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Investigation of partial purified guaiacol peroxidase incherry tomatoe cultivated in Kurdistan of Iran

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ABSTRACT

Peroxidases (EC 1.11.1.7.) belong to a large family of enzymes that are ubiquitous in fungi, plants, and vertebrates. The enzyme usually contains a ferri proto porphyrin IX prosthetic group and oxidizes several substrates in the presence of hydrogen peroxide Peroxidase, extracted from cherry tomatoes was isolated by ammonium sulfate precipitation technique and purified by ion exchange chromatography. The crude enzyme having 36 U/mL activity and 2.4 U/mg specific activity was subjected to ammonium sulfate precipitation technique for partial purification and the resulted activity and specific activity were 28 U/mL and 7.3 U/mg respectively. After ion exchange chromatography through DEAE-cellulose, fraction between 40-60 exhibited maximum activity of 24 U/mL and specific activity of 15 U/mg. The enzyme under discussion was found to be quite active with optimum temperature of 45^{o}C. Optimum pH for the enzyme was 5.5. Thermal treatment of crude extract of cherry tomatoes peroxidase was more stable at pH 5.5.Results showed optimum temperature is 45 ^{o}C for enzyme. Like most chemical reactions, with increase of temperature from 27 ºC, gradually, activity of peroxidase increased so; we reached to maximum of activity at 45 ºC (180%). It was found that enzyme followed the Michealis-Menton mechanism and 42 units/mg.protein and 12mM were the calculated values for Vmax and Km in precence of various concentrations of guaiacol and constant concentration of H2O2. The results showed that cherry tomatoe peroxidase was a thermostable enzyme. After 40 min at 55°C, the remaining activity was 40%.

Key words: kinetics, peroxidase, cherry tomatoes

INTRODUCTION

Peroxidases (EC 1.11.1.7.) belong to a large family of enzymes that are ubiquitous in fungi, plants, and vertebrates. The enzyme usually contains a ferri proto porphyrin IX prosthetic group and oxidizes several substrates in the presence of hydrogen peroxide [1].The enzyme occurs naturally in nearly all plants, animals and microorganisms [2]. It is found primarily in the roots and sprouts of higher plants [3].They have multiple molecular weights and a broad subcellular distribution. During plant development, patterns of the peroxidase isoforms appear to differ depending on source, organ or growth stage of sample being analyzed. Thus, it seems that the expression pattern of peroxidases is tissue specific and developmentally regulated [4]. This enzyme has been widely used as a reagent for organic synthesis and biotransformation as well as in coupled enzyme assays, chemiluminescent assays, immunoassays and treatment of waste waters [5].The documented sources of peroxidase in plants are horseradish, turnip, potato, tomato, carrot, bananas and etc. Peroxidase has been reported to participate in late stages oflignin-forming process [6]. Peroxidase is the most heat-stable enzyme having a wide range of application in health sciences asa diagnostic tool [7]. Several isoperoxidases, notably horseradish and turnip, have been studied in great detail during the past two decades [8]. Studies in our laboratories and by other workers on peroxidase isoenzymes from many different plants indicated that physical and kinetic properties and substrate preference of this isoperoxidase even from a single source might vary significantly [9,10,11].The study of this enzyme in food has attracted interest because of its capacity to modify food in both desirable and undesirable ways. Peroxidase activity has been related

to the existence of cationic and anionic isoenzymes [12]. Generally, the enzyme is found in glycosylated form and associated to membranes.The objectives of this work included the kinetic studies, partial purification and characterization through ion-exchange chromatography from cherry tomatoe.

