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Biochemical Evaluation of Polyphenol Oxidase Activites in Yellow Yam (*Dioscorea cayenensis*) and Water Yam (Dioscorea alata)

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Abstract: Yams (*Dioscore*a species) are annual or perennial tuber-bearing and climbing plants belonging to the family of *Dioscoreaceae*. *Dioscorea cayenensis* (yellow yam) and *Dioscorea alata* (greater yam or water yam) contain various nutrients including anthocyanin and vitamin A. Enzymatic browning occurs as a result of the oxidation by polyphenol oxidase (PPO) of phenolic compounds in many foods of plant origin, which generally cause deterioration in food quality by changing nutritional and organoleptic properties. In this study, the biochemical properties of partially purified PPO were evaluated. The resultshowed that PPO activity of *D. cayenensis* was optimal at pH 7 in the absence and presence of SDS respectively while *D. alata* showed optimal at pH 6 and pH 7 in the absence and presence of SDS respectively. The PPO was effectively stable at 20 hours of incubation in pH 4-8. Optimum temperature at 30°C for both yam species and effective thermal stability at 50°C was observed after 60 minutes of incubation. The kinetics parameter showed high specificity for diphenolics, while ascorbic acid proved to be an effectively nutritional additive.

Key words: *Dioscorea cayenensis* · *Dioscorea alata* · Yellow yam · Water yam Polyphenol oxidase · Kinetics parameter · Physicochemical properties

INTRODUCTION

Yams (*Dioscore*a species) are annual or perennial tuber-bearing and climbing plants belonging to the family of *Dioscoreaceae*. Some species of yam originated from Africa before spreading to other parts of the world while some originated from Asia and have spread to Africa [1]. Today, yams are grown widely throughout the tropics and they have a large biological diversity including more than 600 species worldwide [2] but only six are widely cultivated in West and Central Africa. These cultivated species are *D. alata, D. bulbifera, D. dumetorum* (Pax), *D. esculenta* (Lour), *D. cayenensis* (Lamk) and *D. rotundata* (Poir). West Africa is the leading producer of yam and grows over 90% of the worldwide production (40 tones fresh tubers/year). Nigeria is the world's largest producer of yams followed by Ghana, Ivory Coast and Togo [3]. Yams arealso produced in Jamaica, Tanzania, Sudan, Japan, Papua New Guinea, the Philippines and Panama.

Information on the nutritive value of yam has been highlighted by several authors in their work on yam, [4, 5, 6]. They contain appreciable amount of potassium, a mineral that helps to control blood pressure. Yam is therefore recommended for people with high blood pressure but is not suitable for people with renal failure [7]. Yam is also a good source of manganese, a trace mineral that helps with carbohydrate metabolism and also acts as a cofactor in a number of enzymes important in energy production and antioxidant defenses. It also contains traces of vitamin B-complex [8].

Yam is more importantly used as food in West Africa.There are about 72 morphologies of *D. alata* tuber. Several authors had reported on the nutrient compositions of *D. alata* [9, 10, 11]. Rasper and Coursey [12], reported the predominance of cyanidin-3, 5, di-glucosides in one West African cultivar of *D. alata*and ferullic acid cyanidin-3-gentiobioside ester in a West Indian cultivar of the same species. Some *D. alata* cultivars have cream coloured or light yellow flesh, which may be because of carotene content. Varieties with higher anthocyanin content are often prone to polyphenolic oxidation. The anthocyanin pigment in *D. alata* and *D. trifida* cultivars may impart a pink or purplish-red colourto the tuber tissue, either the entire tuber tissue

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or just beneath the skin of the tuber. An amount of 1.44 mg/100g of β-carotene, a precursor of vitamin A, has been reported in Igangan a cultivar of *D. cayenensis* [9, 13]. Anthocyanins and carotenoids are pigments known to occur in yam to give characteristic colours to the flesh of the tuber. Xantophyll esters and and β- carotene in *D. cayenensis* has been reported to be responsible for the yellow flesh of the species [13]. Asemota *et al*. [11] found phosphorus content to be higher in *D. alata* than in *D. rotundata*and *D. cayenensis*. The amount of this minerals or nutrients in yams depends on the type of soil it was harvested from, moisture content and maturity of the crop. The relationship that existed between *D. cayenesis* and *D rotundata* has been explored [14, 15].

