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ISOLATION & CHARACTERIZATION OF PEROXIDASE FROM PEELS WASTE OF *(CITRUS LIMETTA & CITRUS X LIMON)*

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Abstract—*Peroxidase [PODs,E.C.1.11.1.7] is one of the bio catalytic enzyme belongs to the oxidoreductase family. It is a heme containing enzyme, widely present in plants, animals and microorganisms. It catalyzes the oxidation of H_2O_2 and Guaiacol forming the product tetraguaiacol and water. The present work is focused on the extraction and characterization of peroxidise enzyme from the peel of citrus fruits. Peroxidise enzyme was extracted from peels of Citrus Limetta and Citrus Limon was found to be 5 and 6 respectively. The optimum temperature for activity from both enzyme extract was found to be 30^0C. As increasing the enzyme concentration (20µl - 120µl), the peroxidase activity was increased significantly. EDTA, NaCl & Urea decreased the enzyme activity throughout the reaction. Cations likes Mn^{++} & Cu^{++} increased the activity of peroxidase enzyme. Peroxidase enzyme could be use in waste water treatment removal of phenolic compounds, aromatic amines etc. it can also be employed for the decolorization of dye overloaded in the industrial effluents. Hence, this study could be employ as a cost effective method for waste water treatment of industrial effluents.*

Keywords: *Citrus peels, Peroxidase Enzyme activity, Activator, Inhibitor.*

1. INTRODUCTION

Peroxidase [E.C.1.11.1.7] is a ubiquitous enzyme which belongs to the oxidoreductase class of enzyme and generally catalyzes a reaction between H_2O_2 as electron acceptor and many kinds of substrate by means of O_2 liberation from H_2O_2 [1] and extensively distributed among higher plants, animal and microorganisms. Most of the peroxidises are heme proteins and contain ferriprotoporphyrin IX as the prosthetic group having the molecular weight ranging from 30,000 to 150,000 Da. They catalyze the reduction of peroxidase (H_2O_2) but also oxidase a variety of organic and inorganic compounds.

$$ROOR' + 2e^- + 2H^+ \rightarrow ROH + R'OH$$

Peroxidase is divided into three classes which differ in molecular weight and in absorption spectra i.e. Ferriprotoporphyrinn peroxidase, Verdo peroxidase and Flavoprotein peroxiadse. The peroxidases belong to a family of glycoproteins containing iron atoms as a prosthetic group and different quantities of carbohydrates residues [2]. They are located mainly in the cell wall and in the cells vacuoles of plants; their location varies according to age, species and developmental stages [3].

Peroxiadses have acclaimed a prominent position in biotechnology and associated research areas and they are one of the most extensively studied groups of enzymes. Commercially available peroxiadse is widely employed for removal of phenols and amines from industrial waste water, bleaching of industrial dyestuffs, lignin degradation, fuel and chemical production from wood pulp and in various organic syntheses. It is also used in balancing and controlling the biosynthesis of plant growth hormone, serving as a blanching indicator due to thermal stability under limited heat treatment and is widely employed in microanalysis due to its ability to yield chromogenic products at low concentrations with relatively good stability.

Peroxidase act on the removal of hydrogen atom most usually from the alcohol groups, which are combined with hydrogen peroxide in order to form molecules of water and oxidized phenolic compounds, acting as detoxifying enzymes and as a cell wall crossing linked enzyme during wounding stress [4].

2. METHODOLOGY

2.1 Extraction of peroxidase enzyme from the waste:

Ten gram of peels of citrus limetta and citrus x limon was taken and grinded in mortar for 30min with phosphate buffer and filtered with using filter paper. The filtrate volume is used for the determination of enzyme activity and protein concentration.

2.2 For the Determination of peroxidase activity:

Preparation of reagents: Guaiacol (20µl guaicol in 10ml of distilled water) & hydrogenperoxide solution (550µl hydrogenperoxide in 10ml of distilled water). The activity of peroxiadse is measured according to ***(Whitaker and Bernhard, 1972).***

The substrate solution was prepared by mixing the Guaicol: Hydrogen peroxide Solution : Sodium acetate solution : enzyme solution; 1ml: 1ml: 1ml: 60µl respectively. One blank solution and other with the 60µl of enzyme solution, measured the absorbance after 3min at 470nm.

