Polyphenol Oxidase from Malatya Apricot (*Prunus armeniaca* L.)

Oktay Arslan,*,† Arzu Temur,‡ and İsrafil Tozlu‡

Science and Art Faculty, Sakarya University, Adapazarı, Turkey, and Science and Art Faculty, Yüzüncü Yıl University, Van, Turkey

Polyphenol oxidase (PPO) of Malatya apricot was isolated by $(NH_4)_2SO_4$ precipitation and dialysis. PPO showed activity to catechol, L-dopa, and gallic acid. Catechol was the most suitable substrate for Malatya apricot PPO (lowest K_m value). The optimum pH for the PPO was 8.5. Heating for 40 min at 40 °C did not cause a significant loss of enzymic activity. The times required for 50% inactivation of activitiy at 60 and 80 °C were found to be 47 and 16 min, respectively. The I_{50} values of inhibitors studied on PPO were determined by means of activity percentage [I] diagrams. The values were 2.7×10^{-5}, 5.03×10^{-5}, 3.62×10^{-5}, 3.68×10^{-5}, and 8.30×10^{-4} M for sodium metabisulfite, ascorbic acid, 2-mercaptoethanol, thiourea, and salicylic acid, respectively. Ascorbic acid, 2-mercaptoethanol, sodium metabisulfite, and thiourea inhibited the reaction strongly.

Keywords: *Malatya apricot; Prunus armeniaca L.; polyphenol oxidase; inhibitors*

INTRODUCTION

The browning reaction during storage or processing of fruits and vegetables is a widespread phenomenon. In most cases, the browning reaction is considered detrimental to the quality of the product (Zhow and Feng, 1991). Malatya apricot is one of the best varieties of apricot grown in Turkey, but the fruits are prone to browning during storage. This is commercially undesirable. It is caused primarily by the oxidation of some phenolic substrates to corresponding quinones by polyphenol oxidase (PPO) in the presence of oxygen. It has been related to enzymic browning in several plant tissues (Zhow and Feng, 1991; Sharon and Khan, 1979; TanX, 1984; Galeazzi and Sgarbieri, 1981a,b; Roudsari et al., 1981; Augustin et al., 1985; Wissemann et al., 1985).

PPO from different plant tissues shows different substrate specificities and degrees of inhibition. Therefore, characterization of the enzyme could help to develop more effective methods in controlling browning of plants and products. Our objective was to isolate PPO from Malatya apricot and to characterize the enzyme at different pH values and temperatures. Substrate and inhibitor effects were also studied.

EXPERIMENTAL PROCEDURES

Materials. Malatya apricots (*Prunus armeniaca* L.) were harvested fresh from Malatya, Turkey. All chemicals were purchased from Sigma Chemical Co. Enzyme assays were conducted using an LKB UV−vis spectrophotometer.

Isolation of PPO. Malatya apricots (*P. armeniaca* L.) were harvested fresh (Malatya, Turkey) and stored at 4 °C. For preparing the crude extract, 20 g of fruit was homogenized in 100 mL of 0.5 M phosphate buffer (pH 7) containing 0.5% poly(ethylene glycol) and 10 mM ascorbic acid by using a Waring blender for 2 min. The crude extract was filtered, and the filtrate was centrifuged at 20000*g* for 30 min at 4 °C. The supernatant was brought to 80% $(NH_4)_2SO_4$ saturation with solid $(NH_4)_2SO_4$. The precipitated PPO was separated by centrifugation at 20000*g* for 30 min. The precipitate was dissolved in a small amount of 0.5 M phosphate buffer (pH 7) and dialyzed at 4 °C in the same buffer for 3 h with three changes of buffer during dialysis. The dialyzed sample was used as the PPO enzyme source in the following experiments.

Assay of PPO Activity. Enzyme activity was determined by measuring the increase in absorbance at 420 nm with a spectrophotometer. The sample cuvette contained 2.8 mL of substrate in various concentrations prepared in the homogenization buffer and 0.2 mL of the enzyme. The blank sample contained only 3 mL of substrate solution. Enzyme activity was calculated from the linear portion of the curve. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001/min (Oktay et al., 1995).

Effect of pH. The activity of the enzyme was determined in the pH range 3.5−10 by using 0.1 M phosphate and 0.1 M Tris-HCl buffers. The optimum pH for the PPO was obtained with catechol as substrate.

Heat Inactivation of PPO. The thermal denaturation of PPO was studied at 10, 40, 60, and 80 °C. For the study, 0.5 mL of enzyme in a test tube was incubated at the required temperature for fixed time intervals. At the end of the required time interval, the test tube was cooled by immersion in ice. The activity of the enzyme was then determined (Augustin et al., 1985).