Polyphenol oxidases (PPOs) are copper containing oxidoreductases that catalyze the hydroxylation and oxidation of phenolic compounds in the presence of molecular oxygen. Previous studies has shown that polyphenol oxidase (PPO; 1, 2-benzenediol: oxygen Oxidoreductase; EC 1. 10. 3.1) is widely distributed in plants and microorganisms. In plants, PPOs are located mainly in thylakoid membrane of chloroplasts and mitochondria [16]. Browning is attributed to the oxidation of phenolic substances by the enzyme polyphenol oxidase [17]. Enzymatic browning occurs as a result of the oxidation by PPO of phenolic compounds to quinones and their eventual (non-enzyme-catalyzed) polymerization to melanin pigments [18]. It appears that oxidative browning reactions, proceeding in many foods of plant origin, generally cause deterioration in food quality by changing nutritional and organoleptic properties [19, 20]. These reactions significantly diminish consumer acceptance, storage life and value of the plant product. The major yam species has been reported to contain PPO whose activity varies even within a given species [21, 13]. The biochemical characteristics of PPO has been studied from edible yam (*D. cayenensis-rotundata cv. Kponan*) [22].

Polyphenol oxidases have significant applications in many areas such as food, medicine and industry. In food processes, PPO has been mainly used for enhancement of the flavour in tea, coffee and cocoa. On the other hand, in some food processes, PPO activity is undesirable and plays key role in deterioration of food quality. In this study, the biochemical properties of polyphenol oxidase from *D. cayenensis* (yellow yam) and *D. alata* (water yam) were investigated and compared, toward improving industrialprocess for yam foods such as yam chips snacks, pounded yam flour and many others.

MATERIALS AND METHODS

Preparation of Crude Extract: 150g of each yam species (peeled) was thoroughly homogenized in 450ml of ice cold 25mM phosphate buffer (pH6.8) containing 10mMascorbic acid using a warring blender for 3min with a 60 seconds resting period to avoid local elevation in temperature. Themixture was filtered using four layers of cheese cloth. The filtrate obtained was centrifuged in a refrigerating centrifugeat 6, 000rpm (revolutions per minute) for 30 min at 4°C. The supernatant obtained was stored in a freezer and used as crude extracts for further studies.

Partial Purification of PPO: 175ml of the supernatant obtained from each species was brought to 80% ammonium sulphate $(NH4)_2SO_4$ saturation by slowly dissolving solid ammonium sulphate using magnetic stirrer. The precipitate was separated by centrifugation at 6, 000rpm for 30 minutes. The precipitate was dissolved in 0.1M phosphate buffer (pH 6.8) and dialyzed at 4°C overnight with three changes of buffer. The dialyzed extracts were used as the crude PPO for further analysis.

Protein Determination: Protein concentration was measured according to the method of Lowry *et al.* [23] using bovine serum albumin (BSA) as a standard.

Determination of PPO Activity: Polyphenol oxidase activity was determined according to the method of Coseteng and Lee [24]. 0.7 ml of 10mM 3, 4-dihydrophenylalanine (DOPA) solution in 0.1 M phosphate buffer (pH 6.8) and 0.3 ml of the enzyme solutionat room temperature for 10mins. The blank sample contained only 0.7 ml of 10mM 3, 4-dihydrophenylalanine and 0.3ml of 0.1M buffer solution. Absorbance was measured at 475 nm. One unit of polyphenol oxidase activity was defined as the amount of enzyme that caused a change in absorbance of 0.001 per minute.

Effect of pH on PPO Activity in Presence and Absence of SDS: Effect of pH on polyphenol oxidase activity in presence and absence of SDS was carried out according to the method of Escribano *et al*. [25], in buffer pH range 4.0-9.0, using 0.1 M acetate buffer (pH 4.0–5.0), 0.1 M phosphate buffer (pH 6.0–7.0) and 0.1M Tris/HCl buffer (pH 8.0-9.0). The reaction mixture in presence of SDS contains 0.69mM of the detergent and the PPO activity was assayed according to standard assay procedure earlier described.

Effect of pH on PPO Stability: The effect of pH on stability was determined by incubating 1ml enzyme solution in 3ml of the buffer solution between pH 4.0 - 9 .0 at room temperature. The enzyme activity was measured after mixing using a vortex mixer while the residual PPO activity was determined periodically.

Effect of Temperature on PPO Activity: The assay mixture containing the PPO and DOPA was incubated for 10mins at different temperature of 30 to 80°C at 10°C temperature interval. The absorbance reading was taken at 475nm according to standard assay procedure.

Thermal Stability of PPO: Thermal stability of the enzyme was determined by incubating the enzymes solution in 0.1 M phosphate buffer at pH 6.8 for 1 hour at different temperatures ranging from 30 to 80°C at 10°C. The residual PPO activity was determined at 20mins time interval.

Kinetics Parameter of PPO: Michaelis-Menten constant (K_m) and maximum reaction velocity (V_{max}) were determined using two different substrates (Tyrosine, DL-DOPA). Substrate solutions were prepared in 0.1 M phosphate buffer (pH 6.8) at different initial concentrations (25, 20, 15, 10, 5 and 1 mM). PPO activity was assayed according to standard assay procedure earlier described and the Lineweaver-Burk [26] plot was derived from the data.