Enzyme Activity (U/ml) = $A * V_t / € * V_s * L$

V_t is the total volume of reaction solution, V_s is the enzyme solution volume (µl), L is the light path (cm), € is the extension Coefficient ($mM^{-1} cm^{-1}$).

2.3 For Determination of protein concentration:

Preparation of reagents: BSA stock solution (1mg/ml), Analytical reagents: a) 50ml of 2% sodium carbonate mixed with 50ml of 0.1N NaOH solution. b) 10ml of 1.56% copper sulphate solution mixed with 10ml of 2.37% sodium potassium tartarate solution. prepare analytic reagents by mixing 2ml of b) with 100 ml of a), folin solution(2ml of commercial reagent with 2ml of distilled water).

Different dilution of BSA solution is prepared by mixing stock solution with water (range 0.05 to 1mg/ml). Then take 0.2ml of protein solution in different test tubes and add 1ml of analytic reagent and incubate at room temperature for 30min. then add 0.2ml of folin solution in each test tube and incubate for 30min, then measure the absorbance and determine the concentration of protein.

2.4 Characterization of peroxidase:

2.4.1 Optimum pH of enzyme activity:

Prepare buffer solution of acetate buffer of different pH range (3 – 8). Then mixed 1ml of guaiacol with1ml of hydrogenperoxide and 1ml of different pH buffer (different tubes) & the add 60µl of enzyme extract and observe the absorbance at 470nm.

2.4.2 Optimum temperature of enzyme activity:

Different range of temperature (20^oC – 80^oC) were used to estimate the optimum temperature. The substrate that was prepared was incubated at different temp. for 5 min & then add 60µl peroxidase solution and then measured the absorbance.

2.4.3 Effect on Enzyme Concentration on enzyme activity:

Different ranges of enzyme concentration including (20 - 120µl) were used to estimate the optimal concentration of enzyme activity. Substrates mixed with different concentration of enzyme and then observe the absorbance at 470nm.

2.4.4 Effect of inhibitors on enzyme activity:

Reagents include NaCl, EDTA and Urea with (2-10mM) concentration. The enzyme was incubated with inhibitor solutions at room temp. For 10 min and then the enzymatic activity was measured comparison with the control that represent the untreated enzyme.

3. RESULTS

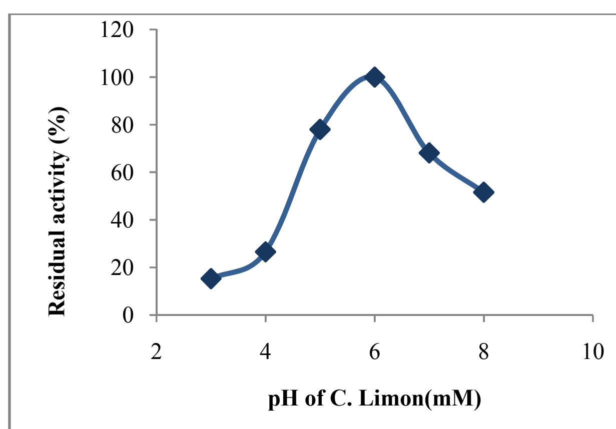
3.1 Determination of peroxidase activity in Citrus Limetta & Citrus x Limon:

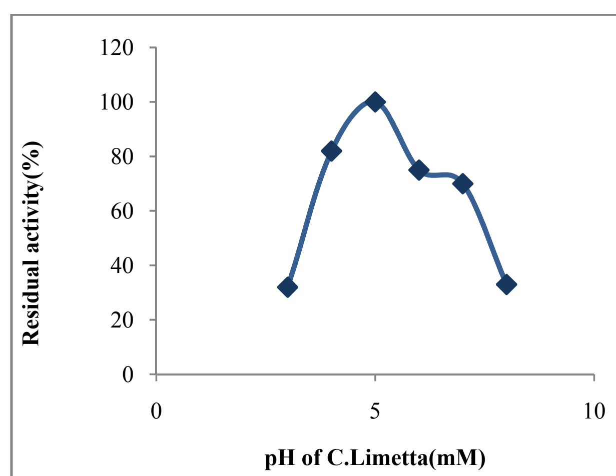
The highest activity and specific activity of Citrus x Limon reach to 1629.69U/ml and 1253.60U/mg protein respectively, compared to Citrus Limetta which gave activity 550.26U/ml and 203.8U/mg protein respectively.

