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 *Brassica oleracea* (Cabbage)

Prerna Jain, Gayathri R*, Vishnu Priya V

Department of Biochemistry, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai - 600 077, Tamil Nadu, India

Article History:

Received on: 14.02.2018
Revised on: 21.05.2018
Accepted on: 28.05.2018

Keywords:

Cabbage,
Peroxidase,
SDS-PAGE,
Temperature

ABSTRACT

Peroxidases, are the enzymes which decomposes hydrogen peroxide and also acts as an antioxidant. The plant peroxidases are involved in various biochemical processes and development and also has a role in defence. *Brassica oleracea* var. capitata (cabbage) being the source of peroxidase have been employed for the remediation of commercial dyes. Pure cabbage extract was prepared by adding buffers, extraction of proteins and protein dialysis was done. The extracted assay and concentrated enzyme was characterized for its optimum pH and temperature. The protein was fractionated using SDS-PAGE electrophoresis to find the approximate molecular weight. Once the research was performed, the optimum pH for peroxidase activity was found to be 6.0 whereas the optimum temperature for peroxidase activity was calculated to be 45°C and the molecular weight of the partially purified peroxidase enzyme is 43kDa. Peroxidase have many possibilities of clinical applications. They can be used as a diagnostic kit for Glucose and in waste water treatment.

* Corresponding Author

Name: Gayathri R
Phone: +91-9710680545
Email: gayathri.jaisai@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i3.1532

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Current bioeconomy depends on the development of reliable environmentally sustainable processes in challengeable tasks. Enzymes are biocatalyst which are stable and specific in their action .Enzymes can be utilized in various industrial processes as an eco-friendly approach. Therefore, there is a current focus in identification of desirable enzymes and its source. (Pandey *et al.,* 2017). When plants are considered, enzymes has a varied role to play such as oxidation of phenolic substances, post-harvest senescence, starch breakdown, softening of plant tissue by post-harvest demethylation of pectic substances. (Rui *et al.,* 2006). Peroxidase (POD) is an enzyme which is commonly found in vegetables that 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 and un- blanched frozen vegetables (Lee, Klein, 1989, Aruoma *et al.,* 1996). Peroxidase is found to play an important role in vital processes of plant growth and development which includes cell wall metabolism, auxin metabolism, lignification, reactive oxygen species (ROS) metabolism,, fruit ripening, plant defence against pathogens etc. (Passardi *et al.,* 2005). Owing to its specificity peroxidase has potential to be used as an industrial enzyme. Peroxidase can be potentially used in biotechnological, immunological and industrial sectors. (Adams, 1997). Hydrogen peroxide is produced by microorganisms in oral cavity. The enzyme peroxidase plays a vital role in removing the peroxidase produced in oral cavity and thus reduces the acidity in the oral cavity and also decreases the plaque deposition, gingivitis and dental caries. A high level of this enzyme is frequently observed in periodontal disease.

(Senthamil Sindhu and Nithya Jagannathan, 2014). Since peroxidase is an oxidoreductase it finds application in diagnosis and biosensors, bioremediation, decolorisation of synthetic dye, downstream processing in paper and pulp industry, polymer synthesis etc. Enzymes which are stable in high temperature, pH changes and also in the presence of heavy metals are more preferred for industrial applications. Thus in view of varied applications of peroxidase, exploring newer sources which can yield enzyme with greater resistance is more desirable (Jia *et al.,* 2002).

Physical or chemical treatments such as adding chemical additives, heating or lowering pH inhibits the enzyme activity in fruits and vegetables (Gunes and Bayindirli, 1996). Methods such as using of acidulates, reducing and chelating agents, and inorganic salts have shown to extend shelf-life of minimally processed fruits and vegetables are now being focused on. But this method could not be achieved because the demands of the consumer is concerned about the use of chemicals in such products. The search for alternative anti browning compounds was given more attention (Daraei Garmakhany *et al.,* 2010). Peroxidase is an oxidoreductase that is directly involved in many plant functions such as hormone regulation, defence mechanisms, indolacetic degradation and lignin biosynthesis (Serrano-Martínez *et al.,* 2008). It catalyses a reaction in which hydrogen peroxide acts as the acceptor and another compound acts as the donor of hydrogen atom. Peroxidase involved in enzymatic browning since diphenols may function as reducing substrate in this reaction (Chisari *et al.,* 2007, Serrano-Martínez *et al.,* 2008). The involvement of Peroxidase in browning is limited by the availability of electron acceptor compounds such as superoxide radicals, hydrogen peroxide and lipid peroxidase. Enzymatic browning is a significant problem in a number of fruits and vegetables such as strawberry, grape, potato and lettuce (Muñ oz *et al.,* 2004, Lee and Park 2007, Gawlik-Dziki *et al.,* 2007). The discolouration in fruits and vegetables by enzymatic browning, resulting from conversion of phenolic compounds to o-quinones which subsequently polymerise to be a brown or dark pigment, the enzyme involved these processes is peroxidase (Jiang *et al.,* 1999). Because peroxidase is the main enzymes involved the phenolic oxidation of many fruits and vegetables, their activities have attracted much attention.

