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RESEARCH ARTICLE

EFFECT OF POLYPHENOL OXIDASE PURIFIED FROM SWEET PEPPER (*Capsicum annuum* L.) IN AGGREGATION OF *STRPTOCOCCUS MUTANS* THAT CAUSE TOOTH DECAY

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Abstract

The present study was conducted to screen three parts (fruit, leaves, stem and seeds) to determine the polyphenol oxidase activity, the fruit part showed the highest specific activity and followed by leave and stem but the seeds reached less specific activity .when catechol was used as substrate. The study was also amid to determine the optimum condition for enzyme extraction. It used three methods to extract enzyme to select the best. finally extraction by 0.1M sodium phosphate buffer ,pH 7.5 , and followed extract by acetone powder while decrease when extract by 0.1M potassium phosphate buffer .

The purification process included ammonium sulfate precipitation by saturation ratio 70%, ion exchange chromatography by using DEAE-cellulose and gel filtration chromatography on the Sepharose 6B column, the characterization studies revealed that the molecular weight of enzyme determined by Sepharose 6-B column was 38355 Da. The optimum pH for enzyme activity and stability were 7.5 and (7-8) respectively. The optimum temperatures for enzyme activity and stability were 30^{o} C and (20-35) ^{o} C, respectively. The effect of metal ions showed decrease in the activity of the enzyme with $HgCl_2$ at 5mM and 10mM while increase in activity with $CuSO_4$ and $CaSO_4$,NaCl,$FeCl_3$ at same concentration . The enzyme inhibition by ascorbic acid, SDS, sodium azid and EDTA .but no effect activity when enzyme incubated with $MgSO_4$, $ZnSO_4$.

INTRODUCTION

Polyphenol oxidase belongs to oxido reductase group, number sequence (1.14.18.1) (12). It spreads wide rang in the plant and some fungi and bacteria. It is responsible of melanin in the animals and interactions of enzymatic browning in the plant (15). And enzymatic browning happened due to this enzyme. (6, 11). The worked of the enzyme initial happen by the imbalance in the safety of the cell when mixed components of plastid with the vacuoles (8, 10). Polyphenol oxidase may have a role in the water stress and damage the optical oxidation.

Enzyme extraction

The pericarp was homogenized by using blender for 2min .Five grams of homogenized pepper pericarp were extracted with 0.1M phosphate buffer pH7 containing 3% PVP using magnetic stirrer for 15min. the homogenate was filtered through Whatman No.41 filter paper and then centrifuged at 2,500rpm for 20min. The supernatant was filtered through Whatman No.42 filter paper and collected as an enzyme extract (4).

Assay of PPO Activity

Enzyme activity was determined with catechol as substrate according to a spectrophotometric procedure (21). The sample cuvett contained 2.9 ml of 0.01M substrate in 0.05M sodium phosphate buffer (pH7.0) and 0.1ml of the enzyme. The blank sample contained only 3ml of substrate solution. One unit of PPO activity was defined as the amount of enzyme that causes an increase in absorbance of 0.001/min.

Protein determination

Protein content was determined according to the dye binding method of Bradford using bovine serum albumin as a standard (7).

Determination activity of PPO in different type (*Capsicum annuum* L.)

Appropriated crud enzyme extraction from parts of sweet pepper, five gram from (fruit, leaves, stem and seeds) concluded each part alone using the way described (4), and then estimated the activity and protein concentration.

Optimization of PPO extraction

Determind the optimal way to extraction of enzyme

Preparation of PPO crud extract

The pepper extract prepared by weighting 5g of fruit section (The most effective part) of sweet pepper by cutting quickly with a clean knife , each piece of 1cm then crushed by using a clean ceramic mortar with poured in the clean tube directly centrifuge 8000 rpm for 15min at 4^{o}C (24). Then separated supernatant (enzyme crud extract) from the sediment used to estimation PPO activity and protein concentration.

