Determination activities of optimal conditions of peroxidase in hot Pepper and Broccoli

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تقدير فعاليات الظروف المثلى لانزيم البيروريكسديز في الفلفل الحا ر والبروكلي

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المستخلص

قدرت فعالية انزيم البيروكسديز من الفلفل الحار والبروكلي بالطريقة السبكتروفوتومترية. استخلص الانزيم من الفلفل الحار والبروكلي مع اضافة 5غم من كل نموذج مع 20 مل حجم معين من دارىء البفر تركيز 0.1 مولار برقم هيدروجيني (pH7. 0) , وتم تقدير الفعالية الانزيمية للبيروكسديز باستخدام الكوايكول كمادة اساس. قدرت تأثيرات ;تركيز مستخلص الانزيم ,تركيزالمادة الاساس ,تركيز بيروكسيد الهيدروجين ,الرقم الهيدروجيني ودرجة الحرارة . لوحظ ان اعلى فعالية لبيروكسديز كانت عند تركيز(2.5 ملغم /مل) و (2ملغم /مل) من الفلفل الحار و البروكلي على التوالي . اما تركيز المادة الاساس فالبنسبة للكوايكول سجلتا اعلى فعالية عند تركيزي 100 و 16 ملي مولار للبيروكسديز من الفلفل الحار و البروكلي على التوالي, واعلى فعالية سجلت لانزيمي البيروكسديز عند الارقام الهيدروجينية 6.0 و 7.0 من الفلفل الحار و البروكلي على التوالي ولوحظت اعلى فعالية للانزيمين عند درجات الحرارة ° 40C و من الفلفل الحار و البروكلي على التوالي° 30 C .هذه الظروف المثلى التي قدرت الفعاليات لكلا الانزيمين في نموذج الفلفل الحار والبروكلي.

الكلمات المفتاحية:البروكلي , البروكسديز ,الفلفل الحار , المثلى

Abstract

The activities of peroxidase (POD) in hot pepper pericarp and broccoli were evaluated using spectrophotometric method. The enzymes were extracted from the pepper pericarp and broccoli 5 g with 20 mL of 0.1 M phosphate buffer solution pH (7. 0). POD activities were determined using guaiacol as a substrate. The effects of the concentration of enzyme extract, substrate concentration, hydrogen peroxide concentration, pH and temperature were investigated. The highest activity of POD was at (2.5 mg /ml) and (2mg/ml) enzyme concentration for hot peper and broccoli respectivily .The highest activity of using guaiacol concentration of 100 mM and 16 mM for hot peper and broccoli respectivily. POD was maximal optimized with guaiacol and 24 mM H_2O_2. The optimum pH was 6.0 and 7.0 for POD for hot peper and broccoli respectivily . The optimum temperature was 40°C and 30 °C for hot peper and broccoli respectivily. These optimum conditions were used to determine the enzyme activities in hot pepper and broccoli sample.

Keyword: Brocoli ; peroxidase ; hot pepper ;optimization

Introduction

Enzymes in plant tissues can have undesirable or desirable effects on the quality of fruits and vegetables such as the post-harvest senescence, oxidation of phenolic substances, starch-sugar conversion and post-harvest demethylation of pectic substances leading to softening of plant tissues during ripening, The major factors which are mostly used to control enzyme activities are temperature, water activity, pH and the use of certain chemicals during processing (1). Peroxidase (POD) is an enzyme commonly found in vegetables, which bind to hydrogen peroxide and produce an activated complex that can react with a wide range of donor molecules and cause off-flavors and colors in raw and unbalanced frozen vegetables (2, 3).

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 POD (4).

Thus, the aim of this study is to determin that POD and PPO activities in hot pepper pericarp. The optimum conditions for determination of both enzyme activities by spectrophotometric method were investigated including the amounts of enzyme extract, substrate concentration, pH and temperature of incubation.

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 provitamin A and carotenoid compounds with well-known antioxidant properties (5 -12).