Enzyme Kinetics and Substrate Specificity. Michaelis constant (K_m) and maximum velocity (V_{max}) were determined using four substrates (L-dopa, catechol, gallic acid, and L-tyrosine) in five different concentrations. The reaction was followed in a spectrophotometer, and data were plotted according to the method of Lineweaver and Burk (1934). To investigate substrate specificity, the same four compounds at various concentrations were tested as substrates for PPO.

Effect of Inhibitors. To determine effects of inhibitors, the reaction assay contained 0.3 mL of 0.1 M catechol, 0.1 mL of enzyme solution, 2.6 mL of 0.1 M phosphate buffer, and inhibitor solution at various concentrations. Inhibitors studied were ascorbic acid, sodium metabisulfite, thiourea, salicylic acid, and 2-mercaptoethanol. To determine the inhibitor concentration that reduced the enzyme activity by 50% (I_{50}), regression analysis graphs were drawn by using percent

* Address correspondence to this author at the Sakarya Universitesi, Fen-Edebiyat Fakultesi, Kimya Bölümü, 54100 Sakarya, Turkey (fax 00.90.264.2762059).

† Sakarya University.

‡ Yüzüncü Yıl University.

S0021-8561(97)00599-2 CCC: $15.00
Published on Web 03/04/1998

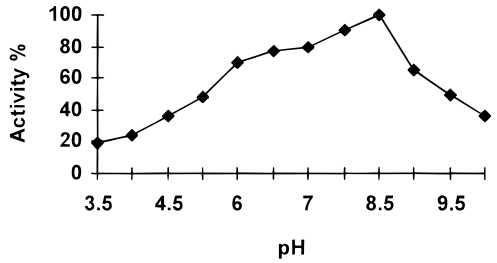

Figure 1. Effect of pH on the activity of PPO from apricot.

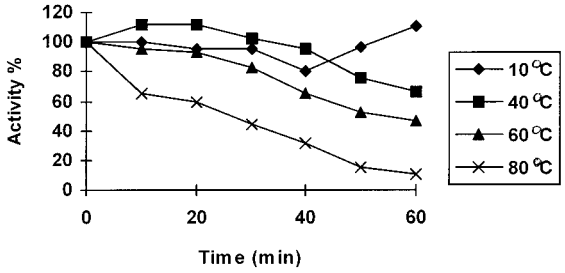

Figure 2. Heat inactivation of apricot PPO at various temperatures.

Table 1. Substrate Specificity and Kinetic Data of Apricot PPO

substrate	K_m (mM)	V_{max} (units/mL·min)
catechol	6.6 ± 0.47	833 ± 9.32
L-dopa	12.5 ± 5.5	500 ± 11.7
gallic acid	20 ± 2.03	1.5 ± 0.04

inhibition values by a statistical packing program on a computer. I_{50} values were determined from the graphs (Arslan et al., 1997).

RESULTS AND DISCUSSION

Effect of pH. The pH profile of the PPO was determined between pH 3.5 and 10 (Figure 1) with catechol as substrate. Only one maximum of pH 8.5 was observed within the pH range used. In general, most plants showed maximum activity at or near neutral pH values (Oktay et al., 1995). However, we found alkaline pH to be optimum for catechol. The optimum pH for PPO activity has been found to vary with the source of the enzyme and substrate in a relatively wide range of pH (Zhow and Feng, 1991).

Heat Inactivation of PPO. A typical thermolability profile of the apricot PPO is shown in Figure 2. Heating for 40 min at 40 °C did not cause a significant loss of enzymatic activity. The times required for 50% inactivation of activitiy at 60 and 80 °C were found to be 47 and 16 min, respectively. It has been noted that heat stability of the enzyme may be related to ripeness of the plant, and in some cases it is also dependent on pH. In addition, different molecular forms from the same source may have different thermostabilities (Park et al., 1985).

Enzyme Kinetics and Substrate Specificity. Malatya apricot PPO showed activity to catechol, L-dopa, and gallic acid (Table 1). The substrate with highest activity was catechol, followed by L-dopa and gallic acid. L-Tyrosine and resorcinol were also tested but were not oxidized by Malatya apricot PPO. The enzyme had no activity toward monophenols. These findinds are similar to those for Yali pear PPO (Roudsari et al., 1981), Bartlett pear PPO (Rivas and Whitaker, 1973), and kiwi fruit PPO (Park and Luh, 1985).

