

Partial Purification and Characterization of Polyphenol Oxidase from Water Yam (*Dioscorea alata*)

Belonwu Chijioke Mmuoasinam¹, Philippa Chinyere Ojimekwe^{2,*}, Evelyn Njideka Bede³

¹Department of Food Science and Technology, Imo State Polytechnic Umuagwo, Ohaji, Nigeria

²Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Nigeria

³Department of Food Science and Technology, Federal University of Technology, Owerri, Nigeria

Email address

philippaco60@gmail.com (P. C. Ojimekwe)

*Corresponding author

Citation

Belonwu Chijioke Mmuoasinam, Philippa Chinyere, Ojimekwe, Evelyn Njideka Bede. Partial Purification and Characterization of Polyphenol Oxidase from Water Yam (*Dioscorea alata*). *World Journal of Biochemistry and Molecular Biology*. Vol. 3, No. 1, 2018, pp. 22-30.

Received: February 25, 2018; Accepted: March 21, 2018; Published: May 10, 2018

Abstract: The aim of this research was to evaluate enzymic browning of *Dioscorea alata* and determine methods of preventing and controlling the browning reaction. Polyphenol oxidase (PPO) was extracted from water yam and partially purified by acetone precipitation and dialysis. Optimal pH activity, substrate specificity and inhibition studies of this enzyme was carried out. The result revealed a 9.9 fold increase in activity after dialysis of acetone enzyme precipitate. Substrate specificity of water yam PPO showed activity for diphenolic compounds- catechol, methylcatechol and L-DOPA while no activity was observed in the presence of monophenolic compound, p-cresol. Optimal pH activity of this enzyme was observed at pH 6.8. Tyrosine, phenylthiourea and hydroquinone inhibited water yam PPO competitively in the presence of catechol while ascorbic acid and 2-mercaptoethanol completely inhibited this enzyme at concentrations used. Activation of catechol oxidation by the water yam PPO was observed in the presence of pyrogallol and garlic acid. The data obtained from this inhibition study may be used to predict prevention of browning in yam tuber using chemical inhibitors.

Keywords: Enzymic Browning, *Dioscorea alata*, Inhibitors, Polyphenol Oxidase

1. Introduction

Browning phenomenon is commonly observed in foods during processing and preservation. Enzymatic browning, a type of browning in foods, is known to be caused predominantly by a group of enzymes, polyphenol oxidases (PPOs), which are naturally distributed from bacteria to mammals. They catalyse the oxidation of phenolics to quinones with eventual production of brown pigments in bruised tissues [1]. Polyphenol oxidase has been isolated from higher plants such as sour cherry [2], lemon balm [3], banana [4], Anna apple [5], white cherry fruit [6], taro [7], mushroom [8], soursop [9] and Chinese water chestnut [10]. In addition to pigment formation in plants, polyphenol oxidase is implicated in oxygen scavenging and defence activities against plant pathogens and predatory insects [11].

Water yam is widely distributed and is being cultivated in the tropical and subtropical parts of Africa, America, Asia and Caribbean [12]. Yams, generally, are considered important food staples in many African countries, comprising Cote d'Ivoire, Ghana, Togo, Burkina Faso and Nigeria [12] [13]. Further to food uses, some yams are used as medicines to prevent diarrhoea and diabetes [14] [15] while few species are popular medicines for indigestion and intestinal colic, soothing of diverticulitis, relieve of dysmenorrhoea, as well as allaying uterine and ovarian pain [15] [16]. In spite of these numerous benefits associated with yam, its processing, storage and distribution is highly affected by the activities of polyphenol oxidase resulting in browning of stored fresh tubers and products made from it.

Hence, this study was aimed at extraction and

characterization of polyphenol oxidase from water yam.

2. Materials and Methods

2.1. Materials

Water yam used for this work was procured from farmers in Ikot Ekpene in Nigeria. Catechol was product of Mikenis Reagent Company; 4-methylcatechol, pyrogallol, 3-4 dihydroxy-L-phenylalanine (L-DOPA), D-tyrosine, Triton X-100, polyvinylpyrrolidone (PVP) and P-cresol were all products of Sigma Chemical Company (Germany) while Disodium hydrogen phosphate and potassium di-hydrogen phosphate were from Guangzhou Jinhua chemical reagent Co. Ltd (China). All other reagents used were the best analytical grade available.

2.2. Methods

2.2.1. Sample Preparation

The water yam tubers were sorted to remove spoilt tubers, washed with water. Subsequently, a wholesome tuber was taken and precooled for a period of 24h.

2.2.2. Extraction of Enzyme

The procedure of Galeazzi *et al.* [17] was used for enzyme extraction. The precooled yam tuber was peeled, and one hundred grams of longitudinally sliced yam tissue, covering the entire length of the tuber, was homogenized for 30 sec with 100ml of 0.2M phosphate buffer (pH 6.8) containing 1.5% polyvinylpyrrolidone (PVP) and 0.5% Triton X-100 at 4°C in a blender. Homogenates obtained were centrifuged at 12000rpm for 15min at 4°C (GenFuge 24D centrifuge) in a cold room. The enzyme remained in the supernatant (crude extract). Polyvinylpyrrolidone, a phenol scavenger, was added to the extraction buffer during isolation, to minimize enzyme denaturation by phenol oxidation products [18]. The activity of the crude enzyme extract was determined using 1ml of 0.05M catechol, 0.05ml of enzyme extract and 0.2M phosphate buffer (pH 6.8) in a final volume of 5ml. The assay was carried out at a temperature of 30°C. One unit (U) of PPO activity was defined as the amount of the enzyme that increased the absorbance by 0.01 min^{-1} under the conditions of the assay.