Broccoli is among the vegetables with the highest POD activity, compared to other rich sources such as horseradish (13) and it is an economically important vegetable for the food industry. Thus, the target of this study was to determined POD activity in hot pepper pericarp and broccoli. The optimum conditions for determination of POD 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).
Fresh broccoli (*Brassica oleracea var*.) was obtained from a local market in Baghdad and washed with distilled water. Broccoli stems and florets were separated. Only the stems were used for peroxidase extraction due to their relatively higher activity of peroxidase as compared to the floret (15). Fresh prepared samples were frozen and stored at -20 °C until used. Broccoli stems

were removed from frozen storage and homogenized at 4 °C for 7 min. using 0.1 M phosphate buffer, pH 7.0, in a ratio of 1: 2 (grams of broccoli per milliliter of buffer). The extract was centrifuged, and the supernatant was used for further steps.

Enzyme assays

POD activity was assayed spectrophotometrically at 470 nm using substrate mixture contained guaiacol as a phenolic substrate with hydrogen peroxide (9). The reaction mixture contained 0.15 mL of 16.3 mM guaiacol, 0.15 mL of 900 mM H_2O_2, 2.66 mL of 0.1 M phosphate buffer pH 7.0 and 40 µL of the enzyme extract. Since peroxidase activity assay using guaiacol as a substrate is very sensitive and rapid, it is important to use the right levels of enzymatic activity in the extract, The enzyme source a reagent blank was prepared with 0.03 ml deionized water instead of antioxidant (control sample). One unit of activity is defined as a change in absorbance of 0.001 min-1(14).

$$\text{Activity (U/ml)} = \frac{(\text{V ml of mix})}{\text{Volume of enzyme} \times 1 \times 6.4} \times \text{slope}$$

V: volume of mix.

Effect of amounts of enzyme extract on enzyme activity

The activity of POD as a function of amounts of enzyme extract was investigated. POD activity was assayed at various amounts of the enzyme extract from (1 ,1.5 ,2, 2.5 , 3 ,3.5) mg/ml . The solution of the reaction mixture contained 0.1 mL of 900 mM H_2O_2,2.78 mL of 0.1 M phosphate buffer pH 7.0 and 0.1 mL of 16. 3mM guaiacol (14).

Effect of substrate concentration on enzyme activity

POD activity was performed using the different concentrations of substrate (10 ,12 ,14, 16 ,18) mM , and activity was assayed by using the mixture containing 40 µL of the extract of enzyme, 0.1 mL of 900 mM H_2O_2, 0.1 M phosphate buffer pH 7.0 and 16 .3 m M guaiacol at a selected volume. The effect of H_2O_2 concentration on POD activity was studied using(100 , 300 , 500 ,700 ,900, 100) m M the reaction system consisted of 40 µL of the enzyme extract, 0.15 mL of 16.3 m M guaiacol, 900 mM H_2O_2 and 0.1M phosphate pH 7.0 at a selected volume, In each measurement, the final volume of the reaction solution was 3 mL in a quartz cuvette (14).

Effect of pH on enzyme activity

The activity of POD were determined at pH values of 3, 4, 5, 6, 7 and 8 using 0.1 M citrate buffer (pH 3-5) and phosphate buffer (pH 6-8). The optimum pH for POD was obtained using guaiacol as substrate. The effect of pH on POD and the reaction mixture contained, 0.15 mL of 900 mM H_2O_2 , 2.66 mL of 0.1 M buffer solution , 0.15 mL of 16.3 mM guaiacol and 40 µL of the enzyme extract (14).

Effect of temperature on enzyme activity

POD activities were determined at 20, 30, 40, 50, 60 and 70 °C. The substrate and buffer solutions were incubated for 5 min at various temperatures from 20 to 70°C before adding of the enzyme extract. Spectrophotometric measurement for 5 min was carried out at 25°C. The activity of POD under optimum temperature was determined by adding 0.15 mL of 900 mM H_2O_2, 0.15 mL of 16.3 mM guaiacol, 2.66 mL of 0.1 M phosphate buffer pH 7.0 and 40 µL of the enzyme extract (14).

Results and Discussion

Optimization conditions for enzyme activity measurements POD are oxidative enzymes which catalyze the oxidation of phenolic substrates mainly due to enzymatic browning (15). In this study, guaiacol was used as the substrate for POD. The effect of various amounts of the enzyme extract on POD activity was studied and the result was shown as the rate of substrate oxidation by the enzymes. The substrate oxidation was found to be dependent on the amounts of the enzyme extract. (Figures 1 , 2) showed the enzyme concentration range assayed (1 , 1.5 , 2, 2.5 , 3 , 3.5) mg/ml. for POD.

