Iraqi Journal of Biotechnology, 2016, Vol. 15, No. 1 , 53-60

Determination the Activity of Some Physical and Chemical Factors on the Polyphenol Oxidase in Hot Pepper and Cabbage *(Brassica oleracea Var)*

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Received: September 7, 2015 / **Accepted**: November 24, 2015

Abstract: The activity of polyphenol oxidase (PPO) in hot pepper pericarp and cabbage was evaluated using spectrophotometric method. The enzyme was extracted from the pepper pericarp with suitable volume of 0.1 M phosphate buffer solution pH (7. 0) .PPO activity was determined using catechol as a substrate. The effects of the enzyme concentration, substrate concentration, pH and temperature were investigated. The highest activity of ppo at (2.5 mg /ml) from hot pepper and the maximum activity of ppo from cabbage was at (1.5 mg /ml) .The highest activity of enzyme catechol concentration was at 100 mM for PPO from hot pepper and cabbage respectively. The optimum pH was 6.0 and 7.0 for PPO from hot pepper and cabbage. The optimum temperatures were 40°C, 30 °C for hot pepper and cabbage respectively. These optimum conditions were used to determine the enzyme activity in hot pepper and cabbage.

Key words: polyphenol oxidase, cabbage, hot pepper, optimization.

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Introduction

The enzymes involved in these processes are polyphenol oxidase (PPO) (1), since PPO and POD are the main enzymes involved in the phenolic oxidation of many fruits and vegetables. Phenolic compounds are a group of chemical substances in plants, which play an important role during enzymatic browning because they are substrates for the browning enzymes. The phenolics are normally complex organic substances, which contain more than one phenolic group. Polyphenolics can be divided into many different subcategories, such as flavonoids and non flavonoid components (2).

Polyphenol oxidase (PPO) (E.C. 1.14.18.1) (3), is a copper-containing enzyme which is probably present in all plants. It is widely distributed enzyme involved in the biosynthesis of melanins in animals and in the browning of plants. The enzyme catalyzes the oxidation of phenolic Compounds to form corresponding quinone intermediates which polymerize to form undesirable pigment. It catalyzes two types of the oxidative reaction involving molecular oxygen: the oxidation of o-diphenols to o-quinones and the

hydroxylation of monophenols to o-diphenols, which lead to the formation of black or brown pigments (4,5). Enzymatic browning is a significant problem in a number of fruits and vegetables such as lettuce (4). The discoloration in vegetables and fruits by enzymatic browning, resulting from conversion of phenolic compounds to o-quinones which subsequently polymerize to be a brown or dark pigment. The enzymes involved these processes are PPO (5). Because PPO are the main enzymes involved the phenolic.

Pepper fruits (*Capsicum annuum* L.) are popular vegetables because of the combination of color, test and nutrition. They are used as foods and spice. Moreover, the red pepper fruit has been used for many years as a source of pigments to add or change the color of foodstuffs. Fresh peppers are good source of vitamin C and E as well as pro vitamin A and carotenoid compounds with well-known antioxidant properties (6, 7, 8, 9,10,11,12 ,13).

Cabbage is a popular cultivar of the species Brassica *oleraceaLinne* (Capitata Group) of the Family Brassicaceae and is vegetable of leafy green. It is a herbaceous, biennial, dicotyledonous flowering distinguished by stem upon which is crowded a mass of leaves green but in some varieties red or purplish, which while immature form a characteristic compact, globular cluster (cabbagehead) (2). Cabbage is an excellent source of vitamin C. It also contains significant amounts of glutamine, an amino acid that has anti-inflammatory properties. Thus, the target of this study was to determined PPO activity in hot pepper pericarp and cabbage leaves. The optimum conditions for determination of ppo enzyme activity by spectrophotometric method were investigated including the amounts of enzyme extract, substrate concentration, pH and temperature of incubation.