Cabbage (*Brassica oleracea*) a green leafy vegetable usually grown throughout the year for its dense leaves. Cabbage, broccoli, cauliflower have been decanted from wild cabbage and are closely related. The most preferred vegetable for food is the smooth leafed firm headed green and red cabbages. cabbages can grow much larger, under conditions of long sunlit days, such as those found at high northern latitudes in summe. (Samec *et al.,* 2011). Analysis of chemical components has shown that the main component of cabbage are carbohydrates, comprising nearly 90% of the dry weight, where approximately one third is dietary fibre and two thirds are low-molecular- weight carbohydrates (Wennberg *et al.,* 2006). Cabbage is consumed either raw or processed in different ways, such as cooked, boiled, steamed or used as salads. Cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal disorders, due to its antioxidant, anti-inflammatory and antibacterial properties (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) as well as in treatment of minor cuts and wounds and mastitis (Samec *et al.,* 2011). Positive effects of cabbage in healing peptic ulcers has been shown by clinical research . A reduction in serum LDL levels was also facilitated by cabbage (Cheney, 1949).

MATERIALS AND METHODOLOGY

Assay of Peroxidase

The procedure starts by taking one gram of fresh Cabbage paste. To that, 5 mL of ice cold 0.5 M calcium chloride solution was slowly added. This slurry was centrifuged at 1000x g for 8 minutes. The supernatant (containing POD) was poured and stored in a clean and labelled test tube and stored on ice. Then, re suspension was done and the cell wall pellet remaining in the centrifuge tube with 2.5 mL of ice cold calcium chloride solution (0.5 M) and centrifuged again. The supernatant was poured in with the first supernatant which was collected and stored on ice for up to two hours prior to performing peroxidase assay. Thus a 10:1 volume: mass dilution was made, by adding 10ml of calcium chloride solution.

Characterisation of Enzyme

Effect of pH On Enzyme Activity

Into a series of clean dry test tubes, 2.5ml of 0.2ml phosphate buffers of varying pH 2-10 was added, along with that 0.5ml of substrate 3% solution and 0.5ml of enzyme was also added. The tubes were incubated at 60°C for minutes. The reaction was stopped by the addition of 10% sodium tungstate and sulphuric acid. Along with these control were also incubated, to which the enzyme was added at the end of 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 calorimeter within 5 minutes.

Effect of Temperature on Enzyme Activity

Into a series of clean dry test tubes 2.5ml of 0.2m phosphate buffer and 0.5ml of substrate and 0.5ml of enzyme was added to all the test tubes. The enzyme was not added in blank test tubes. Tubes were in a at various temperature 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 enzyme was added to the blank and then centrifuged. 0.25ml 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 calorimeter with in 5 minutes.

SDS-Page Gel Electrophoresis

Fractionation and further molecular weight determination of the proteins in sample was done using SDS Page gel electrophoresis. The sample was electrophoresed in gel along with amolecular weight marker. After staining and destaining, molecular weight of the enzyme is determined using molecular weight marker.

RESULTS AND DISCUSSION

Peroxidase has been purified from various sources like sweet potato, horseradish, turnip (Klapper and Hackett, 1965). 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. The enzyme activity determination is based upon the rate of utilization of substrate and in which source it is present. From the study, we obtained the following results as the optimum pH for peroxidase activity was found to be 6.0 (Figure 1), the optimum temp for peroxidase activity was found to be 45ºC (Figure 2. On gel images, it was seen that the approximate molecular weight (Mr) of the partially purified peroxidase enzyme is 43kDa (Figure 3).

