WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

TOTAL PHENOLIC CONTENT AND POLYPHENOL OXIDASE ACTIVITY IN THREE VARIETIES OF *IPOMOEA BATATAS*

Ayibaene Frank-Oputu and Ebizimor Wodu

Department of Biochemistry, Faculty of Basic Medical Sciences, Niger Delta University. Bayelsa State. Nigeria

ABSTRACT

Phenolic content and the activity of polyphenol oxidase extracted from tubers of three varieties of Ipomoea batatas were investigated. Total phenolics were estimated as Gallic acid equivalent per gram of sample. Polyphenol oxidase activity was assayed by monitoring the increase in optical density at 420nm for 3mins. Results revealed that orange flesh variety had the highest phenolic content (89.73 mgGAE/g±2.95), while purple peel variety had the highest activity (1.204 Units ml-1 min-1±0.031) of polyphenol oxidase. Purple peel variety was most efficient in the oxidation of catechol substrate to o-quinone. There was no correlation between the total phenolic content and the polyphenol oxidase activity. Ipomoea batatas is a good source of phenolics and the kinetic parameters of Ipomoea batatas polyphenol oxidase are similar to those from other sources.

Keywords*: Polyhenolics, Polyphenol Oxidase, Ipomoea batatas, Kinetic parameters.*

1. INTRODUCTION

Secondary metabolites in plants play a crucial role in plant adaptation in their environment (Oh et al., 2009). These metabolites also play a crucial role in human health (Bohn et al., 2014; Rasouli et al., 2017). Polyphenols are bioactive secondary metabolites that are essential part of human diet and are of interest because of their biological function (Farzaei et al., 2015; Villano, et al., 2016). They have been proven effective in the prevention of certain chronic diseases like coronary heart diseases, diabetes mellitus and cancers (Asami et al., 2003), because of their free radical-scavenging activities. Phenolic compounds are important constituents of fruits as they contribute to their taste, colour and nutritional properties (Cheynier, 2009). When these phenolic compounds are oxidized mainly by polyphenol oxidases, they adversely affect the taste, colour and nutritional properties of the fruit.

Polyphenols are substrates for polyphenol oxidases and are located in the vacuole (Nicholas et al., 1994; Jukanti, 2017), while polyphenol oxidases are present in the thalakoid membrane and thalakoid lumen (Yoruk and Marshall, 2003). Polyphenol oxidases are found widely in plant and their level and activity depend on the age, species, variety, maturity, and stress status of plants (Mayer, 2006). When senescence or injury results in contact between polyphenol oxidases and polyphenol compounds, the possible reactions that occur are the hydroxylation of monophenols to form diphenols, the oxidation of diphenols to form quinones (Whitaker, 1994; Yoruk and Marshy, 2003), which ultimately leads to the formation of the dark undesirable pigment. The formation of dark pigments is ultimately due to activity of polyphenol oxidase,

This work examines the total phenolic content and the activity of polyphenol oxidases extracted from tubers of three varieties of Ipomoea batatas.

2. MATERIALS AND METHODS.

Preparation of Enzyme Extract:

Freshly harvested tubers of *Ipomoea batatas* were washed to remove dirt. The tubers were cut into tiny sizes. Exactly 30g of the tiny cubes of each variety were homogenized in ice cold 0.2M potassium phosphate buffer (pH 6.8). The homogenate was filtered through double layer of cheese cloth. The filtrate was centrifuged (universal centrifuge, 320R Hectti) at 4000rpm for 25mins at 4°C to obtain the aqueous crude extract.

Acetone Precipitation:

The enzyme was precipitated by adding ice cold acetone in bits to the supernatant in the ratio 1.5:1, with gentle stirring for 60 minutes in ice bath. The mixture was centrifuged at 4000rpm for 25mins at 4°C to obtain the precipitate, which was re-dissolved in 10ml of extraction buffer and was used as the enzyme.

Protein Estimation and Enzyme Assay:

Protein estimation was done in all preparations by the Bradford method (1976). Bovine serum albumin was used as standard.

Polyphenol oxidase activity was assayed by monitoring the increase in absorbance at 420nm. The 3ml reaction mixture contained 2.8ml of 40mM catechol substrate (prepared in 100mM phosphate buffer, pH 6.8) and 0.2 ml of enzyme solution. The increase in absorbance was measured monitored for 3mins at 30sec interval using a UV-spectrophotometer (Spectrum lab 755a). The blank consisted of 3 ml of substrate solution in phosphate buffer. The initial velocity was calculated from the slope of the absorbance versus time graph. One unit of PPO activity was defined as the amount of enzyme that caused a 0.001 increase of absorbance per minute.

