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Purification and Characterization of *Ocimum basilicum* L. Polyphenol Oxidase

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A partial characterization of polyphenol oxidase (PPO) activity in *Ocimum basilicum* L. is described. PPO in *O. basilicum* L. was extracted and purified through $(NH_4)_2SO_4$ precipitation, dialysis, and a Sepharose 4B−L-tyrosine-*p*-aminobenzoic acid affinity column. The samples obtained from $(NH_4)_2SO_4$ precipitation and dialysis were used for the characterization of PPO. At the end of purification by affinity chromatography, 11.5-fold purification was achived. The purified enzyme exhibited a clear single band on sodium dodecyl sulfate−polyacrylamide gel electrophoresis. The molecular mass of the enzyme was estimated to be ∼54 kDa. The contents of total phenolic and protein of *O. basilicum* L. extracts were determined. The total phenolic content of *O. basilicum* L. was determined spectrophotometrically according to the Folin−Ciocalteu procedure and was found to be 280 mg 100 g^{-1} on a fresh weight basis. The protein content was determined according to the Bradford method. The enzyme showed activity to 4-methylcatechol, catechol, and pyrogallol substrates, but not to tyrosine. Therefore, of these three substrates, 4-methylcatecol was the best substrate due to the highest V_{max}/K_m value, followed by pyrogallol and catechol. The optimum pH was at 6, 8, and 9 for 4-methylcatechol, catechol, and pyrogallol, respectively. The enzyme had an optimum temperature of 20, 40, and 50 °C for 4-methylcatechol, catechol, and pyrogallol, respectively. It was found that optimum temperature and pH were dependent on the substrates studied. The enzyme activity with increasing temperature and inactivation time for 4-methylcatechol, catechol, and pyrogallol substrates decreased due to heat denaturation of the enzyme.

KEYWORDS: Polyphenol oxidase; *Ocimum basilicum* L.; purification; substrate specificity; pH; temperature; heat inactivation

INTRODUCTION

Surface discoloration, commonly called “browning” or “phenolic browning”, is a serious problem in marketing and storage. Tissue browning in fruits and vegetables damaged by mechanical injury during harvesting, postharvest storage, or processing is one of the main causes of quality loss and a great problem for the food industry. This reaction, which is mainly catalyzed by polyphenol oxidase (PPO), gives rise to the formation of *o*-quinones, which subsequently polymerize, leading to the appearance of brown pigments (*1*). Polyphenol oxidase (monophenol, dihydroxyphenylalanine:oxygen oxidoreductase, EC 1.14.18.1), also known and reported under various names (tyrosinase, phenolase, catechol oxidase, catecholase, monophenol oxidase, *o*-diphenol oxidase, and orthophenolase) based on substrate specificity, is widely distributed in plants and fungi (*2*, *3*).

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The presence of PPO in plant tissues is of concern to processors and researchers. PPO catalyzes the formation of highly active quinones that react with amino or sulfhydryl groups in proteins or enzymes. These reactions lead to changes in physical, chemical, or nutritional characteristics of proteins and, in many cases, to inactivation of enzymes including PPO (*2*). Quinones also lead to polymerization and condensation reactions between proteins and polyphenols, forming brown pigments. Quinones are very reactive compounds that strongly interact with other molecules, leading to a large variety of dark-colored compounds (*4*, *5*). Because of the importance of this reaction in the food industry, PPO has been intensively studied in several plant tissues such as aubergine (*6*), *Origanum* (*7*), apricot (*8*), *Thymus* species (*9*−*11*), *Salvia* (*12*), spinach (*13*), and tea leaves (*14*). PPO has been found to differ in isoforms, latency, catalytic behavior, molecular weight, isoeletric point, specificity, and hydrophobicity (*15*). There is no investigation related to polyphenol oxidase activity obtained from *Ocimum basilicum* L. in the literature.

