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RESEARCH ARTICLE

EFFECT OF CHEMICAL AND THERMAL TREATMENTS ON INHIBITION OF PEROXIDASE ACTIVITIE OF PURPLE SKIN EGGPLANT (*Solanummelongena* L.)

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Abstract

Peroxidase (POD) associated with the browning of fresh-cut fruits and vegetableswas extracted from purple skin eggplant(*Solanum melongena* L.) and characterised using reliable spectrophotometric methods. Maximal POD activity was found at 35 °C and pH 6.0 with guaiacol as the substrate. The enzyme was stable at his optimal temperature (35 °C) and hisat pH stability was in the range of 5.6 - 6.6.Peroxidase retained its full activity in the presence of ion K^+, Cu^{2+}, Na^+, Pb^{2+} and Ba^{2+} but were inhibited strongly by the ion Fe^{2+} and Mg^{2+} and the reducing agents as sodium thiosulfateand ascorbic acid. Effect of heattreatment on eggplant peroxidase showed that D-values decreased with increasing temperature, indicating faster peroxidase inactivation at higher temperatures.At 60 °C, the D-values ranged from 20.42 to 54.24 min. Hence, heat treatment at 60 °C for 30 min reduced browning of eggplant fruit.These data can be used to predict prevention of browning in the purple skin eggplantby thermal inactivation and the use of chimical agents onthe enzyme.

Introduction:-

Fruits and vegetables are of great importance in terms of nutrition and functional quality of fresh or processed products, provide valuable sources of antioxidants including vitamins A, C and E, carotenoids and flavonoids, as well as minerals, which are known for their health benefits. The Eggplant (*Solanum melongena* L.) genus is a climacteric fruit that belongs to the Solanaceae family and it is originally from India and China. They are an important food both from the economic and nutritional points of view and are cultivated and consumed worldwide. It contains important phytonutrients such as phenolic and flavonoid compounds which have high antioxidant capacities (Cao *et al*., 1996; Jung *et al*., 2011). In the fruits, the antioxidant capacities and phenolic compounds are found in both the pulp and skin (Huang *et al*., 2004). The antioxidant capacity and total phenolic content of purple skin eggplants have been reported to be higher than those of green and white skin cultivars (Junmatong*et al*., 2008). The high antioxidant potential from eggplant help to prevent chronic and degenerative illnesses as shown in clinical studies, and their increased consumption in recent years (Nisha *et al*., 2009; Zaro *et al*., 20014). Despite its

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importance, eggplant is unfortunately hampered by a phenomenon of enzymatic browning when the tissues are cut, peeled or crushed during processing for food or for storage (AnosikeandAyeebene, 1981). Enzymatic browning of fruits and vegetables during postharvest handling and processing degrades the sensory properties and nutritional value and discourages consumer purchase of fresh-cut products. Consequently, enzymatic browning results in significant economic losses for the fresh produce industry. Browning reactions which occur after infliction of a mechanical injury to some live fruitsand vegetable are initiated by the enzymes, polyphenol oxidase, peroxidase and to the production of polyphenols and derived products (Osagie and Opoku, 1984). Peroxidase (POD) (hydrogen-peroxide oxidoreductase E.C.1.11.1.7), is one of the major enzymatic compounds which catalyze redox reactions of a wide range of phenolic and non-phenolic substrates, in the presence of hydrogen peroxide as an electron acceptor, being found in bacteria, fungi, algae, plants and animals (Chakraborty *et al*., 2015). POD is one of the main quality deterioration indicators, such as flavor loss and different biodegradation reactions, being also relevant as a browning enzyme that contributes to the darkening of fruit and vegetable products during processing and storage (Jang and Moon, 2010).

The inactivation of POD is essential in order to minimize losses that are caused by enzymatic browning, in such a manner that several methods and technologies have been studied. Currently, one of the most applied methods for the inactivation of oxidative enzymes is thermal treatment and using chemical agents, which are also being used to ensure product quality in the food industry. This technology provides the possibility to minimize the effect on flavor, color, and nutritional properties in order to obtain food products of high-quality. The data obtained in this studiemay be considered as a reference for the determination of treatment techniques effective thus ensuring the quality of purple skin eggplant (*Solanum melongena* L.).

Material and Methods:-

Plant material and chemicals

Fresh purple skin eggplant (*Solanum melongena* L.) was purchased from tall market, of Lobia (Daloa, Côte d'Ivoire), during September and October 2020.All chemicals and reagents were analytical grade and purchased from the Merck A.G. (Darmstadt, Germany) and from the Sigma Chemical Company (St. Louis, USA).

