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Studies on the Thermal Stability of Peroxidase from Leaf of Oil Palm (*Elaeis guineensis*)

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ABSTRACT: Peroxidase was extracted from leaves of oil palm tree with 0.01M phosphate buffer pH 7.0. It was partially purified using 70% ammonium sulphate ($(NH_4)_2SO_4$) precipitation. This resulted in peroxidase with activity of (26U/ml) and specific activity of 35.8U/mg. Effect of heat on the activity of peroxidase was studied at temperature of 323-363°K. After gel filtration on sephadex G100, the peroxidase activity increased to 27U/ml, with specific activity of 55U/mg .The overall purification fold was 4 with 51.9% enzyme recovery. The peroxidase partially purified from leaves of oil palm tree showed pH and temperature optima of 5.0 and 50°C respectively. High pH and temperature stabilities of pH 5.0 to pH 9.0 and 50°C to 70°C were obtained respectively. Also, the activation energy (Ea) of the reaction was -21.616kj/mol. The free energy changes (ΔG) were 96008.64, 96315.59, 97901.63, 94132.33 and 97146.75kj/mol at 323,333,343,353 and 363°K respectively. It was observed that the D-values were decreasing with increasing temperature with a Z-value of 0.044. The enthalpy results suggest that the reaction was exothermic, non-spontaneous and reversible.

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Peroxidases (EC 1.11.1.7) are hemoproteins that catalyze the hydrogen peroxide (H_2O_2) -dependent oxidation of different substrates including phenolic compounds (Qayyum *et al*., 2010; Omeje and Eze, 2018). They are widely distributed in plants, animals and micro-organisms. It can be found in vacuoles, tonoplast, plasmalemma, cell wall, chloroplast and mitochondria with variable functions (Omeje *et al*., 2017; Idesa and Getachew, 2018). It is involved in plant hormone regulation, defense mechanisms, indole acetic acid degradation during maturation and senescence of fruits and vegetables, and lignin biosynthesis (Falade *et al*., 2019). Peroxidases have wide applications in different areas such as organic synthesis, medicine, pharmaceuticals and biotechnology (Veda *et al*., 2017). Earlier, Agunbiade *et al* (2021) reported the presence of peroxidase in higher plants such as *Moringa oleifera* leaves. African oil palm (*Elaeis guineensis*) leaves are among the tropical underutilized plants that have recently attracted worldwide attention. Nigeria is one of the nations of the world known for its production of palm trees. After harvesting the fruits, other by-products including the leaves are discarded indiscriminately, thereby causing nuisance to the environment. Up till now, there is still a dearth of information in literature on peroxidase from *Elaeis guineensis* (Rodriguez *et al*., 2002).

MATERIALS AND METHODS

Fresh oil palm (*Elaeis guineensis*) leaves used for this study were harvested from Nsukka, Enugu State, Nigeria. All chemicals were of analytical grade and reagents were freshly prepared, unless otherwise stated. O-dianisidine was purchased from Sigma-Aldrich.

Enzyme assay: Enzyme assay was carried out according to the procedure described by Eze *et al*. (2010)

Enzyme purification: Enzyme purification was done using the method described by Rodriguez *et al*. (2002)

Optimum pH and temperature of peroxidase: The optimum pH for peroxidase activity was determined using 0.1M sodium acetate buffer pH 3.0 - 5.5, 0.1M phosphate buffer pH 6.0 – 8.0, 0.1M Tris-HCl buffer pH 8.0 - 9.5 and glycine-NaOH buffer pH 9.5-10.5. The enzyme activity was assayed at different pH and read at absorbance of 460nm with Jenway 6303 spectrophotometer. The optimum temperature was determined by incubating the assay mixture for 20min at 30-90^{o}C. After heating, it was allowed to cool for 5mins and the enzyme was added and absorbance read at 460nm. (Eze, 2012).

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pH and temperature stability of peroxidase: A 2.4ml of the different pH buffers (Na-acetate buffer; 3-5.5, Phosphate buffer; 6-8.5, Tris-HCl buffer and Glycine-NaOH buffer; 10.5) were incubated with 0.2ml of enzyme and assayed at different time for 2hr and absorbance was read at 460nm. Also, temperature stability was determined. In each case, 0.2ml of the enzyme was added in a test tube and heated in a water bath from 20, 40, 60, 80,100 and 120min respectively. The assay mixture was added after each time interval and the absorbance was read at 460nm with Jenway 6303 spectrophotometer (Eze, 2012).