O. basilicum L. is an important medicinal plant and culinary herb. The common or sweet basil (*O. basilicum* L.) is one of

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Table 1. Total Phenolic Contents of Some Vegetables

vegetable	protein content (mg)	ref
fresh turmeric	176	Kaur and Kapoor (*30*)
broccoli	88	
tomato	68	
yam	92	
mint	400	
black carrots	350	
aonla	349	
beet root	323	
artichoke	425	Doğan et al. (*26*)

several aromatic herbs or seed plants, the basils, belonging to the genus *Ocimum* and the mint family Lamiaceae. Species of Lamiaceae are valued for their pharmaceutical properties; for example, the aromatic oils produced in their leaves are used as antioxidants (*16*). It is called as "fesleğen" in Turkey. Fesleğen is widely cultivated for the production of essential oils and is also marketed as an herb, either fresh, dried, or frozen. The essential oil of *O. basilicum* L. possesses antifungal, insect-repelling, and toxic activities (*17*). Sweet basil is grown commercially as a cultivated herb plant in the United States, the Mediterranean region, and many other parts of the world. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food. A number of phenolic compounds with strong anti-oxidant activity have been identified in these plant extracts (*18*).

The aim of this paper was to (i) partially purifiy, kinetically characterize, and determine the molecular mass of *O. basilicum* L. PPO and (ii) to determine the protein and phenolic compound contents of *O. basilicum* L. Therefore, characterization of PPO from *O. basilicum* L. was studied in terms of substrate specificity, optimum pH and temperature, and thermal inactivation in order to help to predict the behavior of the *Ocimum* PPO.

MATERIALS AND METHODS

Materials. *O. basilicum* L. used in this study was harvested directly from a local garden in İzmir and Aydin cities (Turkey) and stored at −70 °C until processed. The chemicals used were obtained from Sigma Chemical Co. and Merck and were of the best grade available. Enzyme assays were measured with the aid of a Cary |1E|g UV−visible spectrophotometer (Varian).

Extraction of PPO. The extraction procedure was adapted from that of Wesche-Ebeling and Montgomery (*19*). *O. basilicum* L. (10 g) was placed in a Dewar flask under liquid nitrogen for 10 min to rupture cell membranes. A 10 g sample was homogenized using a Waring blender for 2 min in 100 mL of 0.1 M phosphate buffer (pH 6.5) containing 10 mM ascorbic acid and 5% poly(ethylene glycol). The 0.1 M concentration was chosen to avoid the influence of enzymatic extract ionic strength on PPO activity, as described by Angleton and Flurkey (*20*). The crude extract was filtered, and the filtrate was centrifuged at 15000*g* for 30 min at 4 °C. The supernatant was brought to 80% $(NH_4)_2SO_4$ saturation with solid $(NH_4)_2SO_4$. Inactive proteins were partially removed by ammonium sulfate precipitation. The precipitated PPO was separated by centrifugation at 15000*g* for 60 min at 4 °C. The pellet then was dissolved in a small volume of 0.1 M phosphate buffer (pH 7.0) and dialyzed at 4 °C in the same buffer for 3 days with three changes of buffer to remove excess ammonium sulfate ions. The dialyzed sample was used as the PPO enzyme source in the following experiments (*11*). After dialysis, the active fraction was purifed with affinity chromatography. The gel used was synthesized according to the method of Arslan and Erzengin (*21*). The purification procedures are summarized in **Table 1** for *O. basilicum* L. PPO. As seen in **Table 1**, finally, PPO was purified up to 11.5-fold for *O. basilicum* L.

Electrophoresis and Molecular Mass Determination. Affinity chromatography was done according to the method of Arslan and Erzengin (*21*). In this method, PPO was purified from *O. basilicum* L. on a Sepharose 4B−L-tyrosine−*p*-aminobenzoic acid affinity column. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the method of Laemmli (*22*). SDS-PAGE was carried out using an SDS marker protein kit as standard (MBI Fermentas). Samples were applied to 10% polyacrylamide gels. The slab gels of 1.5 mm thickness were run at a constant current of 180 mV. Gels were stained for protein using a standard Coomassie Blue method. The molecular mass of the purified enzyme was determined by SDS-PAGE.

