BIOCHEMICAL PROPERTIES OF POLYPHENOL OXIDASE FROM *DAUCUS CAROTA*

Reyhan Gul Guven[1], Kemal Guven[2], Fatma Matpan Bekler[3], Nurten Aslan[1]

[1]Dicle University, Education Faculty, Mathematic and Science Teaching Section, Diyarbakir, Turkey
[2]Dicle University, Science Faculty, Molecular Biology and Genetics Section, Diyarbakir, Turkey
[3]Dicle University, Science Faculty, Biology Section, Diyarbakir, Turkey
Email: kemalg@dicle.edu.tr

Abstract
Polyphenol oxidase (PPO) is a very important enzyme that is responsible for the enzymatic browning of vegetables and fruits, which is undesired process and need to be prevented in food technology. In this study, PPO from *Daucus car*ota pell (carrot) was extracted and some of its biochemical properties were investigated. The optimum temperature and pH of PPO were found to be 40 °C and 8.0, respectively. The Lineweaver – Burk plot analysis of the PPO was carried out and the Km and Vmax values were determined for the substrate catechol. We also found that some inhibitors such as SDS and sodyum azide inhibit the enzyme activity. This is the first study on characterisation of PPO from *Daucus carota* bark that may provide new insight into how to overcome the enzymatic browning.

Keywords: Polyphenol oxidase, *Daucus carota*, inhibition, characterisation.

Introduction

Polyphenol oxidases (PPO; EC 1.14.18.1) belong to a set of copper containing metalloenzymes that are members of oxidoreductases, which catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen (Queiroz, et al. 2008).

Polyphenol oxidases are widely spread in nature. They can be found in almost all living organisms including animals, plants, bacteria and fungi. PPO is important because it is responsible for skin, eye, inner ear and hair melanization, as well as browning in fruits and vegetables (Marín-Zamora et al., 2005). Particularly, when a plant gets a bruise, cut or damage, the enzyme leads to the oxidation of some phenolic compounds to form a polymer structure, resulting in protection of the plant against insects or microorganisms (VanGelder, et al., 1997). This process causing enzymatic browning of fruits and vegetables is undesirable in food technology as it results in loss of quality. In plant tissues, the browning pigments lead to organoleptic and nutritional modifications, thus depreciating the quality of the food product (Friedman, 1996; Sanchez-Ferrer et al., 1995).

Carrots (*Daucus carota*), one of the important root vegetables, are known for their nutrient contents β-carotene besides appreciable amount of vitamins and minerals (Walde et al. 1992). Phenolics in carrots are present throughout the roots but are highly concentrated in the periderm tissue (Mercier et al. 1994) Therefore, the higher level of phenolics and antioxidant properties in carrot peel treated as the waste in the processing industry could be considered for value-added utilization (Oviasogie et al.2009).

The inhibition of PPO activity and thus inhibition of browning is a big challenge for fruit and vegetable industry (Mayer, 2006). The purification and characterisation of this enzyme in many plants would lead to biotechnological control of its activity.

PPO characteristics have been studied in a wide variety of plants such as apple (Aydin et al., 2015), banana (Ünal, 2007), potato (Lourenço et al., 1992), broccoli (Gawlik-Dziki et al., 2007), ispir sugar bean (Sakiroglu et al., 2013), and Ataulfo mango (Cheema & Sommerhalter, 2015).
This is the first report on the purification and characterisation of PPO from *Daucus carota* in order to understand how the enzymatic browning can be prevented in food technology. In the present study, PPO from *Daucus carota* was extracted and the kinetic parameters for catechol substrate was determined.

Material And Methods

Materials and reagents

The *Daucus carota* used in this study was purchased from a local market in Diyarbakir City, Turkey and frozen at -25 °C until used. Catechol was purchased from Merck (Darmstadt, Germany) Co. All chemicals used in this study were of analytical grade.

