REVIEW OF RESEARCH
ISSN: 2249-894X
IMPACT FACTOR : 5.7631(UIF)
UGC APPROVED JOURNAL NO. 48514
VOLUME - 8 | ISSUE - 9 | JUNE - 2019

"THE POLYPHENOL OXIDASE IS A *pH* SENSITIVE ENZYME, EXTRACTED FROM *Actinidia Deliciosa* (KIVI FRUIT)"

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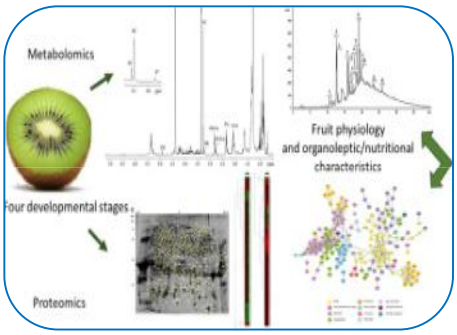
ABSTRACT:



*The **Actinidia deliciosa** (i.e. Kivi fruit) with family **Actinidiaceae** is good for health and shows good medicinal properties. Kivi fruit is also known as Chinese gooseberry which has dark brown color peel (skin). Kivi fruit contain enzyme called Polyphenol oxidase (PPO), which a good oxidizing agent. This paper tell us, the general introduction of Polyphenol oxidase enzyme which is to be extracted from Kivi fruits by using centrifuge technique and perform different Physico-chemical tests like, measurement of PPO activity, decolourization (degradation) studies by using Malachite Green dye. The studies show Kivi fruits contain more, actively Polyphenol oxidase enzymes (PPO). It's also shows that, the variation in PH that affects the activity of Polyphenol oxidase enzyme (i.e. oxidization property of enzyme).*

KEYWORDS: *Kivi fruit, Polyphenol oxidase (PPO), malachite green dye, decolourization reaction etc.*

INTRODUCTION :

The Kivi fruit have more nutritional quality with multiple, minerals and a good source of vitamin A, E and K. The Kivi fruit contains good quantity of vitamin C (Hemila H 2013). The fruit is also associated with some secondary metabolites and source of protein like actinidin (Yoruk R et. al 2003). The browning of the cut surface of some fruits and vegetables is due to the presence of a group of enzymes called Polyphenol oxidase (PPO). These enzymes are released by the broken cells and they catalysis the reaction between colorless molecules called Polyphenol and molecular oxygen (Ahvenainen,R. 1996). This reaction creates colored compound and these new compound can spontaneously cross react with one another to form black-brown complexes called Melanin. PPO has been largely investigated in relation to the commercial importance of browning process (Niranjan Ray S 2012). Polyphenol oxidase plays a key role in plant defense systems. Plant Polyphenol oxidase enzyme is group of cupper (Cu++) containing proteins that are widely distributed from the plants, bacteria to mammals. Polyphenol oxidase plays a major role in browning of plant tissues has been purified from a number of fruit tissues; vegetables tissues and their properties are well studied (Spencer JP et.al 2008). The PPO enzymes have different forms depending up of cupper as (Cu++) cofactor. Each PPO enzyme is substrate specific in nature. Buy the Locally available Kivi fruits from market, perform the extraction and it was used for different tests.

Material and Methods

Plant sample

Buy the Kivi fruits which are locally available in market. The price is variable from city to city, in Nasik city it is about 50Rs per fruit (fig.No.1).

PARTIAL PURIFICATION OF ENZYME FRACTION

Take a Kivi fruit 5g (select the fruit part) were subjected to, homogenization with 50 ml **0.2M** of sodium phosphate buffer (pH 7.0). The homogenate was filtered through two layers of cheese cloth and then the filtered materials were centrifuged at 10,000 rpm for 15 min at 4 C. Solid Ammonium Sulphate ((NH4)2SO4) was added to the supernatant to obtain 70% saturation and then centrifuged at 14,000 rpm for 30 min at 40 C. The precipitates were dissolved in **0.2M** phosphate buffer (pH 7.0). The enzyme extract was extensively dialyzed against the same buffer at **4** ^{c} overnight. The dialyzed samples were used as the PPO enzyme source in the experiments (**E. Y. PARK , B. S. LUH** 1985).

DECOLOURIZATION REACTION (DEGRADATION OF MALACHITE GREEN DYE)

Malachite Green dye (20 ug/ml) was incubated with extracted enzyme source at room temperature. The disappearance of the color by Kivi fruit PPO activity was monitored at 620 nm of the dye solution. The percent degradation was calculated by taking the maximum absorbance of untreated dye solution as control (100%). Degradation was monitored by spectroscopic analysis (Systronic 2800 spectrophotometer) (Nicolaï, B.M.; Beullens 2007). The extent of degradation under the action of PPO enzyme from Kivi fruit was studied. The methodology deal with increasing concentration of dye, the numbers of test tubes was arranged with the increasing amount dye solution (2 mg/100 ml) i.e. 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml). In each test tube 0.5ml of purified PPO enzyme was added and was incubated for 48 hrs at room temperature for observation.