RESULTS AND DISCUSSION

Peroxidase was extracted from the leaf of oil palm tree and partially purified using two purification processes of Ammonium sulphate precipitation and sephadex G-100, gel filtration. Table 1, shows the purification steps of peroxidase from oil palm leaves. The protein concentration of peroxidase decreased from1.220mg/ml for crude enzyme to 0.488mg/ml after gel filtration while peroxidase activity increased from 17U/ml to 27U/ml as the purification step increased, and the specific activity also increased to 55.4U/mg. The protein concentration of the crude extract was 1.220mg/ml, which decreased to 0.727mg/ml after $(NH_4)_2SO_4$ precipitation. After gel filtration on Sephadex G-100, the protein concentration reduced to 0.488 mg/ml. Peroxidase activity of the crude enzyme was 17U/ml, and after $(NH_4)_2SO_4$ precipitation and gel filtration, peroxidase activity increased to 26U/ml and 27U/ml respectively. This shows that peroxidase activity increased at every step of the purification process. This implies that, as the purification step increases, some proteins which serve as impurities were eliminated. Also the high activity of these steps suggested that the precipitation of the crude enzyme at 70% ammonium sulphate saturation did not damage the integral structure of the enzyme. The specific activity of the crude enzyme was 13.90 U/mg, which increased to 35.80 U/mg and 55.46U/mg after $(NH_4)_2SO_4$ precipitation and gel filtration respectively. This result corroborate the report by Omeje *et al.* (2019), who stated that for purification procedure to be successful, the specific activity of the desired enzyme must be greater than the former after each purification step. Peroxidase extracted from oil palm *(Elaeis guineensis*) leaves was purified four folds in this study.

Table 1: Purification table of oil palm leaves peroxidase

Purification step	Volume (ml)	Protein Conc. (mg/ml)	Total protein (mg)	Peroxidase Activity (U/ml)	Total activity (U)	Specific activity (U/mg)	Purification fold	% yield
Crude	600	1.220	732	17	10,200	13.9	1	100
70%$(NH_4)_2SO_4$	60	0.727	43.6	26	1,560	35.8	2.6	15.3
Sephadex G-100 Gel filtration	30	0.488	14.6	27	810	55.4	4.0	51.9

µmole/min = Unit (U)

Table 2: The various thermodynamic parameters used in characterizing the enzyme.

T(°K)	Kd	t½(min)	D-value	ΔH(KJ/mol)	Ea(KJ/mol)	ΔG(KJ/mol)	ΔS(KJ/mol)
323	0.0020	346.57	1151.29	-2642.19	-21.616	96008.64	-305.42
333	0.0054	128.36	426.40	-2790.17	-21.616	96315.59	-297.61
343	0.0088	78.76	261.65	-2873.31	-21.616	97901.63	-293.80
353	0.0865	8.01	26.61	-2956.45	-21.616	94132.33	-275.03
363	0.0793	8.74	29.03	-3039.59	-21.616	97146.75	-275.99