3.2 Characterization of peroxidase:

3.2.1 Optimum pH of enzyme activity:

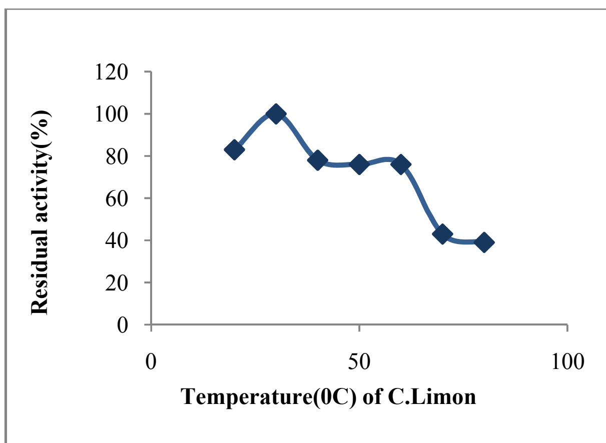
The increasing of activity by increasing the pH value until reaches to maximum activity 1342U/ml at pH 6.0 of Citrus x Limon and for Citrus Limetta maximum activity 537U/ml at pH 5.0 using guaiacol as a substrate of enzyme, then it began to decreased at higher pH value.

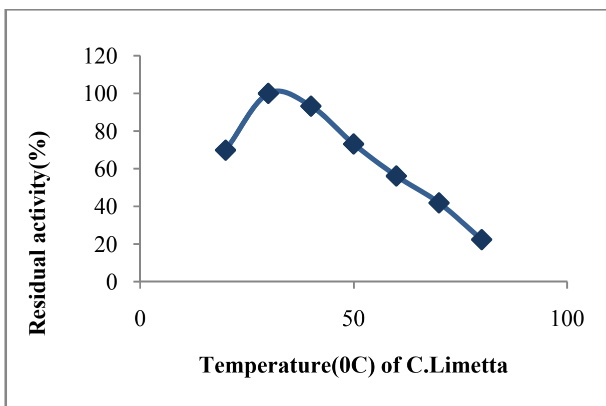




3.2.2 Optimum temperature of enzyme activity:

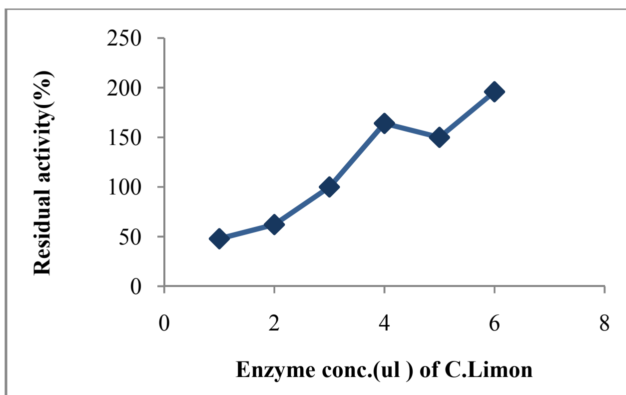
Increased in peroxidase activity by increasing the temperature when it reached to maximum 100% at 30^{0}C in both cases, then it begin to decline with increasing temperature until reached 50% at 70^{0}C in case of Citrus x Limon while it reduced to 50% at 60^{0}C in case of Citrus Limetta.

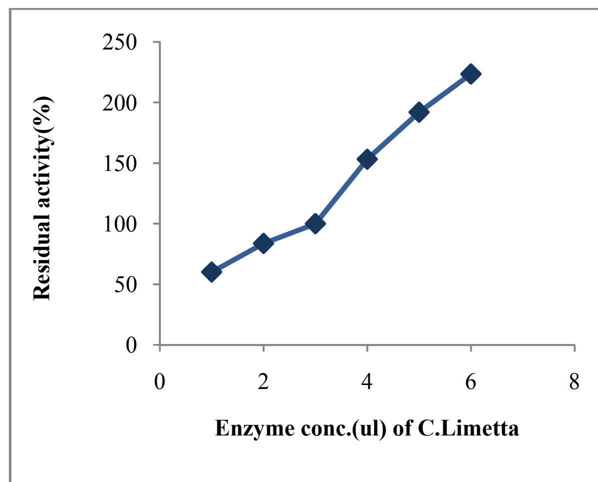




3.2.3 Effect of Enzyme Concentration on peroxidase:

As we increase the enzyme concentration than its activity also increases. The different concentration of enzyme was taken to estimate the optimum enzyme concentration.

