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Evaluation of Peroxidase Activity in Selected Vegetables from Hyderabad, Telangana, India

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Abstract

Present study describes the enzymatic activity of peroxidase in selected vegetables. Peroxidases occur naturally in plants, animals and microorganism. These enzymes catalyze a wide range of substrates by liberating oxygen from H_2O_2. In addition, it is capable of oxidizing a wide range of compounds. It is the oxidation process that might be involved in the loss of color, flavor, and nutritional value of raw and processed foods. Vegetables (cabbage, cauliflower, carrot, green chilli and spinach) were chosen as sources for peroxidase. Results clearly indicated that peroxidase activity was found in all the vegetable samples investigated. Peroxidase activity among different vegetables varied significantly and was found to increase gradually over a period of 5 min. Highest peroxidase activity was observed in the case of cabbage while lowest activity was observed in the case of green chilli and spinach.

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Introduction

Peroxidases (E.C.1.11.1.7), are distributed in plants like, radish, soybean (Ambreen et al., 2000), tomato (Zia et al., 2001), potato, turnip, carrot, wheat, pear, apricot, banana, dates (Reed, 1975), strawberry (Jen et al., 1980) and horseradish (Rehman et al., 1999); animals (leukocyte- Agner, 1943), spleen, lungs, mammary and thyroid glands, bone marrow and intestine (Harris and Loew, 1996) and microorganisms (*Streptococcus faecalis* – Reed, 1975). It is also reported in fruits like oranges (Clemente, 2002), peach (Neves, 2002), pears (Regalado et al., 2004) and apples (Singh et al., 2010).

It is an oxidoreductase, which catalyzes a redox reaction between H_2O_2 as an electron acceptor and many kinds of substrates by means of oxygen liberation from H_2O_2 (Brill, 1996).

$$2H_2O_2 \xrightarrow{\text{Peroxidase}} 2H_2O + O_2$$

Peroxidases of plant origin are capable of oxidizing a wide range of compounds (e.g., guaiacol, pyrogallol, chlorogenic acid, catechin, and catechol (Onsa et al., 2004). This oxidation process may be responsible for losses in color, flavor, and nutritional value of raw and processed foods (Robinson, 1987; Nebesky et al., 1950; Bruemmer et al., 1976; Kampis et al., 1984). Peroxidases from plants exist as isoenzymes and its number may vary from one vegetable source to another. In addition they also differ in terms of thermal stability, optimum pH, substrate specificity, amino acid

composition and their physiological roles in the plant tissues.

Because of its heat stability (it is used to evaluate the heat processing of vegetables (Adams, 1997). Peroxidase finds its application in health sciences as a diagnostic tool (Kwak et al., 1995). In addition, it is also used in the preparation of enzyme conjugated antibodies which are widely used in ELISA and other sensitive analytical techniques (Barnes et al., 1993). It occupies a prominent place in the fields of Biotechnology and associated research areas (such as, Enzymology, Biochemistry, Medicine, Genetics, Physiology, Histo- and Cytochemistry) (Azevedo et al., 2003). Other areas in which peroxides are used includes, chemical synthesis, medicine, analysis of food, chemicals, clinical and environmental samples (Agostini et al., 2002). In recent years it is used in the processes like detoxification and removal of variety of organic pollutants, e.g. aromatic amines, phenols, dyes, etc., from contaminated waste water (Duran and Esposito, 2000; Shaffiqu et al., 2002; Bhunia et al., 2001; Akhtar et al., 2005).

Keeping in view the importance of peroxidase the present study was aimed to evaluate and compare the levels of peroxidase activity in selected vegetables from the city of Hyderabad, Telangana.

Materials and methods

Plant material collection

Fresh vegetables (cabbage, cauliflower, carrot, green chilli and spinach) were purchased from local market in Hyderabad city. Before the extraction procedure, all the samples were thoroughly cleaned with 0.2M Potassium phosphate buffer pH 7.0 to remove any adhering contaminants if present. Peroxidase activity in the vegetable samples was determined on the same day of purchase.

Preparation of extract

Sample (10 g) was homogenized in a blender using 2 mL of 0.2M Potassium phosphate buffer pH 7.0. The extract was then passed through cheese cloth. The filtrate was centrifuged at 10000g for 10 min and the clear supernatant was labeled as crude extract. The extract was subjected to 65 ºC for three min to inactivate any catalase activity present in extract (Rehman et al., 1999). After examining different volumes (10-100 µL) of the crude enzyme for peroxidase activity assay, 20 µL of the crude enzyme was finally selected to examine the peroxidase activity assay. Assay was carried out at room temperature. All the vegetables were similarly treated.