Materials and Methods

Enzyme Extraction

The pepper were washed several times with tap water and homogenized (India) by using a homogenizer for 2 min , Five grams of the homogenized pepper pericarp were extracted with 0.1 M phosphate buffer pH 7.0 containing 5 g of polyvinylpyrrolidone (PVP) using magnetic stirrer for 15 min. The homogenate was filtered through Whatman No.41 filter paper and then centrifuged at 2,500 rpm (1000 series centifugal, Espain) for 20 min. The supernatant was filtered through Whatman No.41 filter paper and collected as an enzyme extract. All the steps of enzyme extraction were carried out at 4°C (14, 15).

The Cabbage leaves *(Brassica oleracea capitata L.*) was purchased at local markets, in winter.. The leaves were washed, cut into small pieces and frozen at −20°C for 24 h. After thawing, leaves were homogenized with 1/2 W/V K_2HPO_4 solution (0.1 M) for 5 min. The homogenate was filtered through chesse clothe and then centrifuged at 10,000 rpm with cooling Centrifuge for 10 min, to remove debris. The supernatant was designated as crude enzyme. All stepes and purification procedures were carried out at 20 -25°C (16).

Enzyme Assays

The PPO activity determined by using a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm (1). 1.95 mL of 0.1 M

Phosphate buffer solution pH 7.0 , 1 mL of 100 m M catechol as a substrate and 50 µL of the enzyme extract were pipetted into a test tube and mixed thoroughly. Then the mixture was rapidly transferred to a 1-cm path length cuvette. The absorbance at 410 nm was recorded continuously at 25°C for 5 min using ultraviolet-visible spectrophoto meter, (Espain) (6) One unit of enzyme activity was defined as the amount of enzyme that causes an increase 0.001 of absorbance per min.

$$\textbf{PPO activity (unit/ml)} = \frac{\triangle A_{410nm}}{0.001 \times 0.05 \times V_{rm}}$$

0.05 = volume of enzyme
RM= reaction mixture (3 ml)

Effect of Enzyme Amounts on Enzyme Activity

The activity of PPO as a function of amounts of enzyme extract was investigated. PPO activity was assayed at various amounts of the enzyme extract included 1 ,1.5 ,2, 2.5 , 3 ,3.5 mg/ml and mixing with 1 mL of 100 m M catechol, and 1.95 mL of 0.1 M phosphate buffer pH 7.0 (9).

Effect of Substrate Concentration on Enzyme Activity

PPO activity using the catechol substrate concentrations 40, 60 ,80, 100 ,120 mM , PPO activity was proceeded by mixing 50 µL of the enzyme extract, 100 m M catechol and 0.1 M phosphate buffer pH 7 at a selected volume , The enzyme activities were measured in a quartz cuvette of 3 mL volume (6).

Effect of pH on Enzyme Activity

The activity of PPO were determined at pH values of 3, 4, 5, 6, 7 , 8 using 0.1 M citrate buffer (pH 3 - 5) and phosphate buffer (pH 6 - 8). The optimum pH for PPO were obtained using catechol as substrate. The effect of pH on PPO activity was observed by using the reaction mixture containing 1 mL of 100 m M catechol, 1.95 mL of 0.1 M buffer solution and 50 µL of the enzyme extract (11).

Effect of Temperature on Enzyme Activity

PPO activities were determined at 20, 30, 40, 50, 60 , 70 °C. The substrate and buffer solutions were incubated for 5 min at various temperatures from 20 to 70°C before adding the enzyme extract. Spectrophotometric measurement for 5 min was carried out at 25°C. The activity of PPO under optimum temperature was determined by adding 1 mL of 100 m M catechol, 1.95 mL of 0.1 M phosphate buffer pH 7 and 50 µL of the enzyme extract (6).

Results and Discussion

The effect of various amounts of the enzyme extract on PPO activity was studied and the result was shown as the rate of substrate oxidation by the enzymes. In this study the highest activity of ppo at (2.5 mg /ml) from hot pepper and the maximum activity of ppo from cabbage was at (1.5 mg /ml) enzyme concentration. (Figures 1,2). The substrate oxidation was found to be dependent on the amounts of the enzyme extract.