Methods:-

Extraction of peroxidase (POD) of the purple skin eggplant

A sample of eggplant (150 g) was crushed in a blender (Moulinex, France) and homogenized for 10 min in 300 ml of NaCl 0.9% (w/v). The resulting homogenate was centrifuged at 8000 g for 10 min at 4°C (Refrigerated centrifuge TGL-16M, China). The collected supernatant was the crude enzymatic extract used for POD activity assays (Gnaguie*t al*., 2009).

Enzyme activity assay

The reaction mixture to determine the POD activity adjusted to 2 mL, consisted of 1.6 mL citrate 100 mM buffer, pH 6.0, 0.2 mL substrate solution (guaiacol 10 mM and dissolved in citrate buffer, pH 6.0), 0.1 mL hydrogen peroxide 3% and 0.1 mL enzyme extract. This reaction mixture was incubated at 25°C for 10 min. After incubation, the activity was determined by measuring the absorbance of the reaction mixture at 480nm. Experiments were performed in triplicate, and the results expressed as units of enzymatic activity per mg of protein. One unit of enzymatic activity (U) was defined as an increase in absorbance of 0.001 per min (Cong *et al*., 2005)

Optimal pH and stability

The optimum pH of POD extracted from purple skin eggplantwas determined by measuring the oxidation of substrate guaiacol in different buffers at various pH values ranging from pH 2.6 to 8.0. The buffers (100 mM concentration) used were acetate from pH 3.6 to 5.6, phosphate from 5.6 to 8.0 and citrate from pH 2.6 to 7.0. The pH stability of peroxidase was studied at a pH range of 2.6-8.0 with 100mM buffers. Buffers used were the same as in pH study. After 2 hours preincubation at 25 °C (room temperature), residual peroxidase activitie was measured at 25 °C for 10 min by adding substrate guaiacol. Experiments were performed in triplicate, and the results expressed as percentage activity of zero-time control of untreated enzyme.

Temperature optimal and Thermostability

The effect of temperature on PODactivitie was performed in 100 mM citrate buffer pH 6.0, after 10 min incubation at temperatures ranging from 10 to 80°C using a water bath under standard test conditions. The thermal inactivation was determined at 37 °C and at enzyme optimum temperature (35°C). Enzyme in appropriate buffer was exposed to

each temperature for 120 min. Then, aliquots were withdrawn at intervals (15 min) and immediately cooled. In the thermal denaturation tests, aliquots of each enzyme solution were preheated at different temperatures at a range of 10-90 °C for 15min. Residual activities, determined at 25 °C under the enzyme assay conditions were expressed as percentage of activity of zero-time control of untreated enzymes (Gnangui*et al*., 2009).

The effect of temperature and the rate constant in a activation process was related according to the Arrhenius equation (Arrhenus, 1889):
$k = Ae(-Ea/RT)$ (1)
Where;
k is the reaction rate constant value,
A is the Arrhenius constant,
Ea is the activation energy (energy required for the activation to occur), R is the gas constant (8.31 $Jmol^{-1}K^{-1}$),
T is the absolute temperature in Kelvin.
The Q_{10} temperature coefficient is a measure of the reaction rate of temperature increase of 10 °Cthe Q_{10} is calculated as:
$Q_{10} = (X_2/ X_1)$ (2)
where:
X_1 represents the lower absorbance (D.O at 10 °C); X_2 represents the higher absorbance (D.O at 20 °C)

Effect of metal ions and chemical agents on enzyme activity
To determine the effect of various compounds as possible activators or inhibitors of the activitie peroxidase each enzyme solution was preincubated at 25 °C for 20 min with the compounds and the activity was assayed under the enzyme assay conditions. Residual activities were expressed as percentage referred to control without chemical agents.

Inactivation kinetics of POD
The thermal inactivation of POD activitie was determined at temperatures ranging from 40 to 80 °C. The crude enzymatic extract in citratebuffer 100 mMpH 6.0 was preincubated at different temperatures. Aliquots were withdrawn at intervals and cooled at room temperature for 10 min. The enzymatic activitie of the aliquots was measured under standard conditions. The Kinetic data analysis of thermal inactivation of the PODactivitie was done from the equation 3 (3). This equation is derived from the equation of first-order reactions (Râpeanu*et al*., 2006).
$\ln [At/A_0] = -kt$ (3)

Where At is the residual enzymatic activity at time t, A_0 is the initial enzymatic activity, and k is the reaction rate constant (min^{-1}) at the temperature studied.