MATERIALS AND METHODS

Preparation of Cherry tomatoe Extract: The peroxidase activity of cherry tomatoes was measured according to Jen et al. (1980)[17]. 500 gram of cherry tomatoes were added to 400 ml distilled water and thoroughly blended for 15 min. Sediments were discarded and supernatants were passed through filter paper. Total volume of prepared extract was 500 ml which was then heated at 65°C for 3 min in water bath to inactivate catalase present in the extract. The crude extract was subjected to partial purification or enzyme by using ammonium sulfate precipitation technique [13]The precipitates were recovered by centrifugation at 15000 *g* for 30 min at 4°C and was dissolved in a small volume of 0.1 M phosphate buffer (pH 6.8), dialyzed against the same buffer. The dialysis solution were applied to a DEAE-cellulose, and the protein was eluted with a linear salt gradient from 0 to 0.3 M NaCl in the above buffer. The fractionsshowing peroxidase activity were pooled, so 60fractions were collected in about 7 hr by a constant drop rate.The protein with peroxidaseactivity was dialyzed again st distilled water for48 handwas stored at - 20°C until use. In order to correct for substrate autoxidation, the reaction mixture, was placed in the sample cuvette while the reference cuvette contained buffer and the substrate. Enzyme activity was calculated from the linear portion of the curve.Enzyme Assay and Protein Estimation: Phosphate buffer of pH 6.5 containing 2 ml guaiacol was used for measuring at 420 nm for peroxidase assay in presence of H2O2[6]. Studies were conducted to determine the protein contents in enzyme extract before and after partial purification by lowry method [14]. Effect of Various Kinetic Parameters investigated on Partially' Purified Peroxidase. Application of Michealis-Menton equation was confirmed by using the partially purified extract of cherry tomatoes containing various concentrations of guaiacol. Crude extract reaction mixtures having pH 1-10 were prepared and analyzed on spectrophotometer at 420 nm after 1 min of reaction [15]. One millilitre of partial purified peroxidase was heated for 5 min at various temperatures and absorbance (OD) was recorded at 420 nm on spectrophotometer of 6305 JENWAY. Again 1ml of crude extract was subjected to a variable thermal treatment. The samples were heated at 60°C for 10 to 60 min and were then placed at 4°C for 5 min. The absorbance was noted at 420 nm.

RESULTS AND DISCUSSION

The partial purification ofperoxidase of cherry tomatoewas done by using ammonium sulfate precipitation [13] and DEAE-cellulose chromatography. To purify the enzyme from cherry tomatoes, firstly it was salted out with (NH4)2S04. The enzyme of interest was earned at precipitation from 30 to 80% saturation. Degree of purification after ammonium sulfate precipitation was found 3. Rehman et al. (1999)[16] reported the degree of purification as 1.93 in horseradish peroxidase, whereas Civelloet al. (1995)[6], reported 2.37 degree of purification from strawberry fruit using the same technique. The most often used cellulosic anion exchanger is DEAE-cellulose [16]. Degree of purification of cherry tomatoes peroxidase was 6.25 fold with DEAE-cellulose chromatography. Specific activity of cherry tomatoes peroxidase in crude extract was 2.4 unit/mg.protein and it increased during the process of purification to 15 (Table I). Protein contents were estimated by lowry method. The absorbance values of crude and partially purified extracts were recorded at specific wavelength of 420 nm after 3 min reaction period. The OD values with respect to time interval were noted (Fig. 1). Guaiacolwas used as a substrate in partially purified extract (Fig. 2). In crudeand partially purified extract, the optimum pH was 5.5at a range 3-10. It was observed that activity of enzyme increased gradually with increasing pH with Its peak at pH 5.5 with guaiacol. The optimum pH depends upon H' donor. It may be changed according to the substratc used (Halpin et al., 1989)[18]. Jen et al. (1980)[17] also found pH 5.5 as optimum with guaiacol while purifying tomato peroxidase. The purification procedure and results by crudeextract, precipitation with ammonium sulfate and ionexchangechromatography are summarizedin Table 1. Table I shows that the specific activity of cherry tomatoe peroxidase was 15U/mg protein and the degree of purification was 6.25. Specific activity of cherry tomatoe peroxidase was 2.4, 3.6, 7.3 and 15U/mg protein for enzyme in stage of crude extract, (NH4)2SO4 30% , (NH4)2SO4 80% and after DEAE-cellulose chromatography, respectively. Activity of cherry tomatoe peroxidase increased in presence of various concentrations of guaiacol and constant concentration of H2O2(10mM). So with increase of guaiacol, activity of enzyme increased until reached to maximum of rate at 42 unit/mg.protein(figure 3). More increase in concentrations of guaiacol accompanied with decrease of activity and showed substrate inhibition of peroxidase. Km of peroxidase calculated 12 mM and catalytic efficiency is 3.5 unit/mg.protein per mM. When different concentrations of H202 were added to cherry tomatoe peroxidase solution at pH 5.5, it was found that the peroxidase activity gradually increased(Fig. 2). So its apparent *Vmax* and *Km* were 2.7 U/mg protein and 10mM respectively in presence of constant concentration of guaiacol(15 mM).Cherry tomatoe peoxidase in presence of guaiacol and H2O2 showed fluctuations in activity with increasing temperature even as high as 90 ^{o}C. The plot for temperature demonstrated that the enzyme was very thermostable between 27 and 70^{o}C. Our results showed optimum temperature is 45 ^{o}C for enzyme. Like most