Preparation and extraction of PPO from *Daucus carota*

Five grams of *Daucus carota* bark were homogenized in the extraction solution (100 ml of 0.1 M phosphate buffer containing 4% PEG at pH 6.5 and 10 mM ascorbic acid) by using a blender for 5 min. The crude extract samples were centrifuged at 15000 g for 20 min at 4ºC. The homogenate was then filtered.

Enzyme activity

PPO activity was determined using a spectrophotometric method based on the initial rate of increase in absorbance at 420 nm. Enzyme activity was assayed in 3 ml of reaction mixture consisting of 0.1 ml substrate (0.1 M catechol) and 0.1 ml enzyme preparation in 0.1 M phosphate buffer (pH 6.5). PPO activity was determined by measuring the absorbance at 420 nm using an spectrometer. The blank consisted of 2.9 ml buffer and 0.1 ml substrate. PPO activity was assayed in triplicate and one enzyme unit was defined as the amount of enzyme that produces a rise of 0.001 absorbance in one minute at 420 nm.

Effect of pH and temperature on PPO activity

 The effect of pH on PPO activity was determined using 0.1 mL of enzyme preparation, 0.1 mL of 0.1 M catechol and finally topped-up to 3 mL with 0.1 M sodium acetate buffer (pH 2-5) or 0.1 M sodium phosphate buffer (pH 6.0-9.0). Enzyme activity was measured spectrophotometrically in this buffering range according to the procedure described for the PPO activity assay. The optimum pH corresponding to the highest PPO activity was used for the study in order to determine the effect of inhibitors and temperature on enzyme activity.   PPO activity was determined at different reaction temperatures in the range of 20-70°C using catechol as substrate. Analyses were performed in triplicate under the standard mixing conditions.

The Effect of Buffer Concentration

The effect of buffer concentration on the PPO enzyme was studied using 0.1-0.4 M concentrations of phosphate buffer using catechol as subsrate at an optimum pH.

Enzyme kinetics and substrate specificity

Michaelis-Menten constant (Km) and maximum velocity (Vmax) values of PPO were calculated using the substrates catechol (1-10 mM), under the optimized pH and temperature conditions. Km and Vmax values of PPO for substrate were obtained from a plot of 1/V versus 1/[S] by the method of Lineweaver and Burk (1934). Measurements were carried out in triplicate.

Effects of inhibitors

The effects of several inhibitors (EDTA, sodium azide, SDS) on PPO activity were studied. PPO activities were measured at (0.5 mM) inhibitor concentrations with substrate concentration of 3.3 mM.

Results And Discussion

Activity of Enzyme

The activity of crude enzyme of *Daucus carota* was 40 units/ml. Sanni (2016) found result with two species of African mango obtaining the activity of crude enzyme values of 86.4 and 100 units/ml.