2.2.3. Partial Purification of Crude Enzyme

Partial purification of the crude extract involved acetone precipitation and dialysis. All purification steps were carried out at 4°C. The acetone precipitate was obtained by addition of 2ml of cold acetone and re-centrifugation at 1200rpm for 15min at 4°C. The precipitate obtained was re-extracted with 1ml, 0.2M phosphate buffer (pH 6.8). The re-extracted solution containing the acetone-precipitated protein was put into a dialysis sack (Sigma Dialysis 'Sacks' D6066-25EA) and dialyzed in 0.2M phosphate buffer (pH 6.8). The dialysis was carried out at 4°C for 12h with two changes of dialysis media. The enzyme activity of the dialyzed extract was determined at a temperature of 30°C.

2.2.4. Protein Analysis

Protein content of extract in each step of purification was determined using Biuret test [19], with bovine serum albumin as the standard.

2.2.5. Enzyme Assay

Activity of partially purified enzyme was determined according to the method of Ikran *et al.* [20] Varying amounts of enzyme extract was added to a mixture of 0.2M phosphate buffer (pH 6.8) containing 1ml of 0.05M catechol in a final volume of 5ml. The assay was carried out at a temperature of 30°C. The reaction rate was calculated from the linear slope of activity curves. One unit (U) of PPO activity was taken as the amount of enzyme that increased the absorbance by 0.001 min^{-1} under the conditions of the assay [20].

2.2.6. Substrate Specificity

Specificity of substrate was determined by preparing 0.05M of catechol, 4-methylcatechol, p-cresol and L-DOPA. The standard mixture for activity measurements contained 1ml of 0.05M various substrates, 4ml of 0.2M phosphate buffer at pH 6.8 and 0.027ml of enzyme solution. The reaction rates were measured at the wavelengths of 420nm (catechol), 420nm (4-methylcatechol), 400nm (p-cresol) and 420nm (L-DOPA). Enzyme activity was determined as $\text{OD} \cdot \text{min}^{-1} \cdot \text{mL}^{-1}$ and compared with the rate of catechol oxidation.

2.2.7. Effect of pH on Enzyme Activity

PPO activity was determined at a pH range of 4.5 – 8.0 in 0.2M phosphate buffer, using standard reaction mixture containing 1ml of 0.05M of catechol, 0.27ml of enzyme and 4ml of buffer. The buffer was changed after each determination. Specific enzyme activities (Umg-1) were plotted against the pH range to obtain the optimum pH.

2.2.8. Chemical Inhibition

L-tyrosine, ascorbic acid, N-phenylthiourea, pyrogallol, garlic acid, hydroquinone and 2-mercaptoethanol were used as PPO inhibitors in the study, and the effects of these inhibitors on PPO were determined using catechol as substrate at 30°C. The PPO activities were determined with and without inhibitors at varying concentrations (0.26 – 1.56mM) of substrate at pH of 6.8. The assay mixture consisted of 0.125ml of 0.01M of inhibitor, varying volumes of 0.05M of catechol and was made up to a final volume of 5.13ml with 0.2M phosphate buffer (pH 6.8). Absorbance was determined according to the method of Anosike and Ojmelukwe [21]

3. Results and Discussion

3.1. Protein Purification

The protein concentration, specific activity and purification fold of crude extract and dialysed fraction of PPO extracted from water yam are shown in Table 1. The dialysis of enzyme extract obtained after acetone precipitation resulted in a 9.9 fold increase in specific

activity. Flurkey and Jen [22] discovered that activity of peach PPO was greatly enhanced by acetone powder preparation. It was suggested that the increased activity of acetone powder peach PPO extract over that of fresh peach PPO extract was due to the presence of inhibitory substances

in fresh extract or possibly because of aggregation of PPO isoenzyme forms during acetone powder preparation [22]. The possibility of these factors affecting yam PPO preparation cannot be ignored.

Table 1. Effect of partial purification on protein content and specific activity of crude extract of yam PPO.

Sample	Stage	Protein Mg ml ⁻¹	Specific activity U mg ⁻¹	Purification Fold
Water yam	Crude extract	27.7	17.1	1
	Dialysed enzyme	3.7	169.65	9.9

3.2. Substrate Specificity

The result of substrate specificity for water yam PPO is presented in figure 1. From the result in Figure 1, the PPO from water yam (Figure 1) exhibited affinity for diphenolic compounds, however, no activity was observed for monophenol (P-cresol). After the first twenty seconds of

oxidation, catechol exhibited the highest level of oxidation with 238.6 EU min⁻¹, followed by methylcatechol (90 EU min⁻¹) while the least activity was recorded by L- DOPA (25 EU min⁻¹); p-cresol was not oxidized. Therefore, catechol was the most preferred substrate, followed by 4-methyl catechol while L-DOPA was the least preferred substrate.

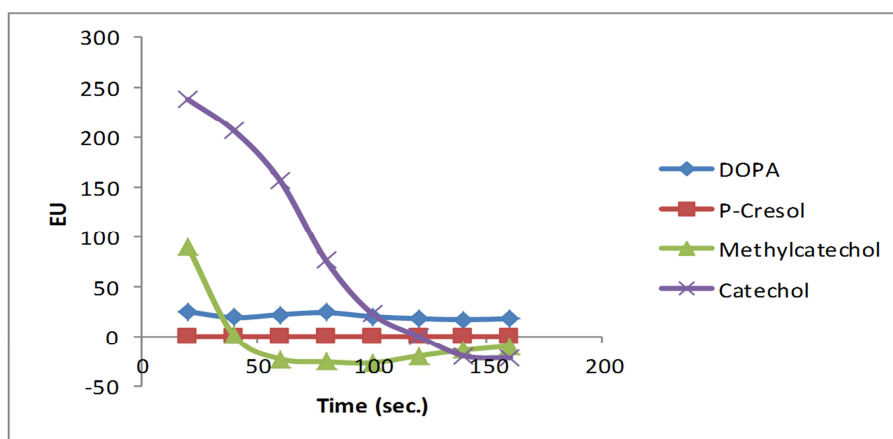


Figure 1. Substrate specificity of water yam PPO.