Determination of Total Phenolic Compound Content. Total phenolics were determined using the Folin−Ciocalteu reagent (*23*). Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in the cold at 10000*g* for 15 min, and the supernatant was saved. The residue was re-extracted twice with 80% ethanol, and the supernatants were pooled, put into evaporating dishes, and evaporated to dryness at room temperature. The residue was dissolved in 5 mL of distilled water. One hundred microliters of this extract was diluted to 3 mL of the water, and 0.5 mL of Folin−Ciocalteu reagent was added. After 3 min, 2 mL of the 20% sodium carbonate was added, and the contents were mixed thoroughly. The color was developed and the absorbance measured at 650 nm in a Carry |1E|g UV−visible spectrophotometer after 60 min using catechol as a standard. The result was expressed as milligrams of catechol per 100 g of fresh weight material.

Determination of Protein Content. Protein content was determined according to Bradford's method using bovine serum albumin as standard (*24*).

Assay of PPO Activity. The enzyme activity was determined by monitoring the formation of quinones spectrophotometrically with time and at constant temperature. The desired temperatures were provided by using a Tempette Junior TE-85 temperature controller attached to the cell holder of the spectrophotometer. The activity was determined, using different substrates, by measuring the increase in absorbance at 420 nm for 4-methylcatechol and catechol, at 484 nm for tyrosine, and at 320 nm for pyrogallol with a spectrophotometer, respectively. Typically, assay mixtures (3 mL total volume) contained the substrate, phosphate buffer, and enzymatic extract solutions. Reference cuvettes contained all of the components except the enzyme extract, with a final volume of 3 mL. The reaction was initiated by adding aliquots of enzymatic extract in the assay medium. The oxidation of phenolic substrate was followed by monitoring the increase in absorbance resulting from the oxidation of substrate in the presence of oxygen. Activity was determined by measuring the maximal slope from the linear part of the curve. Unless otherwise indicated, PPO was added last. Enzyme activity data are averages of duplicate measurements. Monophenolase activity was determined using tyrosine as a substrate. Diphenol oxidase activity was determined using 4-methylcatechol (0.42−3.33 mM), catechol (5.0−20.0 mM), and pyrogallol (1.67−8.33mM) as substrates in 0.1 M sodium phosphate buffer (pH 6.5). One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per minute for 1 mL of enzyme at 25 °C (*6*).

Enzyme Kinetics and Substrate Specificity. The catalytic activity of the enzyme preparation was measured as a function of the substrate. PPO activity was determined by using 4-methylcatechol, catechol, L-tyrosine, and pyrogallol as substrates, and the rate of the PPO reaction was measured at various substrate concentrations in the standard reaction mixture in terms of the increase in absorbance at the wavelength of maximum absorption for the corresponding chromophore. A graphical evaluation of the results was obtained by inserting the data into the Michaelis−Menten equation. To obtain an equation of a straight line and a more reliable determination of V_{max} and K_m, the Michaelis−Menten equation was transformed into the double-reciprocal form (the Lineweaver−Burk plot). Substrate specificity (V_{max}/K_m) was calculated by using the data obtained on a Lineweaver−Burk plot (*25*).

Table 2. Purification of *O. basilicum* L. PPO

type of extract	vol of extract (mL)	protein concn (μg mL^{-1})	activity (EU mL^{-1})	specific activity (EU μg^{-1} of protein)	purification fold
crude extract	75	693	3695	5.33	
$(NH_4)_2SO_4$ precipitation	12.2	2265	7619	3.36	0.6
dialysis	12.1	1831	9438	5.15	1.5
affinity	2.4	8.9	530	59.46	11.5