Extraction by acetone powder preparation

Acetone powder was prepared by extraction 5g of pericarp of fruit pepper from homogenate with 30 ml of cold acetone that had been kept at -15 C by ceramic mortar about ten min in present of ice. The slurry obtained was filtered under vacuum from a Bunchier funnel containing a Whatman No: 1 filter paper. The solids remained on the filter paper was collected and lifted about two hours at laboratory temperature to remove residual acetone (20).

Extraction procedure

The enzyme extraction from acetone powder was prepared by mixing 0.3g of acetone powder with 10 ml of 0.1M potassium phosphate buffer pH 6.0. The mixture were stirring for 60 min at 25^{o}C with magnetic stirrer and the supernatant was separated from the sediment by cooled centrifuge using 8000rpm for 15min at 4C . Finally, enzyme activity and concentration of protein were determined (22).

Effect of extraction ratio

Different ratios (1:4, 1:6, 1:8, 1:10) (w: v) were used to extract enzyme from pericarp of fruit pepper. Enzyme activity and protein concentration was measured.

Effect different buffer on PPO activity

PPO activity were determined at pH values of 4,4.5,5 using 0.1M acetate buffer 6,6.5,7,7.5,8 by using 0.1M sodium phosphate buffer and 8.5,9 using Tris-HCl.enzyme activity and protein concentration was measured.

Effect of buffer concentration on PPO activity

The enzyme extraction at different concentration of buffer (0.05, 0.1, 0.2, 0.4) to determine the optimum concentration.

Effect of time extraction on PPO activity

The enzyme extraction at different time of magnetic stirrer (15, 30, 45, 60).to determine the optimum time to extraction.

Purification of PPO

Enzyme concentration

The enzyme concentration by ammonium sulfate at different saturation ratio to determine the best ratio. The enzyme purified from sweet pepper by using ion-exchange chromatography DEAE-cellulose and gel filtration chromatography to column sepharos 6-B .were prepared according to the instruction of the Pharmacia Fine Chemical Company.

Characterization of PPO

Determination of molecular weight

The molecular weight of enzyme was determined by gel filtration on column Sepharose 6-B.

Effect pH on PPO activity

The substrate (catechol) was prepared at a concentration of 0.1M with different rang of pH (4-9), 0.1ml partially purified enzyme was added to test tubes each contained 2.9ml of catechol solution and the activity was estimated.

Effect pH on PPO stability

Amount of 0.2ml of enzyme was added to test tubes containing 0.2ml of buffer in different rang (4-9) and the tube incubated in a water bath at a temperature 25^{o}C for 60min. The remaining activity was estimated.

Effect of temperature on PPO activity

The enzyme activity was estimate in a range of temperatures (20-80) ° C for 5min.The activity was estimated.

Effect of temperature on PPO stability

The thermal stability studied, the enzyme was incubated at various temperatures for 60min, and rapidly cooled in an ice bath for 5min .remaining activity was estimated.

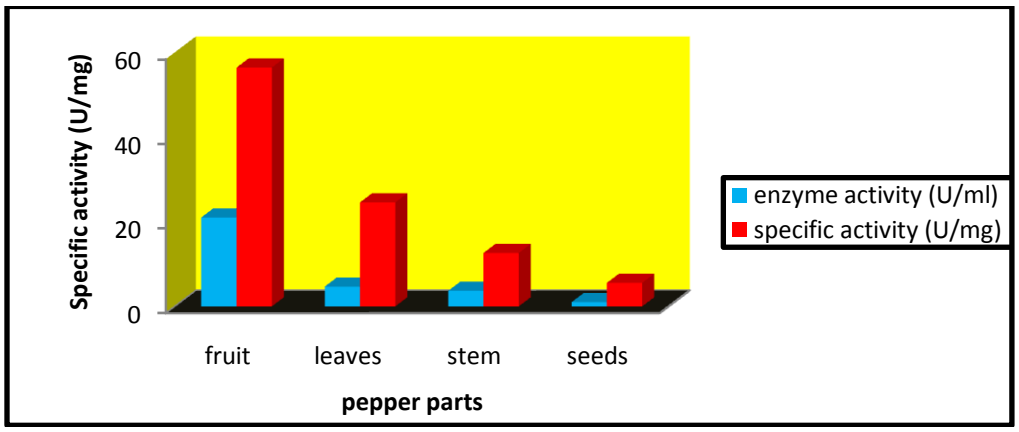
Effect inhibitors on PPO activity

The enzyme incubated with various inhibitors($CuSO_4$,$CaSO_4$,KCl,NaCl,$MgSO_4$,$ZnSO_4$,$FeCl_3$) and chelating agent (EDTA,SDS,Ascorbic acid, Sodium azid) ,was prepared in 0.1M phosphate buffer at pH7.5 ,for 5min at 30°C. remaining activity was estimated .