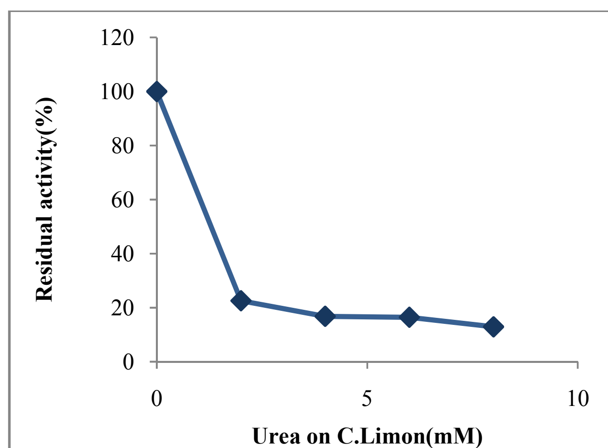


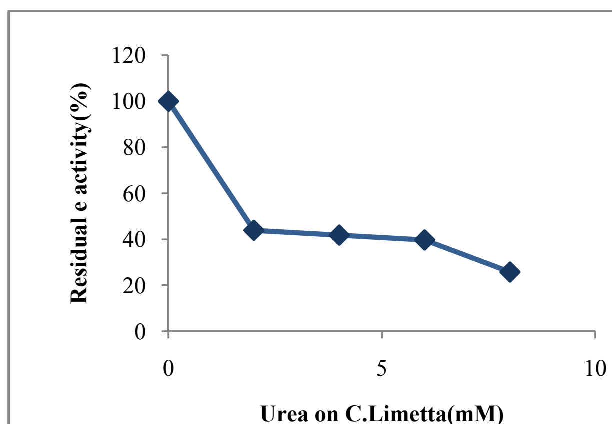


3.2.4 Effect of Inhibitors on peroxidase:

3.2.4.1 Effect of Urea on peroxidase:

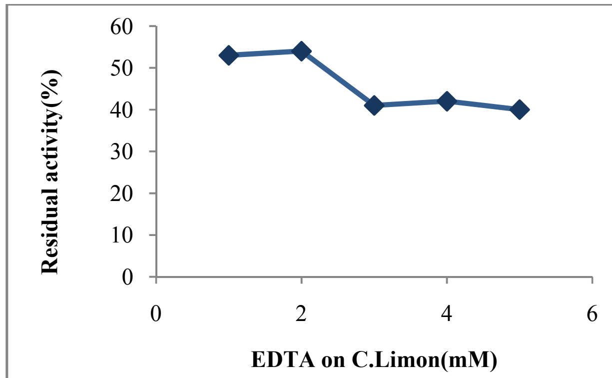
Incubation of Citrus x Limon and Citrus Limetta with 8.0M urea for 60 min resulted in the retention of 23% activity and 42% activity respectively which proved that orange peroxidase to be quite resistant against chaotropic agent.