Effect of pH and Temperature. The pH and temperature optima of *O. basilicum* L. PPO were determined using 4-methylcatechol, catechol, and pyrogallol as substrates. All buffer concentrations were 0.1 M. The buffers used were 0.1 M acetate (pH 4.0−6.0) and 0.1 M phosphate (pH 6.0−9.0) adjusted with 0.1 M NaOH and HNO_3 (*26*). For determining the optimum temperature values of the enzyme, PPO activity was measured at different temperatures in the range of 10−60 °C. The effect of temperature on the activity of PPO was tested by heating the standard reaction solutions (buffer and substrate) to the appropriate temperatures before introduction of the enzyme. The desired temperatures were provided by using a Tempette Junior TE-85 temperature controller attached to the cell holder of the spectrophotometer. Once temperature equilibrium was reached, enzyme was added and the reaction was followed spectrophotometrically at constant temperature at given time intervals. The reaction mixture contained 0.6 mL of substrate, 2.3 mL of 0.1 M buffer solution, and 0.1 mL of enzyme solution. All assays were performed in duplicate (*26*).

Heat Inactivation of PPO. To determine the thermal stability of the PPO, the partially purified enzyme was studied at 35, 55, and 75 °C. For the study, 1 mL of enzyme solution in a test tube was incubated at the required temperature for fixed time intervals. At the end of the required time interval, the enzyme was cooled in an ice bath and brought to room temperature; 0.1 mL of heated enzyme extract was mixed with substrate and buffer, and residual PPO activity was determined spectrophotometrically. The percentage residual PPO activity was calculated by comparison with unheated enzyme (*26*).

RESULTS AND DISCUSSION

Total Phenolic Content. Phenolic compounds are important constituents of plants, vegetables, and fruits. They are easily degraded (e.g., by oxidation or hydrolysis) and may also form covalent products and non-covalent complexes with various types of molecules (*27*). The total phenolic content is generally a major contributing factor to the degree of browning and therefore plays a major role in the browning of *O. basilicum* L. This relationship has been demonstrated in a number of plants, and therefore total phenolic content has been used as a measure of browning potential in several crops such as apples and grapes (*28*, *29*). Browning appears to be a complex process involving several factors including substrate levels, enzyme activity, and the presence of ascorbic acid and other inhibitors or promoters influencing the browning reaction, in addition to tissue damage (*27*). We found that the level of total phenolic compounds in the *O. basilicum* L. extracts was ~280 mg per 100 g of fresh weight. The total phenolic contents of some vegetables have been given in **Table 1**. As seen in **Table 1**, the total phenolic contents of some vegetables such as fresh turmeric, broccoli, tomato, and yam (*30*) are lower than those obtained in *O. basilicum* L. On the other hand, the total phenolic contents of vegetables such as mint, black carrots, aonla, and beet root (*30*) and artichoke (*26*) were higher than those obtained in *O. basilicum* L. Generally, it can be said that *O. basilicum* L. has a rich phenolic compound content.

Molecular Mass Determination. Table 2 shows the purification procedure of *O. basilicum* L. PPO. As seen in **Table 2**, finally, PPO was purified up to 11.5-fold. The molecular mass of PPO was estimated on SDS-PAGE with a single band of 54 kDa (**Figure 1**). **Table 3** shows the molecular mass of PPO purified from various sources. Our results indicate that the molecular mass of *O. basilicum* L. was similar to those of *V. faba* L., sago palm, mushroom, artichoke, and *L. sativa* but different from those of tea leaf, sunflower seeds, apple, banana, cabbage, and field bean seed.

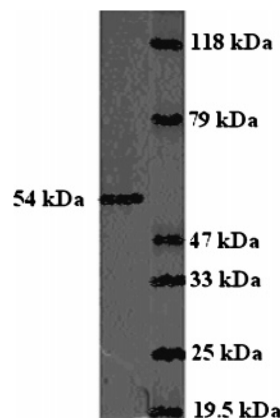

Figure 1. SDS-PAGE of *O. basilicum* L. PPO.