Similar results were observed by Shengmin *et al.* [10], who found the greatest activity for Chinese Water Chessnut (CWC) PPO on catechol which is a diphenol, and no activity for CWC PPO to monophenols. Most PPOs from fruits such as peaches and apples also show similar characteristics [23]. This trend was likewise reported by Yagar and Sagioglu [24] who indicated that quince PPO showed activity towards ortho-diphenols including catechol and L-DOPA but not with monophenols like tyrosine and p-cresol. Cocoa bean [25] and pear [26] have been reported to have similar activities. Ziyan and Pekyardunci [27] reported that PPO from *Ankara* pear showed higher affinity for diphenolic compounds than monophenolic compounds like p-cresol and D-tyrosine. PPO from *Amasya* pear was also reported to have similar results [28]. Cash *et al.* [29] reported only catecholase activity with Concord grape PPO and no activity for monophenolic compounds. Most other researchers have reported activity against ortho-diphenolic compounds in some fruits [30] [31] [32] [33]. The findings in literature indicate that the PPO enzyme systems of most plants are specific for ortho-

diphenolic substrates. Nevertheless, Yue-Ming [34] reported specificity for both diphenols and triphenols.

3.3. pH Profile

The pH activity profile within a pH range of 4.5 – 8.0, by water yam PPO is presented in Figure 2. Here, the optimum pH of activity was observed under room temperature (28°C) to be pH 6.8 (Figure 2).

Most assays of PPO activity that have been reported to have been carried out between 20°C and 35°C. This is because a significant thermal inactivation may occur during the assay at temperatures higher than 40°C [35]. Browning rates above pH 8.0 were not taken into consideration since rapid non-enzymatic browning of substrates may occur at higher pH values [36] [37]. Consideration was also given to the fact that browning due to oxidation of phenols by phenolases may also involve the participation of other enzymes, may be non-enzymatic, or the browning may not involve phenols at all [37].

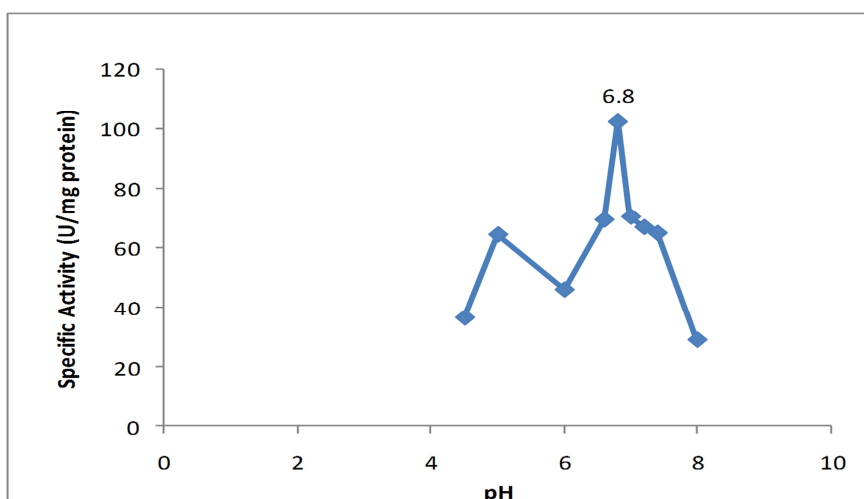


Figure 2. pH profile of the acetone powder PPO extract from water yam.

The pH optima of PPOs differ widely with plant sources but are generally in the range of 4.0 – 8.0 [10]. It has been reported that the PPO systems in fruits are more active at or near neutral pH values [25]. Maximum activity at pH 7.0 was found in aubergine [3] cocoa beans [25] *d'* Anjou pears [26], Yali pear [27], *Amasya* apples [28] raspberry [38] Jerusalem artichoke [39] oil bean seeds [40], burdock [41] and *Anethum graveolens* L.[42]. The pH optima for PPO from banana [17] cherry [30], Longan fruit [34] Clingstone and Halford peaches [43] were found to be between 6.2 and 7.2. pH optima that were reported in other plant PPO sources include 4.7 – 4.8 for Lula avocado [32]; 4.5 for strawberry [44] 6.0 for DeChaunac grape [45], 7.2 for guava [46], 7.5 for *Allium spp.*[47], 8.5 for Dog rose [48]. Sakiroglu *et al.* [48] reported that the optimal pH of pear PPO may vary between 5.8 and 6.4 using catechol as substrate. In tubers, the pH optimum also varies depending on the source of PPO. Earlier studies reported that the optimum pH for PPO extracted from tuber were 7.0 for Jerusalem artichoke [39] and edible yam [41], 4.6 for taro tuber [49], 6.8 for potato [50], 6.5 for rooster potato [51], 7.4 for mustard tuber [52] and 7.5 for cassava [53] using catechol as substrate.

In some species such as egg-plant [54], Hale Haven peaches [55], blueberry fruit [56] etc., two pH optima have been observed. Furthermore, optimum pH values differ in

different parts of the same plant [57]. Xiao-Lin [58] reported that sweet potato leaves had higher value of pH 8.0 because its PPO is concentrated in the plastids. The pH optimum for PPO of different plants may vary depending on the origin of the material, extraction method used, the stage of maturity of the plant and the type of substrate used [59] reported that the optimum pH maximum for PPO activity in plants ranged from 4.0 to 7.0, depending on the purity of the enzyme, the type of buffer used and the substrates used for the assay.

3.4. Inhibition Studies

In this work, inhibition of PPO from water yam was investigated using catechol as substrate. Under the experimental conditions, oxidation of catechol by water yam PPO followed Michaelis-Menten kinetics. Kinetic studies of the enzyme in the presence of L-tyrosine are presented by the Lineweaver-Burk plot shown in Figure 3. The result revealed that water yam PPO was completely inhibited by tyrosine. The function of PPO is to oxidize the diphenol to an ortho-quinone which is used for the synthesis of melanin. Tyrosine is used to produce melanin in humans. Considering both its structure and human functions, therefore; one would expect that tyrosine might inhibit PPO.