Effect of Inhibitors and Activator on PPO Activity: Polyphenol oxidase activity was measured in the presence of reagents (ascorbic acid, EDTA disodium salt, benzoic acid and sodium dodecyl sulfate) at three concentrations (10, 5 and 1 mM) prepared in 10 mM DOPA (pH 6.8). PPO activity was assayed according to standard assay procedure earlier described.

RESULTS

The activity of the crude PPO and partially purified PPO was found to be 23.1 U/mland 124.4 U/ml for *Dioscorea cayenensis* and 9.1 U/ml and 73.5 U/ml for *Dioscorea alata* respectively. The protein concentration, specific activity and yield of partially purified PPO were found to be 0.39 mg/ml, 319 U/mg and 44.3%for *D. cayenensis* and 0.34mg/ml, 216 U/mg and 57.4% for *D. alata.* The optimum pH in presence and absence of SDS was pH 7.0 for PPO of *D. cayenensis* with over 60% residual activity after 20 hours of incubation as shown in Fig. 1 and 3.There was gradual decrease in the activity as the pH increases to the optima before steady decline. SDS significantly raised the activity of PPO in *D. alata* at each pH and shifts the optimal pH from pH 6.0 to pH 7.0, with over 60% residual activity after 20 hours of incubationas shown in Figs. 2 and 3.

A gradual increase in the activity was observed as the temperature increases. The PPO from both *D cayenensis* and *D. alata* has optimum temperature of 30°C

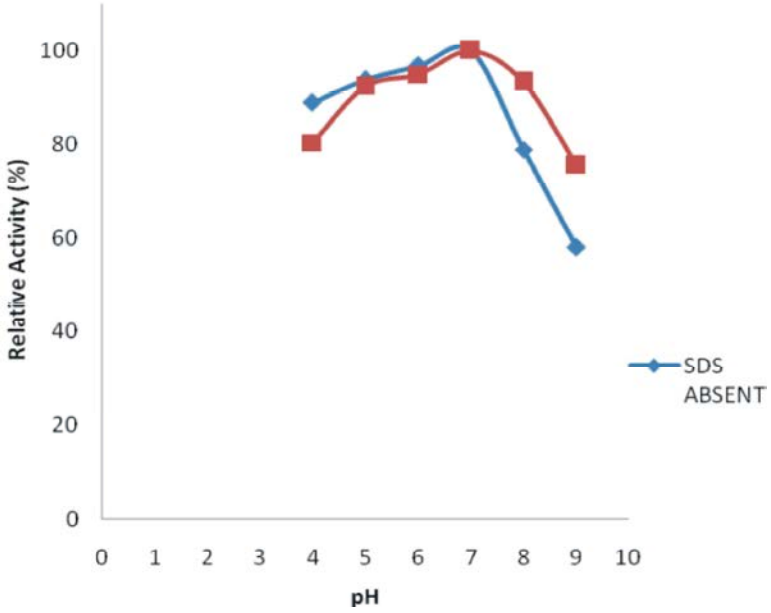


Fig. 1: Effect of SDS on the optimal activity of *D. cayenensis* PPO

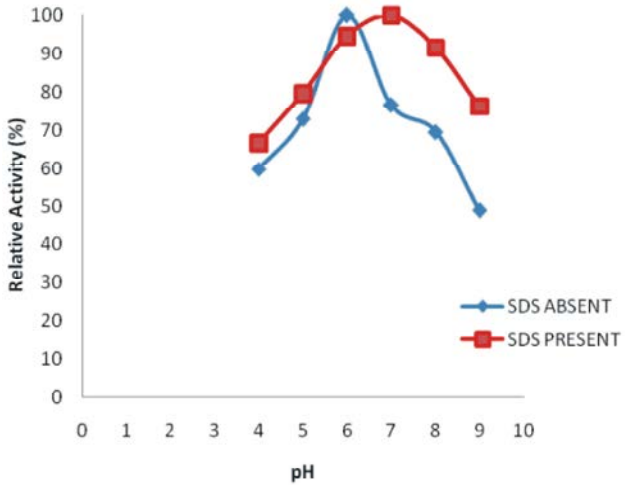
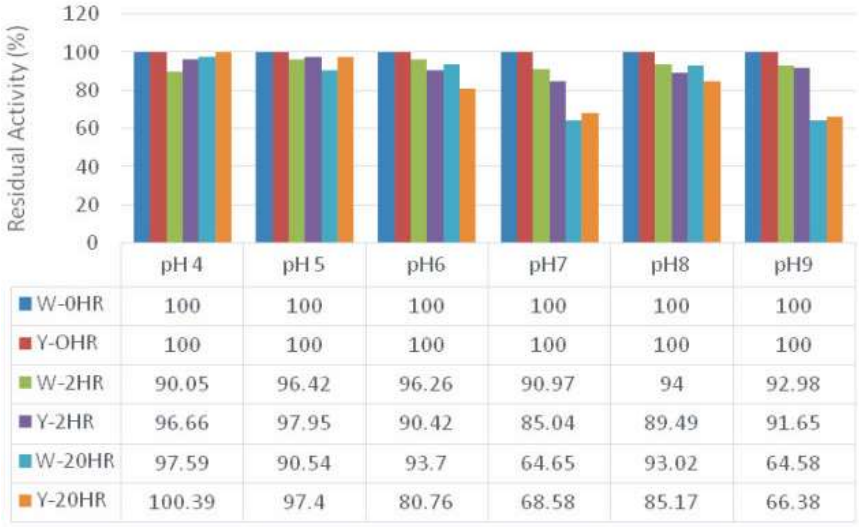