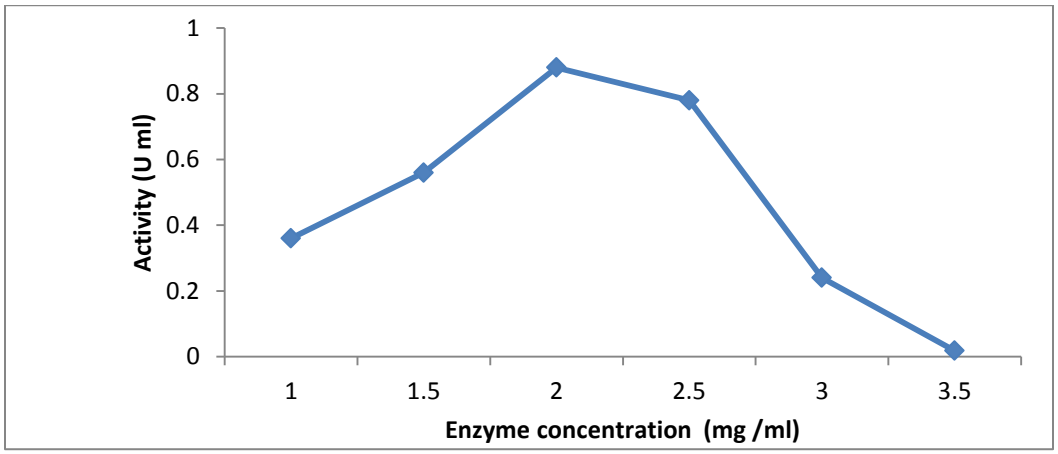

Figure (1): Effect of of the enzyme concentration on POD activity from hot pepper.

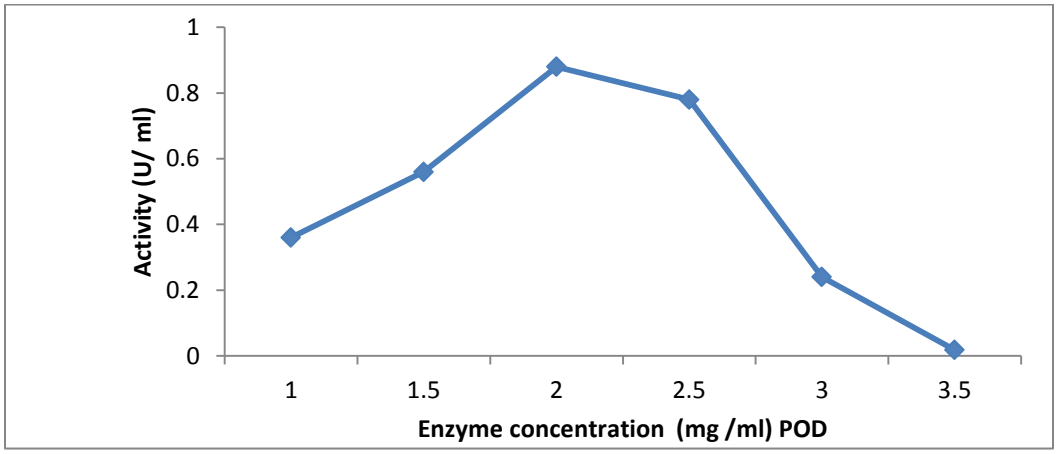

Figure (2): Effect of the enzyme concentration on POD activity.

using different amounts of the substrate (Figure 3 ,4). As expected, an increase in the substrate concentration resulted in an increase in pigment formation. Therefore, the concentration of 100 mM catechol was routinely chosen because at higher concentrations of the substrate did not significantly affect the formation of the o-quinone intermediate. When the oxidation of guaiacol by hot pepper POD was carried out in the presence of H_2O_2, the quinone intermediate formed gave absorbance maximum at 470 nm. The optimal guaiacol concentration was 16 mM determined and the results are shown in (Figure 4).

