



Inactivation Kinetics and Thermodynamics Parameters of Polyphenol Oxidase and Peroxidase Activities in an Extract from of Violet Eggplant (*Solanum melongena L.*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author JCY designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors GSEE and KCY wrote the protocol, managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Enzymatic browning is associated with the action of polyphenol oxidases (PPO) and peroxidases (POD). The products of these enzymes cause undesirable changes of color and flavor of processed eggplant products. The present study aimed to evaluate kinetic properties and thermodynamics parameters of PPO and POD activities for controlling this undesirable process in extract from of violet eggplant. The effect of heat treatment on polyphenol oxidase and peroxidase activities in violet eggplant were studied over a range of 30 to 80 °C. $T_{1/2}$ -values of enzymatic activities are between 6.15 ± 0.03 and 13.27 ± 0.04 min at 80 °C, they decreased with increasing temperature, indicating a difference thermostability of each enzyme. D- and k-values decreased

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and increased, respectively, with increasing temperature, indicating faster of these enzymes inactivation at higher temperatures. Results suggested that polyphenol oxidase and peroxidase were relatively thermostable enzymes with a Z-value which from 50.25 and from 88.33 °C and Ea of 41.21 and of 27.78 kJmol⁻¹. Thermodynamic parameters were also calculated. The Gibbs free energy ΔG values range from 43.24 ± 0,03 to 91.45 ± 0,01 kJ/mol. These kinetic data can be used to predict prevention of browning in the violet eggplant (*Solanum melongena* L.) by thermal inactivation of enzymes.

Keywords: *Violet eggplant; peroxidase; polyphenol oxidase; enzymatic browning; Inactivation kinetics; thermodynamics parameters.*

1. INTRODUCTION

Eggplant fruits (*Solanum melongena* L.) are widely consumed around the world due to their generous composition of nutraceuticals [1]. It is grown as an annual vegetable crop in warm climates and several years in the form of a bushy in tropical climates [2]. It was produced 54.07 million tons in 2018, according to FAO statistic [3]. Eggplant has been found to be among the top ten vegetables with regards to antioxidants claimed to have several health benefits [4,5]. Eggplant (*Solanum melongena* L.) is important for its richness in phytonutrients with antioxidant capacity, mainly phenolic acids such as chlorogenic acid, caffeic acid and *p*-coumaric acid [6,7].

Despite all the advantages, its consumption is limited due browning that occurs after mechanical damage, during long term storage and long-distance transportation, or when it is peeled and crushed in processing [8], which results in the appearance of dark color compounds [9]. Many studies have shown that polyphenol oxidase (PPO) and peroxidase (POD) activities increase in response to biotic and abiotic stresses. On general, enzymatic browning is related to the action of the PPO and POD isozymes, which use phenolic compounds as substrates and cause undesirable changes in the color and flavor of fruits, vegetables and tubers [10].

PPOs (EC 1.14.18.1 monophenol monooxygenase and EC 1.10.3.2 o-diphenol: oxygen oxidoreductase) are proteins that catalyze oxidation reactions of phenolic compounds to quinones, with further polymerization to brown or black pigments known as melanin [11]. The enzyme has great heterogeneity regarding substrate, sensitivity to inhibitors, optimum pH, latency, thermal inactivation, number of isoforms and molecular mass [12]. PODs (EC 1.11.1.7) are enzymes that

catalyze peroxidation, oxidation- catalytic, and hydroxylation reactions [13]. They are the most studied protein in plant, since they have been found in every major plant division [14]. They are involved in ripening and senescence, plant defense, and darkening reactions [13].

The inactivation of the PPO and 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, which is also being used to ensure product quality in the food industry [15]. Short exposures to 70-90°C are mostly sufficient for destruction of the enzymes catalytic function [16]. The enzymes represent quite a challenge for large industrial processors due to their high thermostability even at pasteurization temperatures, which can lead to organoleptic and nutritional properties deterioration of the food. However, knowing the inactivation parameters is an important factor in optimizing a treatment or a combination of treatments, in order to obtain the desired product [15]. The aim of this work was to investigate the thermal inactivation of violet eggplant PPO and POD on a kinetic basis, a method that permits to determine accurate calculations of kinetic and thermodynamic parameters. These results can indicate an adequate choice of temperature conditions to inactivate violet eggplant enzymes and allow to predict the impact of a given heat-treatment and to design processes suitable to obtain the desired product properties.

2. MATERIALS AND METHODS

2.1 Plant Material and Chemicals

Fresh eggplant violet (*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).