The inactivation rate constant k was estimated by linear regression analysis of the logarithm of residual activity versus treatment time.

The time where the residual activity reaches 50% known as the half-life ($t_{1/2}$), was given by the equation 4 (4):
$t_{1/2} = \ln (2)/k$ (4)

The D-value, defined as the treatment time (min) needed to reduce the initial activity of 90% was calculated according to the equation 5 (5) (Espachs-Barroso *et al*., 2006).
$D = 2{,}303/k$ (5)

Statistical Analysis
The Statistical Analysis System (SAS) for the personal computer program (SAS Inst., 1988) was used for the ANOVA; LSD means separation, single, Pearson and stepwise regression analyses.

Results And Discussion:-

Effect of pH and pH stability
pH is a very important parameter that affects the ionization of the exposed amino acids of an enzyme in such a manner that the low stability at very acidic pH values can be attributed to the instability of the binding site of the enzyme (Terefe*et al*., 2014). The peroxidase stability assessed under extreme conditions is a conclusive factor for its

industrial applications (Sonkar*et al*., 2015). The effect of pH on POD from purple skin eggplant was studied in the pH range of 2.6 to 8.0, at 25°C. Regarding the pH, the enzymatic activity of POD, the highest enzyme activity was found at pH 6.0, whereas at pH 5.6, the enzyme retained 88.01 % of the initial activity (Fig. 1). In the acidic pH range of 2.6 to 5.6, the enzymatic activity of POD displayed a progressive increase, while in the alkaline pH range from 7.6 to 8.0, a significant decrease of the enzymatic activity was observed. Since POD activitie from purple skin eggplant was optimal at pH 6.0, his activity could be inhibited when exposed in acidic environment (pH < 5.6). The lowest values of the relative enzymatic activity were recorded at pH values of 2.6 and 8.0, representing 37.99 and 24.74 respectively, compared to values obtained at pH 6.0. Our results are in accordance with the values reported by other studies which indicate the fact that PODs present the maximum activity in the pH range of 4.5-6.5 (Sakharov *et al*., 2001). Sonkar*et al*. (2015)studied the peroxidase extracted from Artocarpus lakoocha in the pH range 0.5-12.0 and registered the highest activity at pH 6.0. Fang *et al*. (2008)extracted kiwi peroxidase and identified the optimum pH of the enzyme as being 6.0. Enachi*et al*. (2019)studied POD extracted from the Plums (prunus domestica) in the range of pH from 3.0 to 9.0, the enzyme showing maximum activity at pH 6.5.

The pH stability of POD from purple skin eggplant was evaluated during a period of 2 h by incubating the enzyme at the appropriate pH values ranging from 2.6 to 8.0. In the pH range, after 2 h, at the values of 5.6 and 7.0, the enzyme retained about 80 %of the initial activity in citrate buffer (Fig. 2). The results are in good agreement with those reported by Fortea*et al*. (2009)who studied the pH stability of POD extracted from table grapes (Crimson Seedless) by using ABTS substrate. Pandey and Dwivedi (2015)determined the pH stability in a wide pH range for the POD extracted from papaya, which exhibited a high stability in the pH range 7.0 - 9.0, while at lower pH values the enzyme activity gradually decreased. Thus, depending on the source from which the enzyme is extracted, the pH stability varies within a wide range of values (Enachi*et al*., 2019).