Total Phenol Content Estimation:

The total phenol of the tuber extract was estimated using the folin-ciocalteau reagent method as described by (Singleton *et al,* 1999) and (Demiray *et al*., 2009). The plant sample [100mgmL,1.0ml] was mixed thoroughly with 5ml Folin-Ciocalteau reagent (diluted tenfold) and after 5 minutes 4.0ml if sodium carbonate (0.7M) was added and the mixture was allowed to stand for 1 hour with intermittent shaking. For colour development the absorbance was measured at 765nM in a spectrometer against a blank. The blank solution contained the solvent used to dissolve the tuber extract. Gallic acid was used as a standard. Serial dilution of 10mg/ml of the standard was made to obtain a calibration curve. Total phenol contained in plant extract was calculated as gallic acid equivalent (GAE).

Kinetic Properties:

Ipomoea batatas polyphenol oxidase activity was determined with catechol as substrate (10mM to 40mM). The enzyme reaction proceeded at pH 6.8 and ambient temperature. The enzyme's kinetic parameters (Km and Vmax) were estimated by the Linewaver-Burk plot (Lineweaver & Burk 1934).

Statistical analysis:

The SPSS statistical analysis system was used for analysis of the data.

3. RESULTS AND DISCUSSION

Polyphenols in plants constitute group of secondary metabolites with free radical scavenging properties, thus they help the human body to fight against diseases. The Mean±SD of total phenolic content in mgGAE/g of sample present in the tubers of the three varieties of *Ipomoea batatas* investigated are presented in table 1. Of the three varieties studied, orange flesh variety had the highest concentration (89.73mgGAE/g±2.95) of phenolics. Purple peel variety had 84.75 mgGAE/g±6.80, while brown peel variety had the lowest concentration of 73.00 mgGAE/g±2.77. The results above indicate that *Ipomoea batatas* varieties are good sources of phenolic compounds. Potatoes have been reported to be good sources of phenolics with total phenolic content higher than some fruits and vegetables such as carrots, onions, or tomatoes (Chun *et al*., 2005).

Activity of polyphenol oxidases extracted from *Ipomoea batatas* (table 1) at 40mM concentration of catechol substrate and at constant concentration of enzyme were 0.820Units ml^{-1} min^{-1}±0.50, 1.204Units ml^{-1} min^{-1}±0.031 and 0.714Units $ml^{-1}min^{-1}$±0.081 respectively for orange flesh, purple peel and brown peel varieties. From the results obtained in the study, there was no correlation between the phenolic content and the activity of polyphenol oxidase in the tubers investigated.

Kinetic parameters (km and Vmax) with which *Ipomoea batatas* polyphenol oxidase catalyzed the oxidation of catechol to the corresponding o-quinone are shown in table 2. Km and Vmax of 52.63mM±2.65 and 1.45Units ml^{-1} min^{-1}±0.11; 25mM±2.06 and 1.67 Units ml^{-1} min^{-1}±0.20; and 40mM±4.12 and 1.45Units ml^{-1} min^{-1}±0.09 were obtained for orange flesh, purple peel and brown peel varieties respectively. The Michaelis-Menten constant values obtained in this investigation compare well with 47mM reported by Zhang and Shao, (2015) for Loquat fruit polyphenol oxidase and 50mM reported by Deepaa and Wong, (2012) for *Ipomoea batatas* polyphenol oxidase (using 4-methylcatechol as substrate). Very high km values of 357.1mM and 384.6mM were also reported for *Ipomoea batatas* polyphenol oxidase (Deepaa and Wong 2012). Also, low km values of 4.87mM and 2.12mM were reported for dormant corm and waking corm of *Gocus sativus* respectively (Esmaeili *et al*., 2017); and 2.4mM for potato polyphenol oxidase (Mahmood *et al*., 2009).