Effect of pH and temperature

As shown in Figures 1 and 2, optimum pH and temperature values were found to be pH 8.0 and 40 °C, respectively using the catechol as substrate. The previous studies also raported that optimum pH values were 7.0 for parsley (Lin et al., 2016), pH 5.5 for ispir sugar bean (Sakiroglu et al., 2013) using catechol as a substrate. It had been previously shown that different plant types exhibited different and similar optimum temperatures, such as 40 ℃ for artichoke (Doğan et al.,2005), and for corn tassel (Gul Guven et al., 2015) using catechol as the substrate.
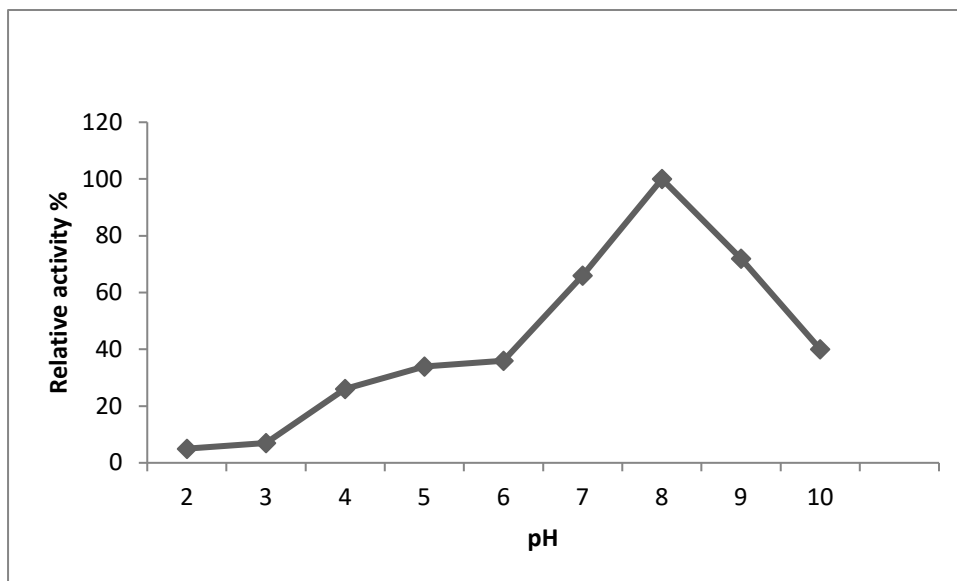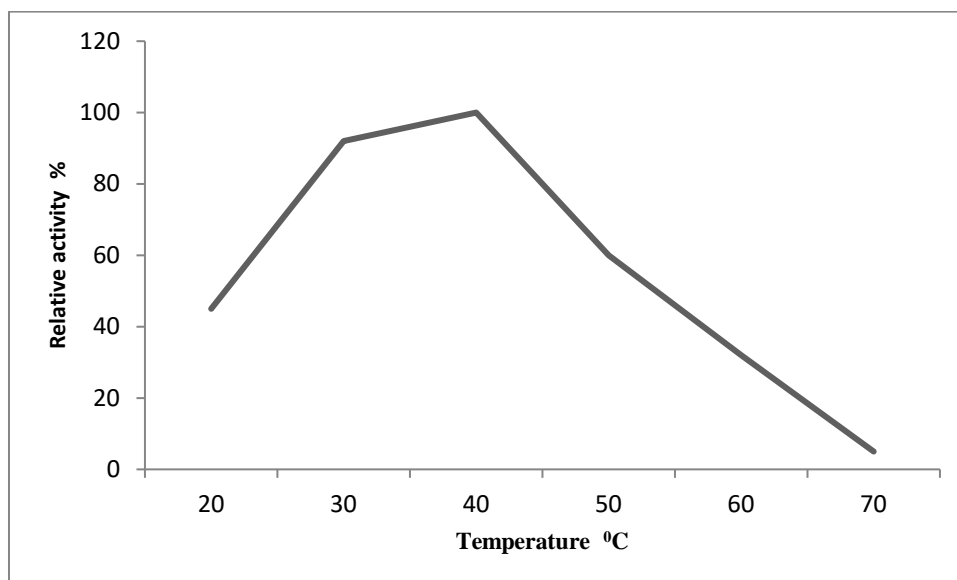
Figure 1. Effect of pH on PPO activity