Table 3. Molecular Mass of PPOs Purified from Various Sources

source	molecular mass (kDa)	ref
Vicia faba L.	59	Lanker et al. (*38*)
sago palm	53	Onsa et al. (*39*)
mushroom	58	Espin et al. (*40*)
Lactuca sativa	56	Fujita and Tono (*41*)
tea leaf	72	Halder et al. (*14*)
sunflower seeds	42	Raymond et al. (*42*)
apple	65	Murata et al. (*43*, *44*)
banana	41; 62 ± 2	Galeazzi et al. (*45*)
cabbage	39	Fujita et al. (*46*)
field bean seed	120 ± 3	Paul and Gowda (*47*)
artichoke	57	Doğan et al. (*26*)

Table 4. Substrate Specificity of *O. basilicum* L. PPO

substrate	V_{max} (EU min^{-1})	K_m (mM)	V_{max}/K_m (EU min^{-1} mM^{-1})	optimum temp (°C)	optimum pH
catechol	6941	2.77	2500	40	8
4-Me-catechol	11586	1.62	7143	20	6
pyrogallol	10369	3.42	3030	50	9

Enzyme Kinetics and Substrate Specificity. Mono- and diphenolic substrates were tested for substrate specificity of *O. basilicum* L. PPO. The diphenolic substrates such as 4-methylcatechol, catechol, and pyrogallol were oxidized significantly by *O. basilicum* L. PPO, whereas the enzyme was unable to oxidize L-tyrosine, which is a monophenolic substrate. Some plant polyphenol oxidases, for example, mushroom, potato, and broadbean, catalyze both the hydroxylation of monophenols and the oxidation of *o*-diphenols. However, many PPOs lack monophenol activity (*31*). Substrate specificities clearly show that the observed activity is diphenolase as reported for PPOs from other plant sources such as artichoke (*6*, *8*, *26*). Substrate saturation curves for each diphenolic substrate indicated that *O. basilicum* L. PPO follows simple Michaelis−Menten kinetics. Regression coefficients are very close to 1 (**Table 4**). Lineweaver−Burk plots for the kinetic analysis of the reaction rates, at a series of concentrations for each substrate (**Figure 2**), resulted in individual V_{max} and K_m values (**Table 4**). Substrate specificities were evaluated by using the V_{max}/K_m ratio as

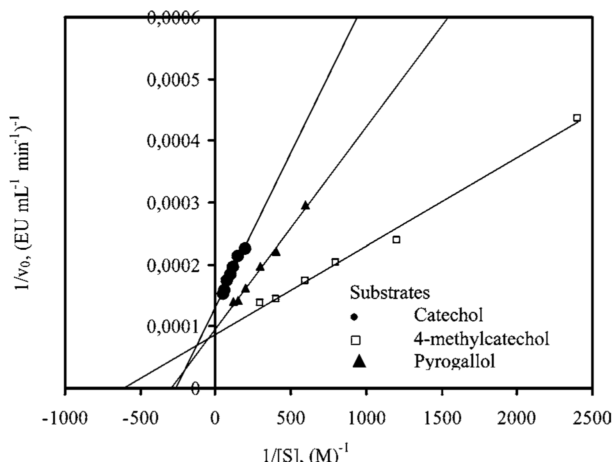

Figure 2. Lineweaver–Burk plots for *O. basilicum* L. PPO.

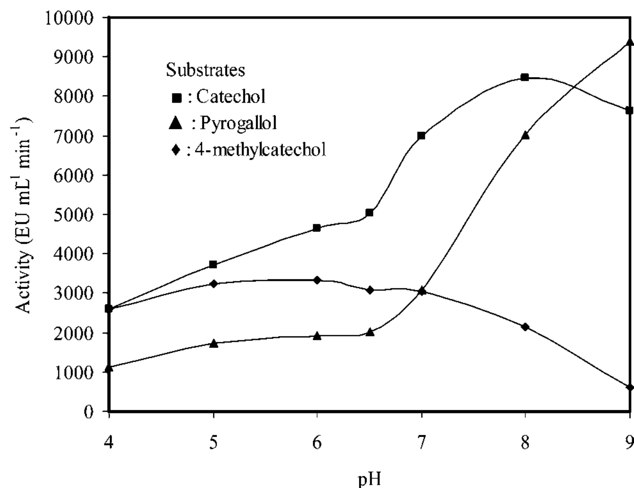

Figure 3. Effect of pH on PPO activity.