2.2 Methods

2.2.1 Extraction of polyphenol oxidase (PPO) and peroxidase (POD)

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 PPO and POD activities assays [17].

2.2.2 Assay of polyphenol oxidase (PPO) and peroxidase (POD) and protein determination

To determine the enzymatic activity of PPO, the reaction mixture adjusted to 2 mL consisted of the 100 µL of the enzyme extract, 1.1 mL of 100 mM phosphate buffer (pH 6.6), supplemented with 0.8 mL of pyrocatechol substrate (10 mM). The blank assays had all the components for the reaction, except the enzymatic extract, which was replaced with buffer [18].

To determine the enzymatic activity of POD, the reaction mixture adjusted to 3 mL, an aliquot of 100 µL of the enzyme extract was added to the reaction medium containing 0.5 mL of guaiacol (1.68%), 1.9 mL of 100 mM citrate buffer (pH 6.0) and 0.5 mL H₂O₂ (1.8%). Blanks were constituted by all components of the reaction medium, except the enzymatic extract, which was replaced with citrate buffer [19].

This reaction mixture was incubated at 25°C for 10 min, and enzymatic activity was analyzed in a spectrophotometer, observing the variation in absorbance units at wavelengths of 480 nm for POD and 420 nm for PPO (standard conditions). 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. Protein concentrations were determined spectrophotometrically at 660 nm by method of Lowry et al. [20]. using bovine serum albumin as a standard.

2.2.3 Thermal inactivation

The thermal inactivation of PPO and POD activities was determined at temperatures

ranging from 30 to 80 °C. The crude enzymatic extract in appropriate buffers [100 mM phosphate pH 6.6 (for PPO) and citrate pH 6.0 (for POD)] was preincubated at different temperatures. Aliquots were withdrawn at intervals and cooled at room temperature for 10 min. The residual enzymatic activity, determined in both cases at 30 °C under the standard test conditions, was expressed as percentage activity of zero-time control of the untreated enzyme.

2.2.4 Kinetic data analysis

The temperature dependence of the reaction rate constant for the studied enzyme served as the basis for fitting to the Arrhenius equation [21]:

$$\ln [A_t/A_0] = -kt \quad (\text{Eq. 1})$$

where;

A_t is the residual enzyme activity at time t (min),

A_0 is the initial enzyme activity,

k (min^{-1}) is the inactivation rate constant at a given condition.

k -values were obtained from the regression line of $\ln (A_t/A_0)$ versus time as slope.

D-value is defined as the time needed, at a constant temperature, to reduce the initial enzyme activity (A_0) by 90 %. For first-order reactions, the D-value is directly related to the rate constant k (Eq. 2) [22,23]:

$$D = 2.303/k \quad (\text{Eq. 2})$$

The Z-value (°C) is the temperature increase needed to induce a 10-fold reduction in D-value [22]. This Z-value follows the Eq. 3:

$$\log [D_1/D_2] = [T_2 - T_1]/Z_T \quad (\text{Eq. 3})$$

where,

T_1 and T_2 are the lower and higher temperatures in °C or K.

Then, D_1 and D_2 are D-values at the lower and higher temperatures in min, respectively.

The Z-values were determined from the linear regression of $\log (D)$ and temperature (T).

2.2.5 Thermodynamic analysis

The Arrhenius equation is usually utilized to describe the temperature effect on the inactivation rate constants and the dependence is given by (Eq. 4 or 5):

$$k = Ae^{(-E_a/RT)} \quad (\text{Eq. 4})$$

Eq. 4 can be transformed to:

$$\ln k = \ln A - E_a/R \times T. \quad (\text{Eq. 5})$$

where,

k is the reaction rate constant value,
 A the Arrhenius constant,
 E_a ($\text{kJ}\cdot\text{mol}^{-1}$) the activation energy,
 R ($8.31 \text{ J}\cdot\text{mol}^{-1}\text{K}^{-1}$) the universal gas constant
 T is the absolute temperature in Kelvin (K).

When the "ln" of "k" is plotted against the reciprocal of the absolute temperature, a linear relationship should be observed in the temperature range studied. The slope of the line obtained permitted to calculate the activation energy and the ordinate intercept corresponds to $\ln A$ [24,25].