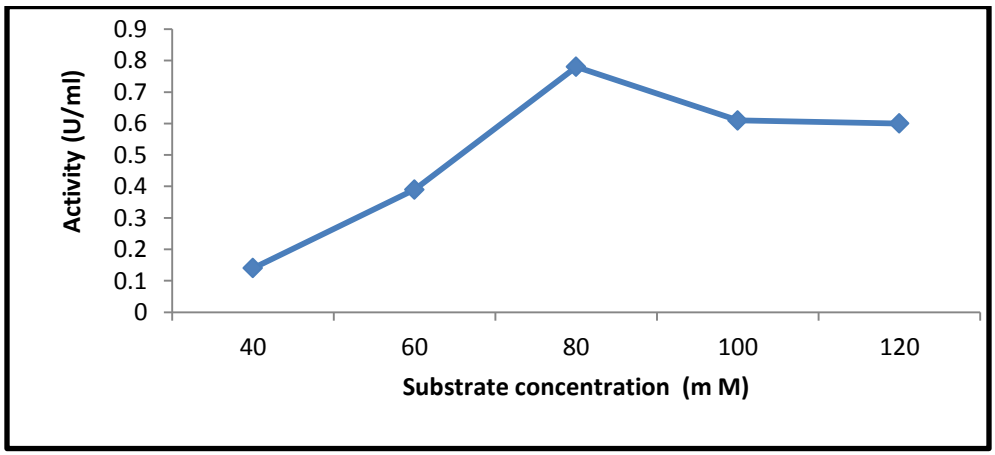

Figure (3): Effect of catechol concentration on the hot pepper PPO activity.

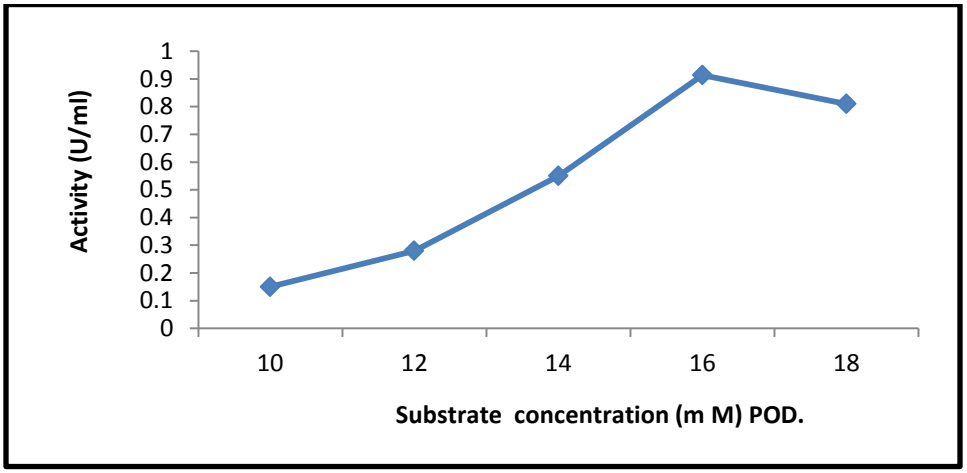

Figure (4): Effect of guaiacol and hydrogen peroxide concentrations on the hot pepper POD activity.

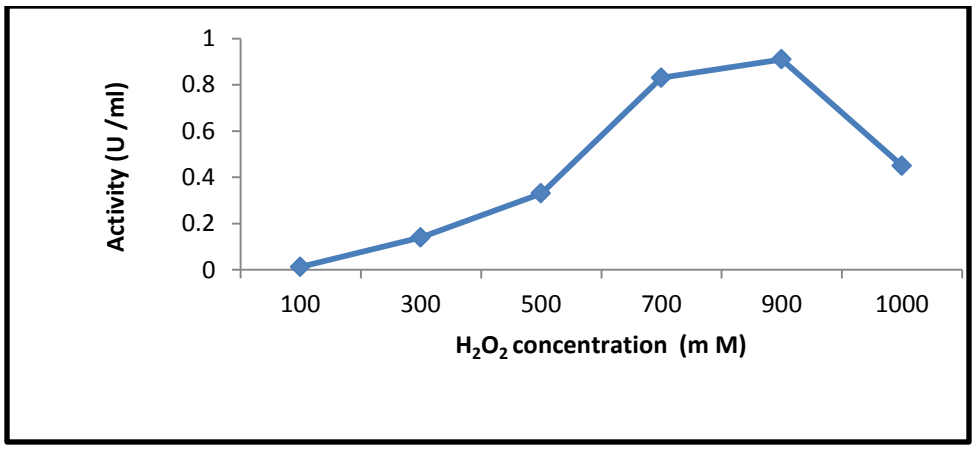

Figure (5): Effect of hydrogen peroxide concentrations on the hot pepper POD activity.