chemical reactions, with increase of temperature from 27 ºC, gradually, activity of peroxidase increased so; we reached to maximum of activity at 45 ºC (180%) (Fig.5). The drop in percentage residual activity at high temperatures can actually be due to the unfolding of the tertiary structure of the enzyme to form the secondary structure. Variations in reaction temperature as small as 5 degrees from 55 to 60 ºC introduce decrease of 40% in the activity. We measured the relative activity of enzymes at constant temperature(55°C) at a fixed pH 5.5and at a concentration of H202 (10mM) and guaiacol 15 mM after incubating for 5-60 min. The results showed that cherry tomatoe peroxidase was a thermostable enzyme. After 40 min at 55°C, the remaining activity was 40% (Fig. 6).

Table1 : Summary of cherry tomatoe peroxidase purification

	Protein (mg/ml)	Activity (U/ml)	Specific activity	Degree of purification
Crude extract	15	36	2.4	1
(NH4)2S04 30%	9	33	3.6	1.5
(NH4)2S04 80%	3.8	28	7.3	3
DEAE- cellulose	1.6	24	15	6.25

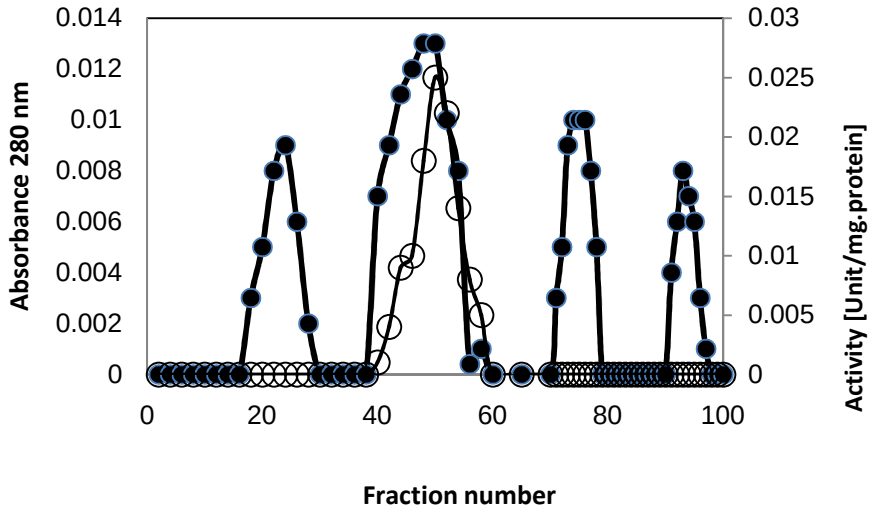

Figure 1: Absorbance of fractions of DEAE-cellulose after chromatography at 280 nm(left - ●) and Peroxidase activity of fractions inpresence of guaiacol (15mM) and H2O2 (10mM)(right-○)