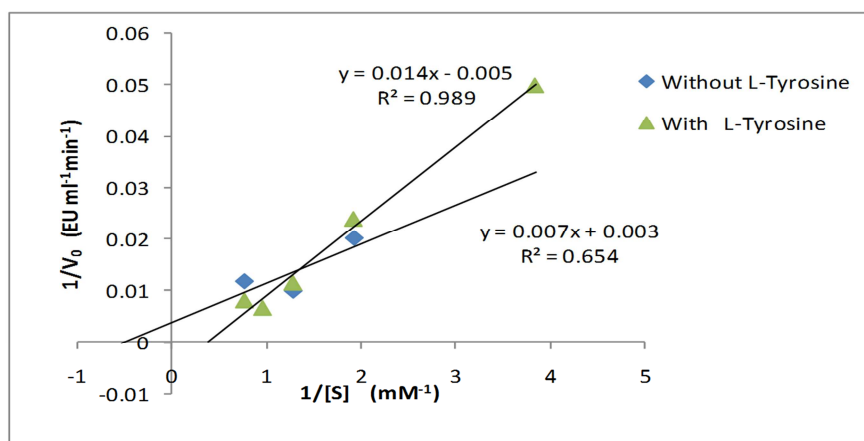


Figure 3. Lineweaver-Burk plots for inhibition of Polyphenol Oxidase – mediated oxidation of catechol by tyrosine in water yam.

The Lineweaver-Burk plot for the inhibited reaction revealed that the curve for the inhibited reaction lies in the first quadrant; however, the V_{max} for the inhibited reaction decreased compared to that of uninhibited reaction which suggests that tyrosine acted as a competitive inhibitor of water yam PPO.

Under the conditions used in this study, oxidation of

catechol by water yam PPO in the presence of ascorbic acid did not follow Michaelis –Menten kinetics. No linear relationship could be established between the plot of $1/V_0$ and $1/[S]$. Here, complete inactivation of enzyme was recorded. This shows that at the concentration of inhibitors used in this work, total inactivation of water yam PPO activity by ascorbic acid was achieved.

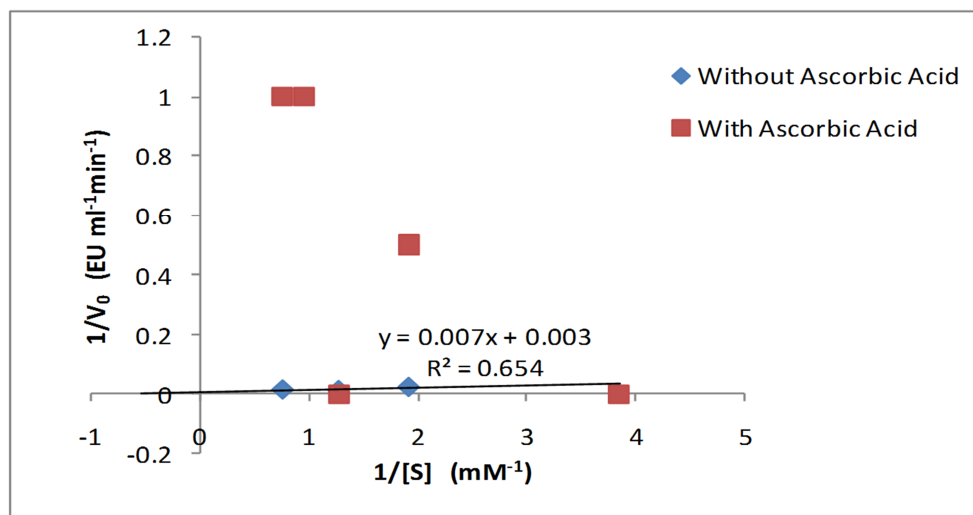


Figure 4. Lineweaver-Burk plots for inhibition of PPO – mediated oxidation of catechol by ascorbic acid in water yam.

Under the condition used in this investigation, oxidation of catechol by water yam PPO tested followed Michaelis-Menten kinetics. Kinetics studies of the enzyme in the presence of phenylthiourea is presented by the Lineweaver-Burk plot shown in Figure 5. The inhibitory behaviour of phenylthiourea was demonstrated to be competitive in water yam PPO.

The Lineweaver-Burk plot for the reactions that have a linear relationship shows that the K_m for the inhibited reaction increased compared to that of uninhibited reaction while the V_{max} of the inhibited reaction seem to decrease relative to the uninhibited reaction which shows that phenylthiourea acted as a competitive inhibitor of water yam PPO.

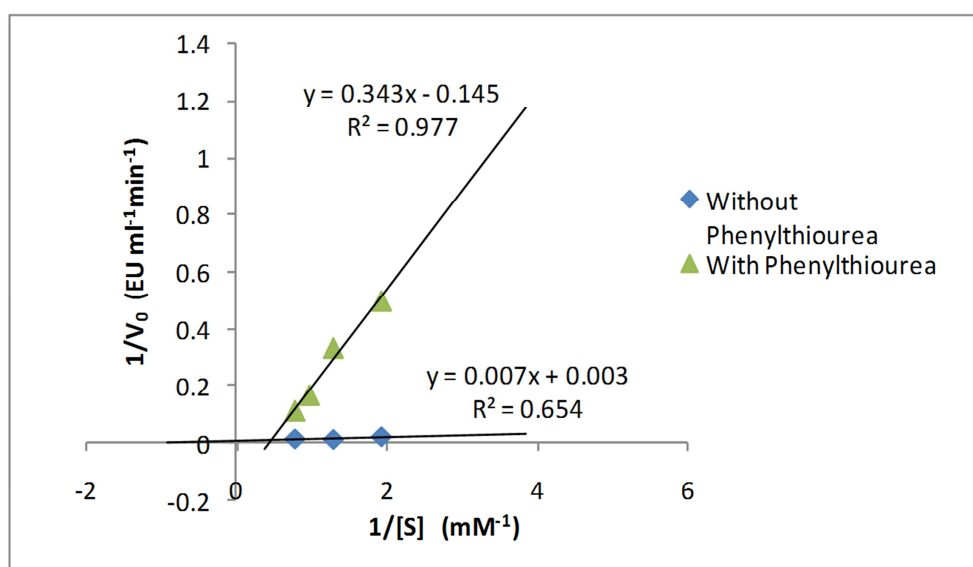


Figure 5. Lineweaver-Burk plots for inhibition of PPO – mediated oxidation of catechol by phenylthiourea in water yam.