Result and Discussion:

Determination of polyphenol oxidase in different parts of (*Capsicum annuum* L.)

Results in figure (1) showed the PPO was present in all tested parts of sweet pepper (fruit, leaves, stem and seeds).The fruit part showed the highest specific activity protein and followed leave. Therefore, the fruit part was selected in this study to extract and purify the enzyme. Studied on polyphenol oxidase from different sours .PPO extract from hot pepper (*Capsicum annuun* L.) (4), Mango (*Mangifera indicia*) (18).



Figer (1) specific activity of polyphenol oxidase in different parts of (*Capsicum annuum* L.).

Optimization of PPO extraction condition

Optimum methods extraction of enzyme:

In figure (2) showed the extraction by 0.1M sodium phosphate buffer containing 3% PVP reached the highest specific activity because ability of PVP to binding with phenol compound and prevent phenols –protein interaction (25). This result is similar to extraction of PPO from Sapodilla plum's (*Achras sapota*) by weightining 5g of Rip fresh were blended for 1 min with 100 ml of 0.1M phosphate buffer ,pH7, and 0.2g of ascorbic acid , different concentration s of PVP and 0.1% of TX100 at 4°C (9).

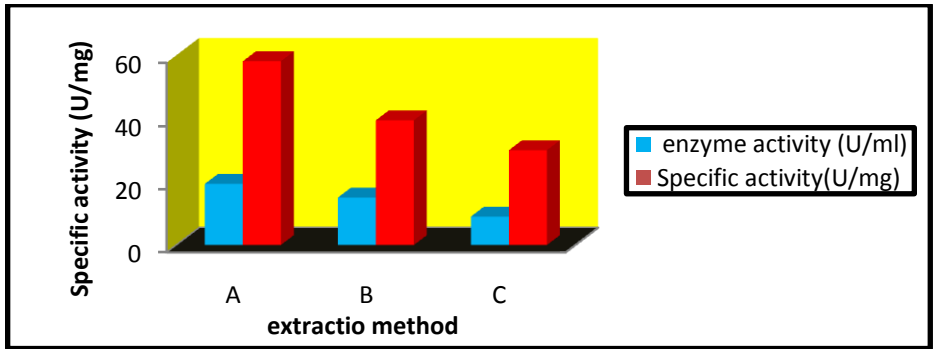


Figure (2) Comparison between extractions of PPO A: Extraction by 0.1M phosphate buffer containing 3% PVP, pH7, B: Acetone powder, C: 0.1M potassium phosphate buffer pH 6.

Effect of extraction ratios:

Result indicated that the PPO activity and specific activity were varied in different extraction ratios (fruit weight: tap water volume) used. The highest specific activity at extraction 1:4 (w: v) and followed by 1:6. This study is similar to extraction ratio of hot pepper (*Capsicum annuum*). The enzyme extraction by 1:4 (w: v) (4).but the enzyme extract from Indian Gooseberry (*Phyllanthus Emblica*) ratio 1:5 (w: v) (2).while the enzyme extract from cut lettuce by ratio 1:3 (3).

