ELSEVIER

Food Chemistry 85 (2004) 431–437

Food Chemistry

www.elsevier.com/locate/foodchem

A diphenolase from persimmon fruits (*Diospyros kaki* L., Ebenaceae)

Arzu Özen, Ahmet Colak, Barbaros Dincer, Saadettin Güner*

Department of Chemistry, Karadeniz Technical University, 61080 Trabzon, Turkey

Received 23 May 2003; accepted 21 July 2003

Abstract

A diphenolase from persimmon fruits (*Diospyros kaki* L.), active against catechol, 4-methylcatechol, *L*-3,4-dihydroxyphenylalanine and 3-(3,4-dihydroxyphenyl)propionic acid, was characterized in detail in terms of pH and temperature optima, stability, kinetic parameters and inhibition behaviour toward some general PPO inhibitors. The substrate specificity of the persimmon PPO was very high for catechol and 4-methylcatechol. The enzyme was extremely stable at its optimum pH of 7.5 in the presence of 4-methylcatechol as the substrate and it retained over 90% of its original PPO activity after 24 h of incubation at 4 °C at that pH. Thermal properties of the persimmon enzyme, together with thermodynamic parameters, show that the persimmon enzyme is very heat-sensitive. Ascorbic acid, metabisulfite, azide and benzoic acid all inhibited the 4-methylcatechol oxidation by persimmon PPO, indicating its sensitivity toward the general PPO inhibitors, especially metabisulfite. All the data support the presence of a highly active diphenolase in persimmon fruits (*Diospyros kaki* L.) having similar properties to other plant polyphenoloxidases.

Keywords: *Diospyros kaki*; Diphenolase; Polyphenoloxidase; Persimmon; Metabisulfite; Ebenaceae

1. Introduction

Polyphenol oxidases (PPO) are a group of copper-proteins, widely distributed phylogenetically from bacteria to mammals (Robb, 1984); in plants, these enzymes are responsible for the enzymatic browning reaction occurring during the handling, storage and processing of fruits and vegetables. PPOs catalyze two different reactions in the presence of oxygen: the hydoxylation of monophenols to *o*-diphenols (monophenolase activity) and the oxidation of *o*-diphenols to *o*-quinones (diphenolase activity). In plant tissues, the quinones are then polymerized to brown, red or black pigments (Zawistowski, Biliaderis, & Eskin, 1991) which lead to organoleptic and nutritional modifications and diminish food product quality (Friedman, 1996).

Persimmon (*Diospyros kaki* L.) is a member of the Ebenaceae family, native to China and Japan and cultivated throughout North-Eastern Turkey for its edible fruits, known as Japanese persimmon locally, and as Trabzon persimmon in Turkey (Yaltirik, 1972). In addition to the nutritional value, persimmon fruit (*Diospyros kaki* L.) is a good source of calcium, potassium and vitamin C (Mowat, 1990). The fruit is characterized by its high level of tannic acid (tannins), which disappears when the fruits are very ripe. Persimmons can be astringent because of soluble tannins contained in the fruits. These compounds are a group of phenolic substrates of PPO, containing gallic acid derivatives and glucose units linked together via glycosidic bonds.

Persimmon is also rich in antioxidant phenolic compounds other than tannins, and it has been demostrated that these compounds may reduce the risk of chronic diseases by protecting tissues against free radical-mediated damage (Gorinstein et al., 1994). It has been reported that persimmon seed extracts have a strong radical-scavenging activity (Ahn, Jeon, Lee, Hwang, Lim, & Park, 2002). Moreover, tannins have been described to have antimutagenic, anticarcinogenic and antioxidant activities (Gali, Perchellet, Klish, Johnson, & Perchellet, 1992; Mukhtar, Khan, Wang, Bik, & Bickers, 1988).

In this work, characterization of PPO from persimmon fruit was studied in terms of substrate specificities,

* Corresponding author. Tel.: +90-462-3772598; fax: +90-462-3253196.

E-mail address: sguner@ktu.edu.tr (S. Güner).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.07.022

thermal activation and stability, pH optimum and stability, and degrees of inhibition by general PPO inhibitors, in order to help predict the behaviour of the persimmon enzyme.

2. Materials and methods

2.1. Plant materials and chemicals

Fresh persimmon fruits were harvested directly from a local garden in Trabzon (Turkey) in December. The fruits were carried into the laboratory in liquid nitrogen, with a nitrogen Dewar flask, and stored deep-frozen at −20 °C for 1–2 months until used.

Subtrates and 3-methyl-2-benzothiazolinone hydrazone (MBTH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the other reagents were of analytical grade and used as obtained.

