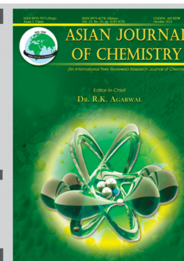
Asian Journal of Chemistry; *Vol. 24, No. 5 (2012), 2159-2161*

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Kinetic Properties of Polyphenol Oxidase Obtained from Various Olives (*Olea europa* L.)

NAHIT GENÇER[1,*], SELMA SINAN[2] and OKTAY ARSLAN[1]

[1]Department of Chemistry, Faculty of Science and Art, Balikesir University, 10145 Balikesir, Turkey
[2]Department of Biology, Faculty of Science and Art, Balikesir University, 10145 Balikesir, Turkey

*Corresponding author: Fax: +266 6121215; Tel: +266 6121278; E-mail: ngencer@balikesir.edu.tr

(*Received*: 17 May 2011; *Accepted*: 16 December 2011) AJC-10849

In this study, the biochemical properties of olive polyphenol oxidase (PPO) which are known to be the primary reason for enzymatic browning, have been investigated. The polyphenol oxidase of *Olea europaea* L. cultivars (Domat, Kiraz, Uslu, Gemlik and Ayvalik) was used for enzyme source. It was found that the optimum pH values were 6.5 with four cultivars except DPPO for catechol as substrate. Optimum pH value was 7.0 for DPPO enzyme. UPPO has the most activity toward catechol, due to the lowest K_M (5.74 mM) and the biggest V_{max}/K_M (1249.93) values. The enzyme had a temperature optimum at 40 ºC and was relatively stable at 50 ºC, with 55 % loss of activity approximately. APPO and KPPO activity lasted until 1 h at 60 ºC. At 60 ºC, heat denaturation of the DPPO, UPPO and GPPO enzymes occurred.

Key Words: Polyphenol oxidase, Olive cultivars, Optimum pH, Heat-denaturation, Renaturation.

INTRODUCTION

Polyphenol oxidase (PPO) is a copper-containing enzyme, widely distributed in nature, responsible for melanization in animals and browning in plants[1,2]. Polyphenol oxidase also catalyzes the *ortho*-hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones[1]. Enzymatic browning of fruits is related to oxidation of phenolic endogenous compounds into highly unstable quinones, which are later polymerized to brown, red and black pigments[3]. The degree of browning depends on the nature and amount of endogenous phenolic compounds, on the presence of oxygen, reducing substances and metallic ions, on pH and temperature and on the activity of PPO, the main enzyme involved in the reaction[4]. Enzymatic browning is also an economic problem for processors and consumers[1]. At least five causes of browning in processed or stored fruits and plants are known: enzymatic browning of the phenols, Maillard reaction, ascorbic acid oxidation, caramelization and formation of browned polymers by oxidized lipids[5]. Browning reactions are major causes of quality loss during harvesting, post-harvest handling/storage and processing of fruits, plants and vegetables in food industry[6]. Enzymatic browning has been studied in several plant tissues such as onion leaves[7], banana[8], mulberry[9], grape[10] and potato[11]. Several methods such as the addition of antioxidants and the exclusion of oxygen as well as thermal processing have been used to inhibit enzymatic browning. For inactivation of PPO, thermal processing has limits like loss of sensory and nutritional quality of food products. Therefore, high pressure treatment has been considered as an alternative[12,13]. Olive is of considerable economic importance for Turkey. The chemical components of olives have been studied extensively and have been found to be a rich source of polyphenolic compounds, with mono- and dicaffeoylquinic acids and flavonoids as the major chemical components[14].

In this study, PPO was partially isolated from five different olive cultivars by a combination of $(NH_4)_2SO_4$ precipitation and dialysis. The contents of phenolic compounds were not determined, neither was the molecular mass of enzyme. Because little information is available on the characterization and purification of PPO from olives, this study has been aimed to assess some of its properties such as optimum pH and temperature, heat-denaturation, renaturation and kinetic values (V_{max} and K_M). Polyphenol oxidase catalyzes the browning reaction occurring during fruit storage. This information will be useful in devising effective methods for inhibiting browning during storage.

EXPERIMENTAL

Olive varieties such as *Olea europaea* L. Domat (D), Kiraz (K), Uslu, (U), Gemlik (G), Ayvalik (A) used in this study were freshly taken in autumn from Akhisar in Turkey and kept for 2 days in a refrigerator at 4 ºC before PPO extraction. Poly-

ethylene glycol, sodium phosphate, ammonium sulphate and catechol used in this study were of analytical grade and these chemicals were obtained from either Sigma or Merck.