Fig. 2: Effect of SDS on the optimal activity of *D. alata* PPO



	pH 4	pH 5	pH6	pH7	pH8	pH9
W-0HR	100	100	100	100	100	100
Y-0HR	100	100	100	100	100	100
W-2HR	90.05	96.42	96.26	90.97	94	92.98
Y-2HR	96.66	97.95	90.42	85.04	89.49	91.65
W-20HR	97.59	90.54	93.7	64.65	93.02	64.58
Y-20HR	100.39	97.4	80.76	68.58	85.17	66.38

Fig. 3: Effect of pH on Stability of *D. cayenensis* (Y) and *D. alata* (W) PPO

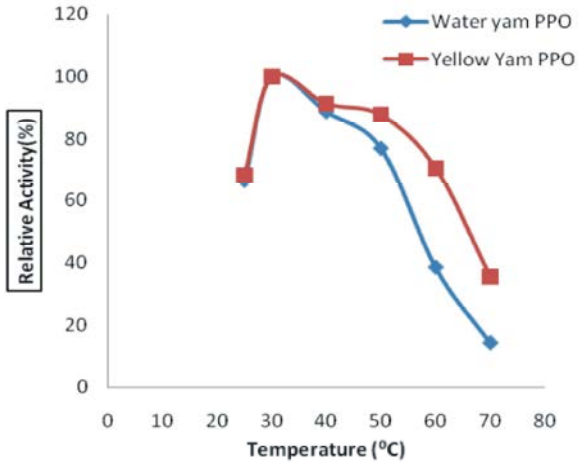


Fig. 4: Effect of temperature on the activity of water yam (*D.alata*)PPO and yellow yam (*D. cayenensis*) PPO.