2.2. Crude polyphenoloxidase preparation

Persimmon fruits (50 g) were placed in a Dewar flask under liquid nitrogen for 10 min, in order to decompose cell membranes. The cold fruits were homogenized by using a blender in 50 ml of 50 mM cold sodium phosphate buffer (pH 7.5), containing 2 mM EDTA, 1mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 6% (w/v) Triton X-114, for 2 min at 4 °C. The homogenate was filtered and kept at 4 °C for 60 min before being centrifuged at 17,000 rpm for 30 min at 4 °C. The supernatant was used as crude enzyme extract which retained PPO activity for at least 1 month at 4 °C.

2.3. Protein determination

Protein content in the enzyme extracts was determined according to the Lowry method with bovine serum albumin as a standard (Lowry, Rosebrough, Farr, & Randall, 1951). The values were obtained by graphic interpolation on a calibration curve at 650 nm and it was found that the crude extracts had an average of 10.4±3.1 mg/ml protein.

2.4. Assay of polyphenol oxidase activity

PPO activity was assayed by measuring the rate of increase in absorbance at a given wavelength using a double beam model ATI Unicam UV2-100 spectrophotometer, as described previously (Dincer, Colak, Aydin, Kadioglu, & Güner, 2002). The activity was determined, using different substrates, by measuring the increase in absorbance at 494 nm for 4-methylcatechol and 500 nm for all other substrates (Espin, Morales, Varon, Tudela, & Garcia-Canovas, 1995). The reaction mixture had various concentrations of substrates (stock 100 mM), an equal volume of 3-methyl-2-benzothiazolinone hydrazone (MBTH, stock 100 mM), and 20 μl dimethylformamide (DMF), and the solution was diluted to 950 μl with buffer and 50 μl enzyme extract were added. One unit of PPO activity was defined as the amount of enzyme causing 0.001 increase of absorbance per minute in 1 ml reaction mixture (Galeazzi & Sgarbieri, 1981).

2.5. Properties of persimmon PPO

2.5.1. pH optimum and stability

PPO activity, as a function of pH, was determined in a pH range of 4.5–5.5 in 50 mM acetate buffer, 6.5–7.5 in 50 mM phosphate buffer and 8.5–9.5 in 50 mM Tris–HCl buffer. PPO activity was assayed using catechol, 4-methylcatechol, *L*-3,4-dihydroxyphenylalanine (*L*-DOPA), 3 - (3,4 - dihydroxyphenyl)propionic acid (DHPPA) as diphenolic substrates and *L*-tyrosine as a monophenolic substrate. The optimum pH obtained for all substrates was used for determining substrate specificies, thermal properties, and other parameters.

The pH stability was determined by incubating 0.05 ml of crude enzyme solution in 0.73 ml buffer solution, ranging from pH 4.5–5.5 in 50 mM acetate buffer, 6.5–7.5 in 50 mM phosphate buffer, and 8.5–9.5 in 50 mM Tris–HCl buffer for 24 h at 4 °C. Residual PPO activity was determined in the form of percent residual PPO activity at the optimum pH by mixing 0.1 ml of 100 mM 4-methylcatechol as a substrate, 0.1 ml of 10 mM MBTH and 0.02 ml DMF with the incubated enzyme solution (Dincer et al., 2002).

2.5.2. Thermal activity and stability

For determining optimum temperatures for the persimmon enzyme, PPO activity was measured at various temperatures over the range of 10–80 °C, using a circulation water bath. The mixtures of buffer and each substrate solution were incubated for 5 min at the different temperatures indicated above, at the optimum pH values obtained for each substrate. The enzyme extract was added to the mixture and the relative activity of PPO was determined spectrophotometrically at 494 nm as rapidly as possible.

In order to determine the thermal stability of the PPO, the enzyme solution in 50 mM phosphate buffer, pH 7.5, within Eppendorf tubes, was incubated in a water bath at various temperatures of 20–75 °C with 5 °C increments for 30 min. After the mixture was cooled in an ice bath and brought to room temperature, 0.05 ml heated enzyme extract was mixed with 0.1 ml of 100 mM 4-methylcatechol, 0.1 ml of 10 mM MBTH, and 0.02 ml DMF, and residual PPO activity was determined spectrophotometrically. The percentage residual PPO activity was calculated by comparison with unheated enzyme.

The data obtained from the thermal stability profile have been used to analyze some thermodynamic parameters related to persimmon PPO activity in the crude extracts. The rate constant for the thermal inactivation reaction (and its temperature-dependence) was calculated by comparing the activity changes upon heat treatment with the unheated enzyme extract, as reported (Amiza & Apenten, 1994; Dincer et al., 2002; Duangmal & Owusu Apenten, 1999).

