BIOCHEMICAL PROPERTIES OF POLYPHENOL OXIDASE FROM
DAUCUS CAROTA

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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 carota 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 sodium 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 4°C. 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 substrate 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 reported 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 °C for artichoke (Doğan et al., 2005), and for corn tassel (Gül 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 strength on the PPO enzyme was studied using 0.1-0.4 M concentrations of phosphate buffer using catechol as substrate at an optimum pH. PPO enzyme at a buffer concentration 0.1 M was determined.
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Figure 3: The effect of buffer concentration

**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 for the 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.

![Lineweaver-Burk plots for PPO activity with catechol as substrate](image)

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

**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, sodium azide) on PPO activities were measured at 0.5 mM inhibitor concentration with 4 mM concentration of catechol.
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 activity. 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**