The values of the activation energy (E_a) and Arrhenius constant (A) allowed the determination of different thermodynamic parameters [26] such as variations in enthalpy, entropy and Gibbs free energy, ΔH , ΔS and ΔG , respectively, according to the following expressions [27]:

$$\Delta H^\# = E_a - RT \quad (\text{Eq. 6})$$

$$\Delta S^\# = R (\ln A - \ln K_B/h_P - \ln T) \quad (\text{Eq. 7})$$

$$\Delta G^\# = \Delta H^\# - T \Delta S^\# \quad (\text{Eq. 8})$$

Where;

K_B is the Boltzmann constant ($1.38 \times 10^{-23} \text{ J/K}$),
 h_P is the Planck constant ($6.626 \times 10^{-34} \text{ J.s}$),
 T is the absolute temperature.

2.2.6 Statistical analysis

All determinations reported in this study were carried out in triplicate. Results were expressed as means \pm standard deviation.

3. RESULTS AND DISCUSSION

The optimum temperatures of polyphenol oxidase (PPO) and peroxidase isolated from violet eggplant (*Solanum melongena* L.) were 25 °C and 35 °C respectively [28]. The residual activities of PPO and POD were studied as a function of temperature ranging from 30 to 80 °C for PPO and 40 to 80 °C for POD to determine their thermodynamic properties. The thermal stability profile of the PPO and POD presented in the form of the residual percentage activity is shown in Table 1. the activities of PPO and POD decreased with increasing temperature and

treatment time (5 - 60 min). Indeed, at temperatures between 30 - 80 °C, heat-denaturation of the enzymes occurred after 5 min of incubation (98.19 to 77.11 %) for PPO and (89.10 to 56.23 %) for POD. POD is not a very heat-stable enzyme as seen in Table 1. After the treatment at 40 °C for 20 min, POD retained 70 % of its activity, whereas at 55, 65, and 75 °C for the same time interval, it retained less than 46 % of its activity. Although heating at 60 °C for 15 min resulted in partial (55.40 %) inactivation. However, the enzyme is completely inactivated after 30 min pre-incubation at 80 °C (97 % loss of activity). On one hand, the decrease of percentage residual activity at temperatures higher than 40 °C was most likely due to the unfolding of the tertiary structure of the enzyme to form the secondary structure and on other hand, it could be explained by the chemical modification [29]. It has been noted that heat stability of the enzyme may be related to ripeness of the plant and molecular forms of enzyme [30].

PPO showed greater stability than POD in a temperature range of 40 - 70°C, displaying 81.87 and 49.66 % of residual activity after 20 min of incubation at 50 and 70 °C, respectively (Table 1), POD activity was found to be completely inactive after 30 min of incubation at 80 °C (91 % loss of activity). The data indicated that high temperature blanching is necessary to control the enzymatic browning caused by PPO and POD in violet eggplant (*Solanum melongena* L.).

The logarithmic linear relationship between the PPO and POD activities and heat treatment time for the temperature range of 30 - 80°C and 40 - 80 °C respectively followed first-order kinetics each (Figs. 1 and 2). These results were consistent with those reported for polyphenol oxidase and peroxidase of Loquat fruit [31] and PPO and POD of potato tubers [32].

The rates of the PPO and POD inactivation, after \ln transformation, decreased linearly with the inverse of temperature (Fig. 2). This relationship was described by the equation: $\ln k = -4959 (1/T) + 11.22 (R^2 = 0.908)$ and $\ln k = -3344 (1/T) + 6.92 (R^2 = 0.86)$ respectively for the PPO and POD, where T represents absolute temperature (K). From 30 to 80 °C, the activation energy (E_a) value for thermal inactivation of the PPO and POD were calculated to be 41.21 kJ/mol and 27.78 kJ/mol respectively for the two enzymes (Table 2).

Table 1. Effect of treatment temperature and time on inactivation of polyphenol oxidase (PPO) and peroxidase from violet eggplant (*Solanum melongena* L.)