2.5.3. Enzyme kinetics and substrate specificity

PPO activity was determined by using catechol, 4-methylcatechol, *L*-tyrosine, *L*-DOPA and DHPPA with MBTH (Espin et al., 1995) in buffers at optimum pH values for each substrate 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 (Espin, Trujano, Tudela, & Garcia-Canovas, 1997).

For each substrate, the kinetic data were plotted as reciprocals of activities versus substrate concentrations. The Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) were determined as the reciprocal absolute values of the intercepts on the *x*- and *y*-axes, respectively, of the linear regression curve (Lineweaver & Burk, 1934). Substrate specificity (V_{max}/K_m) was calculated by using the data obtained on a Lineweaver-Burk plot.

2.5.4. Effect of inhibitors

Sodium azide (10–50 mM), benzoic acid (1–4 mM), sodium metabisulfite (0.01–0.1 mM) and ascorbic acid (0.01–0.1 mM) were used as PPO inhibitors and the effects of inhibitors on persimmon PPO activity were determined by using 4-methylcatechol as a substrate. One milliliter of reaction mixture contained 0.1 ml of 4-methylcatechol at various concentrations in 50 mM phosphate buffer (pH 7.5), 0.05 ml enzyme solution and 0.1 ml of inhibitor solutions at fixed concentrations. I_{50} values were calculated from the plots of inhibitor concentration versus percentage inhibition of 4-methylcatechol oxidation, and inhibition constants (K_i) were deduced from the Dixon plots for each inhibitor.

2.5.5. Native polyacrylamide gel electrophoresis

Native polyacrylamide gel elecrophoresis was performed on a Hoeffer SE 600 Series Electrophoresis dual slab cell unit (California, USA), using preparative 12% polyacrylamide gels (Laemmli, 1970) under native conditions. After electrophoresis, the gels were stained for PPO activity in 24 mM *L*-DOPA in 50 mM sodium phosphate buffer (pH 7.5) at room temperature for 2 h, then in 1 mM ascorbic acid solution until appearance of isoenzyme bands.

3. Results and discussion

The persimmon polyphenoloxidase (PPO) activities were characterized on crude enzyme preparations extracted from persimmon fruits. Native electrophoresis resulted in two isoforms of persimmon PPO, having R_f values of 0.25 and 0.62, respectively, indicating the presence of two PPO isoenzymes in the *Diospyros kaki* L. (Fig. 1). Two to four PPO isoenzymes have been reported for different fruits, such as banana (Palmer, 1963), apple (Constantinides & Bedford, 1967), pear (Rivas & Whitaker, 1973), cherry (Pifferi & Cultrera, 1974), papaya (Cano, Lobo, de Ancos, & Galeazzi, 1996), dog rose (Sakiroglu, Küfrevioglu, Kocacaliskan, Oktay, & Onganer, 1996) and medlar (Dincer et al., 2002).

3.1. pH optimum and stability

The pH optimum profile for persimmon PPO was found to be bell-shaped for each substrate (Fig. 2). Although the enzyme is active at a narrow pH range in the case of 4-methylcatechol and DHPPA, having pH optimum of 7.5 and 5.5, respectively, the enzyme catalyzes the oxidation of catechol and *L*-DOPA over a wider pH range of 6.5–8.5 with a pH optimum of 7.5 for each substrate. It seems that the persimmon enzyme interacts with DHPPA much more efficiently and catalyzes its oxidation at much lower pH values than the

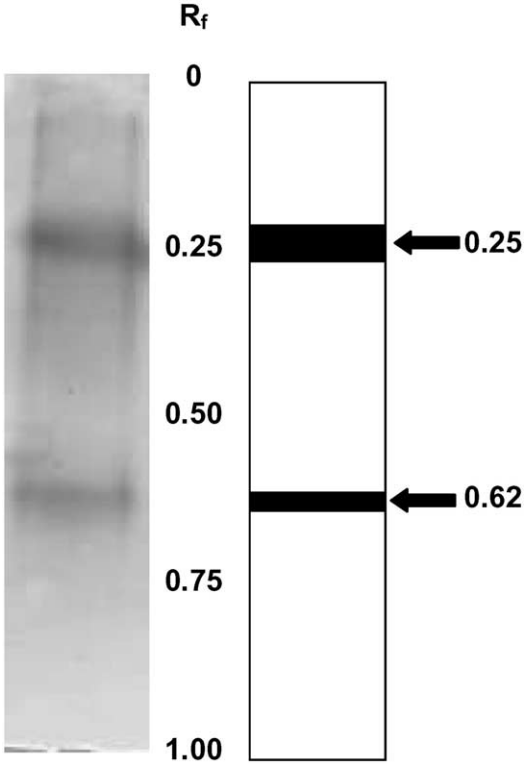

Fig. 1. Activity staining for PPO from persimmon fruit with *L*-DOPA.