From the present study, its shown that peroxidase are more active and stable in acidic pH. This means that at pH 6.0 and optimum temperature 45ºC, it is in its most active and stable state and can be used in various ways. Peroxidase can be used in various fields in today's world. To quote some, ELISA is usually developed to detect antigens or antibodies by producing an enzyme like peroxidase catalysed colour changes (Ramakrishna *et al.,* 1990). Coming to the biosensors, enzyme based biosensors have advantage over other analytical techniques due to the high selectivity and high sensitivity. The performance of these biosensors depends upon the amount and bioactivity of enzyme immobilized onto the electrodes (Jia *et al.,* 2002). The peroxidase has capability to produce stable chromogenic products, thus, they are suitable candidate enzyme for the manufacturing of various diagnostic kits based on enzyme conjugated antibody technology (Krell, 1991). In view of the wide applications like clinical biochemistry, immunology, biotechnology, environment and industry, peroxidases are considered as one of the important industrial enzyme.

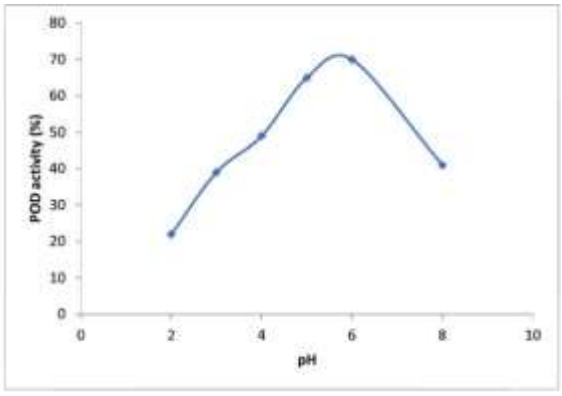

Figure 1: Activity of peroxidase at various pH condition

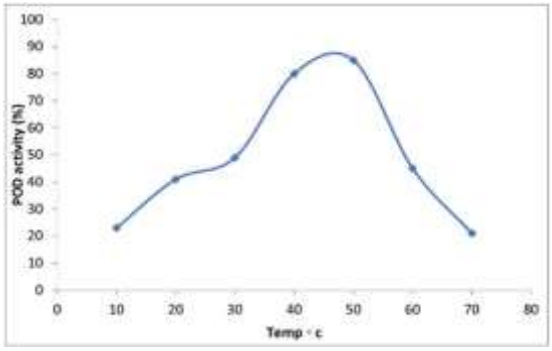

Figure 2: Activity of peroxidase at various temperature condition

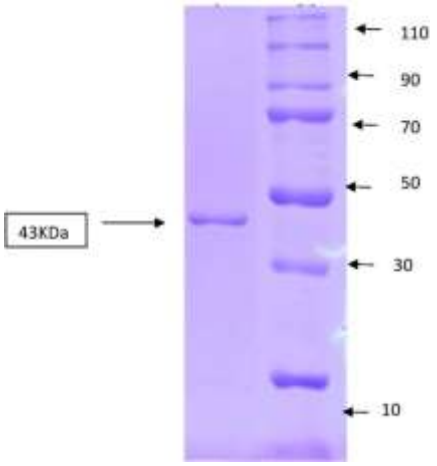

Figure 3 Determination of molecular weight of peroxidase

CONCLUSION

Peroxidase based biosensors are widely used in analytical system for determination of hydrogen

peroxide. Therefore, this enzyme can be a potential and economical source. Peroxidase have many possibilities of clinical applications. They can be used as a diagnostic kit for estimating glucose in waste water treatment. Thus, studies show that with further advancement in purification method these plant peroxidase enzymes can be used as a potential source for various biotechnological applications in the near future

REFERENCES

Adams JB. Regeneration and the kinetics of peroxidase inactivation. Food Chem, 1997, 60(2), 201-206.

Aruoma OI, Spencer JPE, Rossi R, Aeschbach R, Kahn A, Mahmood N, Munoz A, Murcia A, Butler J, Halliwell B. An evaluation of the antioxidant and the antiviral action of extracts of rosemary and provencal herbs. Food Chemical Toxicology, 1996, 34(5), 449-456.

Brassica oleracea L. Cabbage. United States Department of Agriculture. Retrieved 2012-08-10.

Cheney G. Rapid healing of peptic ulcers in patients receiving fresh cabbage juice. California Med, 1949, 70(1), 10-15.