Assay of peroxidase (POX) activity

Assay of peroxidase was carried out according to the method of Sarika et al. (2015). Enzyme activity was determined in triplicates and results are represented as Mean ± SD. The substrate used was hydrogen peroxide (H_2O_2)/phenol/4-aminoantipyrine solution (Porstmann, 1981). H_2O_2 rapidly reacts with 4-aminoantipyrine-phenol solution in the presence of peroxide to produce a quinoneimine chromogen (http://www.amano-enzyme.co.jp) which shows intense pink colour with a maximum absorbance at 510 nm. Aminoantipyrine and phenol were used at concentrations of 0.0025M and 0.17M respectively. In this method the amount of quinoneimine (Fig. 1) formed was influenced by the amount of peroxidase present. Data is represented as mean ± SD for each of the vegetable sample investigated.

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{quinoneimine dye} + 4H_2O$$

Chemical structure of the quinone–imine dye produced in the reaction is presented in Fig. 1 (Trinder and Webster, 1984).

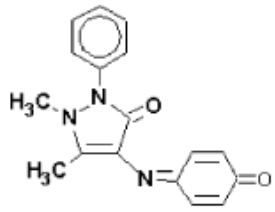

Fig. 1: Quinone-imine product.

Table 1. Assay of peroxidase activity.

S. No.	Vol. of Enzyme (µL)	Vol. of PO_4. buffer (µL)	Volume of 4 – amino anti pyrine – H_2O_2 (mL)	Absorbance at 510 nm				
				1’	2’	3’	4’	5’
1	20	80	2.9					

Results and discussion

Vegetables (cabbage, cauliflower, carrot, green chilli and spinach) were chosen as sources for peroxidase. During the peroxidase enzyme assay change in absorbance value was recorded at a time interval of 1 min over a total period of 5 min. The change in

absorbance values at 510 nm versus time is summarized in Table 2 and depicted in Fig. 2. Results presented in Table 2 and Fig. 2 clearly indicates that peroxidase activity was found in all the samples of vegetables investigated. The presence of peroxidase activity in different fruits and vegetables has been observed by many investigators (Gorin and Hemidema, 1976; Haard, 1977; Müftügil, 1985; Meclellon and Robinson, 1987; Rhotan and Nicolas, 1989; Miesle et al., 1991; Neves, 2002; Llano et al., 2003). Peroxidase activity among the different vegetables varied significantly and enzyme activity was found to increase gradually over a period of 5 min (Table 2). Highest peroxidase activity was observed in the case of cabbage while lowest activity was observed in the case of green chilli and spinach (Table 2). These results are in accordance with those reported by Müftügil (1985). He observed that peroxidase activity was found in all the samples of fresh vegetables investigated. According to Müftügil cabbage and green beans had high enzyme activities.

Table 2. Evaluation of peroxidase activity in selected vegetables.

Vegetable	Time (min)				
	1	2	3	4	5
Cabbage	0.5 ± 0.03	0.91 ± 0.06	1.2 ± 0.09	1.53 ± 0.07	1.7 ± 0.06
Cauliflower	0.17 ± 0.04	0.32 ± 0.11	0.48 ± 0.15	0.63 ± 0.17	0.76 ± 0.21
Carrot	0.15 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.22 ± 0.02
Green chilli	0.11 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.15 ± 0.01	0.17 ± 0.01
Spinach	0.11 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.01

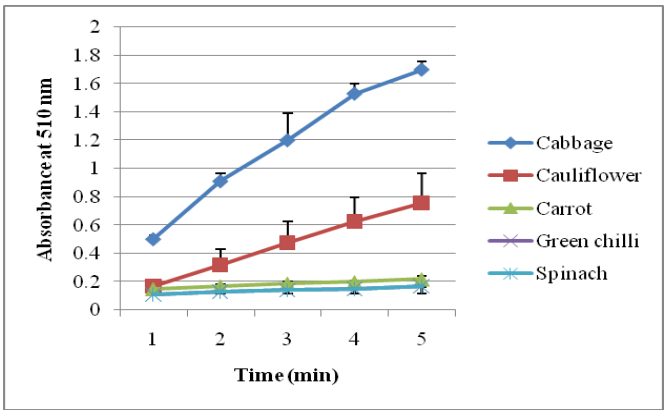

Fig. 2: Evaluation of peroxidase activity in selected vegetables.