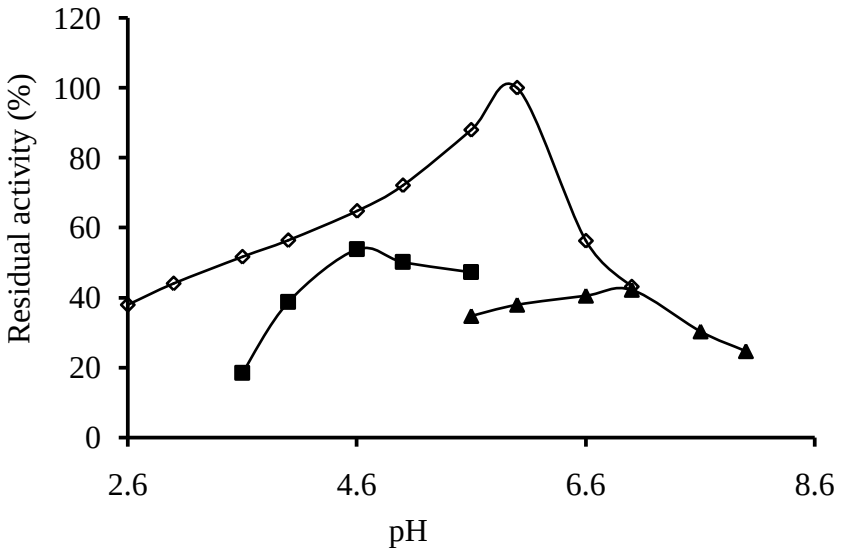

Figure 1: Effect of pH on the activities of peroxidase from purple skin eggplant (*Solanummelongena* L.). Citrate buffer (◊), acetate buffer (■), phosphate buffer (▲)

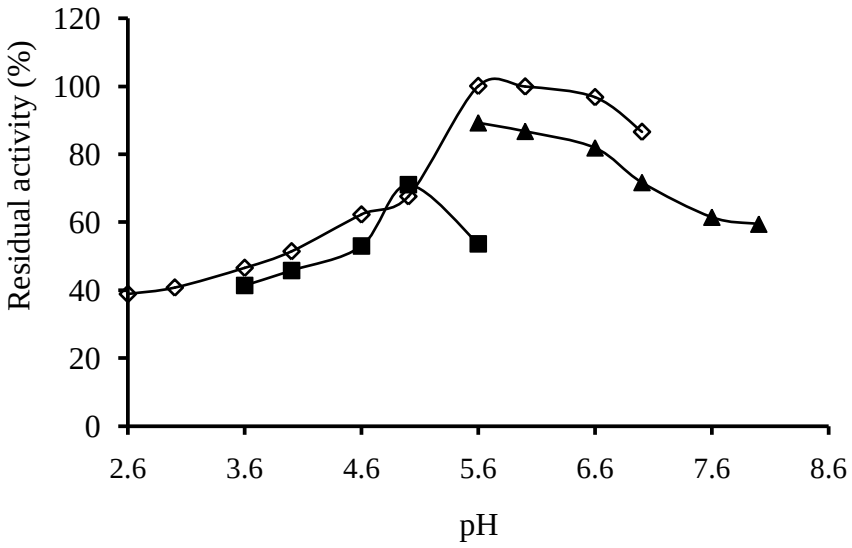

Figure 2: Stability of pH on the activities of peroxidase from purple skin eggplant (*Solanum melongena* L). Citrate buffer (◊), acetate buffer (■), phosphate buffer (▲).

Optimum temperatureandactivation energy

To determine the optimum temperature of POD from purple skin eggplant, the activity was assessed at different temperatures ranging from 10 °C to 80°C. POD presented the highest activity at the temperature of 35° C (Fig. 3). The result obtained for POD from eggplantis in superiorto that of Yadav *et al*. (2012)who investigated the POD from banana (Musa paradisiaca) in the 15 – 35°C temperature range, and reported the optimum temperature of 25°C. In contrast, Rani and Abraham (2006)determined the optimum temperature of 55°C for the activity of POD from Eupatorium odoratum, whereas Pandey and Dwivedi (2015)studied POD from papaya and assessed an optimum temperature of 40°C.

From Arrhenius plot, value of 39.32 ± 2.04 kJ/mol was obtained as the activation energy of POD from purple skin eggplant. This activation energie are lower than that obtained (86,20 ± 5,57 kJ/mol) for the POD from Pumpkin (*Cucurbita Maxima* L.) (Gonçalves*et al*., 2007). Value of activation energy indicate the relative tendency of a failure mechanism to be accelerated by temperature. In this respect, the studied peroxidase should be top-grade tools for various catalyzing reactions since it is well known that enzymes are biocatalysts that speed up chemical reactions by lowering the required activation energy (Koffi*et al*., 2010).

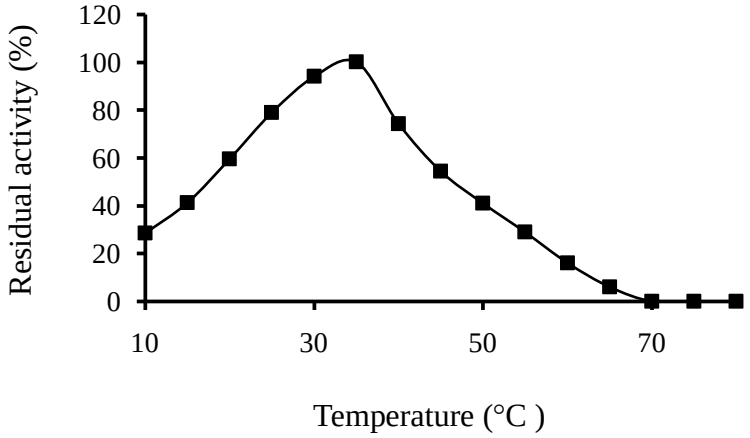

Figure 3: Effect of temperature on the activities of peroxidase from purple skin eggplant (*Solanum melongena* L.)

