophilic surface properties of silicates and glass [7]. The surface modification of glass is carried out using variety silane-coupling agents having organofunctional groups with di-tri-alkoxy structures and the reactions are performed either in gaseous or liquid phase [8]. The silanol groups then condense with the surface residues to form siloxane linkages. In the case of tri-alkoxysilanes, the presence of three silanol residues in the hydrolysis product can lead to the possibility of multiple surface attachments. Aminosilanes as 3-aminopropyltriethoxysilane (APTS) are attractive coupling agents for immobilization of enzymes. Amino groups have catalytic activity formation of siloxane bonds with silanols on the surface the glass [9].

Pectolytic enzymes play a crucial role in food processing industries, e.g., in the production of fruit juices, soft drinks, and liquors. These enzymes are also used in the maceration, liquefaction and extraction of vegetable tissues. They also help to reduce the viscosity of fruit pulp which, in turn, improves filtration and clarification of fruit juices and wood aging [5]. Therefore the immobilization of these enzymes is very important for their usage at these industrial areas. Pectinesterase enzyme (PE; pectinmethylesterase, pectin pectilhydrolase, EC 3.1.1.11) is present in many higher plants and can be found in different plant tissues as well as in pathogenic fungi and bacteria, particularly those contained in fruits. This enzyme catalysis the hydrolysis of methoxyl groups of methylated galacturonic residues in pectin molecules [10].

The determination of PE activity has been reported as a way to obtain information about the degree of fruit juices and concentrates stability [11–14]. The PE assays are commonly based on spectrophotometry using chemical [13,15] or enzymic derivatisation [16,17], or on spectrofluorimetry with enzymic derivatisation [18].

In this work, glass beads were selected as immobilization matrix for the immobilization of the partially purified PE enzyme from Malatya apricot. Glass beads can easily be modified by a variety of functional groups and their mechanical properties are excellent. The partially purified PE enzyme was immobilized on porous glass beads by the chemical method. The characterization of the immobilized enzyme was carried out by determining kinetic constants, optimum pH and temperature, activation energy, thermal and storage stability.

2. Experimental

2.1. Materials

Pectinesterase enzyme used in this work was isolated from apricot harvested from Malatya region in Turkey and purchased at commercial maturity from a local market and stored at −20 °C. Porous glass beads for enzyme immobilization, membranes cut off 12,000 (Sigma Chem. Co., St. Louis, MO) used for dialysis, pectin, KH_2PO_4 (99.0%), DEAE-Sephadex were purchased from Sigma Chem. Co. (St. Louis, MO) (3-aminopropyl) triethoxy silane (APTS) $(NH_4)_2SO_4$, Na_2HPO_4 and H_2SO_4 were purchased from Fluka (Riedel-de Haën, Sigma–Aldrich Laborchemikalien, Seelze, Germany), sodium azid, potassium tartarate, sodium tungstate, sodium molybdate, $CuSO_4{\cdot}5H_2O$, Li_2SO_4, NaOH, HCl, NaCl were purchased from Merck (Schuchardt OHG, Hohenbrunn, Germany). All chemicals used in this study were of analytical grade and were used without further purification.

Spectrophotometric measurements were carried out with Shimadzu UV–vis-1601 spectrophotometer. pH measurements were carried out with Orion 720A model pH-meter, Centrifugation was made by using Beckman Optima XL-100K model, the measurements related to temperature were carried out with mixed and thermostated water bath (Thermolyne Cimarca) and the mixing procedure was made by using vortex (Nüve N 110).