Table 1: PPO activity and polyphenol content in three varieties of *Ipomoea batatas*

Substrate	PPO Activity (Units min^{-1})	Phenolic Content (mgGAE/g)
Orange Flesh	0.820±0.050	89.73±2.95
Purple Peel	1.204±0.031.	84.73±6.80
Brown Peel	0.714±0.081	73.00±2.77

Values are recorded as Mean±SD of triplicate determination

Table 2: Kinetic parameters of *Ipomoea batatas* PPO

Sweet potato variety	Km (mM)	Vmax (Units ml^{-1} min^{-1})	Specificity (Vmax /Km)
Orange flesh	52.63±2.65	1.45±0.11	0.036±0.001
Purple peel	25.0±2.08	1.67±0.20	0.067±0.001
Brown peel	40.0±4.12	1.45±0.09	0.028±0.000

Values are recorded as Mean±SD of triplicate determination

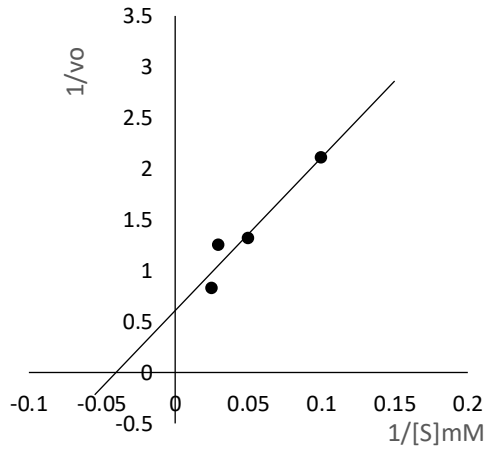

Fig 1 Lineweaver-Burk plot for kinetic constsnt estimation for purple peel sweet potato polyphenol oxidase

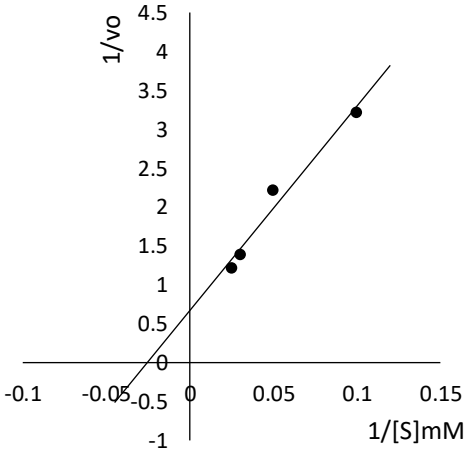

Fig 2. Lineweaver-Burk plot for kinetic constsnt estimation for Orange flesh sweet potato polyphenol oxidase

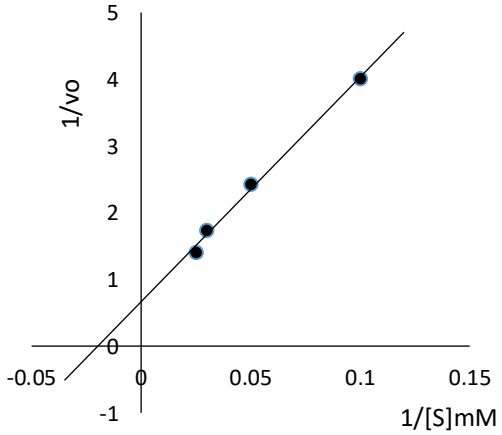

Fig 3. Lineweaver-Burk plot for kinetic constsnt estimation for brown peel sweet potato polyphenol oxidase

The catalytic power which is Vmax/km ratio (table 2) shows the enzyme that is very efficient in the oxidation of catechol to the corresponding o-quinone. The results showed that purple peel variety had the highest Vmax/km ratio of 0.067±0.001, hence polyphenol oxidase extracted from it is most efficient in the oxidation of catechol to the corresponding o-quinone. Also, that purple peel variety had the lowest km of 25.00mM±2.08 indicates that it has the highest affinity for catechol substrate than polyphenol oxidase from the other two varieties.

4. CONCLUSION

In conclusion, the results of this work are not only in agreement with earlier published works on the presence of phenolics in *Ipomoea batatas* tubers but that they possess higher phenolic contents that some regular fruits and vegetables. Polyphenol oxidase from purple peel variety is most efficient in the oxidation of catechol compared to the orange flesh and brown peel varieties.

REFERENCES

[1] Asami, D. K., Hong, Y. J., Barret, D. M. and Mitchell, A. E. (2003) Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. J. Agric. Food Chem. 51, 1237-1241.

[2] Bohn, S. K., Croft, K. D., Burrows, S., Puddey, I. B., Mulder, T. P., Fuchs, D., Woodman, R. J. and Hodgson, J. M. (2014) Effects of Black Tea on Body Composition and Metabolic Outcomes Related to Cardiovascular Disease Risk: A Randomized Controlled Trial. Food Funct. 5(7), 1613–1620. 10.1039/C4FO00209A.