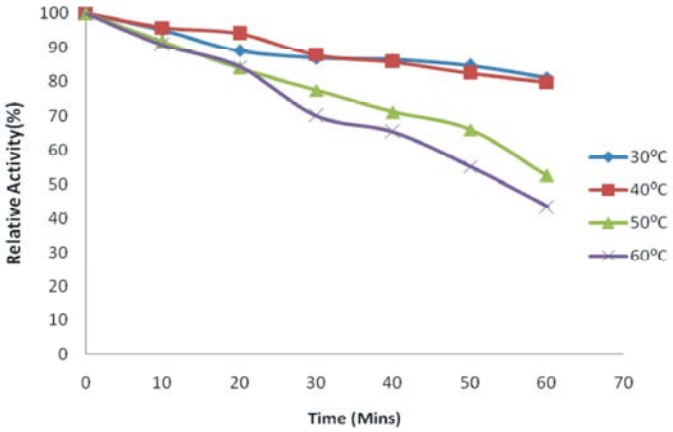


Fig. 5: Effect of temperature on stability of *D. alata* PPO

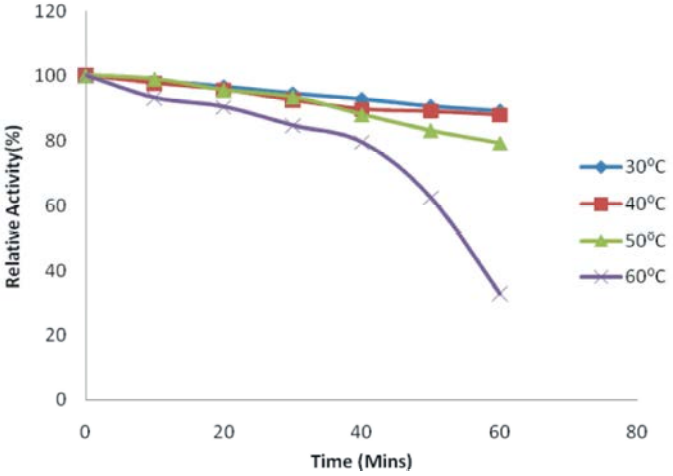


Fig. 6: Effect of temperature on stability of *D. cayenensis* PPO

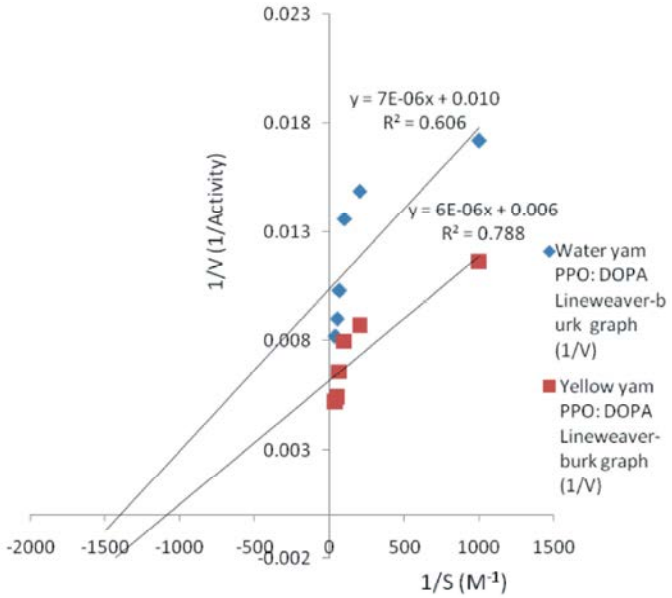


Fig. 7: Double reciprocal plot for water yam (*D. alata)* and yellow yam (*D. cayenensis*) PPO using DOPA as substrate

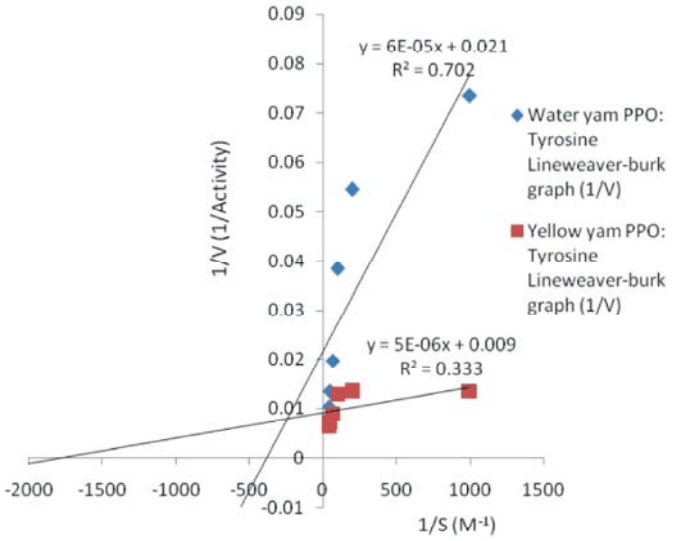


Fig. 8: Double reciprocal plot for water yam (*D. alata)* and yellow yam (*D. cayenensis*) PPO using tyrosine as substrate.

Table 1: Effect of inhibitor on *D. alata* and *D. cayenensis*PPO

	Residual Activity (%) Water yam PPO			Residual Activity (%) Yellow yam PPO		
INHIBITORS	10 mM	5 mM	1 mM	10 mM	5 mM	1 mM
Ascorbic acid	14.42	18.23	20.82	54.82	59.49	65.60
Benzoic acid	58.78	69.39	86.67	71.30	86.17	96.70
EDTA	70.88	85.59	95.78	59.73	78.30	95.18
SDS	177.41	153.06	126.12	134.08	120.18	102.89

(Fig. 4) and effectively stable at 60°C with over 50% residual activity at 30 minutes of incubation (Figure 5 and 6). Km and Vmax for *D. cayenensis* PPO were 1.0 mM and 166.67 U/min for L-DOPA and 0.56mM and 111.11 U/min for tyrosine respectively (Fig. 7). While Km and Vmax for *D. alata* PPO were 0.7mM and 100 U/min for L-DOPA and 2.86mM and 47.62 Umin for tyrosine respectively (Fig. 8).

The results showed that ascorbic acid, benzoic acid and EDTA were effective inhibitors of PPO from both yam species (Table 1), while SDS increases the activity of polyphenol oxidase. However ascorbic acid and benzoic acid gave a higher percentage inhibition of 45.18% and 28.7%. respectively for *D. cayenensis* and 85% and 41.2% respectively for *D. alata.*

DISCUSSION

Polyphenol oxidase activity detected in *D. cayenensis*and *D. alata* conformed to report that major yam species contain polyphenol oxidase activity with variation among species [21]. Different authors had reported the occurrence of polyphenol oxidase in plants; edible yam (*Dioscorea opposite* thunb.) [27], rooster potato [28], cucumber [29], grape [30] and sweet potato [31]. Ammonium sulphate has previously been described as an activator of polyphenol oxidases [32], by removing the high molecular weight proteins and sugars and to concentrate the sample [33].