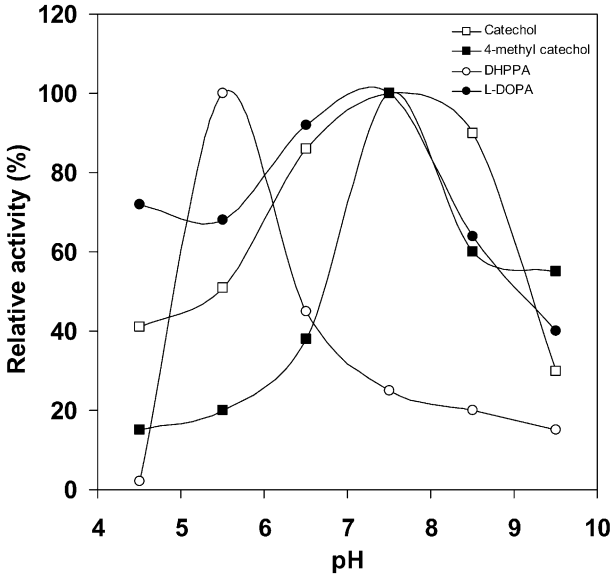

Fig. 2. pH-activity profile for persimmon PPO in 50 mM acetate buffer (pH 4.5–5.5), in 50 mM phosphate buffer (pH 6.5–7.5) and in 50 mM Tris-HCl buffer (pH 8.5–9.5).

other three substrates. The optimum pH, depending upon the substrate for PPO activity in the fruits, differs among plant sources. PPO activity was optimal for eggplant (Fujita & Tono, 1988) and cherry (Fraignier, Marques, Fleuriert, & Macheix, 1995) at pH 4.0–5.0, for banana pulp (Yang, Fujita, Ashrafuzzaman, Nakamura, & Hayashi, 2000) and for medlar PPO (Dincer et al., 2002) at pH 6.5, for Jonagored apple PPO at pH 5.0 and 7.5 (Rocha & Morais, 2000), and for apricot PPO at pH 7.0–8.5 (Arslan, Temur, & Tozlu, 1998).

The residual percentage activity of the enzyme from persimmon fruits for various pH values, from 4.5–9.5 in the presence of 4-methylcatechol as a substrate, is shown in Fig. 3. The pH-stability profile for the enzyme has shown that the PPO activity was fully retained at its pH optimum value. While 90% of the original PPO activity observed at acidic pH values was retained after 24 h of incubation at 4 °C, the enzyme activities decreased to below 70% of original values at basic pH values.

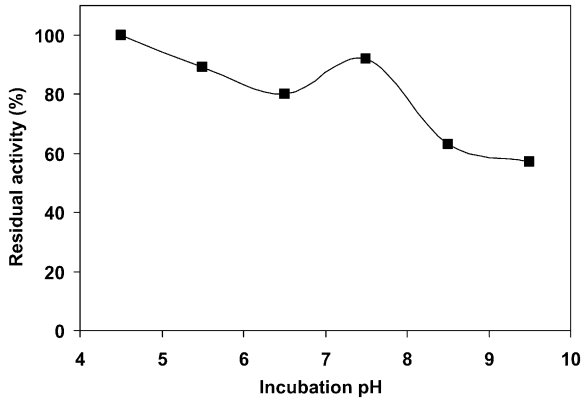

Fig. 3. pH stability of persimmon PPO for catechol as a substrate.

Table 1
Optimization and kinetic characterization of persimmon PPO-catalyzed oxidation reactions of various diphenolic substrates (4-MC: 4-methyl catechol, DHPPA: 3-(3,4-dihydroxyphenyl)propionic acid, *L*-DOPA: *L*-3,4-dihydroxyphenylalanine)

Substrate	pH optimum	Temperature optimum (°C)	V_{max} (μM/min)	K_m (mM)	V_{max}/K_m (min^{-1})	Hill constant
4-MC	7.5	40	49.5	14.6	0.0034	0.96
Catechol	7.5	20	55.2	12.4	0.0044	0.91
DHPPA	5.5	60	17.2	12.8	0.0013	0.96
L-DOPA	7.5	10	13.1	8	0.0016	0.87

3.2. Thermal activity and stability

Thermal activity data for crude PPO from persimmon fruit for different substrates are presented in Table 1. Optimum temperatures for persimmon PPO activity were 20 °C for catechol, 40 °C for 4-methylcatechol, 10 °C for *L*-DOPA in 50 mM phosphate buffer at pH 7.5 and 60 °C for DHPPA in 50 mM acetate buffer at pH 5.5. It is clear that the optimum temperatures for PPO are quite species- and substrate-dependent. PPO from peach (Jen & Kahler, 1974), grape (Cash, Sistrunk, & Stutte, 1976) and plum (Siddiq, Sinha, & Cash, 1992) possessed optimum temperatures of 20, 25 and 37 °C, respectively. It was previously reported that the greatest activity of medlar PPO was observed at 35 °C for 4-methylcatechol, 55 °C for catechol (Dincer et al., 2002), and of dog-rose PPO at 20 °C for 4-methylcatechol, 25 °C for catechol (Sakiroglu et al., 1996).