Thermal denaturation and thermal stability

The thermal denaturation shows that the POD from purple skin eggplant was fairly stable theoptimal temperature 35 °C. At higher temperatures, thermostability decreased progressively and the enzyme were completely inactivated at 80 °C (Fig. 4).

The peroxidase was fully active for more than 2 hours at his optimal temperature (35 °C) in citrate buffer pH 6.0 indicating a thermal stability at his optimal temperature (Fig. 5). However, his catalytic activitie was abruptly affected after 15 min incubation at 37 °C (Fig. 5). This result is similar to those obtained by Terefe*et al*. (2010) who have determined the temperature stability of POD extracted from strawberry puree in the temperature range 25-100°C and of Al-Senaidy and Ismael (2011) whodetermined the thermal stability of POD extracted from persimmon by incubating the enzyme at different temperatures for 60 min. In this context, running biotechnological processes at moderate temperatures would be advantageous for application of this enzyme.

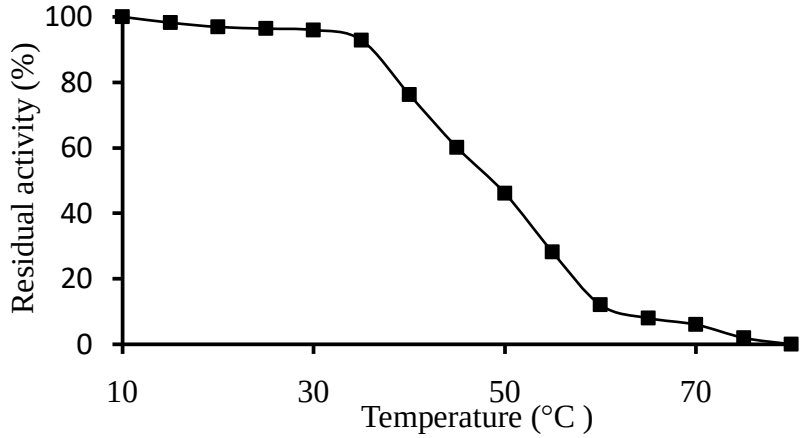

Figure 4: Thermal denaturation of the activities of peroxidase from purple skin eggplant (*Solanum melongena* L.)

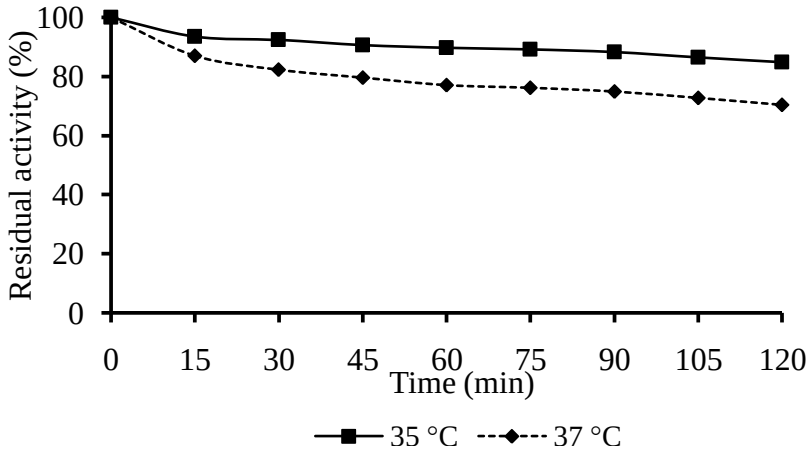

Figure 5: Thermal inactivation of the activities of peroxidase from purple skin eggplant (*Solanum melongena* L.)