This study indicated that the PPO of *D. cayenensis* and *D. alata* showed activity toward ortho-diphenols but low activity toward the monophenolic compound. Several authors have reported that polyphenol oxidase shows differences in activity for diphenol and monophenol substrate [34, 35, 36, 37, 38]. However, this observation may be due to presence of different isoforms in this plant tissue.

In this study, a decrease in activity was observed at pH 4 in the presence of SDS than when no SDS and other pH have high activity in presence of SDS. This drop in activity at pH 4.0 may be the inhibitory effect of SDS on this polyphenol oxidase. Resistance of polyphenol oxidase to SDS is perhaps due to presence of disulfide bonds strengthening polyphenol oxidase structure [39]. The anionic detergent, SDS activated the yellow yam PPO at pH 7 and 9 by 14.94% and 49.78%, while water yam PPO was activated by 70.04% and 102.29%. SDS has been widely used as an activator of latent polyphenol oxidase from several plant sources [40]. Resistance of polyphenol

oxidase to SDS is perhaps due to presence of disulfide bonds strengthening polyphenol oxidase structure [39]. In general, maximal activation by SDS is attained above pH 4.0 and there is no detectable activation or complete inhibition below 4.0 [40, 25].

For example, it was reported that optimum pH values are6.5 for lemon balm [41], 7.0 for purslane [42], 7.0 for snake gourd [43], 7.0 for sweet potato, [15], 8.0 for *Averrhoa carambola* fruit [44] and 8.5 for Dog rose [45], using catechol as substrate.

Polyphenol oxidase from bothyam species were found to be most stable at pH 4 to 6, maintaining greater than 80% activity at 20 hours of incubation. Duangmal and Owusu [46] found romano potato polyphenol oxidase to retained over 80% activity at pH 4.5–8.0 after 30 min incubation but to be almost inactive after incubation at pH 4.0. Fujita *et al*. [27] found pH stability of 4 to 10 for quince polyphenol oxidase.

Effect of temperature on polyphenol oxidase from both yam species showed optimum temperature of 30°C, with optimum stability at 50°C. This result was similar to that of another yam species; edible yam (*Dioscorea opposite* Thunb.) [27], *Dioscorea bulbifera* [47], rooster potato [28]. Maximum PPO activity at an optimum temperature of 40 °C has been reported for lemon balm [41], 30°C for snake gourd and sweet potato [43, 15], 40°C for *Averrhoa carambola* fruit [44], 50 °C for purslane [42], using catechol as substrate.The polyphenol oxidase activity remained greater than 50% after heat treatment at 30, 40 and 50°C for 60min. The result indicated that the enzyme has relatively high thermal stability.

It was observed that the PPO of this *D. cayenensis* and *D. alata* has more affinity for DOPA than tyrosine, which indicates that they have more diphenolase activity. As shown in Table 1, among the inhibitors used, Ascorbic acid was found to be the most effective inhibitor, followed by Benzoic acid, EDTA but SDS appeared to activates the enzyme. Strong inhibitory effect of ascorbic acid has been reported for PPO of potato, avocado, Chinese cabbage, sweet potato [15]. Thus, at high concentration above 10mM, ascorbic acid could be use as nutrient additive.

CONCLUSIONS

This study of the polyphenol oxidase isolated from *Dioscoreacayenensis*and *Dioscoreaalata*showed high activity toward diphenolics. The enzyme has biochemical characteristics similar to several other plant polyphenol oxidases. Thus, to prevent browning in the industrial processing of these two yam species for economically finished products, control of the activity polyphenol oxidase by operating against its optimal conditions would be necessary, as well as avoidance of possible known additives that are its activator, otherwise reduction in the degree of enzyme inactivation could result in shorter shelf-life, nutritional and functional value.

REFERENCES

1. Hahn, S.K., D.S.O. Osiru, M.O. Akoroda and J.A. Otoo, 1987. Yam production andits future prospects. Outlook on Agriculture, 16(3): 105-110.
2. Burkill, I.H., 1960. The organography and the evolution of Dioscoreaceae, the family of the yams. The Journal of the Linnean Society of London, Botany, 56: 319-412.
3. Baah Faustina Dufie, 2009. Characterization of water yam (*dioscoreaalata*) for existing and potential food products. PHD Thesis, A Thesis submitted to the department of Food Science and Technology Faculty of Bioscience, College of Science, Kwame Nkrumah University of Science and Technology, Ghana.
4. Opara, L.U., 1999. Yam storage. In: CGIAR Handbook of Agricultural Engineering Volume IV Agro Processing. Bakker-Arkema (ed). The American Society of Agricultural Engineers, St. Joseph, M. I, U. S.A., pp: 182-214.
5. Alves, R.M.L., 2000. Caracterizacao de ingredients obtidos de cara (*Dioscoreaalata)* epotencialaplicao industrial. PhD Thesis, UniversidadeEstadual de Londrina, Brazil.
6. Afoakwa, E.O. and S. Sefa-Dedeh, 2001. Chemical composition and quality changes in trifoliate yam *Dioscoreadumetorum*tubers after harvest. Food Chemistry, 75(1): 88-91.
7. Osagie, A.U. and O.U. Eka, 1998. Chemical Composition of Root and Tuber crops. In: Nutritional quality of plant foods. Department of biochemistry, University of Benin, Benin City Nigeria.
8. Barquar, S.R. and O.L. Oke, 1977. Mineral constituents of Nigerian yams. Nutr. Rep. Int., 15(3): 265-272.
9. Osagie, A.U., 1992. The Yam in Storage. Postharvest Research Unit, University of Benin, Nigeria.
10. Moorthy, S.N., 1994. Tuber crop starches. Technical bulletin series 18. Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India.