3. Methods

3.1. Isolation and partial purification of Malatya apricot pectinesterase

500 g Malatya apricots was stored at −20 °C during 1 week and kept at +4 °C for dissolving the frosted fruit. Then, the apricots were halved, cored and weighed (465 g). Then, apricot tissue was homogenized with 931 mL of 2N NaCl by using Waring blender for 5 min and homogenate was filtered with cheese cloth. The homogenate called as crude enzyme extract was centrifuged at 24.000 × *g* for 30 min at 4 °C. The enzyme solution from the supernatant was fractionated with solid ammonium sulphate and the precipitate of 40–80% saturation was collected by centrifugation at 24.000 × *g* for 30 min at 4 °C. For the ammonium sulphate precipitation, the addition of solid ammonium sulphate was carried out little and slowly by providing completely dissolving it after each addition during 1 h. The pellet was redissolved in 120 mL homogenization buffer (0.2 M sodium phosphate buffer, pH 7.5) and dialysed at 4 °C against the same buffer in cellulose dialysis tubing (mol. wt. cut off 12 000–14 000 Da). Dialysis buffer was changed five times with 8 h intervals. The dialysed solution was kept in stoppered test tubes at −20 °C. The partially purified enzyme was used for the characterization of apricot pectinesterase enzyme because it showed higher activity.

3.2. Immobilization procedure

Porous glass beads were first activated by (3-aminopropyl) triethoxy silane (APTS) to immobilize pectinesterase enzyme isolated from Malatya apricot (*Prunus armeniaca* L.) on them. The silanization procedure was performed as described by Weethall [35]. The α-amino groups of lysine on enzyme molecules were attached to the aminopropyl glass beads via glutaraldehyde (2.5%) (Fig. 1).

6.0 g glass beads were washed several times with distilled water and then suspended in 20 mL of 10% APTS solution for silanization process. The pH of the medium was adjusted between pH 3.0 and 4.0 with concentrated HCl and then it was refluxed gently (150 rpm) via magnetic stirrer at 75 °C for 5 h. The glass beads activated with APTS were washed with distilled water for three times. The aminopropyl glass derivative was suspended in 150 mL of 2.5% glutaraldehyde solution (prepared in sodium phosphate buffer, 0.2 M, pH 7.5) and it was agitated at 200 rpm at room temperature for 1 h. The beads were separated and washed with the same buffer. They were added to 10 mL of partially purified pectinesterase enzyme isolated from Malatya apricot, which was prepared in 0.2 M sodium phosphate buffer (pH 7.5) with gentle shaking and incubated at 4 °C overnight with magnetic stirrer. The immobilized enzyme conjugate was centrifugated at 12 000 rpm for 15 min and then in order to remove unbound proteins it was washed with the same buffer and dried at 4 °C. For each centrifugation procedure, the supernatant was analyzed for remaining pectinesterase activity by our modified method determined by Versteeg et al. [19] as described above. The protein assay of the each supernatant was carried out by Bradford protein assay method [20].

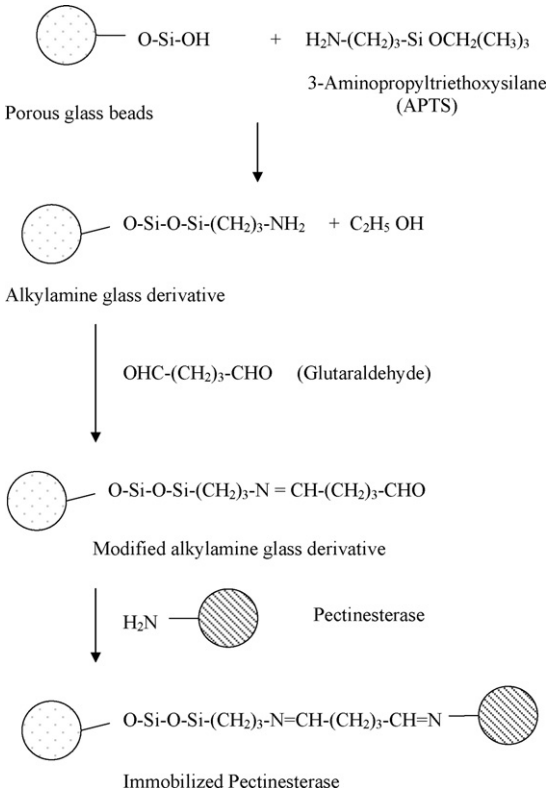

Fig. 1. Immobilization of Malatya apricot pectinesterase on porous glass beads.

3.3. Protein determination

Protein concentrations were determined by Bradford method [20] using bovine serum albumin as standard. The amount of bound protein was calculated from the difference between the amount of protein introduced into the reaction mixture and the amount of protein in the filtrate and washing solutions after immobilization.