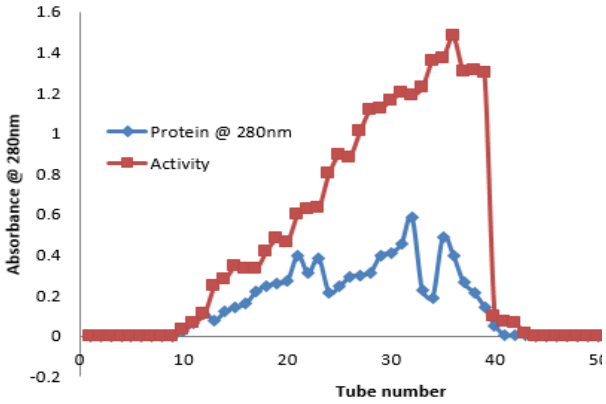

Fig 1 Elution profile of oil palm leaves peroxidase

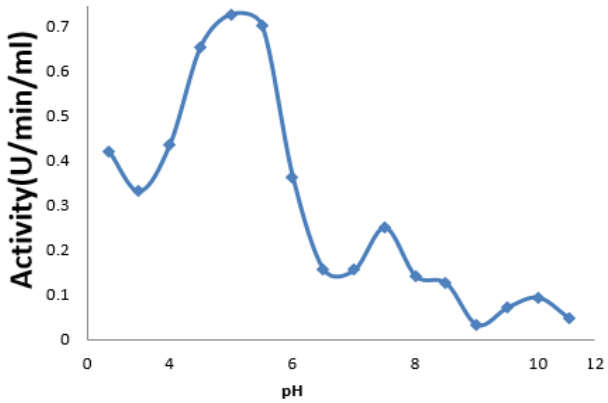

Fig 2: Optimum pH on activity of peroxidase from *E.guineens*

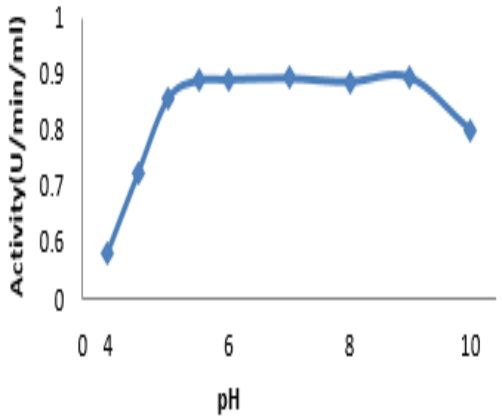

Fig 3: pH stability of peroxidase from oil palm leaf

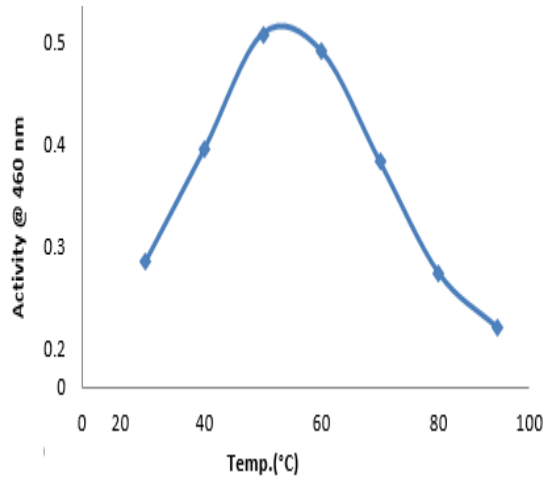

Fig 4: Optimum temperature of peroxidase from oil palm leaves

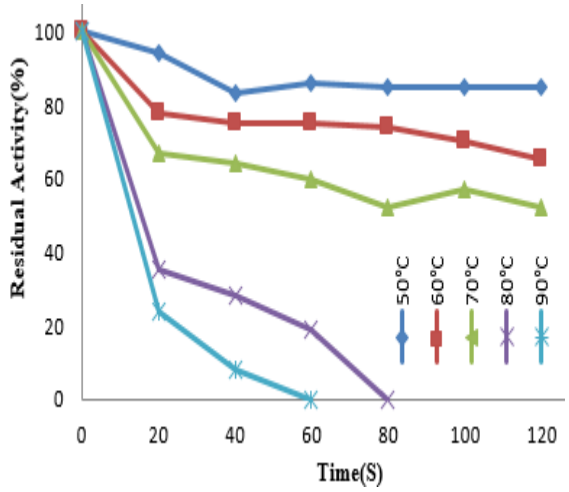

Fig 5: Temperature sensitivity of peroxidase from oil palm leaves

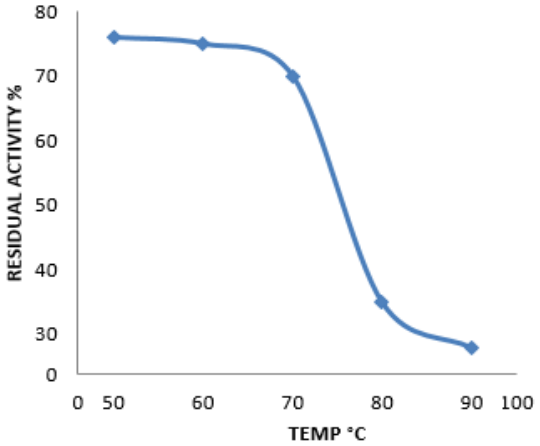

Fig 6: Temperature stability of peroxidase from oil palm leaves

Table 2 shows the various thermodynamic parameters used in characterizing the enzyme. The half-life was decreased as the temperature increased from 30-50°C. At 60°C the temperature increased to 8.74min showing that the time taken for loss of 50% of the enzyme activity was higher than that obtained for 50°C. It was also observed that the D-values (the time required to reduce the peroxidase activity by 90%) decreased with increase in time of incubation. Entropy is the measure of the degree of randomness of a system. +ΔS indicates increase in entropy while –ΔS indicates decrease in entropy. A higher rate constant (Kd) of denaturation would imply a less stable enzyme. ΔH is the amount of heat required to denature the enzyme. A large and positive ΔH could be associated with a more stable enzyme. Gibb's free energy (ΔG) indicates if a reaction will be energetically favourable. Negative free energy (–ΔG) indicates that the reaction is thermodynamically favourable in the direction indicated (without energy input). The change in free energy (ΔG) can be used to predict the direction of a reaction at constant temperature and pressure (Enachi *et al*., 2019).

Conclusion: Peroxidase from oil palm (*Elaeis guineensis*) leaves was under-studied. Result from the present study showed that peroxidase from *Elaeis guineensis* had its optimum pH of 5.0 and optimum temperature of 50°C. It was also stable at pH of 5 and temperature of 50°C.

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