Effect of Metal Ions and Chemical Agents on Enzyme Activity

From an industrial point of view, the possibility to identify the most potent inhibitory compound that can prevent enzymatic browning is very important in improving the sensorial properties not only for fresh fruit and vegetables, but also for their derived products. Based on the studies available in the literature, the process of enzymatic browning catalyzed by POD may be prevented by inhibition of the enzyme. In this context, the effect of some chemicals on the peroxidase activity was examined (Table 1). From Table 1, it can be seen that ions K^+, Cu^{2+}, Na^+, Pb^{2+} and Ba^{2+}had almost no effect on the POD from purple skin eggplant, while Fe^{2+} and Mg^{2+}showed an inhibitory effect on the peroxidase activity at concentration of 1mM and 5 mM.These cations should not be included in the

enzyme preparation. The most effective inhibitors of the POD from purple skin eggplant were ascorbic acid and sodium thiosulfate, since these compounds induced a high degree of inhibition, even at the lowest concentration used. Enachi*et al*. (2019) have also reported similar effect of these chemical agents on inhibition of peroxidase and the browning control of from plums (*Prunus domestica*).

Table 1:- Activity of peroxidase from purple skin eggplant (*Solanum melongena* L.) preincubated with some cations and chemical agents.

Reagent	Concentration (mM)	Relative Activity (%)
Control	0	100
K^+	1	104.16 ± 2.1
	5	100.92 ± 0.6
Na^+	1	120.64 ± 1.36
	5	107.22 ± 2.05
Cu^{2+}	1	128.24 ± 2.1
	5	144.29 ± 1.5
Pb^{2+}	1	155.09 ± 1.89
	5	131.51 ± 2.9
Ba^{2+}	1	112.5 ± 3.9
	5	102.07 ± 1.1
Fe^{2+}	1	33.88 ± 3.1
	5	26.59 ± 2.8
Mg^{2+}	1	65.74 ± 1.8
	5	83.69 ± 2.8
Chemical agents		
EDTA	1	67.12 ± 1.1
	5	89.13 ± 2.3
Acideascorbique	1	34.33 ± 1.2
	5	28.39 ± 2.3
Acidecitrique	1	93.5 ± 2.7
	5	91.33 ± 1.1
Thiosulfate de Sodium	1	57.31 ± 1.20
	5	42.5 ± 2.5

Note. Values given are the averages of at least three experiments.

Thermal inactivation

As concerned the effect of temperature on POD from purple skin eggplant, the optimal temperature, half-life ($t_{1/2}$-value) and D-value which are important parameters for enzyme thermal stability evaluation were determined as shown in Table 2. The increase in temperature from 40 to 80 °C resulted in a decrease in PODactivitie, hence the decrease of $t_{1/2}$-values and D-values. At 60 °C, the $t_{1/2}$-value of the studied enzymatic activitiedecreased to value 16.34 min. This value was lower than those obtained by Muhammad *et al*. (2013) who reported a $t_{1/2}$-value of 514.0 min at 60 °C for the POD of grape juice. Thus, the low $t_{1/2}$-value obtained suggested that POD from purple skin eggplant is strongly inactivated at temperature from 60 °C. With respect to D-values, a 72 % reduction in activity of POD from purple skin eggplant was observed at time from54.24 min at 60 °C. The results for POD extracted from purple skin eggplant are consistent with those obtained by Suha*et al*. (2013) who studied the POD extracted from potatoes, carrots, eggplants, and tomatoes and concluded that the rate of inactivation of the enzyme increased with the increasing temperature and time of treatment. POD inactivation extracted from purple skin eggplant was performed at 80°C for 4 to10 minutes.

Table 2:-Kinetic parameters of peroxidase (POD) activities of the purple skin eggplant (*Solanum melongena* L.).

T (°C)	Parameters	
	$t_{1/2}$ (min)	**D (min)**
40	28.17± 1.5	93.49± 1.1
45	23.98± 1.02	79.58± 1.6
50	21.39± 1.5	70.98± 1.0
55	17.72± 0.8	58.82± 1.1
60	16.34± 1.3	54.24± 1.2
65	14.90± 1.02	49.46± 1.0
70	13.80± 0.8	45.81± 1.5
75	12.29± 1.3	40.78± 1.6
80	6.15± 0.9	20.42± 1.1

Note. Values given are the averages of at least three experiments.

Conclusion:-

Based on the present study, the results are very important because they offer valuable information regarding the behavior of POD from purple skin eggplant under different thermal or chemical agents processing conditions, with a great impact on the food industry. Moreover, the peroxidase activity is sensitive to the heat and some of general inhibitors, especially to ascorbic acid and sodium thiosulfate . This work may help in Côted'Ivoire studies for the food industries and processed food using eggplant.

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