3.4. Assay of enzyme activity

Pectinesterase activity was determined manually by our modified method proposed by Versteeg et al. [19]. Briefly, the method involves measurement of releasing rate of carboxyl groups in a pectin solution in 0.1N NaCl (1%, w/v) at room temperature. Pectin (1%) in 0.1N NaCl (1%, w/v) was prepared and stored according to the procedure described by [21]. The reaction was started by the addition of 0.05 g immobilized PE on porous glass beads dissolved in 0.5 mL sodium phosphate buffer (pH 7.5) enzyme to 4 mL of 1% pectin solution in 0.1N NaCl (1%, w/v) and the reaction pH was adjusted and maintained manually at optimum pH of the enzyme by the addition of 0.1 and/or 0.01N NaOH solution. After the pH was fixed, 0.2 mL 0.01N NaOH was added and provided a sudden increase in pH. The returning 'time' to adjusted pH from suddenly increased pH is recorded. PE activity was calculated by the following formula [22–24]:

$$\text{PE units/mL} = \frac{(\text{mL NaOH})(\text{N NaOH})(1000)}{(\text{time})(\text{mL sample})}$$

The method was also carried out at various temperatures and pH values with pectin substrate for characterization of the enzyme. One unit of PE activity was defined as the amount of enzyme that released 1 μmol of carboxyl group produced in min at room temperature. Experiments were triplicated.

3.5. Effect of pH on immobilized enzyme activity

The effect of pH on free and immobilized pectinesterase activities were assayed at different pH values between 4.0 and 9.5. 0.05 g enzyme dissolved with 0.5 mL 0.2N phosphate buffer (pH 7.5) was added into 4 mL 1% pectin in 0.2N NaCl. The pH influence was studied manually with 0.2 mL 0.01N NaOH after adjusting the pH of the reaction solution to one of the pH values (4.0–9.5) tested. The optimum pH value obtained from these assays was used in all the other experiments.

3.6. Effect of temperature on PE activity

The effect of assay temperature on free enzyme and immobilized enzyme were tested manually with 1% pectin concentration at pH 9.0 and PE activity as a function of temperature under standard assay conditions were determined by using temperatures from 10 to 90 °C controlled by means of a circulating water bath. To determine relative activity of PE at a specific temperature, 0.05 g enzyme dissolved with 0.5 mL 0.2N phosphate buffer (pH 7.5) was incubated in 15 min in circulating water bath, cooled and added to 1% pectin.

3.7. Thermal stability of apricot PE

Thermal stability studies of free and immobilized pectinesterase were performed between 60 and 90 °C temperatures manually under standard assay conditions by measuring the residual activity of the enzyme in 0.2 M sodium phosphate buffer (pH 7.5). 0.05 g enzyme dissolved with 0.5 mL 0.2 M sodium phosphate buffer (pH 7.5) in three different test tubes was incubated in 45 min in circulating water bath at the specified temperature. Enzyme was withdrawn from each tube at various time intervals during 45 min. After cooled, it was added into 1% pectin for various time intervals (15–45 min). The remaining activity of the enzyme was determined.

3.8. Determination of activation energy (E_a)

Activation energy (E_a) of free and immobilized Malatya apricot PE were estimated from the slope of the Arrhenius plot obtained by plotting the logarithm of reaction rate constants ($\ln k$) versus the reciprocal of the absolute temperature ($1/T$). Thermal inactivation rate constants were calculated by comparing the activity changes upon heat treatment with the unheated enzyme extracts as reported by [25].

3.9. Kinetic constants of apricot PE

Determination of K_m and V_{max} values of free and immobilized enzyme was carried out by measuring activities for both pectinesterases in the presence of various substrate concentrations (0.05, 0.075, 0.125, 0.25, 0.5, 0.75, and 1 mM). Michael–Menten constant (K_m) values and the maximum velocities (V_{max}) were determined using the Lineweaver–Burk double reciprocal plot [26], in which the reciprocals of the initial velocities of the pectinesterase activity were plotted against the reciprocals of the concentration of pectin used.