Figure 2. Effect of temperature on PPO activity

Determination of Ionic Strength

The effect of ionic strenght on thr PPO enzyme was studied using 0.1-0.4 M concentrations of phosphate buffer using catechol as subsrate at an optimum pH. PPO enzyme at a buffer concentration 0.1 M was determined.
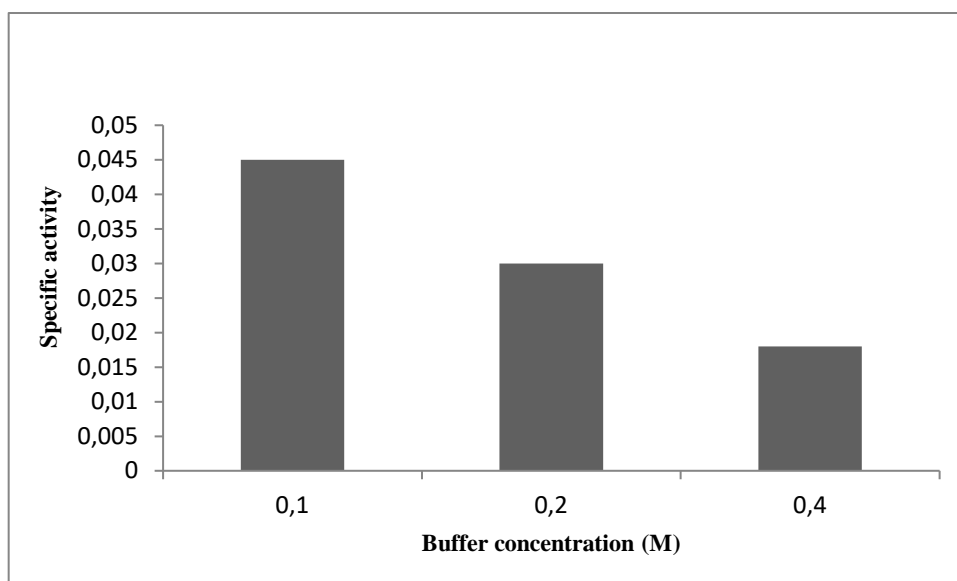
Figure 3: The effect of buffer concenration

Kinetic characteristics of PPO

The variation in the *Daucus carota* PPO activity was determined as a function of substrate (catechol) concentration. For the determination of Michaelis-Menten constant (Km) and maximum velocity (Vmax) values of the enzyme, PPO activities were measured using the concentrations of catechol (1-10 mM) as substrates under optimized pH and temperature conditions. A plot of 1/V versus 1/[S] was drawn by the method of Lineweaver and Burk (1934) to calculate Km and Vmax values of purslane PPO for each substrate. Substrate specificity (Vmax/Km) was also calculated by using the data obtained on the Lineweaver-Burk plot. The Km and Vmax values obtained from the plot analysis of PPO were found as 21 mM and 1701 abs/min. for catechol.

There have been many studies reported on the kinetics of PPO in different plant species, using catechol as substrate. The Km value is a measure of the affinity of the enzyme fort he substrate. A smaller Km value means higher affinity of the enzyme with the substrate and vice versa.   Km values were found to vary in Hemşin apple (Aydin et al., 2015), mamey (Palma- Orozco et al., 2014) and Chinese Toon (Wang et al., 2013) as 6.8 mM, 44 mM and 10.059 mM, respectively.
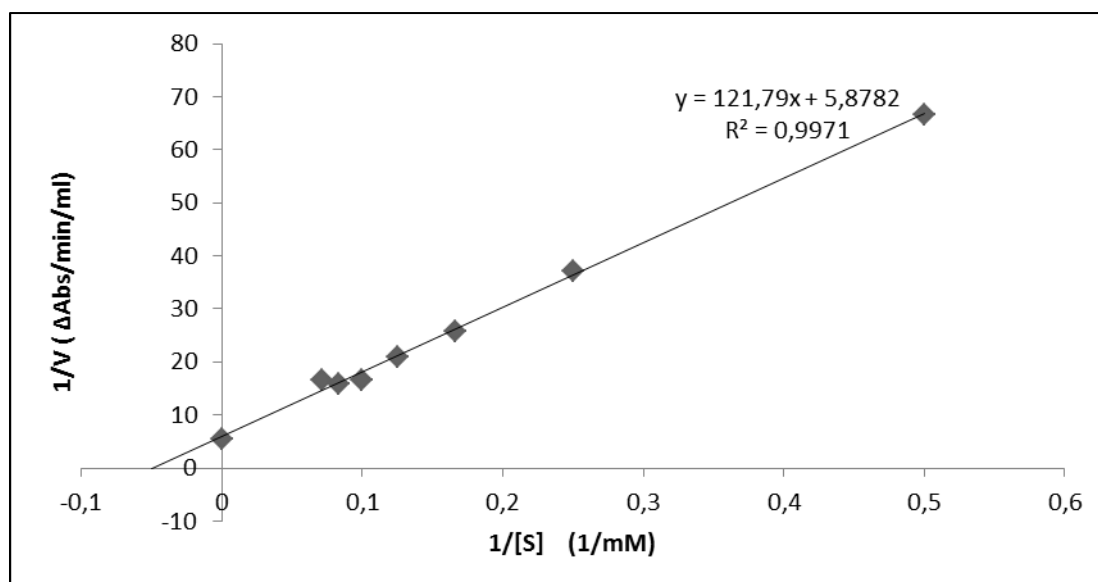