When the value of K_i is less than the value of K_{is} , it indicates that the affinity of the inhibitor for the free enzyme is stronger than that for the enzyme-substrate complex [67]

(Bar, 2001). This behaviour will suggest that this compound can bind both the free enzyme and to the enzyme-substrate complex, and that the equilibrium constant for these two

interactions are different. In this case, mixed-type I inhibition implies that the inhibitor affects the affinity of the enzyme for its substrate, yet it does not bind at the active site for the substrate [68] (Macrae and Duggleby, 1968).

Under the conditions used in this work, oxidation of

catechol by water yam followed Michaelis-Menten kinetics. Kinetics studies of the enzyme in the presence of pyrogallol is shown by the Lineweaver-Burk plot presented in figure 6. Activation of catechol oxidation by PPO in the presence of pyrogallol was observed.

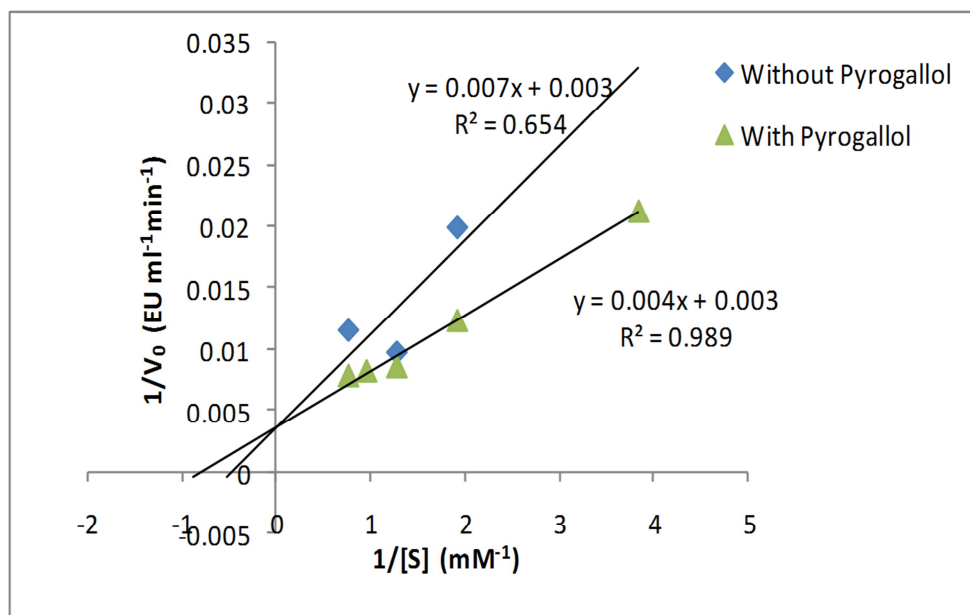


Figure 6. Lineweaver-Burk plots for inhibition of PPO – mediated oxidation of catechol by pyrogallol in water yam

Under the conditions used in this work, oxidation of catechol by water yam PPO followed Michaelis-Menten kinetics. Kinetics studies of the enzyme in the presence of garlic acid is shown by the Lineweaver-Burk plot presented in figure 7. Activation of catechol oxidation by PPO in the presence of garlic acid was observed in water yam (Figure 7).

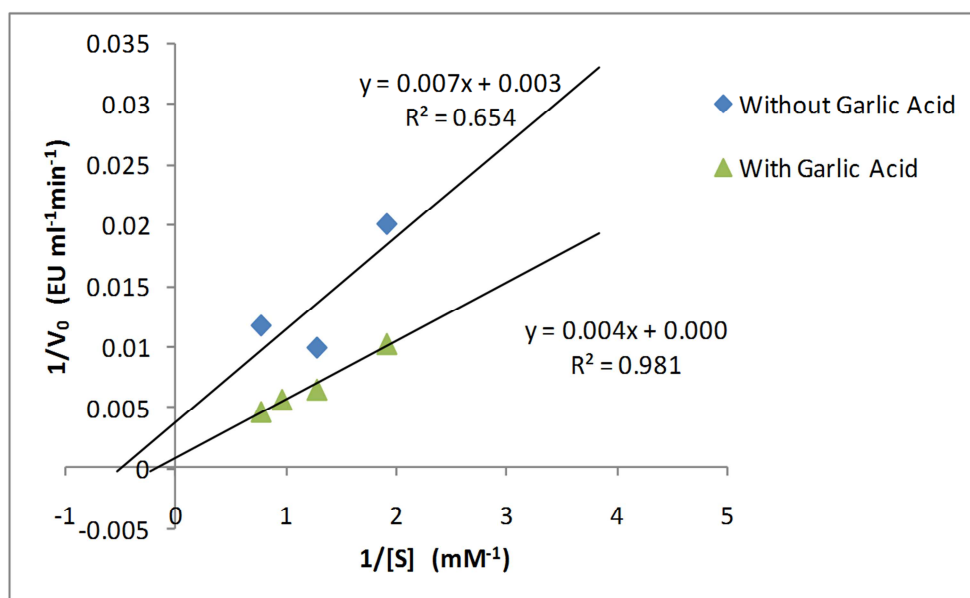


Figure 7. Lineweaver-Burk plots for inhibition of Polyphenol Oxidase – mediated oxidation of catechol by garlic acid in water yam.