11. Asemota, H.N., M.A. Wellington, A.A. Odutaga, and M.A. Ahmad, 1992. Effect of short-term storage on phenolic content, *o*-diphenolase and peroxidase activities of cut yam tubers (*Dioscorea*sp.). J. Sci. Food Agric., 60: 309-312.
12. Rasper, V. and D.G. Coursey, 1967. Properties of starches of some West African yams. J. Sci. Food Agric., 18: 240-244.
13. Martin, F.W. and R. Ruberté, 1976. The polyphenol of *Dioscoreaalata* (yam) tubers associated with oxidative browning. J. Agric. Food Chem., 24(1): 67-70.
14. Martin, F.W. and A.M. Rhodes, 1978. The relationship of Dioscorea Cayenensis and D. rotundata. Trop. Agric., 55(3): 193-206.
15. Manohan, D. and W.C. Wai, 2012. Characterization of polyphenol oxidase in sweet potato (*Ipomoea Batatas* (L.)). Journal for the Advancement of Science and Arts, 3: 14-30.
16. Sanni, D.M., T.H. Fatoki and O.V. Omotoyinbo, 2017. Comparative Evaluation of Computational and Experimental Analysis of Polyphenol Oxidase from Cocoa (*Theobroma cacao* L.). J. Microbiol. Biotech. Res., 7(1): 18-25. DOI: 10.24896/jmbr.2017714.
17. Yoruk, R. and M.R. Marshall, 2003. Physicochemical properties and function of plant polyphenol oxidase: a review. Journal of Food Biochemistry, 27: 361-422.
18. Whitaker, J.R., 1996. Polyphenol oxidase. In Food chemistry (O.R. Fennema, ed.) pp: 492-494, Marcel Dekker New York
19. Zawistowski, J., C.G. Biliaderis and N.A.M. Eskin, 1991. Polyphenol oxidase. In *Oxidative Enzymes in Foods*, (D.S. Robinson and N.A.M. Eskin, eds.) pp: 217-273, Elsevier Science Publishing, New York.
20. Martinez, M.V. and J.R. Whitaker, 1995. The biochemistry and control of enzymatic browning. Trends Food Sci. Technol., 6: 195-200.
21. Muzac-Tucker, I., H.N. Asemota and M.H. Ahmad, 1993. Biochemical Compositionand Storage of Jamaican Yams (*Dioscorea*sp). Journal of the Science of Food and Agriculture, 62: 219-224.
22. Yapi Jocelyn Constant, Sophie Nadège Gnangui, Amedée Pascal Ahi, Jean Didier Kouassi-Koffi and Lucien Patrice Kouamé, 2014a. Biochemical Characterization of Two Polyphenol Oxidase Purified from Edible Yam (*Dioscoreacayenensis-rotundata cv. Kponan*) Cultivated in Côte d'Ivoire. Annual Research & Review in Biology, 4(23): 3532-3548.
23. Lowry, O.H., N.J. Rosebrough, L. Farrra and R.J. Randall, 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265-275.
24. Coseteng, M.Y. and C.Y. Lee, 1978. Changes in apple polyphenol oxiodase and polyphenol concentrations in relation to degree of browning. Journal of Food Science, 52: 985.
25. Escribano, J., J. Cabanes and F. Garcia-carmona, 1997. Characterization of Latent Polyphenol Oxidase in Table Beet: Effect of Sodium Dodecyl Sulphate. J. Sci. Food Agric., 73: 34-38.
26. Lineweaver, H. and K. Burk, 1934. The determination of enzyme dissociation constants (1934). J. Am. Chem. Society, 56: 658-666.
27. Fujita Shuji, Yun-Zhe Han, Chie Kouno, Tomoko Matsuo, Megumi Yamashita, Yasuko Haraguchi, Ying-Jie Li, Nobuyuki Hayashi and Chang-Peng Yang, 2006. Purification and characterization of polyphenol oxidase from edible yam (dioscoreaoppositathunb.), Food Sci. Technol. Res., 12(3): 235-239.
28. Ni Eidhin, D., P. Degn and D. O'beirne, 2010. Characterization Of Polyphenol Oxidase From Rooster Potato (*SolanumTuberosum*Cv Rooster), Journal Of Food Biochemistry, 34: 13-30.
29. Miller, A.R., T.J. Kelley and C.V. Mujer, 1990. Anodic peroxidase isoenzymes and polyphenol oxidase activity from cucumber fruit: tissue and Substrate specificity. Phytochemistry, 29: 705-709.
30. Cash, J.N., W.A. Sistrunk and C.A. Stutte, 1976. Characteritics of concord grape polyphenol oxidase involved in juice colour loss. J. Food Sci., 41: 1398-1402.
31. Golbeck, J.H. and K. Cammarata, 1981. Spinach thylakoid polyphenoloxidase. Plant Physiol., 67: 977-984.
32. Sanchez-Ferrer, A., R. Bru and F. Garcia-Carmona, 1989. Novel procedure for extraction of a latent grape polyphenoloxidase using temperature-induced phase separation in Triton X-114. Plant Physiol., 91: 1481-1487.
33. Xu, J., T. Zheng, S. Meguro and S. Kawachi, 2004. Purification and characterization of polyphenol oxidase from Henry chestnuts (*Cataneahenryi*). J. Wood Sci., 50: 260-265.
34. Jaworski, A.W. and C.Y. Lee, 1987. Fractionation of HPLC determination of grape phenolics.J.Agric. Food. Chem., 35: 257-259.