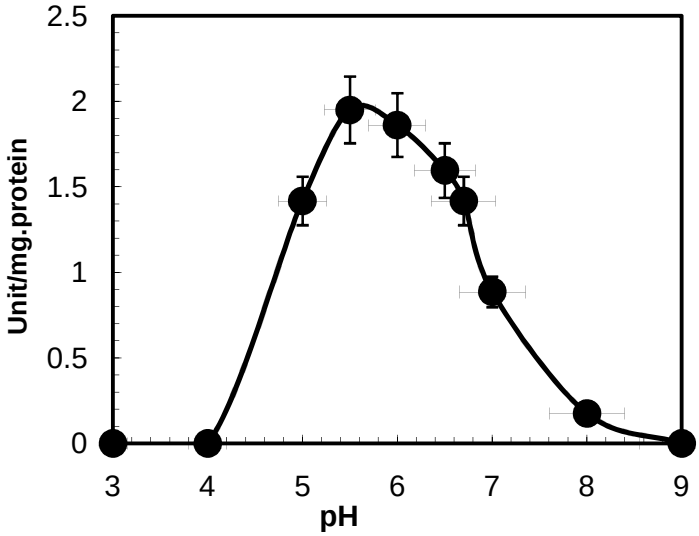

Figure 2: Effect of pH value on the peroxidase activity partial purified from *solanum lycopersicum*

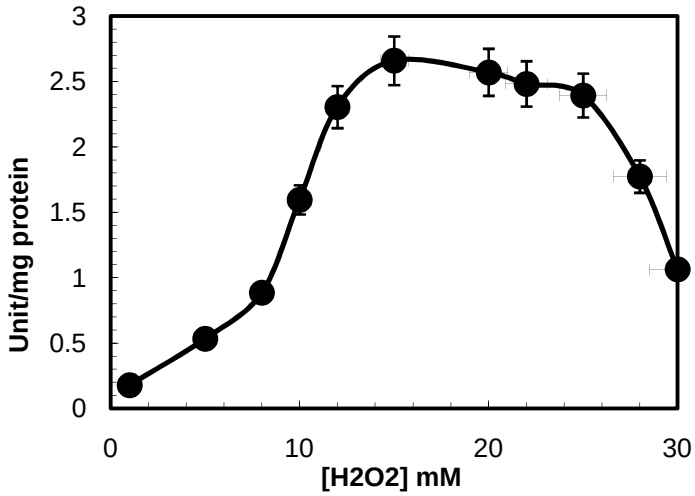

Figure 3: Activity of cherry tomatoe peroxidase in presence of constant concentration of guaiacol (15mM) and different concentration of guaiacol

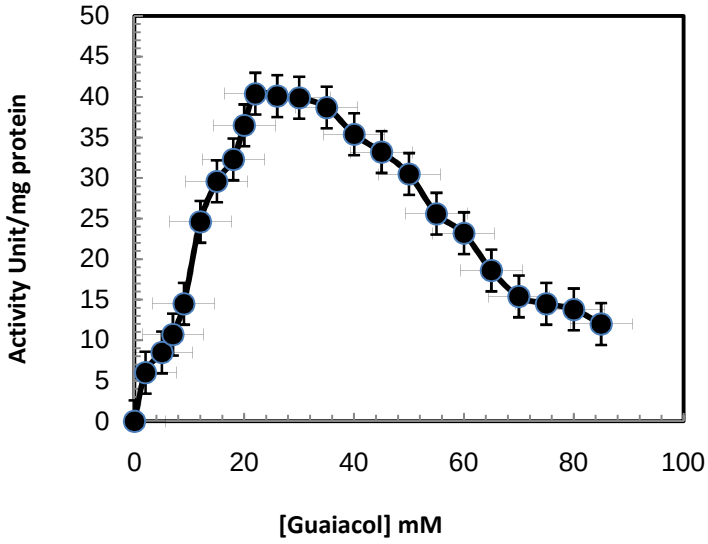

Figure 4: Activity of cherry tomatoe peroxidase in presence of constant concentration of H2O2(10mM) and different concentration of guaiacol