Under the conditions used in this investigation, oxidation of catechol by water yam PPO followed Michaelis-Menten kinetics. Kinetics studies of the enzyme in the presence of hydroquinone are presented by the Lineweaver-Burk plot

shown in figure 8. The inhibition of hydroquinone was competitive in water yam PPO (Figure 8) under the conditions used in this investigation.

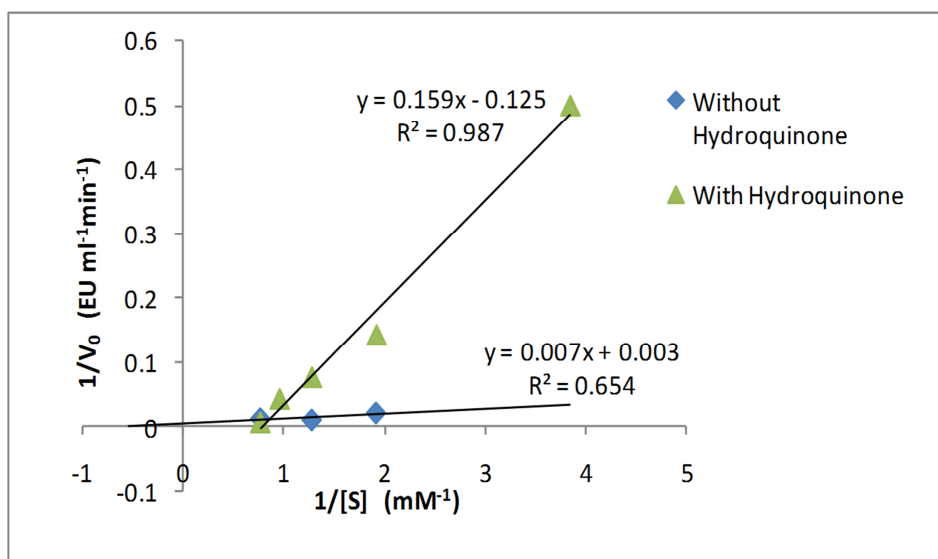


Figure 8. Lineweaver-Burk plots for inhibition of PPO – mediated oxidation of catechol by hydroquinone in water yam.

Under the conditions used in this present work, oxidation of catechol by water yam PPO followed Michaelis-Menten kinetics. Kinetic study of the enzyme in the presence of 2-Mercaptoethanol is presented in the Lineweaver-Burk plot

shown in figure 9. Complete inhibition of catechol oxidation by PPO in the presence of 2-mercaptoethanol was observed in water yam (Figure 9).

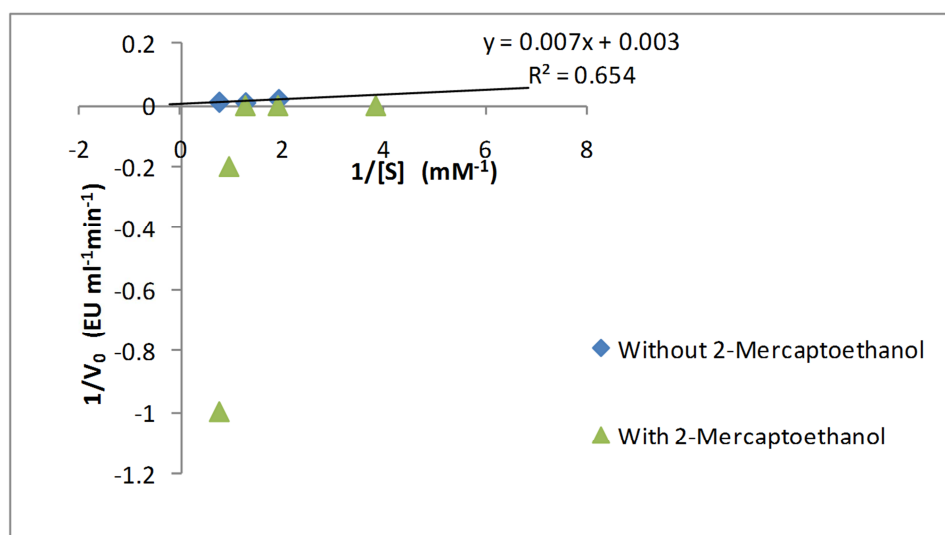


Figure 9. Lineweaver-Burk plots for inhibition of Polyphenol Oxidase – mediated oxidation of catechol by 2-mercaptoethanol in *Dioscorea alata* (abana l).

4. Conclusion

From this research it was concluded that polyphenol oxidase from *Dioscorea alata* has a pH optimum of pH 6.8 and has no affinity for monophenols such as p-cresol. Browning in *Dioscorea alata*. Enzymatic browning in this tuber can be effectively controlled by the use of ascorbic acid.

References

- [1] Christiane, Q., Lopes M. L. M., Fialho, E., and Valente-Mesquita, V. L. (2008). Polyphenol Oxidase: Characteristics and Mechanisms of Browning Control. *Food Rev Intl*, 24: 361-375.
- [2] Jia G., Wang B., xiaoyuan F. Haoru T., Wensheng L. L. and Kaichun Z. (2011). Partial Properties of Polyphenol Oxidase in sour Cherry (*Pyunus cerasus* L. 7.0 for Jerusalem artichoke and edible yam CAB) Pulp. *World J Agric Sci*. 7 (4): 444-449.
- [3] Dogan, M., Arslan, O., and Dogan, S. (2002). Substrate specificity, heat inactivation and inhibition of polyphenol oxidase from different aubergine cultivars. *Int J Food Sci Technol*, 37: 415-423.
- [4] Chaisakdanuagull C., Theerakukait C. and wrolstad R. E. (2007): Pineapple Juice and its fractions In Enzymatic Browning Inhibition of Banana (*Musa* (AAA Group)) Gros Michel). *J. Agric. Food Chem*. 55, 4252-4257.
- [5] Trejo-Gonzalez, A. and Soto-Valdez, H. (1991). Partial characterization of PPO extracted from ÒAnnaÕ apple. *J Amer Soc for Horticultural Sci*, 116: 672-675.