Methods

Enzyme extraction and isolation: The fruits were cut in half and the stones were removed and 50 g sample of olive fruits was homogenized using a Waring blender for 2 min in 100 mL of 0.1M phosphate buffer (pH 7.3) containing 5 % polyethylene glycol. The 0.1M concentration was chosen to avoid the influence of enzymatic extract ionic strength on PPO activity, as described by Angleton and Flurkey[15]. The homogenate was filtered and the filtrate was centrifuged at 15.000 g (Sigma centrifuge) for 0.5 h at 4 ºC. The supernatant obtained was used as crude extract. This enzyme isolation procedure was carried out one by one olive cultivars.

Assay for PPO activity: Polyphenol oxidase activity was determined using a spectrophotometric method based on the initial rate of increase in absorbance at 420 nm[16]. Unless otherwise stated, 2.4 mL of 0.1M phosphate buffer (pH 7.3), 0.5 mL of 0.1M catechol as substrate and 0.1 mL of the enzyme extract were taken by pipette and mixed in a quartz cuvette of 3 mL volume. In each measurement, the volume of solution in the cuvette was kept constant at 3 mL. The 0.1M concentration was chosen to avoid the influence of enzymatic extract ionic strength on PPO activity. A portion of the mixture was rapidly transferred into a 1 cm path length cuvette. Absorbance was recorded immediately and at 10 s intervals, at 20 ± 1 ºC with a Cary |1E|g UV-visible spectrophotometer (Varian). The instrument was zeroed using the same mixture without enzyme. The assay mixture was repeated twice using the same stock of the enzyme extract. Enzyme activity was calculated from the linear portion of the curve. One unit of PPO activity was defined as amount of enzyme that causes an increase in absorbance of 0.001/min for 1 mL enzyme at 420 nm and 25 ºC.

Enzyme kinetics: For determination of Michaels constant (K_M) and maximum velocity (V_{max}) values of the enzyme, PPO activities were measured with the catechol at various concentrations. K_M and V_{max} values of PPO, for catechol substrate, were calculated from a plot of 1/V *versus* 1/S by the method of Lineweaver and Burk.

Effect of pH: The optimum pH for all varieties of PPO activity was determined at pH values of 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0, respectively, using 0.1M acetate (pH: 4-6) and 0.1M phosphate (pH: 6-9) buffer adjusted with 0.1M NaOH or 0.1M HNO_3. The optimum pH value for PPO activity obtained from different varieties was obtained using catechol as substrate. As mentioned above, each assay mixture was repeated twice using the same stock of the enzyme extract.

Heat-inactivation of polyphenol oxidase: The effects of temperature and incubation time on polyphenol oxidase activity were determined. Enzyme extracts (0.1 mL) were subjected to 40-80 ºC using a water bath, for times ranging from 10-60 min. They were then transferred into buffer solutions containing catechol (0.1M) that were prewarmed to the corresponding temperatures. Reaction rates of these enzymes were assayed as previously described in 1 cm cuvette around which water circulated at the respective temperatures of reaction.

RESULTS AND DISCUSSION

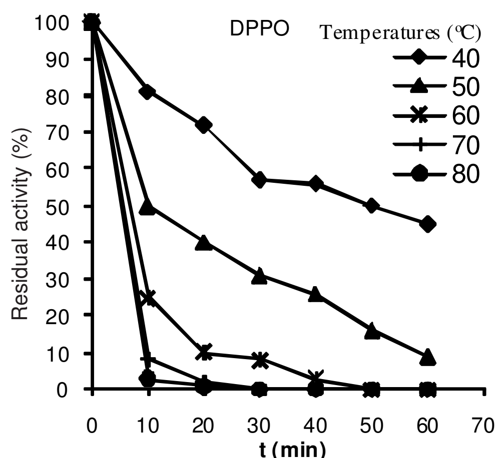
Characterization of the specific enzyme is necessary for effective control of enzymatic browning. Thus, the aim of the present work is to evaluate the activity, kinetic behaviour and thermal inactivation kinetics of olive PPO. Lineweaver-Burk graphs were drawn to calculate the K_M and V_{max} values for cultivar olive fruits. The highest PPO activity can be determined according to K_M and V_{max}/K_M values. The lower K_M and the higher V_{max} are the higher PPO activity. According to this value (Table-1), Uslu variety of olive is the cultivar with the highest PPO activity, followed by Domat olive cultivar. On the contrary, Ayvalik cultivar showed a little PPO activity. Optimum pH values for olive cultivars PPO were determined in the pH range of 4-9. As seen in Table-1, it was found that optimum pH values for GPPO, APPO, UPPO and KPPO were 6.5 and for DPPO were 7.0 for catechol as a substrates. Fig. 1 shows the heat-stability of the enzyme at optimum pH. The APPO enzyme was activated at 40 and 50 ºC. KPPO and UPPO were relatively stable at 40 and 50 ºC. The activation effect of heating was dependent not only on temperature but also on exposure time of the enzyme to various temperatures. However, DPPO and GPPO lost their activity depending time at 40 and 50 ºC. The time required for 50 % inactivation of APPO and KPPO activities at 60 ºC were found to be about 20 min. Fig. 3 shows the renaturation of the enzyme at optimum pH. The DPPO enzyme was renaturated at 40 ºC. KPPO, GPPO, APPO and UPPO were relatively renaturated at 40 and 50 ºC.