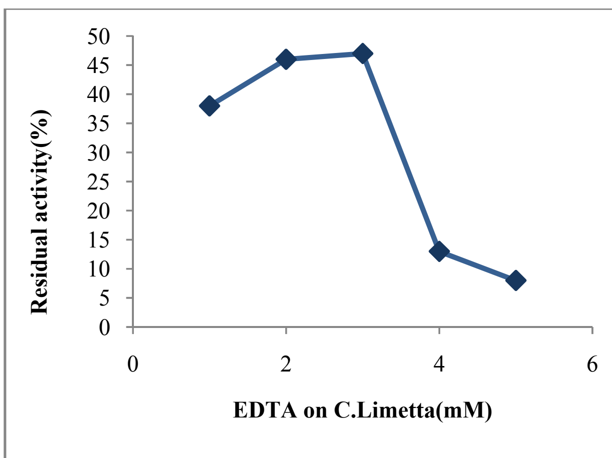




3.2.4.2 Effect of EDTA on peroxidase:

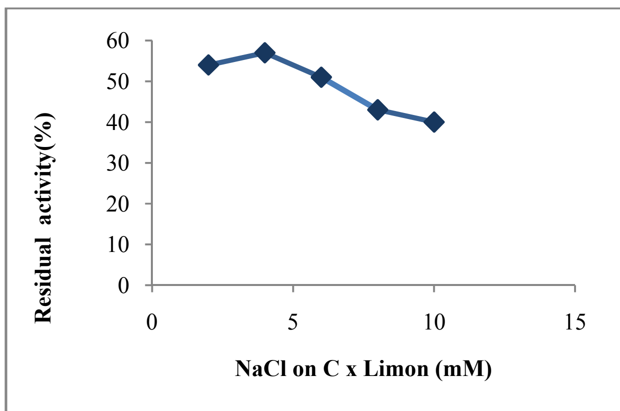
The peroxidase activity decreased to 29% when it treated with chelating agents such as EDTA with 10mM concentration, while only 23% from activity of enzyme remained when it treated with 50mM from EDTA, that confirmed that the enzyme was from metalloenzymes.

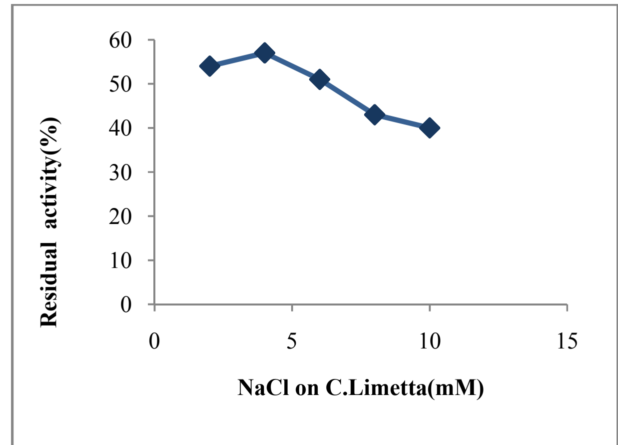


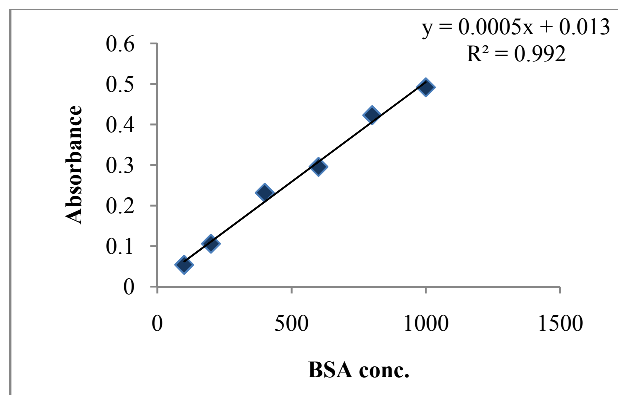


3.2.4.3 Effect of NaCl on peroxidase:

The effect of enzyme concentration on peroxidase; as we increase the concentration of NaCl than its activity gets decreased.







4. CONCLUSIONS

Peroxidase has been detected in crude of plant citrus Limetta or Citrus X Limon extract from the peel of Citrus fruits. The enzymatic activity was found to be higher in Citrus X Limon than Citrus Limetta. The guaiacol is used as substrate in the detection of enzymatic activity of peroxidase. The optimization of extraction process was done by controlling the type and concentration of buffer, pH of the buffer used and the ratio of extraction. The sodium acetate buffer with 100mM and pH 7.0 was found to be the best buffer for extraction of peroxidase. The specific activity was found to be 1253.60U/mg of Citrus x Limon and 203.8U/mg of Citrus Limetta.

Characterization results demonstrate that the optimal pH for activity of Citrus Limetta & Citrus x Limon is 5.0 & 6.0 respectively, the optimum temperature for activity is 30^{0}C for both sources.

EDTA and Urea decreased the peroxidase activity and work as a inhibitor while NaCl does not any effect. The effect of enzyme concentration was studied; it shows that by increasing the concentration of enzyme activity of peroxidase enzyme increased.

The activity of purified peroxidase was observed at absorbance 470nm by using reaction mixture such as acetate buffer, guaiacol and H_2O_2.

5. ACKNOWLEDGEMENT

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