Table 2. Effects of Some Inhibitors on the Activity of PPO from *Anethum graveolens* L.

inhibitor	concn (M)	% inhibition	$I_{50} \times 10^{-5}$
sodium metabisulfite	8.30×10^{-6}	36	2.7
	1.66×10^{-5}	44	
	3.30×10^{-5}	50	
	6.60×10^{-5}	74	
ascorbic acid	1.66×10^{-5}	35	5.03
	3.30×10^{-5}	41	
	6.60×10^{-5}	48	
	8.30×10^{-5}	60	
2-mercaptoethanol	1.60×10^{-5}	36	3.62
	3.30×10^{-5}	41	
	5.00×10^{-5}	58	
	6.60×10^{-5}	83	
thiourea	1.60×10^{-5}	34	3.68
	3.30×10^{-5}	48	
	5.00×10^{-5}	54	
	6.63×10^{-5}	76	
salicylic acid	3.30×10^{-4}	24	83.7
	5.00×10^{-4}	30	
	6.60×10^{-4}	33	
	8.30×10^{-4}	50	

Effect of Inhibitors. Table 2 shows I_{50} values obtained with various compounds as inhibitors using catechol as substrate. The values were 2.7×10^{-5}, 5.03×10^{-5}, 3.62×10^{-5}, 3.68×10^{-5}, and 8.30×10^{-4} M for sodium metabisulfite, ascorbic acid, 2-mercaptoethanol, thiourea, and salicylic acid, respectively. Ascorbic acid, 2-mercaptoethanol, sodium metabisulfite, and thiourea inhibited the reaction strongly. The prevention of enzymic browning by a specific inhibitor may involve a single mechanism or be the result of an interplay of two or more mechanisms of inhibitor action (Roudsari et al., 1981). Ascorbic acid and 2-mercaptoethanol act by reducing the quinones formed by PPO to polyphenols while they are themselves oxidized.

LITERATURE CITED

Arslan, O.; Şekeroğlu, R.; Çelik, İ.; Tarakçı, M. The inhibition effects of some pesticides on the in vitro activity of five serum enzymes. *J. Environ. Sci. Health* **1997**, *32*, 361−365.

Augustin, M. A.; Hasanah, M. G.; Hasimah, H. Polyphenoloxidase from guava (*Psidium guajava* L.) *J. Sci. Food Agric.* **1985**, *36*, 1259−1265.

Ben-Sholom, N.; Khan, J.; Harel, E.; Mayer, A. W. Catechol oxidase from green olives: properties and purification. *Phytochemistry* **1977**, *16*, 1153−1158.

Galeazzi, M. A. M.; Sgarbieri, V. C. Isolation, purification and physicochemical characterization of polyphenoloxidase from a dwarf variety of banana (*Musa cavendishii* L.). *J. Food Sci.* **1981a**, *46*, 150.

Galeazzi, M. A. M.; Sgarbieri, V. C. Substrate specifitiy and inhibition of pholyphenoloxidase from a dwarf variety of banana (*Musa cavendishii* L.) *J. Food Sci.* **1981**b, *46*, 1404−1406.

Lineweaver, H.; Burk, D. The determination of enzyme dissociation constants *J. Am. Chem. Soc.* **1934**, *56*, 685−693.

Matheis, G. Enzymatic Browning of Foods. *Z. Lebensm Unters. Forsch.* **1983**, *176*, 454−462.

Mayer, A. M. Polyphenoloxidases in Plants−Recent Progress. *Phytochemistry* **1987**, *26*, 11−20.

Oktay, M.; Küfrevioğlu, Ö. İ.; Kocaçalışkan, İ.; Şakiroğlu, H. Polyphenoloxidase from Amasya Apple. *J. Food Sci.* **1995**, *60*, 495−499.

Park, E. Y.; Luh, B. S. Polyphenoloxidase of kiwifruit. *J. Food Sci.* **1985**, *50*, 679−684.

Rivas, N. J.; Whitaker, J. R. Purification and some properties of two polyphenoloxidase from Bartlett pears. *Plant Physiol.* **1973**, *52*, 501−507.

Roudsari, M. H.; Signoset, A.; Crovzet, J. Epplant Polyphenoloxidase: purification, characterization, and properties. *Food Chem*. **1981**, *7*, 227−235.

Sharon, O.; Khan, V. Browning potential, PPO catalase and acid phospates activities during ripenning of nonchilled and chilled Avocado. *J. Sci. Food Agric.* **1979**, *30*, 634−638.

TanX, L. P. Y. The partial purification and properties of pholyphenoloxidase from the pericarp of litchi (*Litchi chinensis*). *Acta Phytophysiol. Sin*. **1984**, *10*, 339−345.

Wissemann, K. W. Purification of d'Anjou Pear (*Pyrus communis* L.) Polyphenol Oxidase. *Plant Physiol.* **1985**, *78*, 256.

Zhow, H.; Feng, X. Polyphenoloxidase from Yali pear (*Pyrus bretschneideri*). *J. Sci. Food Agric.* **1991**, *57*, 307−313.

Received for review July 11, 1997. Revised manuscript received January 6, 1998. Accepted January 6, 1998.

JF970599V