35. Ullah, M.R., 1991. Tea. In food Enzymology (P.F Fox, ed.) pp: 163-187, Elsevier Science publishing, New York.
36. Murata, M., M. Tsurutani, M. Tomita, S. Homma and K. Kaneko, 1995. Relationship btw apple ripening and browning-changes in polyphenol content and polyphenol oxidase. J. Agric Food Chem., 43: 1115-1121.
37. Sanchez-Ferrer, A., F. Laveda and F. Garcia-Carmona, 1993. Partial purification of soluble potato polyphenol oxidase by partitioning in aqueous two-phase system. J. Agric. Food Chem., 41: 1219-1224.
38. Zhou, P., N.L. Smith and C.Y. Lee, 1993. Potential purification and some properties of Monroe apple peel polyphenol oxidase. J. Agric. Food Chem., 41: 532-536.
39. Marques, L., A. Fleuriet and J. Machiex, 1995. Characterization of multiple forms of polyphenol oxidase from apple fruit. Plant Physiol. Biochem., 33: 193-200
40. Moore, B.M. and W.H. Flurkey, 1990. Effect ofsodium dodecyl sulfate on enzymatic and physical characteristics of purified broad bean polyphenoloxidase. Journal of Biological Chemistry, 265(9): 4982-4988.
41. Doğan Serap, Yasemin Ayyildiz, Mehmet Doğan, Ümran Alan and Mehmet Emin Diken, 2013. Characterisation of polyphenol oxidase from *Melissa officinalis*L. subsp. *officinalis*(lemon balm). Czech J. Food Sci., 31(2): 156-165.
42. Guven Reyhan Gul, Kemal Guven, Fatma Matpan Bekler, Dmer Acer, Hüseyin Alkan and Mehmet Ddgru, 2017. Purification and characterization of polyphenol oxidase from purslane. Food Science and Technology Campinas. 1-7. DOI: httpI://dx.doi.org/10.1590/1678-457X.03216.
43. Sanni, D.M. and P.A. Dabesor, 2016. Extraction, Purification and Characterization of Polyphenol oxidase from Snake Gourd (*Trichosanthescucumerina*). The FASEB Journal, 30(1): lb116.
44. Murillo Elizabeth, Juan Guillermo Aristizábal, Walter Murillo, Albert Ibarz, Jonh Jairo Méndez and José Fernando Solanilla, 2017. Preliminary characterization of the enzyme polyphenol oxidase and rheological behavior from *Averrhoa carambola* juice. Rev. Fac. Nac. Agron., 70(1): 8099-8113. doi: 10.15446/rfna.v70n1.61769.
45. Sakiroglu, H., O.I. Kufrevioglu, I. Kocacaliskan, M. Oktay and Y. Onganer, 1996. Purification and characterization of Dog-rose (Rosa dumlisRechst.) polyphenol oxidase. Journal of Agriculture and Food Chemistry, 44: 2982-2986.
46. Duangmal, K. and A.R.K. Owusu, 1999. A comparative study of polyphenoloxidases from taro (*Olocasiaesculenta*) and potato (*Solanumtuberosum* var. Romano). Food Chem., 64: 351-359.
47. Ikediobi, C.O. and E. Oti, 1983. Some biochemical changes associate with post-harvest storage of white yam (Dioscorearotundata) tubers. Journal of the Science Food and Agriculture, 34: 1123-1130.