RESULT

The Kivi fruit enzyme was partially purified by using centrifuge technique i.e. crude extract which is used for PH variation studies. The ***Actinidia deliciosa*** (Kivi fruit) extract has higher Polyphenol Oxidase activity in the pH 5.8 as compare to pH 7 and pH 8. Indicating that in pH 5.8 PPO activity has great potential of detoxification of phenolic waste compounds. The decolourization was monitored using enzyme concentration for Malachite Green dye **(Fig. No.2)**. When the partially purified PPO incubated with different concentrations of Malachite Green dye in variation in pH5.8, pH 7and pH 8

Fig. No. 1

Sr.No	PH measured	Degradation of Malachite Green dye	Absorbance at 620 nm
1	5.8	+++++	0.325
2	7	+++	0.125
3	8	++	0.115

Observation table No. 1

Fig No.2: Decolorization Reaction

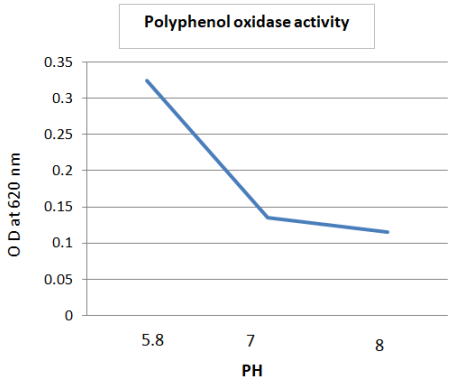


Fig No.3: PPO enzymatic activity

showed decolourization of Malachite Green dye was higher at pH 5.8 than at pH7,pH8 within 48 hours. The spectrophotometer shows the maximum absorption at pH 5.8 **(Fig.NO.3).** The pH variation affects the activity of PPO enzyme. It indicates that the PPO is more sensitive to pH.

REFERENCE

Hemila H, Chalker E (2013) Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev (1):Cd000980.

Yoruk R., Marshall M.R. Physicochemical properties and function of plant polyphenol oxidase: A review. J. Food Biochem. 2003;27:361–422. doi: 10.1111/j.1745-4514.2003.tb00289.x

AHVENAINEN, R. 1996. New approaches in improving the shelf life of minimally processed fruits and vegetables. Trends Food Sci. Technol. 7, 179-200.

Niranjan Ray S., Sarosh B.R., Shetty H.S. Induction and accumulation of polyphenol oxidase activities as implicated in development of resistance against pearl millet downy mildew disease. Funct. Plant Biol. 2006;33:563–571.

Spencer JP, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. Br J Nutr. 2008;99:12–22.

E. Y. PARK , B. S. LUH Polyphenol Qxidase of Kiwifruit. 1985.

Nicolaï, B.M.; Beullens, K.; Bobelyn, E.; Peirs, A.; Saeys, W.; Theron, K.I.; Lammertyn, J. Nondestructive measurement of fruit and vegetable quality by means of nir spectroscopy: A review. Postharvest Biol. Technol. 2007, 46, 99–118.

Boeing H, Bechthold A, Bub A, Ellinger S, Haller D, Kroke A, Leschik-Bonnet E, Müller MJ, Oberritter H, Schulze M, Stehle P, Watzl B (2012) Critical review: vegetables and fruit in the prevention of chronic diseases. Eur J Nutr 51:637–663.

Saba M.K., Moradi S. Internal browning disorder of eight pear cultivars affected by bioactive constituents and enzyme activity. Food Chem. 2016;205:257–263. doi: 10.1016/j.foodchem.2016.03.022.

Belcarz, A., Ginalska, G., Kowalewska, B. and Kulesza, P. 2008. Spring cabbage peroxidase – Potential tool in biocatalysis and bioelectrocatalysis. Phytochemistry 69: 627-636.

Jiang, Y-M. 1999. Purification and some properties of polyphenol oxidase of longan fruit. Food Chemistry 66: 75-79.

Sun, J. Y., You, E. G., Long, X., & Wang, J. (2010). Biochemical properties and potential endogenous substrates of polyphenoloxidase from chufa (Eleocharis tuberosa) corms. Food Chemistry, 118(3), 799-803. http://dx.doi.org/10.1016/j.foodchem.2009.05.065.

Chatterjee, S., Niaz, Z., Gautan, S., Adhikari, S., Pasad, S.V. and Sharma, A. 2007. Antioxidant activity of some phenolic comstituents from green pepper (Piper nigum L.) and fresh nutmeg mace (Myristica fragrans). Food Chemistry 102: 515-523.

Fraignier, M.; Marques, L.; Fleuriet, A.; Macheix, J. Biochemical and immunochemical characteristics of polyphenol oxidase from different fruits of Prunus. J. Agric. Food Chem. 1995, 43, 2375–2380.

Martinez, M.V.; Whitaker, J.R. The biochemistry and control of enzymatic browning. Trends Food Sci. Technol. 1995, 6, 195–200.