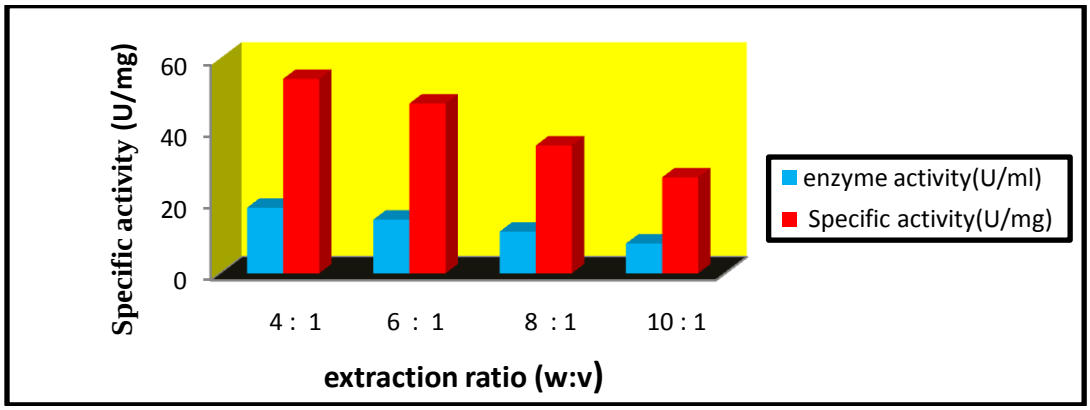


Figure (3) Effect extraction ratio in specific activity of PPO extract from (*Capsicum annuum* L.).

Effect of buffers

Data in finger (4) showed that 0.1M phosphate buffer at pH 7.5 gave maximum specific activity, while minimum specific activity was recorded by 0.1M Tris-HCl .this study is similar to extraction of PPO from *Allium* sp. The optimum pH was 7.5 (5), while the extraction from Mustard tuber and the optimum pH7.4 at the same buffer (13).

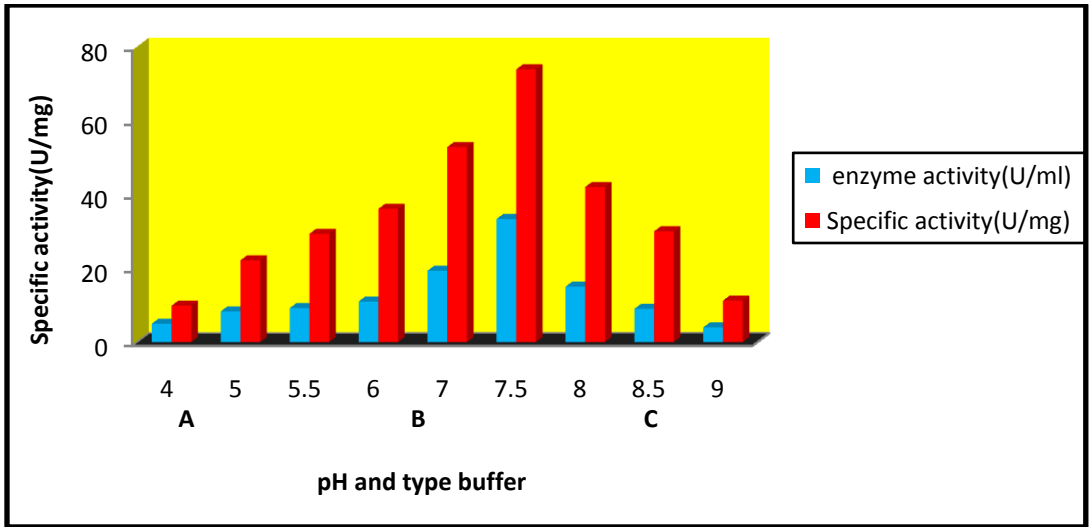
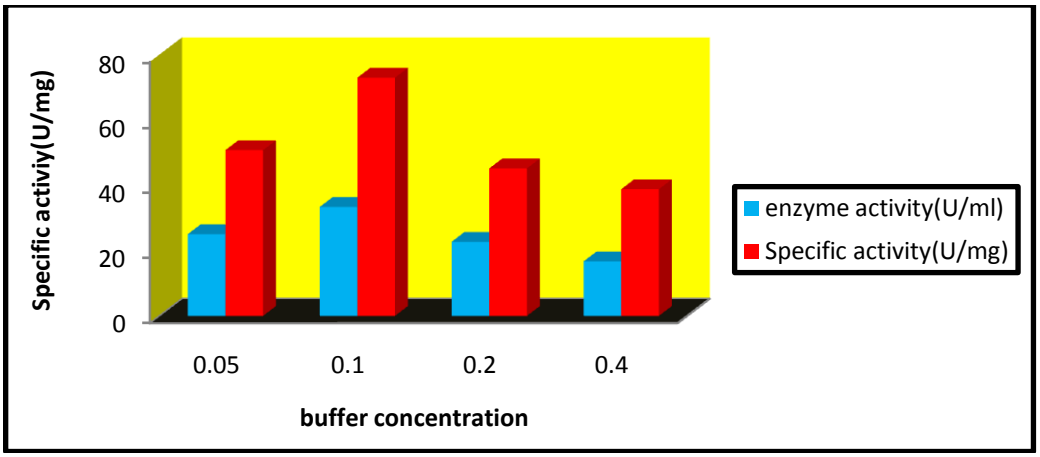