Crude persimmon PPO showed a typical temperature-dependent inactivation profile in the presence of 4-methylcatechol as substrate (Fig. 4). The enzyme was quite stable for 30 min at 20–45 °C and it fully retained its activity at 35 °C within this period. The residual

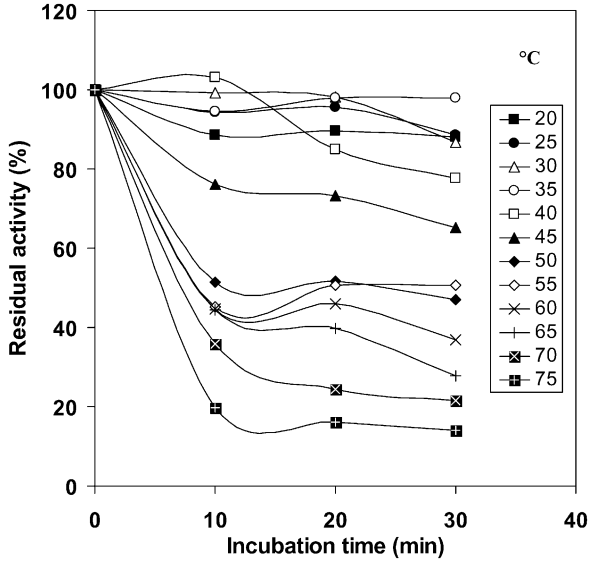

Fig. 4. Thermal stability of persimmon for catechol as substrate.

activity dramatically decreased to below 50% at the end of 10 min incubation above 50 °C. The additional 20 min of incubation did not affect the thermal stability of the enzyme. It is clear that at higher temperatures than 65 °C, heat-denaturation of the enzyme occurred after 10 min of incubation. It has been reported that the drop in percentage residual activity at high temperatures results first in some conformational changes in the tertiary structure and then almost complete inactivation of the persimmon enzyme (Dincer et al., 2002; Duangmal & Owusu Apenten, 1999). Thermal properties of the persimmon enzyme, together with thermodynamic parameters (Table 2) indicate heat-inactivation of the enzyme. It is also clear from these data that the persimmon enzyme has similar thermodynamic properties to medlar enzyme (Dincer et al., 2002). Similar observations have also been reported for other plant PPOs (Buchelli & Robinson, 1994; Dincer et al., 2002; Mazzafera & Robinson, 2000; Robinson, Loveys, & Chacko, 1993). These data also indicate that both medlar (Dincer et al., 2002) and persimmon PPOs have greater heat-sensitivities than other plant PPOs (Duangmal & Owusu Apenten, 1999).

3.3. Substrate specificity

All of the diphenolic substrates, such as catechol, 4-methylcatechol, *L*-DOPA and DHPPA, were oxidized significantly by the persimmon PPO whereas the enzyme was unable to oxidize *L*-tyrosine. Substrate specificities clearly show that the observed activity is a diphenolase as reported for PPOs from other plant sources (Cash et al., 1976; Dincer et al., 2002; Ding, Chachin, Ueda, & Imahori, 1998; Perez-Gilabert & Carmona, 2000; Siddiq et al., 1992). Substrate saturation curves for each diphenolic substrate indicated that the persimmon enzyme follows simple Michaelis–Menten kinetics. Lineweaver-Burk plots for the kinetic analysis of the reaction rates, at a series of concentrations for each substrate, resulted in individual V_{max} and K_m values (Table 1). Substrate specificities were evaluated by using V_{max}/K_m ratio as catalytic efficiency (Palmer, 1995). Substrate binding affinities were within the range of 8–15 mM for the substrates examined. Catalytic efficiency was very low but substrate binding affinity was the highest for *L*-DOPA. On the contrary, catechol was oxidized by persimmon PPO at the highest rate with a much higher K_m value. The catalytic efficiency values, V_{max}/K_m, indicated that catechol and 4-methylcatechol were the most suitable phenolic substrates for persimmon PPO (Table 1). These results are consistent with the previous reports indicating that small *o*-diphenols, such as 4-methylcatechol and catechol, are very efficient substrates for diphenolases from several sources (Cano et al., 1996, Dincer et al., 2002; Ding et al., 1998; Perez-Gilabert & Carmona, 2000). Hill plots gave very similar Hill coefficients (h) for each substrate; 0.96 for 4-methylcatechol, 0.91 for catechol, 0.96 for DHPPA and 0.87 for *L*-DOPA (Table 1), indicating that the persimmon PPO enzyme possesses a single binding site and, therefore, there is a possible lack of cooperation, since all the Hill coefficients are very close to one (Fig. 5).