Table 5. K_m Values of PPO Obtained from Various Sources

source	substrate	K_m (mM)	ref
Amasya apple	4-Me-catechol	3.1	Oktay et al. (*48*)
strawberry		2.71	Wesche-Ebeling and Montgomery (*19*)
Starking apple		4.0	Rocha et al. (*49*)
Thymus		9.8	Doğan et al. (*9*)
artichoke		11.6	Doğan et al. (*26*)
Yali pear	catechol	5.5	Zhou and Feng (*50*)
apricot		6.6	Arslan et al. (*8*)
Amasya apple		34.0	Oktay et al. (*48*)
Starking apple		180.0	Rocha et al. (*49*)
Thymus		18.0	Doğan and Doğan (*11*)
cabbage		682.5	Nagai and Suzuki (*51*)
Stanley plum		20.0	Siddiq et al. (*52*)
artichoke	pyrogallol	5.2	Doğan et al. (*26*)
Thymus		5.5	Doğan and Doğan (*11*)
Yali pear		2.6	Zhou and Feng (*50*)
tea leaf		17.8	Halder et al. (*14*)
Amasya apple		27.0	Oktay et al. (*48*)
cabbage		15.4	Nagai and Suzuki (*51*)
spinach		15.7	Golbeck and Cammarata (*13*)

Table 6. Optimum pH Values for PPO Obtained from Various Sources

source	substrate	optimum pH	ref
strawberry	4-Me-catechol	4.5	Wesche-Ebeling and Montgomery (*19*)
aubergine		6.0	Doğan et al. (*6*)
dog-rose		8.5	Şakiroğlu et al. (*36*)
artichoke		5.0	Doğan et al. (*26*)
dog-rose	catechol	7.0	Şakiroğlu et al. (*36*)
Amasya apple		8.6	Oktay et al. (*48*)
artichoke		8.0	Doğan et al. (*26*)
strawberry	pyrogallol	5.5	Wesche-Ebeling and Montgomery (*19*)
DeChaunac grape		6.0	Lee et al. (*53*)
Amasya apple		7.0	Oktay et al. (*48*)
Anethum graveolens L.		7.0	Arslan and Tozlu (*54*)
aubergine		7.0	Doğan et al. (*6*)
Allium sp.		7.5	Arslan et al. (*55*)
dog-rose		8.5	Şakiroğlu et al. (*36*)
artichoke		7.0	Doğan et al. (*26*)

catalytic efficiency (*11*). Substrate binding affinities were within the range of 1.62−3.42 mM for the substrates examined. **Table 5** shows the K_m values of PPO obtained from various sources. In this study, the values of K_m for PPO obtained from *O. basilicum* L. for the substrates assayed were similar to those of Amasya apple, strawberry, and Starking apple for 4-methylcatechol as a substrate; for Yali pear and apricot using catecol as a substrate; and for artichoke, *Thymus*, and Yali pear using pyrogallol as a substrate. The K_m value of *O. basilicum* L. PPO is lower than those of *Thymus* and artichoke with 4-methylcatechol as a substrate; of Amasya apple, Starking apple, *Thymus*, cabbage, and Stanley plum with catechol as a substrate; and of tea leaf, Amasya apple, cabbage, and spinach with pyrogallol as a substrate. 4-Methylcatechol was oxidized by *O. basilicum* L. PPO at the highest rate with a much lower K_m value. 4-Methylcatechol has a lower K_m value by ~2-fold compared with that when pyrogallol was used as substrate. 4-Methylcatechol was clearly a good substrate for the enzymes, but, surprisingly, catechol was a much poorer substrate. The catalytic efficiency values, V_{max}/K_m, indicated that 4-methylcatechol was the most suitable phenolic substrate for *O. basilicum* L. PPO (**Table 4**). Similar results were found for aubergine (*6*) and medlar fruits (*32*). These results are consistent with previous studies indicating that *o*-diphenols, such as 4-methylcatechol and catechol, are very efficient substrates for diphenolases from several sources (*6*, *7*, *32*).