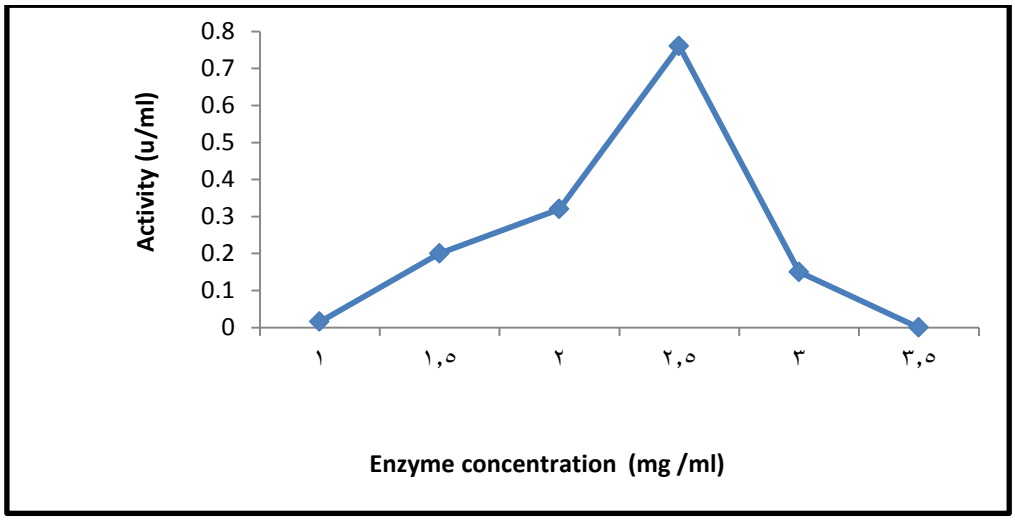

Figure 1: Effect of enzyme concentration on PPO activity from hot pepper

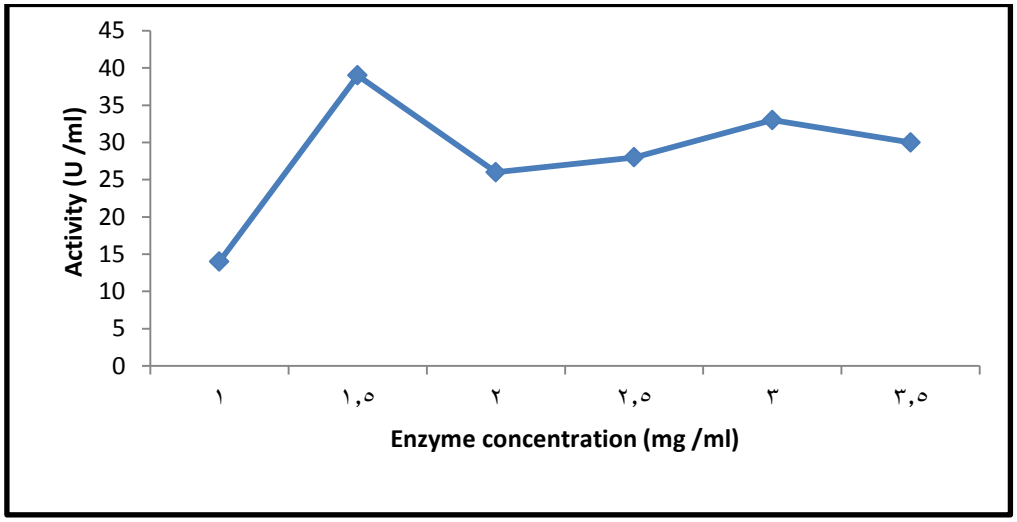

Figure 2: Effect of enzyme concentration on PPO activity from cabbage

In this study, different amounts of the substrate catechol was used as the substrate for PPO , (Figure 3 ,4). As expected, an increase in the substrate concentration resulted in an increase in pigment formation. The rate of stayed practically constant at saturating catechol concentration. Therefore, the concentration of 80 and 100 mM catechol was routinely chosen because at higher concentrations of the substrate did not significantly increase the formation of the o-quinone intermediate.