Table 2
Thermodynamic parameters for thermal inactivation of persimmon PPO[a]

Temperature (°C)	ΔG (J mol^{-1})	ΔH (J mol^{-1})	ΔS (J mol^{-1} K^{-1})
20	304,902	44,454	−260,477
25	310,013	44,412	−265,518
30	315,671	44,371	−271,185
35	315,873	44,329	−271,656
40	327,682	44,288	−283,265
45	334,342	44,246	−290,016
50	341,153	44,204	−296,837
55	346,202	44,163	−302,088
60	352,580	44,121	−308,358
65	358,617	44,080	−311,467
70	364,476	44,038	−320,362
75	370,563	43,996	−326,567

[a] E_a was calculated to be 46,890 J mol^{-1} K^{-1} from the plot of 1/T vs ln k and used for the calculation of ΔH.

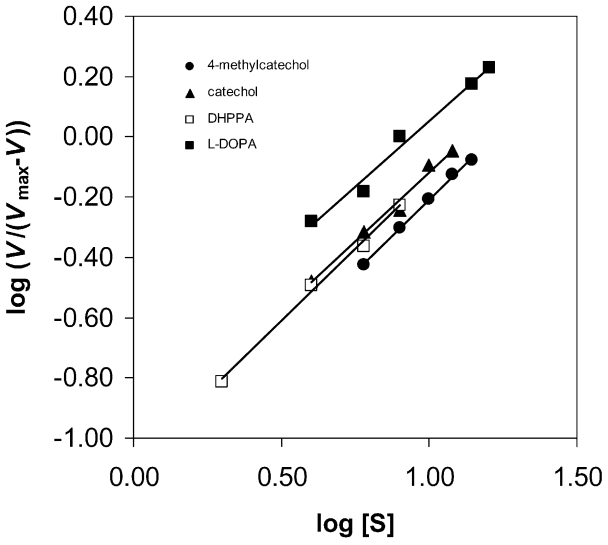

Fig. 5. Hill plots for the oxidation of diphenols by persimmon PPO. Hill coefficients (h) were calculated from the slopes of the linear relationship of log ($V/V_{max}-V$) against log [S] values.

Table 3
Sensitivity of persimmon PPO catalyzed oxidation of 4-methylcatechol to some common PPO inhibitors

Inhibitor	K_i (mM)	I_{50} (mM)
Ascorbic acid	0.01	0.031
Sodium metabisulfite	0.04	0.038
Benzoic acid	2	5
Sodium azide	11	6

3.4. Effect of inhibitors

The behaviour of persimmon PPO toward general PPO inhibitors was examined by treatment with four inhibitors. The compounds, ascorbic acid (10–100 μM), sodium azide (10–50 mM), benzoic acid (1–4 mM) and sodium metabisulfite (10–100 μM) were used at their effective inhibiting concentrations for other PPOs. Their potential for the inhibition of the persimmon PPO was shown as the percentage inhibition of 4-methylcatechol oxidation (Table 3). Inhibition data indicate that the binding affinity of ascorbic acid is the greatest, when compared with others. The inhibition by metabisulfite, with an I_{50} of 0.038 mM, is extremely strong which is consistent with the earlier reports indicating that thiol compounds are potent inhibitors of PPOs (Ding et al., 1998; Duangmal & Owusu Apenten, 1999; Friedman & Bautista, 1995; Yang et al., 2000). Benzoic acid alone competitively inhibited the persimmon PPO activity among all the inhibitors applied. Ascorbic acid and sodium azide exhibited nearly complete inhibition of 4-methylcatechol oxidation by the persimmon PPO at 0.1 mM and 50 mM, respectively. It has been reported that the action of the PPO inhibitors may differ, depending on the compound used (Duangmal & Owusu Apenten, 1999; Martinez & Whitaker, 1995; Rescignio, Sollai, Pisu, Rinaldi, & Sanjust, 2002; Sapers, 1993).