The optimal concentration of guaiacol was found to be 900 mM. In addition, when the H_2O_2 concentration was increased at a fixed saturating concentration of guaiacol, POD exhibited the highest activity at 50 mM of H_2O_2 (Figure 5). The activity of POD were measured at different pH values using guaiacol as substrate, respectively. As shown in Figure (6 , 7) the optimum pH 7 .0 , P H 6.0 of POD 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-methycatechol and catechol as substrates, respectively (16), pH 6.0 - 8.5 for kiwifruit POD using p-phenyllene diamine as substrate,(17) and pH 6.0 for spring cabbage POD using guaiacol as substrate (18).

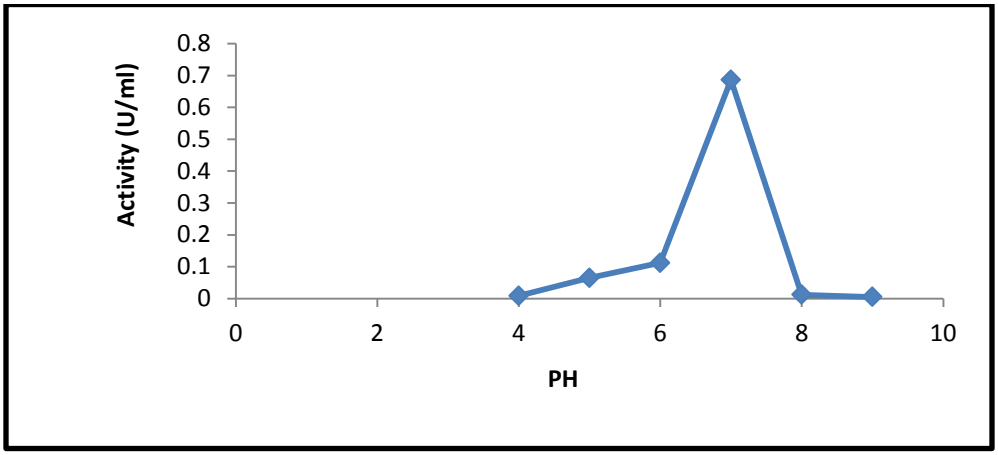

Figure (6): Effect of pH on the hot pepper PPO activity

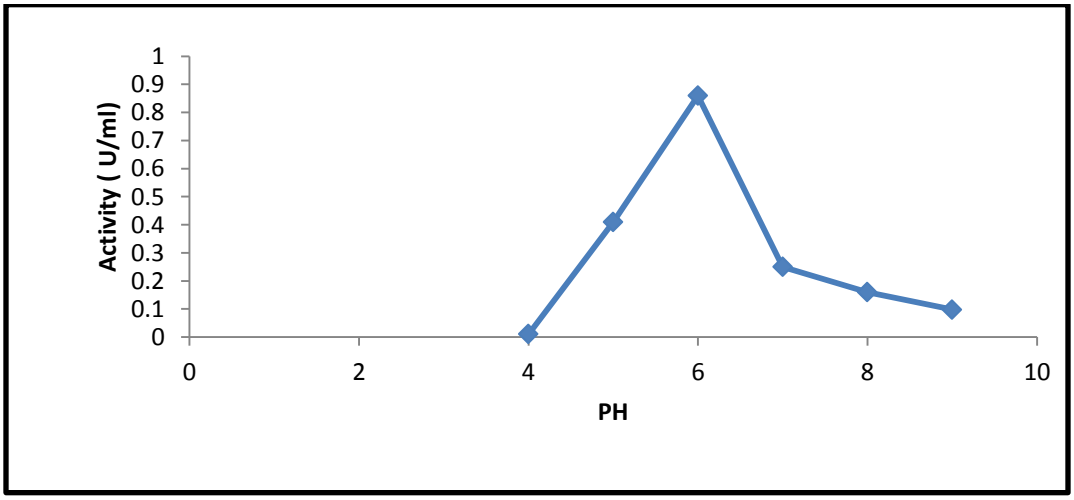

Figure (7): Effect of pH on the hot pepper POD activity.