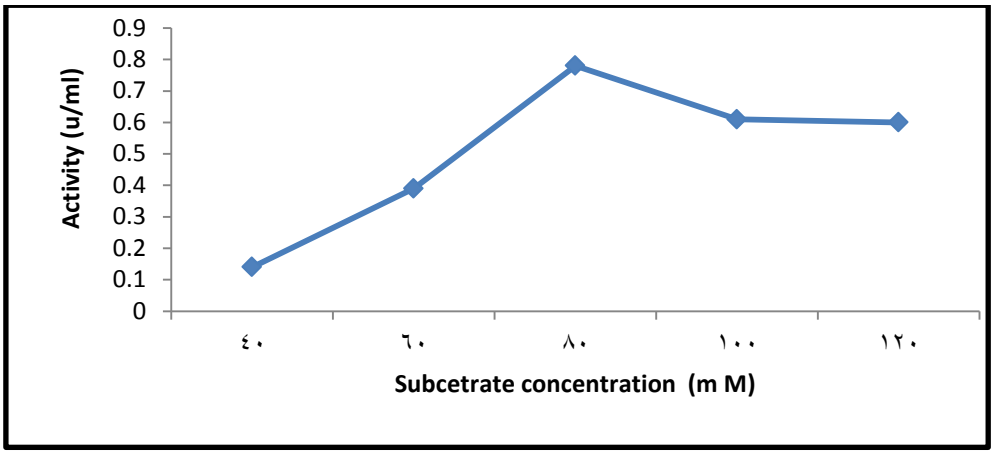

Figure 3: Effect of catechol concentration on the PPO activity from hot pepper

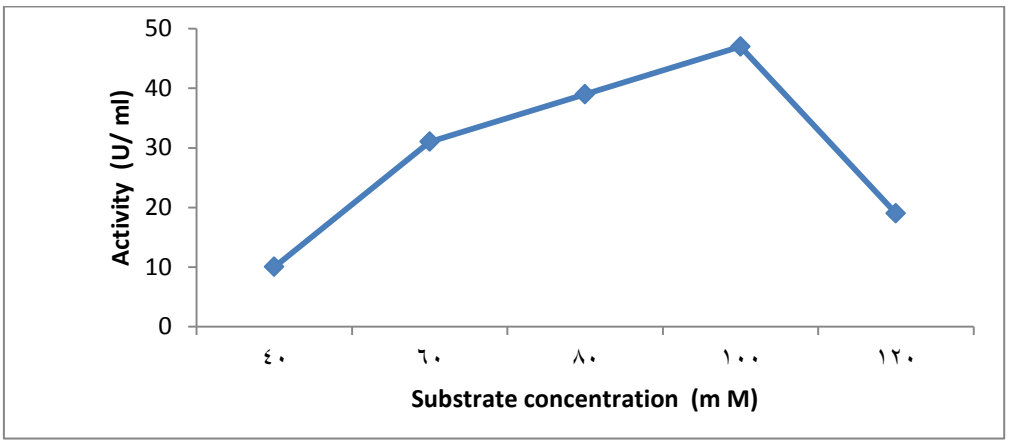

Figure 4: Effect Effect of catechol concentration on the PPO activity from cabbage

The activity of PPO from hot pepper and cabbage was measured at different pH values using catechol as substrate. As shown in Figure (5, 6) the optimum pH 7.0 , pH 6.0 of both enzymes PPO from hot pepper and cabbage respectively were obtained. It is known that the optimum pH for any enzymes depends on substrate and plant materials in the activity assay. In general, most plants show maximum enzyme activity at or near neutral pH. Different optimum pH values for both enzymes obtained from various sources and substrates used have been reported. The optimum pH values are 6.8 and 5.5 for butter lettuce PPO using 4-methylcatechol and catechol as substrates, respectively (15).