- [6] Unal U. M., Yabaci S. N. and Sener A. (2011): Extraction, Partial Purification and Characterization of Polyphenol Oxidase from Tea Leaf (*Camellia sinensis*). *GIDA*. 36 (3): 137-144.
- [7] Yemenicioglu, A. and Cemeroglu, B. (1998). Characteristics of polyphenol oxidase in Hale Heaven peaches. *Turkish J Agric and Forestry*, 22: 261-265.
- [8] Gouzi H, Coradin T, Delicado EM, Ünal MU, Benmansour A (2010). Inhibition kinetics of *Agaricus bisporus* (J. E. Lange) Imbach polyphenol oxidase. *Open Enzyme Inhibition J*. 3: 1-7.
- [9] Bora P. S., Holschuh H. J., da Silva Vasconcelos, M. A. (2004): Characterization of Polyphenol Oxidase of Soursop (*Annona muricata* L.) fruit And A Comparative Study of its Inhibition In Enzyme Extract and In Pulp. *Cienc. Technol. Aliment*. 4 (4): pp. 267-273.
- [10] Shengmin L., Gangping T., Ying L., and Hao F. (2006). Partial Purification and Characterization of Polyphenol Oxidase from Fresh-Cut Chinese Water Chestnut. *J Food Biochem*, 30: 123-137.
- [11] Constabel, C. P., Lynn, Y., Patton, J. J., and Christopher, M. E. (2000). Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. *Plant Physiol.*, 124: 285-295.
- [12] Kouakou, M. D., Dabonne, S., Guehi, T., and Kuoame, L. P. (2010). Effects of post-harvest storage on some biochemical parameters of different parts of two yams species (*Dioscorea* spp). *African J Food Sci and Tech*, 1: 1-9.
- [13] Amanze, N. J., Agbo, N. J., Eke-Okoro, O. N., and Njoku, D. N. (2011). Selection of yam seeds from open pollination for adoption in yam (*Dioscorea rotundata* Poir) production zones in Nigeria. *J Plant Breeding and Crop Sci* 3 (4): 68-73.
- [14] Chou, S., Chiang, B., Chung, Y., Chen, P., and Hsu, C. (2006). Effects of storage temperatures on the anti-oxidative activity and composition of yam. *Food Chem*, 98: 618-623.
- [15] Mignouna, H. D., Abang, M. M. and Asiedu, R. (2008). Genomics of Yams, a Common Source of Food and Medicine in the Tropics. *Plant Genetics and Genomics I*: 549-570.
- [16] Dweck, A. C. (2002). The Wild Yam – review of *Dioscorea* species. *Personal Care Magazine*. 3 (3): 7-9.
- [17] Galeazzi, M. A. M., Sgarbieri, V. C., and Constantinides, S. M. (1981). Isolation, purification and physicochemical characterization of polyphenol oxidase (PPO) from a Dwarf variety of banana (*Musa Cavendishii*, L). *J. Food Sci.*, 46: 50-155.
- [18] Stelzig, D. A., Akhtar, S., and Ribeiro, S. (1972): Catechol oxidase of red delicious apples. *Phytochem.*, 11: 535-539.
- [19] Nicole, K. K. and Michael, W. A. (2009): The Biuret method for the determination of total protein using an evolution array 8-position cell changer. Thermo Fisher Scientific Inc., Madison, WI, USA. www.thermo.com.
- [20] Ikran M., Pat M. L., and Lee K. H. (2011). Partial purification and characterisation of polyphenol oxidase from *Hibiscus rosasinensis* L 2nd International Conference on Biotechnol Food Sci IPCBEE vol. 7 211-220.
- [21] Anosike, E. O and Ojmelukwe, P. C 1982. Partial purification and characterization of polyphenol oxidase from cocoyam (*Xanthosoma sagittifolium*). *Journal of Experimental Botany*. 33 (134): 487-494.
- [22] Flurkey, W. H. and Jen, J. J. (1978): Peroxidase and PPO Activities in Developing Peaches. *J. Food Sci*. pp. 1826-1831.
- [23] 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. *Critical Rev Food Sci Nutr*, 34: 109-157.
- [24] Yagar, H. and Sagiroglu, A. (2002). Partially Purification and Characterization of Polyphenol Oxidase of Quince *Turkish. J. Chem.*, 26: 97-103.
- [25] Lee P. M, Lee K. H, Karim M. I. A. (1991): Biochemical Studies of Cocoa Bean Polyphenol Oxidase. *J. Sci. Food Agric*. 55: 251-260.
- [26] Halim, D. H. and Montgomery, M. W. (1978): Polyphenol oxidase of d' Anjou Pears (*Pyrus communis*, L.). *J. Food Sci.*, 43: 603-608.
- [27] Ziyan, E. and Pekyardimci, S. (2004). Purication and Characterization of Pear (*Pyrus communis*) Polyphenol Oxidase. *Turk J. Chem.*, 28: 547-557.
- [28] Oktay, M., Kufrevioglu, I., Kocacaliskan, I., and Sakiroglu, H. (1995). Polyphenol oxidase from Amasya apple. *J Food Sci*, 60, 494-496.
- [29] Cash, J. N., Sistrunk, W. A., and Stutte, C. A. (1976). Characteristics of Concord grape polyphenol oxidase involved in juice color loss. *J Food Sci*, 41: 1398-1402.
- [30] Benjamin, N. D. and Montgomery, M. W. (1973). Polyphenol oxidase of royal ann cherries: purification and characterization. *J Food Sci*, 38: 799-806.
- [31] Rivas, N. J. and Whitaker, J. R. (1973): Purification and Some Properties of Two Polyphenol Oxidases from Bartlett Pears. *Plant Physiol*. 52, 501-507.
- [32] Kahn, V. (1977): Some Biochemical Properties of Polyphenol oxidase from Two Avocado Varieties Differing in Their Browning Rates. *J. Food Sci*. 42: 38-43.
- [33] Vamos-Vigyazo, L. (1981). Polyphenoloxidase and peroxidase in fruits and vegetables. *Critical Rev Food Sci Nutr*, 15: 49-127.
- [34] Yue-Ming, J. (1999). Purification and some properties of polyphenol oxidase of logan fruit. *Food Chem*. 66: 75-79.
- [35] Vamos-Vigyazo, L. and Gajzago, I. (1978). Substrate specificity of the enzymic browning of apples. *Acta Alimentaria*, 7: 79-90.
- [36] Lee P. M, Lee K. H, Karim M. I. A. (1991): Biochemical Studies of Cocoa Bean Polyphenol Oxidase. *J. Sci. Food Agric*. 55: 251-260
- [37] Li Guo, Ying Ma, John Shi, Sophia Xue. (2006). The purification and characterisation of polyphenol oxidase from green bean (*Phaseolus vulgaris* L.). *Food Chem*, 117: 143-151.
- [38] Gonzalez, B, De Ancos, MP., and Cano, S (1999). Partial characterization of polyphenol oxidase activity in raspberry fruits. *J Agric Food Chem* 4: 4068-4072.
- [39] Ziyan, E, Pekyardimci, S (2003). Characterization of Polyphenol oxidase from Jerusalem artichoke (*Helianthus tuberosus*). *Turkish Journal of Chem* 27 (2): 217-226.