The optimum temperature for enzyme activity usually depends on experimental conditions. Generally, the reaction rate decreases 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 (8, 9). It was found that the highest activity of PPO and POD were obtained at 30°C and 40°C, respectively. PPO showed the highest activity at 30°C, and its activity decreased slightly between 40 and 70°C. The POD activity increased when the temperature was increased from 20 to 40°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 (19). In case of POD, the enzyme was highly active up to 40°C and lost its activity at higher temperatures (12, 19, 20). 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).

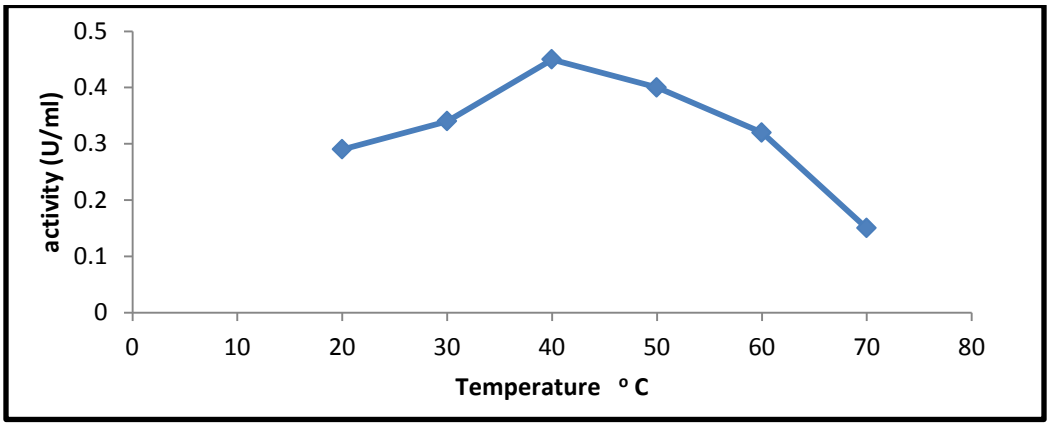

Figure (8): Effect of temperature on the hot pepper PPO activity.

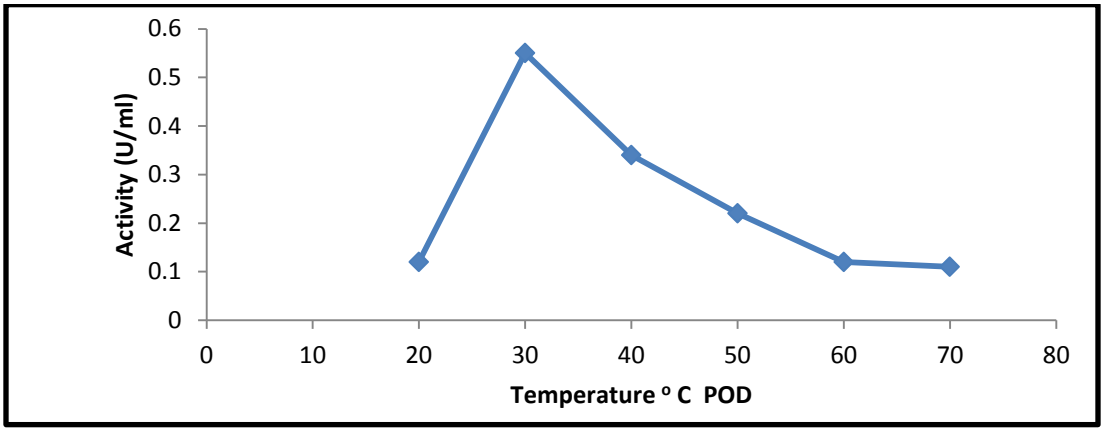

Figure (9): Effect of temperature on the hot pepper POD activity.

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