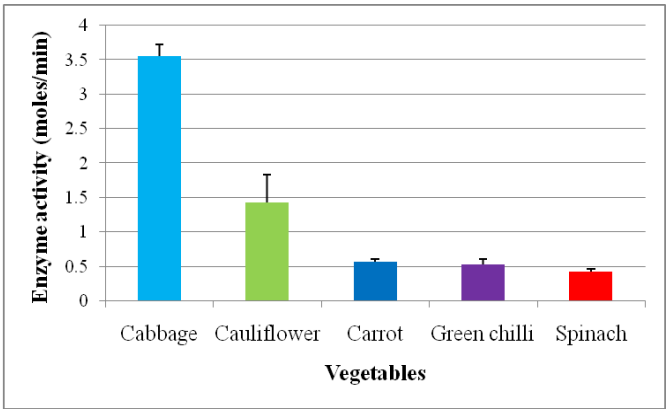

Fig. 3: Peroxidase activity in selected vegetables (moles/min).

Peroxidase activity in terms of moles/min (Table 3 and Fig. 3) in different vegetables samples were: cabbage (3.55 ± 0.18) > cauliflower (1.43 ± 0.41) > carrot (0.57 ± 0.04) > green chilli (0.53 ± 0.08) > spinach (0.43 ± 0.04) respectively.

Table 3. Peroxidase activity in selected vegetables (moles/min).

Vegetable	Mean ± SD
Cabbage	3.55 ± 0.18
Cauliflower	1.43 ± 0.41
Carrot	0.57 ± 0.04
Green chilli	0.53 ± 0.08
Spinach	0.43 ± 0.04

Conclusion

All the vegetable samples investigated exhibited peroxidase activity. Peroxidase activity among the different vegetables varied significantly. Highest peroxidase activity was observed in the case of cabbage while lowest peroxidase activity was observed in the case of green chilli and spinach**.**

Conflict of interest statement

Authors declare that they have no conflict of interest.

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References

Adams, J. B., 1997. Regeneration and the kinetics of peroxidase inactivation. Food Chem. 60, 201-206.

Agner, K., 1943. Verdoperoxidase. Adv. Enzymol. 3, 137-148.

Agostini, E., Hernandez-Ruiz, J., Arnao, M. B., Milrand, S. R., Tigier, H. A., Acosta, M., 2002. A peroxidase isoenzyme secreted by turnip (*Brassica napus*) hairy-root culture inactivation by hydrogen peroxide and application in diagnostic kits. Biotechnol. Appl. Biochem. 35, 1-7.

Akhtar, S., Khan, A. A., Husain, Q., 2005. Partially purified bitter gourd (*Momordica charantia*) peroxidase catalyzed decolorization of textile and other industrially important dyes. Bioresour. Technol. 96, 1804-1811.

Ambreen, S., Rehman, K., Zia, M. A., Habib, F., 2000. Kinetic studies and partial purification of peroxidase in soybean. Pak. J. Agric. Sci. 37(3-4), 119-122.

Azevedo, A. M., Martins, V. C., Prazeres, D. M., Vojinovic, V., Cabral, J. M., Fonseca, L. P., 2003. Horseradish peroxidase: A valuable tool in biotechnology. Biotechnol. Annu. Rev. 9, 199-247.

Barnes, C.S., Upadrashta, B., Pacheco, F., Porton, J., 1993. Enhanced sensitivity of immunoblouing with POD conjugated antibodies using an adsorbed substrate method. J. Chem. 61(2), 281-288.

Bhunia, A., Duran, S., Wangikar, P. P., 2001. Horseradish peroxidase catalyzed degradation of industrially important dyes. Biotechnol. Bioeng. 72, 562-567.

Brill, A.S., 1996. Peroxidase and Catalase Comprehensive Biochemistry. Vol. XIV, Elsevier Pub. Co. Amsterdam. pp.447-479.

Bruemmer, J. H., Roe, B., Bowen, E. R., 1976. Peroxidase reactions and orange juice quality. J. Food Sci. 41, 186.

Clemente, E., 2002. Peroxidase from oranges [(*Citrus sinenses* (L.) Osbeck]. Eur. Food Res. Technol. 215, 164-168.

Duran, N., Esposito, E., 2000. Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review. Appl. Catal. B: Environ. 28, 83-99.

Gorin, N., Heidema, F. T., 1976. Peroxides activity in Golden Delicious apples as a possible parameter of ripening and senescence. J . Agric. Food Chem. 24, 200-201.