It can be concluded that the crude extracts prepared from the persimmon fruits possess a diphenolase having greatest substrate specificity to 4-methyl catechol or catechol. The enzyme appears to share some biochemical characteristics of several plant PPOs in terms of substrate specificity, pH and temperature optima, stability and kinetic parameters. Moreover, the persimmon PPO activity was very sensitive to some of the general PPO inhibitors, especially to metabisulfite.

Acknowledgements

This work was financially supported by the Research Fund of Karadeniz Technical University to S.G. (97.111.002.1).

References

Ahn, H. S., Jeon, T. I., Lee, J. Y., Hwang, S. G., Lim, Y., & Park, D. K. (2002). Antioxidative activity of persimmon and grape seed extract: in vitro and in vivo. *Nutrition Research*, *22*, 1265–1273.

Amiza, M. A., & Apenten, R. K. O. (1994). Thermal inactivation parameters for alkaline proteinases from North Sea cod (*Gadus morhua*) and bovine α-chymotrypsin. *Journal of the Science of Food and Agriculture*, *66*, 389–391.

Arslan, O., Temur, A., & Tozlu, I. (1998). Polyphenol oxidase from Malatya apricot (*Prunus armeniaca* L.). *Journal of Agricultural and Food Chemistry*, *46*, 1239–1241.

Buchelli, C. S., & Robinson, S. P. (1994). Contribution of enzymic browning to colour in sugarcane juice. *Journal of Agricultural and Food Chemistry*, *42*, 257–261.

Cano, M. P., Lobo, M. G., de Ancos, B., & Galeazzi, M. A. M. (1996). Polyphenol oxidase from Spanish hermaphrodite and female papaya fruits (*Carica papaya* Cv. sunrise, solo group). *Journal of Agricultural and Food Chemistry*, *44*, 3075–3079.

Cash, J. N., Sistrunk, W. A., & Stutte, C. A. (1976). Characteristics of Concord grape polyphenol oxidase involved in juice color loss. *Journal of Food Science*, *41*, 1398–1402.

Costantinides, S. M., & Bedford, C. C. (1967). Multiple forms of phenoloxidase. *Journal Food Science*, *32*, 446–450.

Dincer, B., Colak, A., Aydin, N., Kadioglu, A., & Güner, S. (2002). Characterization of polyphenoloxidase from medlar fruits (*Mespilus germanica* L. Rosaceae). *Food Chemistry*, *77*, 1–7.

Ding, C.-K., Chachin, K., Ueda, Y., & Imahori, Y. (1998). Purification and properties of polyphenol oxidase from loquat fruit. *Journal of Agricultural and Food Chemistry*, *46*, 4144–4149.

Duangmal, K., & Owusu Apenten, R. K. (1999). A comparative study of polyphenoloxidases from taro (*Colocasia esculenta*) and potato (*Solanum tuberosum* var. Romano). *Food Chemistry*, *64*, 351–359.

Espin, J. C., Morales, M., Varon, R., Tudela, J., & Garcia-Canovas, F. (1995). A continuous spectrophotometric method for determining the monophenolase and diphenolase activities of apple polyphenol oxidase. *Analytical Biochemistry*, *43*, 2807–2812.

Espin, J. C., Trujano, M. F., Tudela, J., & Garcia-Canovas, F. (1997). Monophenolase activity of polyphenol oxidase from Haas Avocado. *Journal of Agricultural and Food Chemistry*, *45*, 1091–1096.

Fraignier, M. P., Marques, L., Fleuriert, A., & Macheix, J. J. (1995). Biochemical and immunochemical characteristics of polyphenol oxidases from different fruits of prunus. *Journal of Agricultural and Food Chemistry*, *43*, 2375–2380.

Friedman, M., & Bautista, F. F. (1995). Inhibition of polyphenol oxidase by thiols in the absence and presence of potato tissue suspensions. *Journal of Agricultural and Food Chemistry*, *43*, 69–76.

Friedman, M. (1996). Food browning and its prevention: an overview. *Journal of Agricultural and Food Chemistry*, *44*, 631–653.

Fujita, S., & Tono, T. (1988). Purification and some properties of polyphenol oxidase in eggplant (*Solanum melongena*). *Journal of the Science of Food and Agriculture*, *46*, 115–123.

Galeazzi, M. A. M., & Sgarbieri, V. C. J. (1981). Substrate specificity and inhibition of polyphenoloxidase from a dwarf variety of banana (*Musa cavendishii*, L.). *Journal of Food Science*, *46*, 1404–1406.

Gali, H. U., Perchellet, E. M., Klish, D. S., Johnson, J. M., & Perchellet, J. P. (1992). Hydrolyzable tannins: potent inhibitors of hydroperoxide production and tumor promotion in mouse skin treated with 12-O-tetradecanoylphobol-13-acetate in vivo. *International Journal of Cancer*, *51*, 425–432.