Figure 4: Lineweaver-Burk plots for PPO activity with catechol as subsrate

Effect of inhibitor

The present study is the first report on the inhibition of PPO activity in *Daucus carota*. The effects of several inhibitors (SDS, EDTA, sodyum azide) on PPO activities were measured at 0.5 mM inhibitor concentration with 4 mM concentration of catechol.
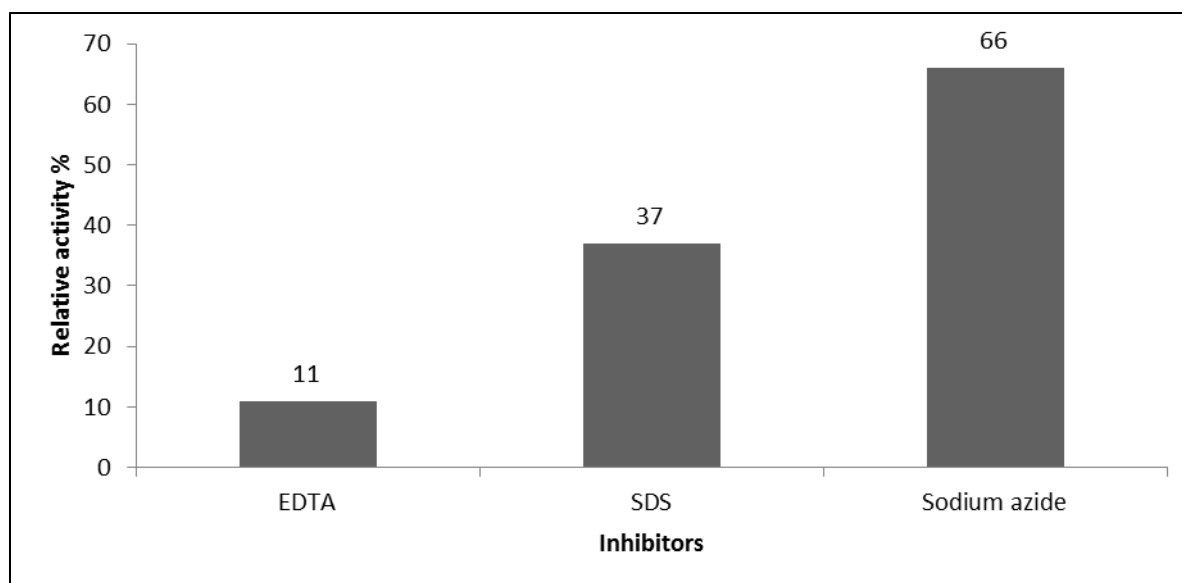
Figure 5: The inhibition of PPO

Conclusions

In conclusion, PPO is a very important enzyme that is responsible for the enzymatic browning of vegetables and fruits, which is undesired process and need to be prevented in food technology. This work reports the extraction and characterization of PPO from *Daucus carota* the first time. The pH and temperature optima were found to be 8.0 and 40 °C for catechol. In this work, sodium azide, SDS and EDTA were found to inhibit the enzyme activity by 66 %, 37 % and 11 %, respectively. A buffer concentration of 0.1 M at was determined for PPO activitye. The Km and Vmax values obtained from the plot analysis of PPO were found as 21 mM and 1701 abs/min. for catechol, respectively.

References

Aydin, B., Gulcin,I., Alwasel S.H. (2015). Purification and Characterization of Polyphenol Oxidase from Hemşin Apple (Malus communis L.). International Journal of Food Properties, 18:12, 2735-2745.

Cheema, S., Sommerhalter, M.(2015) .Characterization of polyphenol oxidase activity in Ataulfo mango. Food Chemistry, 171, 382-387.