Chisari M, Barbagallo RN, Spagna G. Characterization of polyphenol oxidase and peroxidase and influence on browning of cold stored strawberry. J of Agri Food Chem, 2007, 55(9), 3469-3479.

Daraei Garmakhany A, Mirzaei HO, Aghajani N, Kashiri M. Investigation of natural essential oil antioxidant activity on peroxidase enzyme in selected vegetables. J Agri Sci Technol, 2010, 4, 78-82.

Gawlik-Dziki U, Złotek U, Swieca M. Characterization of polyphenol oxidase from butter lettuce (Luctuca sativa var. capitata L.). Food Chem, 2007, 107, 129-135.

Gunes B, Bayindirli A. Peroxidase and lipoxygenase inactivation during blanching of green beans, green peas and carrots. J of LWT, 1996, 26(), 406-410.

Jia J, Wang B, Cheng G, Dong S. A method to construct a third generation horseradish peroxidase biosensor: self-assembling gold nanoparticles to three dimensional sol-gel network. Anal Chem, 2002, 74(9), 2217-2223.

Jiang YM. Purification and some properties of polyphenol oxidase of long an fruit. Food Chem, 1999, 66, 75-79.

Klapper MH, Hackett DP. Investigation on the multiple components of commercial horseradish peroxidase. Biochem Biophys Acta, 1965, 96, 272-278.

Lee HC, Klein BP. Evaluation of combined effects of heat treatment and antioxidant on peroxidase activity of crude extract of green peas. Food Chemistry, 1989, 32, 151-158.

Lee MK, Park I. Studies on inhibition of enzymatic browning in some foods by Du- Zhong (*Eucommia uimoides* Oliver) leaf extract. Food Chem, 2007, 114, 154-163.

Lobarzewsky J, Greppin H, Penel C, Gasparm T (eds) University M Curie, Lublind Poland and University of Geneva, Geneva Switzerland, 1991, 469-478.

Maheswari E, Pradeep Kumar R, Meignana Arumugham I, Sri Sakthi D, Lakshmi T, Pradeep Kumar R. Evaluation of salivary flow rate, pH, buffering capacity, total calcium, protein, and total antioxidant capacity level among caries-free and caries-active children: A systematic review. J Adv Pharm Edu Res, 2017, 7(2), 132-136.

Muñ oz O, Sepú lveda M, Schwaerz M. Effects of enzymatic treatment on anthocyanin pigments from grapes skin from Chilean wine. Food Chem, 2004, 87, 487-490.

Pandey VP, Awasthi M, Singh S, Tiwari S, Dwivedi. A Comprehensive Review on Function and Application of Plant Peroxidases. Biochem, 2017, 6:308.

Passardi F, Cosio C, Penel C, Dunand C. Peroxidases have more functions than a Swiss army knife. Plant Cell Rep, 2005, 24(5), 255-265.

Ramakrishna N, Lacey J, Candish AAG, Smith JE, Goodbrand IA. Monoclonal antibody-based enzyme linked immune-sorbent assay of aflatoxin B1, T-2 toxin and ochratoxin-A in barley. J Assoc Anal Chem, 1990, 73, 71-77.

Rui MSC, Vieira MC, Silva CLM. Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*), Journal of Food Engineering, 2006, 72(1), 8-15.

Samec D, Piljac-Zegarac J, Bogovi M. Antioxidant potency of white (*Brassica oleracea* L. *var. capitata*) and Chinese (*Brassica rapa* L. var. *pekinensis* (Lour.)) cabbage: The in uence of development stage, cultivar choice and seed selection. Sci Hort, 2011, 128, 78-83.

Senthamil Sindhu, Nithya Jagannathan. Saliva: A Cutting Edge in Diagnostic Procedures. J Oral Dis, 2014, 6, 33-37.

Serrano-Martínez A, Fortea FM, Del Amor FM, Nú ñ ez-Delicado E. Kinetic characterization and thermal inactivation study of partially purified red pepper (*Capsicum annuum* L.) peroxidase. Food Chemistry, 2008, 107 (1), 193-199.

Wennberg M, Ekvall MJ, Olsson K, Nyman M. Changes in carbohydrate and glucosinolate composition in white cabbage (*Brassica oleracea* var. Capitata) during blanching and treatment with acetic acid. Food Chemistry, 2006, 95(2), 226-236.