ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Production and characterisation of peroxidase from *Raphanus raphanistrum*

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Article History:

Received on: 11.01.2018
Revised on: 16.05.2018
Accepted on: 19.05.2018

Keywords:

Characterization, Electrophoresis, Peroxidase, *Raphanus sativum,* SDS-PAGE

ABSTRACT

Peroxidases being one of the key antioxidant enzymes are involved in the essential physiological process. They play an essential role in medicinal, biochemical, industrial and environmental applications. They can catalyse degradation/transformation of aromatic dyes either by precipitation or by opening the aromatic ring structure. Radish being the source of peroxidase have been employed for the remediation of commercial dyes. Pure Radish extract was prepared by adding buffers and proteins were precipitated. The enzyme was dialysed, assayed and characterized for the optimum pH and temperature. The protein was fractionated using SDS-PAGE electrophoresis to find the approximate molecular weight. The optimum pH for peroxidase activity was found to be 5.8. The optimum temperature for peroxidase activity was found to be 50°C The molecular weight of the partially purified peroxidase enzyme is 43kDa. The study proves that with further advancement in purification method these plant peroxidase can be used as a potential source for various biotechnological applications.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i3.1549

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INTRODUCTION

Peroxidase is one of the key antioxidant enzyme widely distributed in microorganisms, animals and plants. It binds to hydrogen peroxide and produces an activated complex that can react with a wide range of donor molecules and cause off-flavours and colours in raw frozen vegetables (Lee, Klein, 1989, Aruoma *et al.,* 1986). It is essential for various physiological processes, hormonal regulation, lignin biosynthesis, and other defence mechanisms (Serrano-Martínez *et al.,* 2008). A high level of this enzyme is present in people suffering from periodontal disease. It removes hydrogen peroxide secreted by oral flora and helps in a decrease of acid production (Senthamil Sindhu, Nithya Jagannathan, 2014). So it inhibits the growth of microorganisms in the oral cavity because of its presence in saliva (Pradeep Kumar, 2017). The peroxidase enzyme is present in the cell cytoplasm in a soluble form, and the insoluble form is ionically or covalently bound to the cell wall. A temperature dependent reaction can convert the insoluble into a soluble form (Ponce *et al.,* 2004). Various factors such as the temperature, pH, water activity, and other chemicals can control the enzymatic activity (Rui *et al.,* 2006). The current bio-economy is in need of an eco-friendly and environmentally sustainable process rather than ones that pollute the environment. The roles of peroxidase come into play here because of its several applications. They have been used in bioremediation of contaminating environmental pollutants such as phenols (Sergeyeva et al., 1999, Adams, 1997). Apart from this, it is used in various biotechnological, medical, agricultural sectors as well. They are also used in paper and pulp industry, and microanalytical immunoassay. Immense potential for decolourisation of industrial effluents and widespread application as biosensors through

peroxidase are seen nowadays (Jia *et al.,* 2002). Studies have shown that they are used for the biotransformation of organic molecules, synthesis of various aromatic compounds fruit ripening, and enzymatic browning with polyphenol oxidase activity (Torres *et al.,* 1997).

Peroxidase is one of the constituents in Radish (*Raphanus sativa).* Radish is a root vegetable brown all over the world and a part of the human diet. It is rich in sugars, proteins, carbohydrates, fluorides, fats, water-soluble vitamins and minerals (Khattak *et al.,* 2011). It is used as a home remedy to treat diseases like jaundice, gastric pains, indigestion etc. (Jeong *et al.,* 2005, Shukla *et al.,* 2011). Isothiocyanates, glucosinates, glucoraphanin are some of the bioactive compounds present in white radish (Ishida *et al.,* 2015). Research studies suggest that it has anti-cancerous, anti-inflammatory and antidiabetic properties as well (Kim *et al.,* 2014). The aqueous extract of radish also inhibited alpha-amylase and alpha glycosides enzyme in vitro (Rubilar *et al.,* 2011). This enzymatic inhibition reduces the amount of glucose absorption and so is effective in the management of diabetes (Baenas *et al.,* 2016). Flavonoids and vitamin C present in radish inhibit lipid peroxidation and XOD activities, promote live and RBC catalase. This feature can help in the treatment of cardiovascular diseases (Jin, Kyung, 2011). Major organic acids like oxalic, malic, linoleic, linolenic and also present in radish (Shyamala, Singh, 1987). Studies of peroxidase enzyme from many different plants including radish have been performed in detail. The physical, kinetic properties and substrate preferences of peroxidase vary from source to source. Hence, the study aims to investigate the peroxidase activity of radish by purification, assay and characterisation.