4. Results and discussion

4.1. Extraction and partial purification of PE

In food technology, the presence of pectinesterase in fruit is a problem that has been intensively studied, particularly in relation to cloud loss in juice [27]. PE is of significance to the fruit juice industry since it has been definitively established as the causative

Table 1
Purification of pectinesterase enzyme isolated from Malatya apricot

Purification step	Volume (mL)	Total activity (unit)	Total protein (mg)	Specific activity (unit/mg)	Yield (%)	Purification *n*-fold
Crude extract	1060	1742.6	298.9	5.83	100	1.0
40–80% $(NH_4)_2SO_4$ precipitation	125.5	880.76	29.1	30.27	50.5	5.2
Dialysis	122.5	1666.7	23.5	70.92	95.6	12.2

Table 2
Immobilization of PE from Malatya apricot on porous glass beads at 20 ± 5 °C

Free enzyme		Immobilized enzyme				
Protein (mg/2.15 g glass beads) (*x*)	Specific activity (unit/mg)	Protein in washing (mg) (*y*)	Bound protein (mg) (*x*–*y* = A)	Activity (unit)	Specific activity (unit/mg)	Protein yield (%) ((*A*/*x*) × 100)
1.92	5.83	0.199	1.721	4.651	2.702	89.64

agent of juice clarification and gelation of frozen concentrates. The enzyme catalyzes the hydrolytic cleavage of the methylester moieties on pectin molecules, resulting in the release of methanol and partially de-esterified pectin.

Initially, the isolation and partially purification of pectinesterase enzyme from Malatya apricot pulp were carried out by using 2 M NaCl prepared with 0.2 M sodium phosphate buffer (pH 7.5). For the isolation and partially purification procedure, it was carried out homogenization with NaCl, ultrafiltration at 4 °C, saturation with ammonium sulphate and dialysis, respectively. The optimum concentration of NaCl used for adjusting ionic strength of the solution in this study was similar to that found by us in extracting pectinesterase from papaya [36]. The precipitate obtained from 40% to 80% ammonium sulphate fractionation of crude extract of 420 g of apricot pulp dissolved and dialysed with 0.2 M sodium phosphate buffer (pH 7.5). In Table 1, it was seen that 5.2-fold and 12.2-fold purification were obtained with 80% saturation of solid ammonium sulphate and dialysis, respectively. Dialysis membrane is used to remove salts and low molecular weight inhibitor(s) used in isolation and purification procedures of enzyme solution. Immobilization procedure was carried out with the dialysed enzyme named partially purified enzyme.

The most important criterion for selecting a carrier material for using enzyme immobilization for industrial application is the stability and the cost of the carrier. In this study pectinesterase enzyme isolated from Malatya apricot pulp was covalently immobilized on commercially available inexpensive and renewable porous glass beads. Covalent immobilization method requires chemical modification of the glass surface, so that functionally inert silanols (Si—OH) of a glass surface are modified to posses either nucleophilic or electrophilic functionalities that react with the enzyme reactive functional groups (—NH_2) [8]. Inorganic carriers employed in biotechnology are usually modified bifunctional organosilanes: in particular 3-aminopropyltriethoxysilane. Such modified carriers, especially porous glasses having controlled pore dimensions are widely applied in biotechnology, predominantly for enzyme immobilization [6].

After surface modification of the glass beads were carried out with a silane-coupling agent (3-aminopropyl triethoxysilane), enzyme immobilization achieved. After this modification procedure, the surface of modified glass beads is reacted with glutaraldehyde followed by covalent attachment of the PE enzyme to the newly introduced functional group on the surface. The procedure used for covalently enzyme immobilization on the activated glass bead surface is shown in Fig. 1.

The specific activity and protein concentration for immobilized enzyme were calculated 2.702 IU/mg protein and 1.721 mg, respectively. The immobilization results are summarized in Table 2. Some properties of the immobilized enzyme were investigated and also demonstrated and compared with the free enzyme.