pH Optimum. The pH value of the medium affects significantly the enzyme activity. The enzyme activity with increasing pH values reaches a maximum value and then drops to zero in the alkaline region. Optimum pH values for *O. basilicum* L. PPO were determined in pH ranges of 4−9. The pH optimum profile for *O. basilicum* L. PPO is given in **Figure 3**. Generally, although the enzyme is active over a large pH range, in the case of 4-methylcatechol, having a pH optimum of 6.0, the enzyme catalyzes the oxidation of catechol and pyrogallol over a wider pH range of 8 and 9 for each substrate, respectively. It seems that *O. basilicum* L. PPO interacts with 4-methylcatechol much more efficiently and catalyzes its oxidation at a much lower pH value than the other two substrates. The optimum pH, depending on the exraction method, the substrates used for assay, and the localization of the enzyme in the plant cell for PPO activity in the plants, differs among plant sources (*33*). Different optimum pH values for PPO obtained from vairous sources are reported in the literature (**Table 6**). It is clear, from previous papers, that the pH optimum for PPOs is highly dependent on the enzyme source and the nature of the substrate used (*34*).

Optimum Temperature. Temperature−activity profiles of *O. basilicum* L. PPO for different substrates are presented in **Figure 4**. It is clear that the enzyme possesses activity at

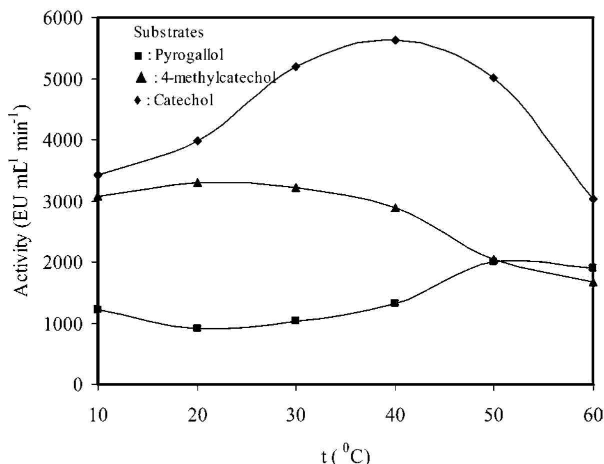

Figure 4. Effect of temperature on PPO activity.

temperatures ranging from 10 to 60 °C. The enzyme showed a temperature optimum at 20, 40, and 50 °C for 4-methylcatechol, catechol, and pyrogallol substrates, respectively. Similar temperature optima were reported for mulberry (*35*) and for dog-rose (*36*) using 4-methylcatechol as a substrate and for *Thymus* (*11*) using catechol as a substrate. It is clear that the optimum temperatures for PPO are substrate-dependent. It was previously reported that the greatest activity of medlar PPO was observed at 35 °C for 4-methylcatechol and at 55 °C for catechol (*32*) and that of dog-rose PPO at 25 °C for catechol (*36*).

Thermal Inactivation. *O. basilicum* L. PPO showed a typical temperature-dependent inactivation profile in the presence of 4-methylcatechol, catechol, and pyrogallol as substrates (**Figure 5**). The enzyme was incubated at different temperatures for 60 min at pH 6.5, and after cooling, the residual enzyme activity was measured using 4-methylcatechol, catechol, and pyrogallol as substrates. PPO showed similar behavior for 4-methylcatechol, catechol, and pyrogallol substrates. The enzyme activity decreased due to heat denaturation of the enzyme with increasing temperature and inactivation time for 4-methylcatechol, catechol, and pyrogallol substrates. The enzyme was quite stable at the examined time range at 35 °C, and it retained its activity at 35 °C within this period. The enzyme activity for three substrates dramatically decreased with time at 55 and 75 °C. Enzyme activity decreased to about 50% at the end of 30 min of incubation at 55 °C and to about 55, 80, and 70% for 4-methylcatechol, catechol, and pyrogallol substrates at 75 °C, respectively. This indicated that the enzyme was rapidly denatured at higher temperatures. The additional time of incubation affected the thermal stability of the enzyme at 55 and 75 °C. It is clear that at temperatures >55 °C, heat denaturation of the enzyme occurred with incubation time, and the diphenolase activities were reduced at higher inactivation temperatures because of heat denaturation of the enzyme or some conformational changes in the tertiary structure due to the increasing temperatures (*34*). It is obvious from these data that *O. basilicum* L. PPO is heat-sensitive. It has been previously reported that heat sensitivity of PPOs may depend on ripeness of the plant or different molecular forms of enzyme from the same plant source (*37*).