MATERIALS AND METHOD

All chemicals used were obtained from a commercial source.

Plant material collection and extraction of proteins

As a source of peroxidase, Raphanus raphanistrum was collected and was homogenised thoroughly in Tris-HCl buffer pH 7.2 using sterile glass powders in an ice bath. This is now centrifuged at 1000 rpm for 10-15 minutes at 4'C. The proteins are precipitated using 10% TCA (150-200 Ul). Ammonium sulphate is added for salting out proteins and kept at -20'C for an hour. After 10 minutes of centrifugation, the precipitate was washed with 5ml of 80% acetone residue TCA. The centrifugation step is repeated.

Assay of peroxidase

To one gram of fresh *Raphanus raphanistrum* paste, 5 mL of ice-cold 0.5 M calcium chloride solution is added. Centrifugation of this slurry at 1000 x gravity for 8 minutes is done, and the supernatant (containing POD) is poured into a clean and labelled test tube and stored on ice. The cell wall pellet remaining in the centrifuge tube with 2.5 mL of ice-cold calcium chloride solution (0.5M) was resuspended and centrifuged again. The supernatant was poured in with the first supernatant that was collected and is stored (crude extract) on ice for up to two hours before conducting the POD assay. The total amount of calcium chloride solution used was 10 mL, thus a 10:1 volume: mass dilution was made.

Characterization of Peroxidase

Effect of pH on Enzyme Activity

Into a series of clean, dry test tubes, 2.5ml of 0.2m phosphate buffers of varying pH 2,4,6,8 and10 is added. This is followed by 0.5ml of substrate 3% solution and 0.5ml of the enzyme. Tubes were incubated at 60 degrees for 15 minutes. The reaction was stopped by the addition of 10% sodium tungstate and sulphuric acid.

Along with these controls were also incubated, to which the enzyme was added at the end of the incubation period, after arresting the reaction centrifuged and 0.25 ml of supernatant was transferred to other tubes. 0.5ml of Nessler's reagent was added and made up to 5ml with distilled water. The colour developed was read at 495nm in a colourimeter within 5minutes. A graph was drawn by plotting the PH on the x-axis and the colourimeter reading on the y-axis.

Effect of Temperature on Enzyme Activity

Into a series of clean, dry test tubes, 2.5ml of 0.2m phosphate buffer, 0.5ml of substrate and 0.5ml of the enzyme was added. The enzyme is not added in blank test tubes. Tubes were incubated at various temperatures 10- 800c for fifteen minutes. The reaction was stopped by the addition of 10% sodium tungstate and sulphuric acid. At the end of incubation period, 0.5ml of the enzyme was added to the blank and then centrifuged. 0.25ml of the supernatant was transferred to other tubes; 0.5ml of Nessler's reagent was added and made up to 5ml with distilled water. The colour developed was read at 495nm in a colourimeter within 5 minutes. A graph was drawn by plotting the temperature on the x-axis and the colourimeter reading on the y-axis.

SDS page electrophoresis

The proteins in the sample were fractionated using SDS-PAGE electrophoresis (Laemmli, 1970). The

30µg protein sample was run on12% reduced SDS-PAGE. After the run, the gel was fixed in the staining solution for 30 min at room temperature. Subsequently, the gel was destained for 45 min and then photographed. In order to find out the approximate molecular weight of partially purified peroxidase enzyme, SDS-PAGE was run with a protein marker.