4.2. Effect of pH and temperature on PE activity

The effect of pH on the activity of free and immobilized pectinesterase was studied within the pH range of 4.0–9.5 at room temperature. The enzyme activities obtained are presented in Fig. 2. The maximum activity was observed at pH 9.0 for free pectinesterase while that of the immobilized enzyme was towards 1.0 pH unit to the acidic region. This is explained that secondary interactions such as ionic and polar interactions, hydrogen bonding between the enzyme and the support matrix are possibly occurred [28]. pH shift to acidic region is also explained in considering with the amino groups occurred after silanization. Strong interactions between enzyme and support will affect the intra-molecular forces responsible for maintaining the conformation of the enzyme that would lead to a change activity. The polar groups of porous glass beads may have interacted with the functional groups of pectinesterase changing the pH characteristics of the enzyme. The change depends on the enzyme reaction as well as on the structure and the charge of the matrix. In the optimum pH of α-amylase immobilized on glass beads, changed towards the acidic direction have been observed [9]. Teke et al. [29] was determined that the optimum pH of immobilized phospholipase A_2 enzyme on porous glass beads was 8.5. There was no shift from the pH optima of the free enzyme.

The optimum pH of an enzyme in solution can change depending on the surface and residual charges of the solid matrix and the nature of the enzyme-bound pH value in the immediate vicinity of the enzyme environment when the same enzyme is immobilized

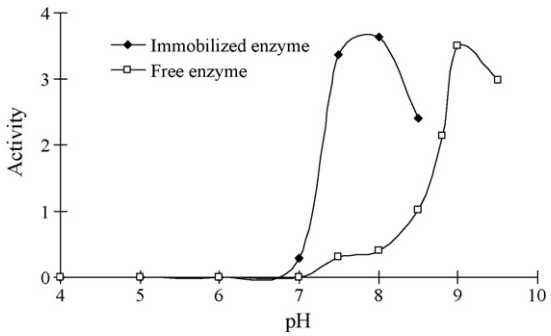

Fig. 2. The effect of pH on the activity of free and immobilized apricot PE. Reactions were made with 0.5 mL 0.2N phosphate buffer (pH 7.5) into 4 mL 1% pectin in 0.2N NaCl.

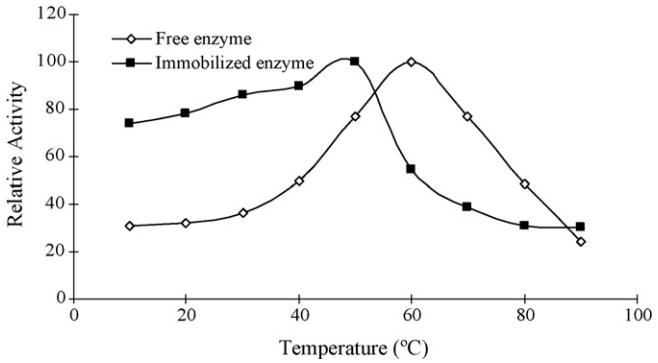

Fig. 3. The effect of temperature on the activity of free and immobilized apricot PE. Reactions were made with 1% pectin concentration at pH 9.0.

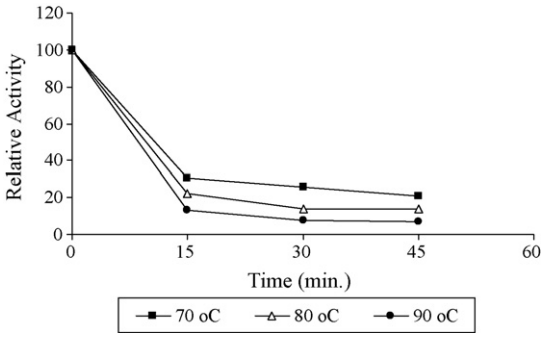

Fig. 5. The thermal stability profile for free apricot PE. Reactions were made with 0.5 mL enzyme in 0.2N phosphate buffer (pH 7.5) into 4 mL 1% pectin in 0.2N NaCl at pH 9.0.

on a solid matrix. The change in the optimum pH normally results in insolubilization of enzymes, depending upon the polymer used as support.