Haard, N. F., 1977. Physiological roles of peroxidase in post-harvest fruit and vegetables. In: Enzymes in Food and Beverages Processing (Eds.: Ory, R. L., St. Angelo, A. J.). ACS. 143p.

Harris, D.L., Loew, G.H., 1996. Identification of putative peroxide intermediates of peroxidase by electronic structure and spectra calculations. J. Chem. Sci. 118, 10588-10594.

Jen, J. J., Seo, A., Flurkey, W.H., 1980. Tomato peroxidase: purification via hydrophobic chromatography. J. Food Sci. 45, 60-63.

Kampis, A., Bartuczkovacs, O., Hoschke, A., Aosvigyazo, V., 1984. Changes in peroxidases activity of broccoli during processing and frozen storage. Lebensm. Wiss. Technol. 17, 293-295.

Kwak, S., Kim, S., Lee, K.H., Jung, I.H., Jin, J.R., 1995. Acidic peroxidases from suspension culture of sweat potato. Phytochem. 39(5), 981-984.

Llano, K. M., Haedo, A. S., Gerschenson, L. N., Rojas, A. M., 2003. Mechanical and biochemical response of kiwifruit tissue to steam blanching. Food Res. Int. 36(8), 767-775.

Miesle, T. J., Proctor, A., Lagrimini, L. M., 1991. Peroxidase activity isoenzymes, and tissues localization in developing high bush bluberry fruit. J. Amer. Soc. Hortic. Sci. 116, 827-830.

Müftügil, N., 1985. The peroxidases enzyme activity of some vegetables and its resistance to heat. J. Sci Food Agric. 36, 877-880.

Nebesky, E. A., Esselen, W. B. Jr., Kaplan, A. M., Fellers, C. R., 1950. Thermal destruction and stability of peroxidase in acid foods. Food Res. 15, 114.

Neves, V. A., 2002. Ionically bound peroxidase from peach fruit. Braz. Arch. Biol. Technol. 45, 7-16.

Onsa, G. H., Saari, N., Selamat, J., Bakar, J., 2004. Purification and characterization of membrane-bound peroxidases from *Metroxylon sagu*. Food Chem. 85, 365-376.

Porstmann, B., 1981. Comparison of chromogens for the determination of horseradish peroxidase as a marker in enzyme immunoassay. J. Clin. Chem. Clin. Biochem. 19, 435-439.

Reed, G., 1975. Oxidoreductase: Enzymes in Food Processing. Academic Press, USA. 216p.

Regalado, C., Garcia-Almendárez, B. E., Duarte-Vázquez, M. A., 2004. Biotechnological applications of peroxidases. Phytochem. Rev. 3, 243-256.

Rehman, K., Yaqub, M., Sheikh, M. A., Arshad, M., 1999. Extraction and evaluation of peroxidases from various vegetable sources. Int. J. Agric. Biol. 1(3), 170-173.

Rhotan, C., Nicholas, J., 1989. Changes in acidic and basic peroxidase activity during tomato fruit ripening. Hortic. Sci. 24, 340-342.

Robinson, D. S., 1987. Scavenging enzyme and catalases. In: Biochemistry and Nutritional Value (Ed.: Robinson, D. S.). Longman Scientific and Technical, Harlow, U.K. pp.459-465.

Sarika, D., Ashwin Kumar, P.S.S., Shaik Arshad, Sukumaran, M.K., 2015. Purification and evaluation of horseradish peroxidase activity. Int. J. Curr. Microbiol. Appl. Sci. 4(7), 367-375.

Shaffiqu, T. S., Roy, J. J., Nair, R. A., Abraham, T. E., 2002. Degradation of textile dyes mediated by plant peroxidases. Appl. Biochem. Biotechnol. 102–103, 315-326.

Singh, J., Dubey, A., Diwakar, S. K., Rawat, S. K., Batra, N., Josh, A., 2010. Biochemical characterization of peroxidases from the fruits of *Mallus pumilus*. Int. Res. J. Biotechol. 1(14), 50-58.

Trinder, P., Webster, D., 1984. Determination of HDL-cholesterol using 2,4,6-tribromo-3-hydroxybenzoic acid with a commercial CHOD-PAP reagent. Ann. Clin. Biochem. 21, 430-433.

Zia, M.A., Rehman, K., Saeed, M.K., Ahmed, A., Ghaffar, A., 2001. Partial purification of peroxidase from tomato. Science. 1(6), 404-406.

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