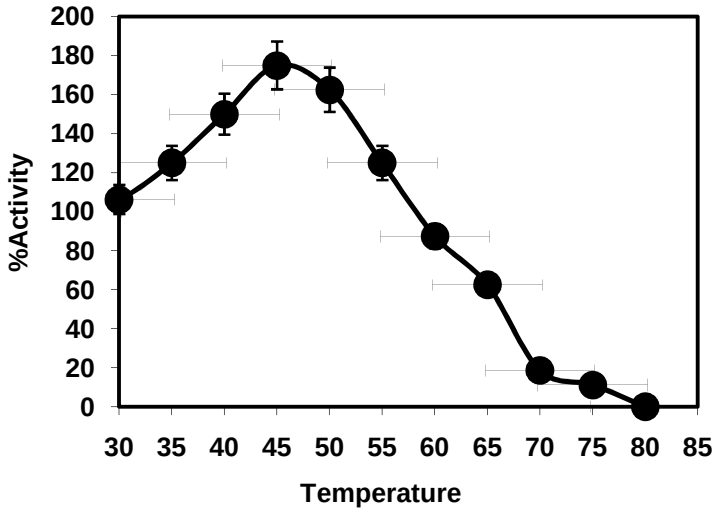

Figure 5: Effect of different temperature (30- 90)˚C on peroxidase activity purified from *solanum lycopersicum*

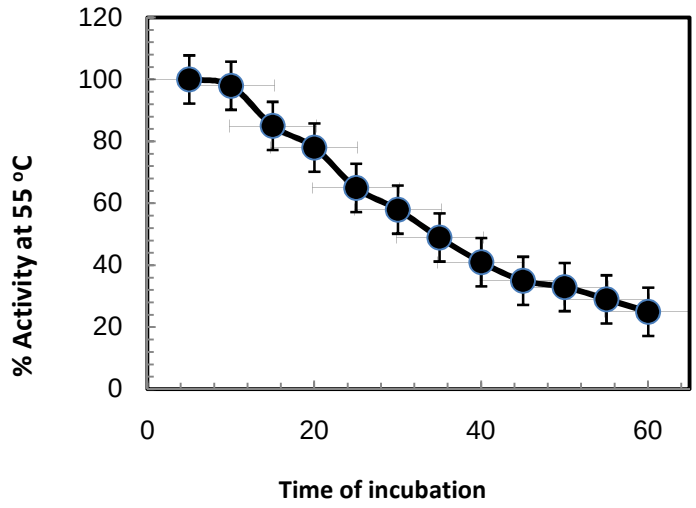

Figure 6: Effect of time incubation of cherry tomatoe peroxidase on activity at 60°C

CONCLUSION

The crude enzyme having 36 U/mL activity and 2.4 U/mg specific activity was subjected to ammonium sulfate precipitation technique for partial purification and the resulted activity and specific activity were 28 U/mL and 7.3 U/mg respectively. After ion exchange chromatography through DEAE-cellulose, fraction between 40-60 exhibited maximum activity of 24 U/mL and specific activity of 15 U/mg. The enzyme under discussion was found to be quite active with optimum temperature of 45°C. Optimum pH for the enzyme was 5.5. Thermal treatment of crude extract of cherry tomatoes peroxidase was more stable at pH 5.5. Results showed optimum temperature is 45 °C for enzyme. Like most chemical reactions, with increase of temperature from 27 ºC, gradually, activity of peroxidase increased so; we reached to maximum of activity at 45 ºC (180%) . It was found that enzyme followed the Michealis-Menton mechanism and 42 units/mg.protein and 12 mM were the calculated values for Vmax and Km in precence of various concentrations of guaiacol and constant concentration of H2O2. The results showed that cherry tomatoe peroxidase was a thermostable enzyme. After 40 min at 55°C, the remaining activity was 40%.

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