The activities of free and immobilized pectinesterase were assayed at various temperatures (10–90 °C). The effect of temperature on the activity of free and immobilized apricot PE is shown in Fig. 3. It was showed that the maximum catalytic activity was obtained at 60 °C for free enzyme and 50 °C for immobilized enzyme. After optimum temperature, the stability of free enzyme reduces rapidly compared to those of immobilized form with the temperature increases. The immobilized pectinesterase retained 35% of its optimum activity whereas the free pectinesterase was 85% at 70 °C. The free and immobilized pectinesterases retained 40% and 30% of their optimum activities at 80 °C. Although the optimum temperature of free enzyme is higher than those of immobilized enzyme, immobilized enzyme is more thermostable than free one. The immobilization procedure probably helps to maintain the oligomeric forms (mainly octameric and hexameric aggregates) of the enzyme prevailing in the free pectinesterase enzyme [30]. The stability of immobilized pectinesterase is significantly improved over those of free form at lower and higher temperatures.

4.3. Thermal stability of free and immobilized apricot PE

It has been reported that the thermal stability of many enzymes was increased after its immobilization on a support because the support material is supposed to preserve the tertiary structure of the enzyme. In addition, it is pointed out that the thermal stability of an enzyme may indicate the efficiency of the immobilization method and reflect the delicate balance between the acquired conformational stability and the resulting microenvironment created around the enzyme [28]. The authors demonstrated that the thermal stability of enzymes might be drastically increased if they are attached to a complementary surface of a relatively rigid support in a multipoint [31,32].

Thermal stability experiments were carried out with free and immobilized enzyme, samples of which were incubated in the absence of substrate at various temperatures. Figs. 4 and 5 show the heat inactivation curves between 70 and 90 °C for immobilized and free pectinesterase, respectively. During a 45 min incubation period, at 70 °C, free and immobilized enzyme retained 30% and 40% activity, respectively. Free and immobilized pectinesterase enzymes were lost about 95% and 80% of the original activity at 90 °C for 45 min, respectively. At all temperatures, the immobilized enzyme inactivated at a much slower rate than the free form. Free enzyme lost on a large scale their initial activity at 90 °C after 45 min. The data obtained from the thermal stability profile were used to analyze some thermodynamic parameters related to apricot PE activity.

The thermal inactivation rate constants k were calculated from the slope of the linear part of the curve at each temperature. The temperature dependence of k was evaluated by using the Arrhenius equation. From a plot of $\ln k - 1/T$ (Fig. 6), E_a (activation energy) was calculated from the slope of the straight line and found to be 0.9 kcal mol^{-1}.

The thermal inactivation rate constants for free and immobilized enzyme were determined from the percentage residual activity versus time, at three different temperatures; the results are presented in Table 3. The thermal inactivation rate constants (k) for free and immobilized PE at 70 °C were 2.55×10^{-3} and 2.46×10^{-3} min^{-1}, respectively. The inactivation rate constant of the free enzyme is bigger than those of immobilized one while the half-life of the immobilized PE is longer than free one (Table 3). Being the inactivation rate constant higher means thermostability of an

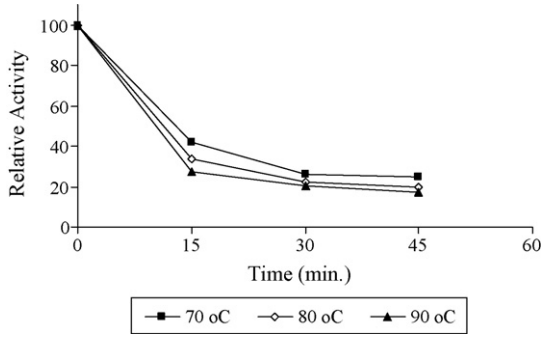

Fig. 4. The thermal stability profile for immobilized apricot PE. Reactions were made with 1% pectin concentration and 0.05 g immobilized enzyme dissolved with 0.5 mL 0.2 M sodium phosphate buffer (pH 7.5) at pH 9.0.

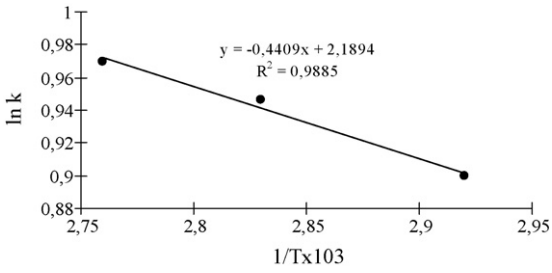

Fig. 6. Arrhenius plot for heat inactivation of immobilized apricot PE.