Dogan, S.; Turan, Y.; Erturk, H.; Arslan, O. Characterization and purification of polyphenol oxidase from artichoke (Cynara scolymus L.). Journal of Agriculture and Food Chemistry 2005; 53, 776–785.

Friedman, M. (1996).Food browning and its prevention: An overview. J.Agric.Food Chem.,44,631–653.

Gawlik-Dziki, U., Szymanowska, U., Baraniak, B. (2007). Characterization of polyphenol oxidase from broccoli (Brassica oleracea var. botrytis italica) florets. Food Chem, 105,1047-1053.

Gul Guven, R., Aslan, N., Guven, K., Matpan Bekler, F., Acer, O. (2016). Purification and characterization of polyphenol oxidase from corn tassel.Cellular and Molecular Biology.62,13,6-11.

Lin, H., Ng, A.W.R., Wong, C. W. (2016). Partial purification and characterization of polyphenol oxidase from Chinese parsley (Coriandrum sativum). Food Science and Biotechnology, 25(S): 91-96.

Lineweaver, H., Burk, D.(1934). The determination of enzyme dissociation constants. Journal of American Chemical Society , 56, 658-666.

Lourenço E.J., Neves V.A., Da Silva M.A. (1992). Polyphenol Oxidase from Sweet Potato: Purification and Properties. J Agric Food Chem, 40:2369-2373.

Marín-Zamora, M.E., Rojas-Melgarejo, F., García-Cánovas, F., García-Ruíz, P.A.(2005). Cinnamic ester of D-sorbitol for immobilization of mushroom tyrosinase. Journal of Chemical Technology and Biotechnology, 80,1356–1364.

Mayer, A. M. (2006). Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry, 67, 2318-2331.

Mercier JJ, Arul J, Julien C. Effect of food preparation on the isocoumarin 6-methoxymellein content of UV-treated carrots. Food Res Int. 1994;27:401–404.

Oviasogie OP, Okoro D, Ndiokwere CL. Determination of total phenolic amount of some edible fruits and vegetables. Afr J Biotechnol. 2009;8:2819–2820.

Palma-Orozco, G., Marrufo-Hernández, NA., Sampedro, JG., Nájera, H. (2014). Purification and partial biochemical characterization of polyphenol oxidase from mango (Mangifera indica cv. Manila).J Agric Food Chem., 8;62(40):9832-9840.

Queiroz, C., Lopes, M. L. M., Fialho, E., Valente-Mesquita, V. L. (2008). Polyphenol oxidase: Characteristics and mechanisms of browning control. Food Reviews International,24 (4):361-375

Sakiroglu, H., Yılmaz, E., Erat M., Öztürk, A.E.(2013).Selected properties of polyphenol oxidase obtained from ispir sugar bean .International Journal of Food Properties, 16, 1314–1321.

Sanchez-Ferrer, A., Rodriguez-Lopez, JN., Garcia-Carnovas, F., Garcia-Carmona, F. (1995).Biochim Biophys Acta,1247,1–11.

Sanni, D.M. (2016). Isolation, partial purification and characterization of polyphenoloxidase from two species of African Mango seeds (Irvingia gabonensis and Irvingia wombolu).Advances in Biochemistry. 4(4): 47-52.

Unal, M.U. (2007). Properties of polyphenol oxidase from Anamur banana (Musa cavendishii). Food Chem, 100: 909-913.

VanGelder, C. W. G., W. H. Flurkey., & H. J. Wichers. (1997). Sequence and structural features of plant and fungal tyrosinases. Phytochemistry,45 (7),1309-1323.

Walde, S.G., Math, R.G., Chakkarvarthı, A,. Rao, D.G.(1992). Preservation of carrots by dehydration techniques. A review. Indian Food Packer 46, 37–42.

Wang, C., Zhang, J.-F., Zhang, Y., & Cheng., Bing. (2013). Characterization and Inhibitors of Polyphenol Oxidase from Chinese Toon. Food Biotechnology,27 (3), 261-278.