Figure (5) Effect of different buffer on specific activity of PPO from (*Capsicum annuum* L.).
A: acetate buffer pH values 4, 4.5,5
B: phosphate buffer values 6, 6.5, 7, and 7.5
C: Tris-HCl buffer values 8.5,9.

Effect of buffer concentration

Result in figure (6) indicated that the specific activity of PPO was decreased with concentration increased to 0.2. the highest specific activity by extract 0.1 M phosphate buffer , therefore the best in extraction .this result is similar to extracted of PPO from peel and pulp of tomato by 0.1M phosphate buffer pH 6.5 (16).



Figer (6) effect buffer concentration on specific activity of PPO from (*Capsicum annuum* L.).

Purification of enzyme

The enzyme purified from sweet pepper including three steps. the first step precipitation by ammonium sulfate at saturation ratio 70% , it is used because of the high salt dissolve and not to damage protein(14).and followed ion – exchange chromatography by DEA-cellulose, collected the effective parts and passed on column gel filtration by using Sepharos 6-B. studied description of enzyme , the molecular weight was 39355 Da .

Characterization of PPO

Optimum pH and stability:

Showed in figure(7) the optimum pH was 7.5 to purified enzyme from PPO by using catechol as substrate .this result is similar to purified enzyme from leave cassava (*Manihot esculenta*) using catechol as substrate (23),and the enzyme stability values (7-8) . This result is similar to purified enzyme from Pacific White shrimp (*Litopenaeus vannamei*) this enzyme was stable from natural to basic (17).

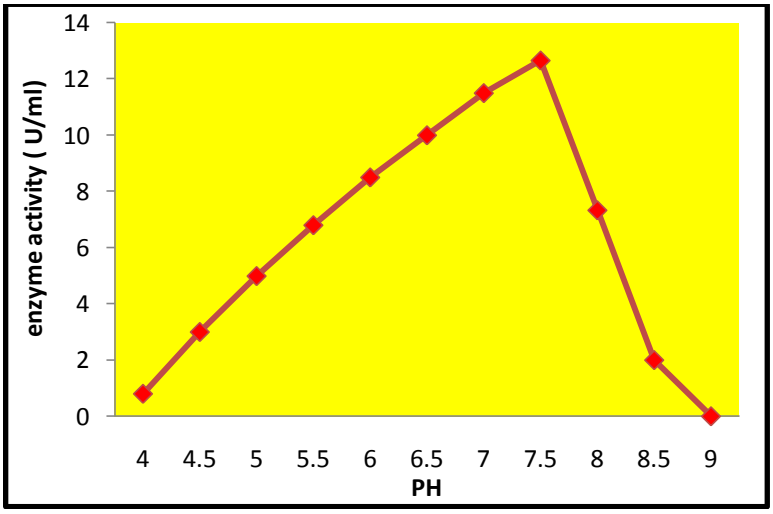


Figure (7) effect pH on PPO activity from (*Capsicum annuum* L.).