Gorinstein, S., Zemser, M., Weisz, M., Halevy, S., Deutsch, J., Tilus, K., Feintuch, D., Guerra, N., Fishman, M., & Bartnikowska, E. (1994). Fluorometric analyses of phenolics in persimmons. *Bioscience, Biotechnology and Biochemistry*, *58*, 1087–1092.

Jen, J. J., & Kahler, K. R. (1974). Characterization of polyphenol oxidase in peaches grown in Southeast. *Hortscience*, *9*, 590–591.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Lineweaver, H., & Burk, D. (1934). The determination of enzyme dissociation constant. *Journal of American Chemical Society*, *56*, 658–661.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Martinez, M., & Whitaker, J. R. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science and Technology*, *6*, 195–200.

Mazzafera, M., & Robinson, S. P. (2000). Characterization of polyphenol oxidase in coffee. *Phytochemistry*, *55*, 285–296.

Mowat, A. (1990). *Charting the future, proceedings of the first national non-astringent persimmon industry workshop*. Gatton College: The University of Queensland.

Mukhtar, H., Khan, W. A., Wang, Z. Y., Bik, D. P., & Bickers, D. R. (1988). Exceptional activity of tannic acid among naturally occuring plant phenols in protecting against 7-12-dimethylbenz(a)anthracene-, benzo(a)pyrene-, 3-methylcholanthrene-, and N-methyl-N-nitrosourea-induced skin tumorogenesis in mice. *Cancer Research*, *48*, 2361–2365.

Palmer, J. K. (1963). Banana polyphenol oxidase: preparation and properties. *Plant Physiolology*, *38*, 508–513.

Palmer, T. (1995). Kinetics of single-substrate enzyme catalysed reactions. In *Understanding enzymes* (pp. 107–127). Hertfordshire: Prentice Hall/Ellis Horwood.

Perez-Gilabert, M., & Garcia-Carmona, F. (2000). Characterization of catecholase and cresolase activities of eggplant polyphenol oxidase. *Journal of Agricultural and Food Chemistry*, *48*, 695–700.

Pifferi, P. G., & Cultrera, R. (1974). Enzymatic degradation of anthocyanines: the role of sweet cherry polyphenol oxidase. *Journal of Food Science*, *39*, 786–791.

Rescignio, A., Sollai, F., Pisu, B., Rinaldi, A., & Sanjust, E. (2002). Tyrosinase inhibition: general and applied aspects. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *17*, 207–218.

Rivas, N. J., & Whitaker, J. R. (1973). Purification and some properties of two polyphenoloxidases from bartlett pears. *Plant Physiology*, *52*, 501–507.

Robb, D. A. (1984). Tyrosinase. In R. Lontie (Ed.), *Copper proteins and copper enzymes, vol. 2* (pp. 207–241). Boca Raton, FL: CRC Press.

Rocha, A. M. C. N., & Morais, A. M. M. B. (2000). Characterization of polyphenoloxidase extracted from Jonagored apple. *Food Control*, *12*, 85–90.

Robinson, S. P., Loveys, B. R., & Chacko, E. K. (1993). Polyphenol oxidase enzymes in the sap and skin of mango fruit. *Australian Journal of Plant Physiology*, *20*, 99–107.

Sakiroglu, H., Küfrevioglu, Ö. I., Kocacaliskan, I., Oktay, M., & Onganer, Y. (1996). Purification and characterization of dog-rose (*Rosa dumalis* Rechst.) polyphenol oxidase. *Journal of Agriculture and Food Chemistry*, *44*, 2982–2986.

Sapers, G. M. (1993). Browning of foods: control by sulfites, antioxidants and other means. *Food Technology*, *47*, 75–81.

Siddiq, M., Sinha, K., & Cash, J. N. (1992). Characterization of polyphenol oxidase from Stanley plums. *Journal of Food Science*, *57*, 1177–1179.

Yaltirik, E. (1972). *Diospyros* L. In P. H. Davis (Ed.), *Flora of Turkey and the East Aegean Islands, vol. 6* (pp. 142–143). Edinburgh: Edinburgh University Press.

Yang, C.-P., Fujita, S., Ashrafuzzaman, M. D., Nakamura, N., & Hayashi, N. (2000). Purification and characterization of polyphenol oxidase from banana (*Musa sapientum* L.) pulp. *Journal of Agriculture and Food Chemistry*, *48*, 2732–2735.

Zawistowski, J., Biliaderis, C. G., & Eskin, N. A. M. (1991). Polyphenol oxidase. In D. S. Robinson, & N. A. M. Eskin (Eds.), *Oxidative enzymes in foods* (pp. 217–273). London: Elsevier.