Table 3
Inactivation rate constants of free and immobilized apricot PE

Temperature (°C)	Inactivation rate constant, $k(\times 10^3)$		$t_{1/2}$ (ln 2/k) (min)	
	Free PE	Immobilized on porous glass beads	Free PE	Immobilized on porous glass beads
70	2.55 ± 0.2	2.46 ± 0.4	271.8 ± 0.08	281.7 ± 0.7
80	2.63 ± 0.07	2.59 ± 0.3	263.5 ± 0.9	267.6 ± 0.3
90	3.007 ± 0.4	2.65 ± 0.09	230.5 ± 0.5	261.5 ± 0.6

Table 4
Properties of free and immobilized apricot pectinesterase on porous glass beads at 20 ± 5 °C

Enzyme	K_m (mM)	V_{max} (μmol min^{-1} mg^{-1})
Free	0.77	1.75
Immobilized	0.71	0.64

enzyme less [33]. These results suggest that the thermostability of immobilized enzyme increased after its covalent immobilization on porous glass beads. The activity of the immobilized enzyme, especially in a covalently bound system is more resistant to heat and denaturing agents than that of the soluble form. If the thermal stability of an enzyme were increased some more by immobilization, the potential utility of such enzymes would be extensive [28].

4.4. Enzyme kinetic studies

The kinetic constants (K_m and V_{max} values) for free and immobilized pectinesterase enzyme were determined by using pectin as a substrate at 20 ± 5 °C (Table 4).

K_m and V_{max} values of both enzymes were calculated from the intercepts on x and y axes of the Lineweaver–Burk plots for the free and immobilized PE, respectively. For the free enzyme K_m was 0.77 mM and the apparent K_m value of covalently immobilized pectinesterase was 0.71 mM. Both K_m values were almost equal. For the free enzyme V_{max} was 1.75 U μmol min^{-1} mg^{-1}, but upon covalent immobilization of the enzyme on porous glass beads V_{max} decreased a little to 0.64 μmol min^{-1} mg^{-1}.

It may be possible that immobilization of the enzyme also occurred inside the porous space of glass beads, increasing mass transfer resistance of substrate and product. During the covalent immobilization, structural changes in the enzyme molecule procedure can occur and cause the change in the kinetic parameters of the immobilized enzyme [28–34]. In any case, immobilized pectinesterase showed lower specificity constants compared to its free counterpart.

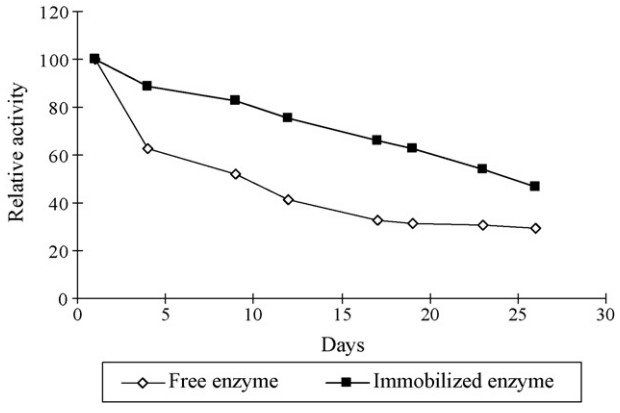

Fig. 7. Storage stability of free and immobilized PE from Malatya apricot pulp at 4 °C.

4.5. Storage stability studies

The storage stability of free and immobilized enzymes, the enzyme preparations were stored at 4 °C and measured for a period of 30 days. Results are shown in Fig. 7. During the first 5 days, the activity lost of 40% and 15% for free and immobilized apricot PE enzymes were observed, respectively. Activities are decreased slowly after 10 days and continued to decrease until day 30. The free enzyme lost about 75% of all its initial activity while the immobilized enzyme retained about 50% of its initial activity after 30 days. The porous glass beads and the immobilization method provide higher shelf-life compared to that of free enzyme since the covalent bonds formed between enzyme and support enhance the conformational stability of the immobilized enzyme.

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