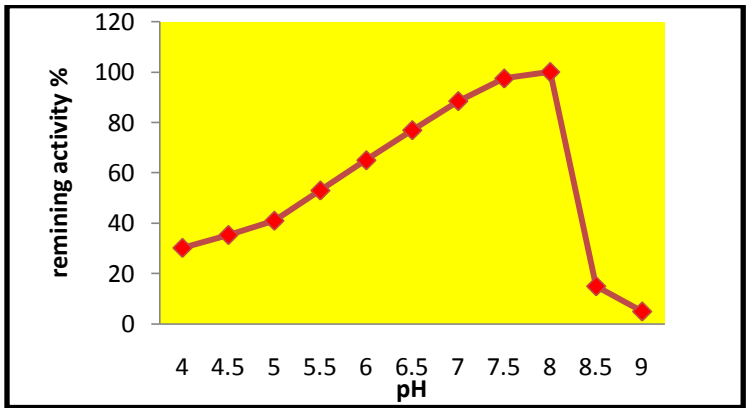


Figure (8) effect pH on PPO slability from (*Capsicum annuum* L.).

Optimum temperature and stability

The activity of PPO was measured at different temperatures at pH7.5 for 5min.The enzyme showed highest activity at 30°C (Figure 8). This value was different from those of tea leave (*Camellia sinensis*) (1) and *Mucuna pruriens* L.) and (*Mucuna prurita* H.) (19). Sweet pepper PPO was found to be most stable at (20-35) °C.

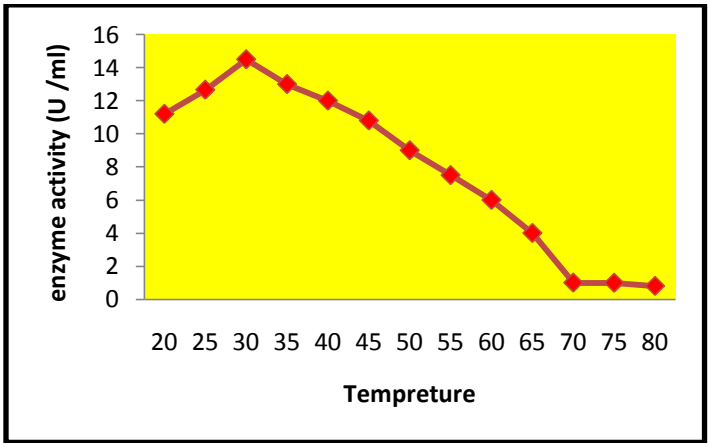
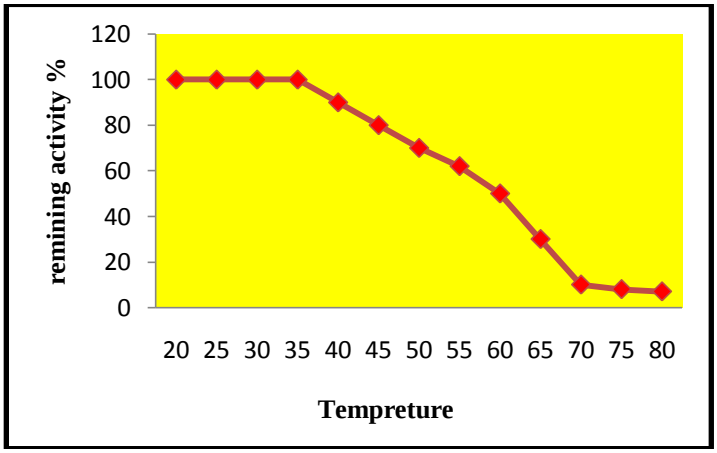


Figure (9) effect temperature on activity of PPO from (*Capsicum annuum* L.).



Figue (10) effect temperature on stability of PPO from (*Capsicum annuum* L.).

Effect inhibition

Studied and evaluated effect of seven metal ions ($CaSo_4$,$CuSo_4$,$ZnSo_4$,$MgSo_4$,NaCl.KCl,$FeCl_3$) SDS,EDTA, ascorbic acid, sodium azide.the results indicates that *Capsicum annuum* PPO is a copper containing enzyme , copper sulphate and zinc sulphate (5,10Mm) serves as activator for its activity . SDS, EDTA, Ascorbic acid, Sodium azide (5,10Mm) showed inhibitory effects on the activity of PPO.

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