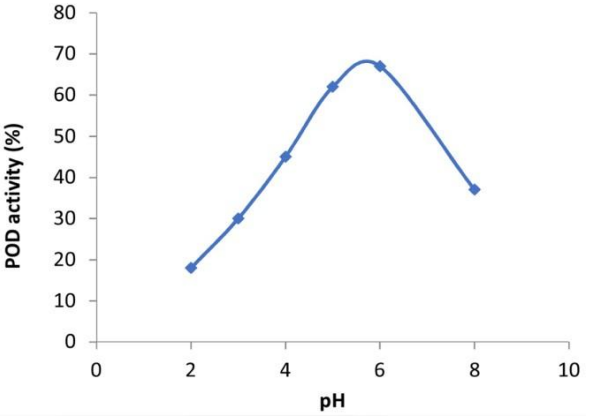

Figure 1: Effect of pH on peroxidase

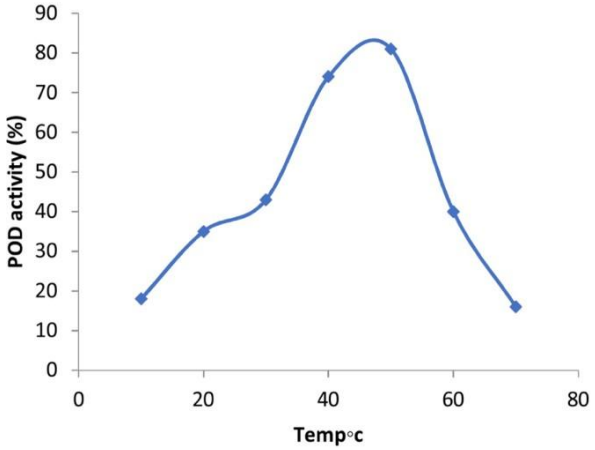

Figure 2: Effect of Temperature on peroxidase

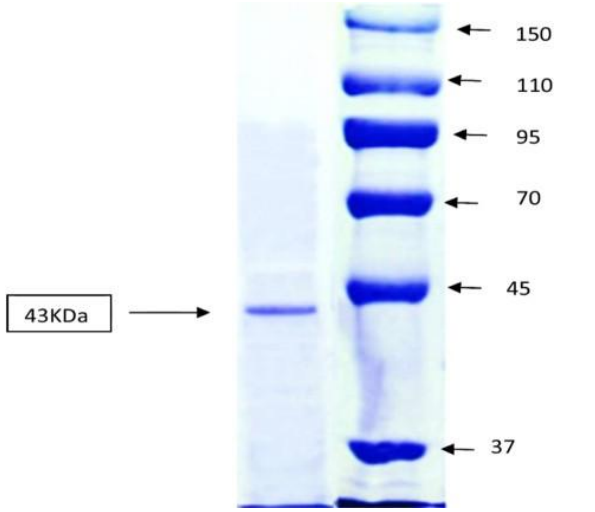

Figure 3: Electrophoresis on peroxidase

RESULTS AND DISCUSSION

The optimum pH for peroxidase activity to be 5.8. Peroxidase activity to be 50°C. On gel images, it was seen that the approximate molecular weight (M.et) of the peroxidase enzyme is 43kDa. From the above results, it has shown that peroxidases are more active and stable in acidic pH. This means that at pH 5.8 and an optimum temperature of 50°C, it is in its most active and stable state and can be used in various ways.

Water pollution has become a major issue nowadays, and industrial effluents are one of the reasons. A decolourisation technique, removal of dyes and colours from the industrial wastewater by various physical, chemical and biological means has become the current interest for research purpose. Peroxidase enzyme can be used for this purpose as a less expensive and eco-friendly alternative (An *et al.,* 2002). A large number of reports regarding the development of enzyme immunoassays using peroxidase as reporter enzyme to detect toxins, pathogens, and other analyses are available. ELISA is usually developed to detect antigens or antibodies by producing an enzyme like peroxidase catalysed colour changes (Ramakrishna *et al.,* 1990).

The peroxidase can produce stable chromogenic products. Thus, they are suitable candidate enzyme for the manufacturing of various diagnostic kits based on enzyme conjugated antibody technology. Coming to the biosensors, enzyme-based biosensors have an advantage over other analytical techniques with regards to high selectivity and high sensitivity. The performance of these biosensors depends upon the amount and bioactivity of enzyme immobilised onto the electrodes (Jia *et al.,* 2002). Because of the wide applications like clinical biochemistry, immunology, biotechnology, environment and industry, peroxidases are considered as one of the important industrial enzymes.

CONCLUSION

The results show that radish produces copious levels of POD, which was partially purified and characterised by homogeneity. The extracted enzyme showed better thermal stability indicating its wider applications. Being an economical and easily available source, further research can be continued to make use of its application such as various chemical and clinical diagnostics, removal of phenolic compounds from wastewaters, industrial decolourisation and in the food industry as well.

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