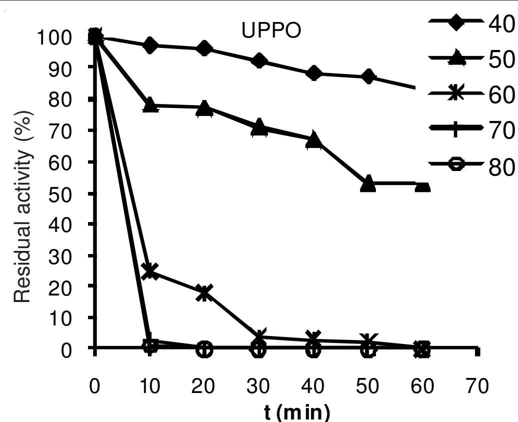
TABLE-1
V_{max}, K_m AND V_{max}/K_m VALUES CALCULATED FOR PPO ACTIVITY OBTAINED FROM ORGANS OF DIFFERENT OLIVES CULTIVARS USING CATECHOL AS A SUBSTRATE

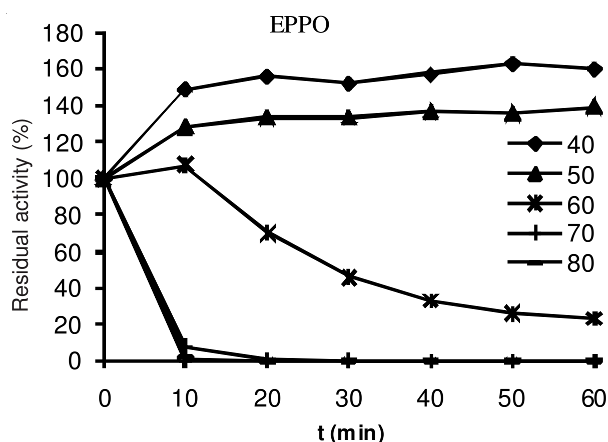
Olea europea cultivars	Optimum pH	K_m (mM)	V_{max} (EU mL^{-1} min^{-1})	V_{max}/K_m (min^{-1})
GPPO	6.5	22.491	3681.890	163.71×10^3
EPPO	6.5	13.691	810.180	59.18×10^3
UPPO	6.5	5.747	7183.392	1249.94×10^3
DPPO	7.0	6.098	5325.381	873.30×10^3
KPPO	6.5	5.251	538.474	102.55×10^3

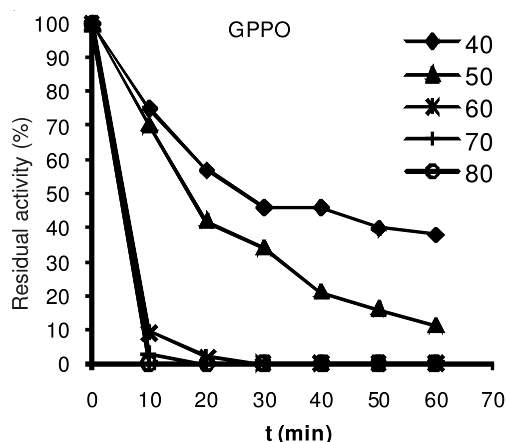
Olive is the most suitable food, with its pleasant appearance in the Turkish markets. K_M values for different cultivars of olives varied from 5.2-22.4 mM. These values are smaller compared to other vegetables such as Chinese cabbage (K_M: 682.5 mM)[17], but higher than values obtained for *Anethum graveolens* (K_M: 1.6 mM)[15] and beetroot (K_M: 0.45 mM)[18]. V_{max} values of different olives of *Olea europae* L. studied in this study were from 538.4-7.183 EU/mL/min. K_M and V_{max} values for PPO activity varied with the type of substrate, buffer, food sources and purity of the enzyme extract as previously stated[17].