Conclusions. The characterization and purification of *O. basilicum* L. PPO is reported for the first time. *O. basilicum* L. had a rich phenolic compound content. PPO from *O. basilicum* L. was purified to 11.5-fold to apparent homogeneity using a three-step procedure. The enzyme existed as a single isoform of M_r 54 kDa. We found that *O. basilicum* L. PPO was a diphenol oxidase and that the most effective substrate was

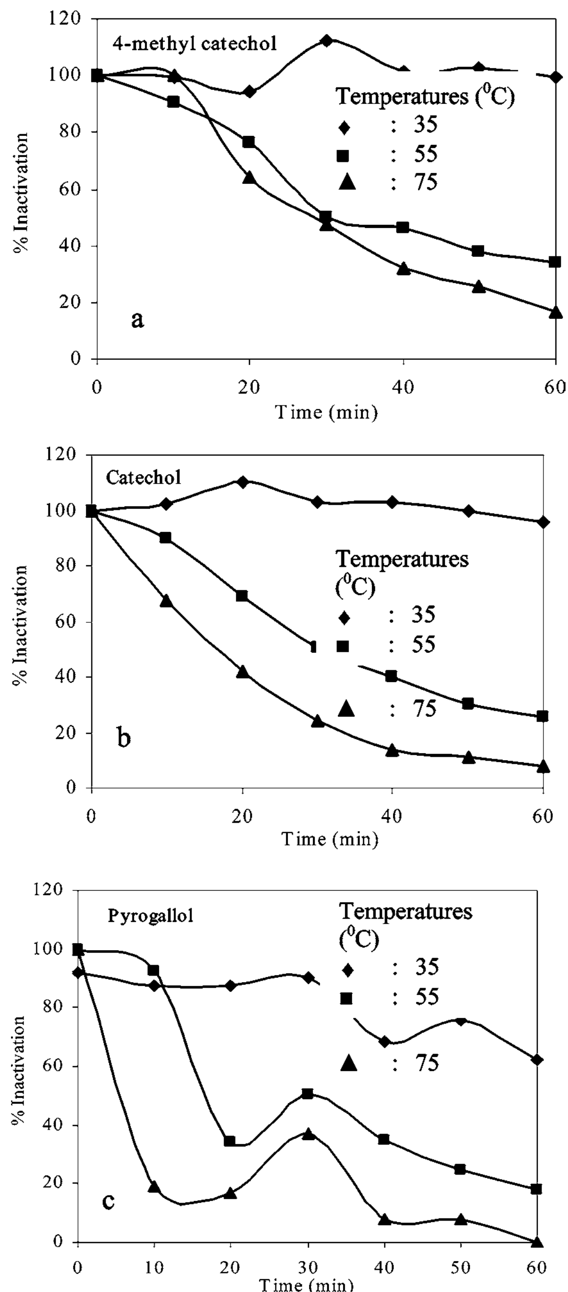

Figure 5. Change of PPO activity as a function of temperature and time.

4-methylcatechol, followed by pyrogallol and catechol. The Michaelis−Menten constants, K_m, for 4-methylcatecol, catechol, and pyrogallol were 1.62, 2.77, and 3.42 mM, respectively. Apparent pH optima were 6.0 for 4-methylcatechol, 8.0 for catechol, and 9.0 for pyrogallol as substrates. The enzyme showed a temperature optimum at 20, 40, and 50 °C for 4-methylcatechol, catechol, and pyrogallol substrate, respectively. The enzyme activity decreased due to heat denaturation of the enzyme with increasing temperature and inactivation time.

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