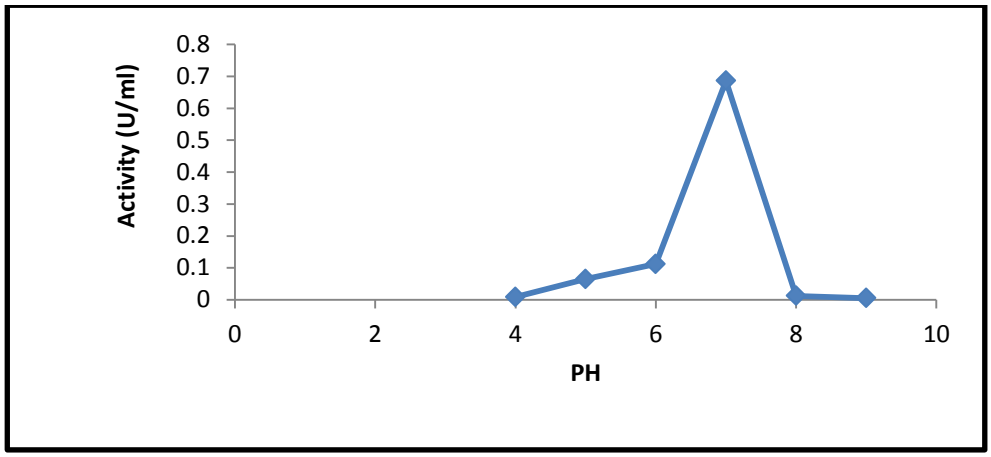

Figure 5: Effect of pH on the PPO activity from hot pepper

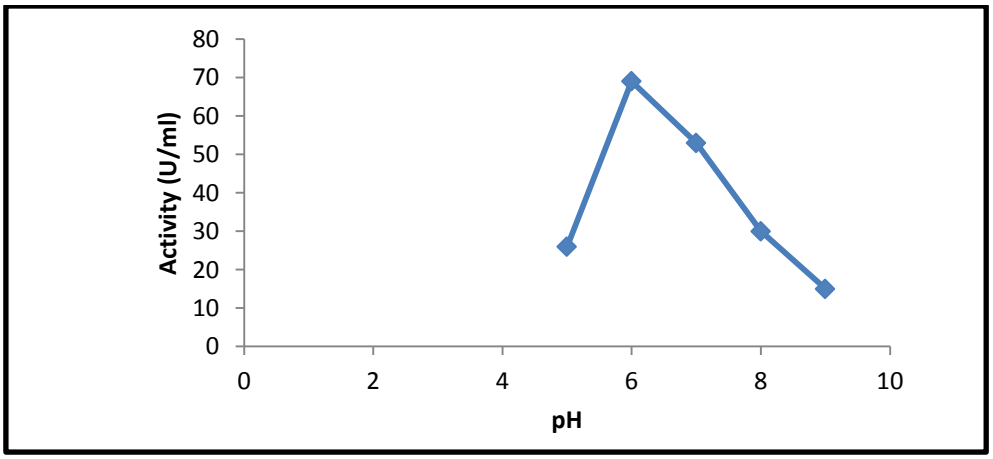

Figure 6:Effect of pH on the PPO activity from cabbage

The optimum temperature for enzyme activity usually depends on experimental conditions. Generally, the reaction rate decreases of high temperature because of thermal denaturation when the temperature is increased, this situation is similar for most enzymes. Temperature dependence in the enzyme activities is presented in Figure (7,8). It was found that the highest activity of PPO from hot pepper and cabbage were obtained at 40 °C and 30°C, respectively. PPO showed the highest activity at 40°C, and its activity decreased slightly between 50 and 70°C from hot pepper. The highest PPO activity from cabbage at 30 °C, and then decreased probably due to denaturation of the enzyme at higher temperatures. From previous studied, the temperature at which PPO showed the highest activity was in the range of 25 - 30°C, and then decreased at temperature above 40°C (17) . From the obtained results, the optimum temperature of the both enzymes was found between 30°C and 40°C. Thus, we determined the enzyme activities of pepper samples at ambient temperature (30 ± 3 °C) (17, 18 , 19).