Enzyme activity exhibits a significant dependency on the pH value of the medium. With rising pH values, the activity increases to a maximum (pH optimum) and drops to zero in the alkaline region, which is expressed in a bell-shaped optimum curve. Different optimum pH values for PPO obtained from various sources are reported in the literature. For example, it is reported that optimum pH values 4.5 for strawberry[19] and 8.5 for Dog rose[20] using 4-methylcatechol









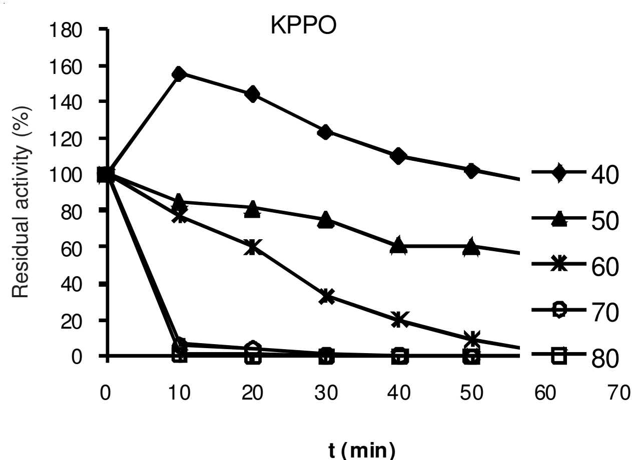


Fig. 1. Renaturation of PPO activity as a function time at 25 °C

as substrate; and 5.5 for strawberry[19], 6.0 for DeChaunac grape[21], 7.0 for Amasya apple[22], *Anethum graveolens* L.[16] and mulberry[6] using catechol as a substrate, respectively. Alyward and Haisman[23] reported that the optimum pH for maximum PPO activity in plants varies depending on the extraction method, the substrates used for assay and the localization of the enzyme in the plant cell. It was reported that polyphenol oxidase was inhibited by kojic acid and thioglycolic acid[24,25].

REFERENCES

1. L.R. Gowda and B. Paul, *J. Agric. Food Chem.*, **50**, 1608 (2002).
2. K.S. Shellby and H.J.R. Popham, *J. Insect Sci.*, **13**, 2442 (2006).
3. M. Blumenthal, A. Goldberg and J. Brinckman, American Botanical Council, Austin, TX (2000).
4. E. Nunez-Delicado, A. Sanchez-Ferrer, F.F. Garcia-Carmona and J.M. Lopez-Nicolas, *J. Food Sci.*, **70**, 74 (2005).
5. F. Pizzocaro, D. Torreggiani and G. Gilardi, *J. Food Process. Preserv.*, **17**, 21 (1993).
6. O. Arslan, M. Erzengin, S. Sinan and O. Ozensoy, *Food Chem.*, **88**, 479 (2004).
7. A.S. Goswami-Giri and N.A. Sawant, *Asian J. Chem.*, **23**, 2212 (2011).
8. E. Karakus and S. Pekyardimci, *Asian J. Chem.*, **21**, 3138 (2009).
9. A. Colak, Y. Kolcuoglu, O. Faiz, A. Ozen and B. Dincer, *Asian J. Chem.*, **19**, 2961 (2007).
10. H. Coban, *Asian J. Chem.*, **19**, 4020 (2007).
11. D.B. Patil and A.A. Kshirsagar, *Asian J. Chem.*, **18**, 3170 (2006).
12. M. Asaka and R. Hayashi, *Agric. Biol. Chem.*, **5**, 2439 (1991).
13. D. Knorr, *Food Technol.*, **47**, 156 (1993).
14. H. Ebrahimzadeh, N. Motamed, F. Rastgar-Jazii, S. Montasser-Kouhsaiu and E.H. Shokraii, *J. Food Biochem.*, **27**, 181 (2003).
15. O. Arslan and I. Tozlu, *Italian J. Food Sci.*, **3**, 249 (1997).
16. J.C. Espin, M. Morales, R. Varon, J. Tudela and F. Garcia-Canovas, *Anal. Biochem.*, **43**, 2807 (1995).
17. T. Nagai and N. Suzuki, *J. Agric. Food Chem.*, **49**, 3922 (2001).
18. J. Escribano, F. Gandia-Herrero, N. Caballero and M.A. Pedreno, *J. Agric. Food Chem.*, **50**, 6123 (2002).
19. P. Wesche-Ebeling and M.W. Montgomery, *J. Food Sci.*, **55**, 1320 (1990).
20. H. Sakiroglu, I.O. Kufrevioglu, I. Kocacaliskan, M. Oktay and Y. Onganer, *J. Agric. Food Chem.*, **44**, 2982 (1996).
21. C.Y. Lee, N.L. Smith and A.P. Pennesi, *J. Sci. Food Agric.*, **34**, 987 (1983).
22. M. Oktay, O.I. Kufrevioglu, I. Kocacaliskan and H. Sakiroglu, *J. Food Sci.*, **60**, 495 (1995).
23. F. Alyward and D.R. Haisman, *Advan. Food Res.*, **17**, 1 (1969).
24. R. Sariri, J. Mahmoodian, K. Khaje and R.H. Sajedi, *Asian J. Chem.*, **18**, 337 (2006).
25. R. Sariri, J. Mahmoodian, K. Khaje and R.H. Sajedi, *Asian J. Chem.*, **18**, 15 (2006)