- [40] Chilaka, FC, Anosike, EO, and Egbuna, P. C. (1993). Purification and Properties of Polyphenol Oxidase from Oil Bean (*P. macrophylla* Benth) seeds. *J. Sci. Food Agric.* 61 (1): 125-127.
- [41] Shuji F., Yun-Zhen, H., and Tomoko, M. (2006). Purification and Characterization of polyphenol oxidase from edible yam (*Dioscorea opposita*). *J Food Sci Technol Res*, 12: 235-239.
- [42] Sakiroglu, H., Ozturk, A E, Pepe, AE, Erat, M. 2008. Some kinetic properties of Polyphenol oxidase obtained from dill (*Anethum graveolens*). *J. Enzyme Inhibition & med Chem* 23 (3): 380-385.
- [43] Luh, BS. and Phithakpol, B. (1972). Characteristics of polyphenol oxidase related to browning in cling peaches. *I. Food Sci.* 37: 264-268.
- [44] Wesche-Ebeling, P. and Montgomery, MW. (1990). Strawberry polyphenol oxidase: extraction and partial characterization. *J Food Sci*, 55: 1320-1325.
- [45] Lee, CY, Smith, NL, and Pennesi, AP. (1983). Polyphenol oxidase from de Chaunac grapes. *J Sci Food Agric*, 34: 987-991.
- [46] Augustin, M., Ghazeli, HM., Hashim, H. 1985. Polyphenol oxidase from guava (*Psidium guajava* L) *J Sci Food Agric* 36 (12): 1259-1265.
- [47] Arslan, O., Temur, A. and Tozlu, I. (1997). Polyphenol oxidase from *Allium* sp. *Journal of Agriculture and Food Chemistry*, 45: 2861-2863.
- [48] Sakiroglu, H., Kufrevioglu, OI., Kocacaliskan, I., Oktay, M., and Onganer, Y. (1996). Purification and characterization of Dog-rose (*Rosa dumalis* Rechst.) polyphenol oxidase. *J Agric Food Chem*, 44: 2982-2986.
- [49] Kiattisak, D, Richard, K., and Owusu, A. (1999). A comparative study of polyphenol oxidases from taro (*Colocasia esculenta*) and potato (*Solanum tuberosum* var. Romano). *J Food Chem*, 64: 351-359.
- [50] Corsini, DL, Pavek, JJ, and Dean, B. (1992). Differences in free and protein-bound tyrosine among potato genotypes and their relationship to internal blackspot resistance. *J Amer Chem Soc*, 69: 423-434.
- [51] Ni Eidhin, DM, Murphy, E., and O'Beirne, D. (2010). Characterization of Polyphenol oxidase from rooster potato (*Solanum tuberosum* CV rooster) *J Food Biochem* 34 (1): 13-30.
- [52] Liu Zhu, M. (2005). Characterization of polyphenol oxidase from mustard tuber. *J Hubei Univ*, 4: 625-712.
- [53] Barthet, VJ. and Veronique, D (1997). Polyphenol oxidases from cassava (*Manihot esculenta* C.) root: extraction, purification and characterization. *J Food Sci Agric Chem*, 4: 179-198.
- [54] Fujita, S. and Tono, T. (1988). Purification and some properties of polyphenol oxidase in eggplant (*Solanum melongena*). *J Sci Food Agric*, 46: 115-123.
- [55] Yemenicioglu, A., Özkan, M. Cemeroglu, B., Heat Inactivation Kinetics of Apple Polyphenol Oxidase and Activation of its Latent Form, *J. Food Sci.*, 62, 508-510, 1997.
- [56] Kader, F., Rovel, B., Girardin, M., and Metche, M. (1997). Mechanism of browning in fresh high bush blueberry fruit (*Vaccinium corymbosum* L.). Partial purification and characterisation of blueberry polyphenol oxidase. *J Sci Food Agric*, 73: 513-516.
- [57] Matheis, G. and Whitaker, JR. (1987). Modification of proteins by polyphenol oxidase and peroxidase and their products. *J Food Biochem*, 8: 137-162.
- [58] Xiao-Lin MA., Xue, BAI., Hui-Jun, LI. SongHe, XUQin, R. 2013. Changes of Polyphenol oxidase activity in potato leaves after potassium supply and aphid infestation. *Acta Entomologica Sinica* 56 (12): 1413-1417.
- [59] Alyward, F. and Haisman, DR. (1969). Oxidation system in fruits and vegetables-their relation to the quality of pressured products. *Adv Food Res*, 17: 1-76.
- [60] Macrae, AR. and Duggleby, RG. (1968). Substrates and inhibitors of potato tuber phenolase. *Phytochem*, 7: 855-61.