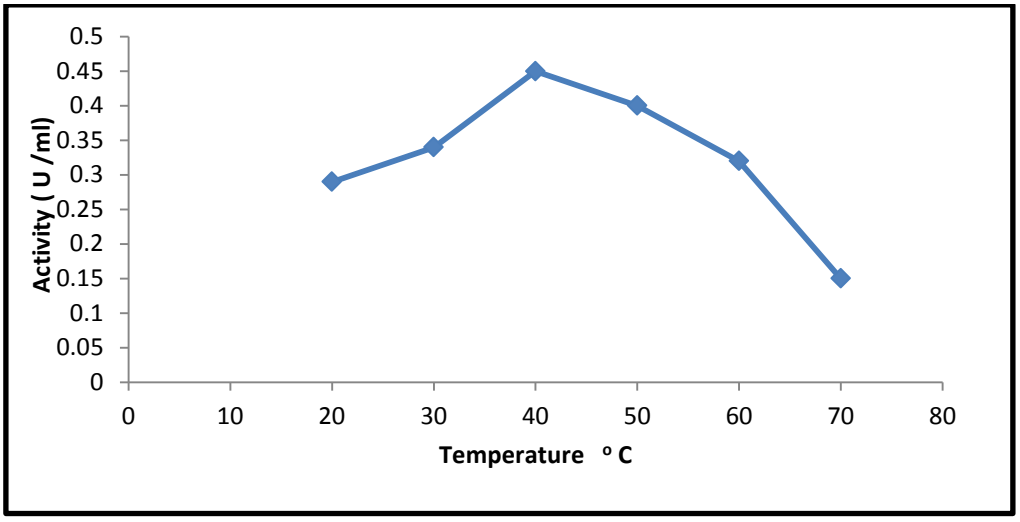

Figure 7: Effect of temperature on the PPO activity from hot pepper

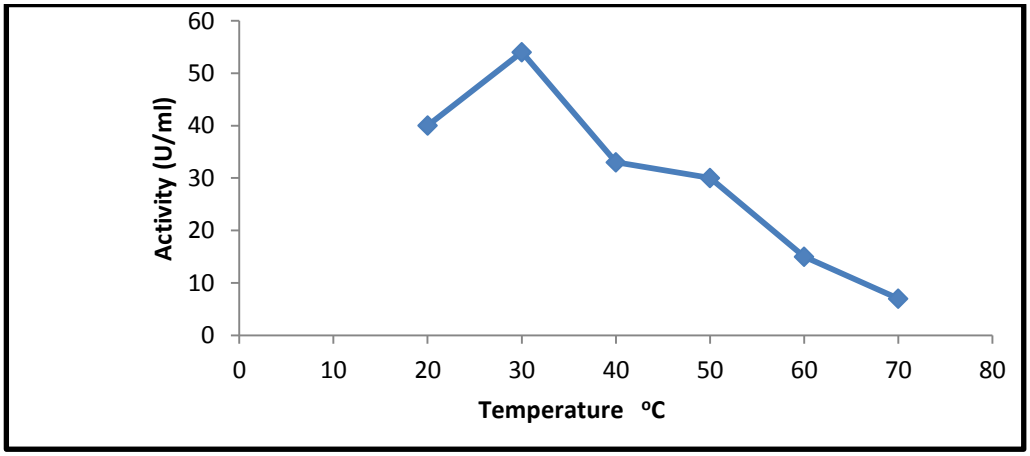

Figure 8: Effect of temperature on on the PPO activity from cabbage

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