[3] Bradford, M. M. (1976) A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein binding. Anal. Biochem. 72: 248-254

[4] Bravo, K. and Osorio, E. (2016) Characterization of polyphenol oxidase from Cape gooseberry (Physalis peruviana L.) fruit. Food Chem. 197 (Pt A):185-190. doi: 10.1016/j.foodchem.2015.10.126.

[5] Cheynier, V. (2005). Polyphenols in foods are more complex than often thought. The American Journal of Clinical Nutrition, 81(Suppl), 223S–229S.

[6] Chun, O., Kim, D., Smith, N., Schroeder, D., Han, J. and Lee, C. (2005) Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. J. Sci. Food Agric. 85, 1715–1724.

[7] Deepaa, M. and Wong, C. W. (2012) Characterization of Polyphenol Oxidase in Sweet Potato (Ipomoea batatas (L.)). Journal for the Advancement of Science & Arts. 3 (1) 14-31.

[8] Demiray, S., Pintado, M. E. and Castro, P. M. L. (2009). Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: Tilia argentea, Crategi folium leaves and Polygonum bistorta roots. World Academy of Science, Engineering and Technology, 54.

[9] Esmaeili, N., Ebrahimzadeh, H. and Abdi, K. (2017) Correlation Between Polyphenol Oxidase (PPO) Activity and Total Phenolic Contents in Crocus sativus L. Corms During Dormancy and Sprouting Stages. Pharmacogn. S519 – S524.

[10] Farzaei, H., Rahimi, M. R. and Abdollahi, M. (2015) The Role of Dietary Polyphenols in the Management of Inflammatory Bowel Disease. Curr. Pharm. Biotechnol., 16(3), 196–210. PMID: 25601607.

[11] Jukanti A. (2017) Polyphenol Oxidases (PPOs) in Plants. Singapore: Springer Nature.

[12] Lineweaver, H. and Burk, D. (1934). The determination of Enzyme Dissociation Constants. Journal of American Chemical Society. 56:658-666.

[13] Mahmood, W. A., Sultan, H. S. and Hamza, S. R. (2009) Extraction and Characterization of Polyphenol Oxidase from Apricot, Apple, Eggplant and Potato. Mesopotamia J. of Agric. 37 (4) 1-8.

[14] Mayer, A. M. (2006) Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry. 67, 2318–2331.

[15] Nicolas, J. J., Richard-Forget, F. C., Goupy, P. M., Amiot, M. J. and Aubert, S. Y. (1994) Enzymatic browning reactions in apple and apple products. Crit Rev Food Sci Nutr. 34(2):109-157. doi:10.1080/10408399409527653.

[16] Oh, M. M., Trick, H. N. and Rajashekar, C. (2009) Secondary Metabolism and Antioxidants are Involved in Environmental Adaptation and Stress Tolerance in Lettuce. J. Plant Physiol. 166(2), 180–191. 10.1016/j. jplph.2008.04.015.

[17] Rasouli, H., Hosseini-Ghazvini, S. M. B. and Adibi, H. (2017) Differential A-Amylase/A-Glucosidase Inhibitory Activities of Plant-Derived Phenolic Compounds: A Virtual Screening Perspective for the Treatment of Obesity and Diabetes. Food Funct. 8, 1942–1954. 10.1039/C7FO00220C.

[18] Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, 299, 152-178. http://dx.doi.org/10.1016/S0076-6879(99) 99017-1.

[19] Villaño, D., Vilaplana, C., Medina, S., Algaba-Chueca, F., Cejuela-Anta, R., Martínez-Sanz, J., Ferreres, F. and Gil-Izquierdo, A. (2016) Relationship between the Ingestion of a Polyphenol-Rich Drink, Hepcidin Hormone, and Long-Term Training. Molecules. 21(10), 1333. 10.3390/molecules21101333.

[20] Whitaker, J. R. (1994) Principles of Enzymology for the Food Sciences. 2nd ed. New York: Marcel Dekker.

[21] Yoruk, R, and Marshall, M. R. (2003) Physicochemical properties and function of plant polyphenol oxidase: a review. J Food Biochem. 27(5):361-422. doi:10.1111/j.1745-4514. 2003.tb00289. x.

[22] Zhang, X. and Shao, X. (2015): Characterisation of polyphenol oxidase and peroxidase and the role in browning of loquat fruit. C*zech J. Food Sci*., 33: 109–117.