Temperature (°C)	Residual activity (%) at each time (min) of heat treatment											
	5		10		15		20		25		30	
	PPO	POD	PPO	POD	PPO	POD	PPO	POD	PPO	POD	PPO	POD
30	98.19±3.1 ^a		95.78±5.3		93.24±2.3		91.39±3.6		88.55±1.5		86.94±3.2	
35	97.64±2.5		95.21±1.8		92.31±0.7		90.48±4.1		87.88±1.4		85.27±2.2	
40	95.12±2.8	89.10±4.1	90.48±2.5	77.62±2.5	86.07±1.5	70.79±4.1	81.87±5.1	61.54±2.3	78.66±2.7	53.70±1.2	75.58±1.8	47.44±1.1
45	88.69±3.5	85.11±3.3	81.87±1.6	74.13±2.1	74.08±3.1	65.22±3.5	67.29±3.4	56.23±0.7	61.26±1.2	48.98±1.3	54.34±1.3	41.69±1.6
50	88.69±1.3	83.18±2.4	78.66±0.6	72.44±1.2	71.18±1.2	60.26±2.3	64.40±2.7	52.48±2.3	57.06±0.8	44.67±0.9	52.20±0.6	38.02±0.7
55	86.94±0.8	78.26±1.6	77.88±1.2	67.61±2.1	69.77±2.1	56.23±1.1	62.50±2.1	45.71±4.5	55.99±2.3	38.02±2.1	49.66±0.8	30.90±1.1
60	84.37±1.2	77.62±1.4	75.58±2.1	64.75±1.1	67.31±1.7	55.40±1.2	58.86±1.9	42.66±2.3	52.20±2.5	33.88±1.6	45.84±1.1	28.18±1.3
65	83.53±2.1	75.86±3.5	71.89±0.9	61.68±0.5	60.65±1.1	50.12±2.3	52.20±1.6	39.81±2.6	45.38±1.8	30.90±0.9	39.06±2.5	25.12±1.1
70	81.87±1.7	74.13±2.9	70.47±1.3	58.88±1.8	59.45±1.8	47.86±1.1	49.66±2.3	37.15±1.7	42.54±0.8	28.18±1.1	37.16±1.0	22.39±0.7
75	79.45±3.2	74.13±1.7	65.05±2.2	54.95±2.1	52.73±0.6	42.66±2.7	43.60±3.1	32.36±2.0	35.67±1.3	24.55±1.3	29.82±1.1	18.62±1.0
80	77.11±1.2	56.23±0.7	60.65±1.1	30.90±2.1	45.38±1.1	19.05±0.6	34.99±2.1	10.47±1.4	27.25±0.8	5.93±0.6	8.80±0.3	3.47±0.4

^aMean (±SD) for triplicate experiments**Table 3. Thermodynamic parameters of polyphenoloxidase (PPO) and peroxidase (POD) from violet eggplant (*Solanum melongena* L.) under heat treatment between 30 and 80°C (assuming a 1st-order kinetic model)**

Temperature (°C)	Thermodynamic parameters					
	$\Delta H^\#$ (kJ.mol ⁻¹)		$\Delta S^\#$ (J.mol ⁻¹ K ⁻¹)		$\Delta G^\#$ (kJ.mol ⁻¹)	
	PPO	POD	PPO	POD	PPO	POD
30	38.69 ± 0,02 ^a			-151.74 ± 0,02		43.24 ± 0,03
35	38.65 ± 0,01			-151.87 ± 0,01		43.96 ± 0,02
40	38.60 ± 0,01 ^t	25.18 ± 0,02	-152.01 ± 0,03	-187.66 ± 0,03	44.68 ± 0,04	83.92 ± 0,04
45	38.56 ± 0,04	25.14 ± 0,01	-152.14 ± 0,04	-187.79 ± 0,04	45.41 ± 0,12	84.86 ± 0,12
50	38.52 ± 0,02	25.10 ± 0,01 ^t	-152.27 ± 0,02	-187.92 ± 0,02	46.13 ± 0,01	85.80 ± 0,01
55	38.48 ± 0,02	25.06 ± 0,04	-152.40 ± 0,01	-188.05 ± 0,01	46.86 ± 0,03	86.74 ± 0,03
60	38.44 ± 0,03	25.02 ± 0,02	-152.52 ± 0,03	-188.17 ± 0,03	47.59 ± 0,02	87.68 ± 0,02
65	38.40 ± 0,01	24.97 ± 0,01	-152.65 ± 0,02	-188.30 ± 0,02	48.32 ± 0,01	88.62 ± 0,01
70	38.35 ± 0,02	24.93 ± 0,02	-152.77 ± 0,04	-188.42 ± 0,04	49.05 ± 0,01	89.56 ± 0,01
75	38.31 ± 0,04	24.89 ± 0,04	-152.89 ± 0,01	-188.54 ± 0,01	49.78 ± 0,02	90.50 ± 0,02
80	38.27 ± 0,01	24.85 ± 0,01	-153.01 ± 0,03	-188.66 ± 0,03	50.51 ± 0,01	91.45 ± 0,01
Mean	38.48 ± 0,03	25.02 ± 0,01	-152.39 ± 0,02	-188.17 ± 0,02	48.87 ± 0,01	87.68 ± 0,01

^a Mean (±SD) for triplicate experiments

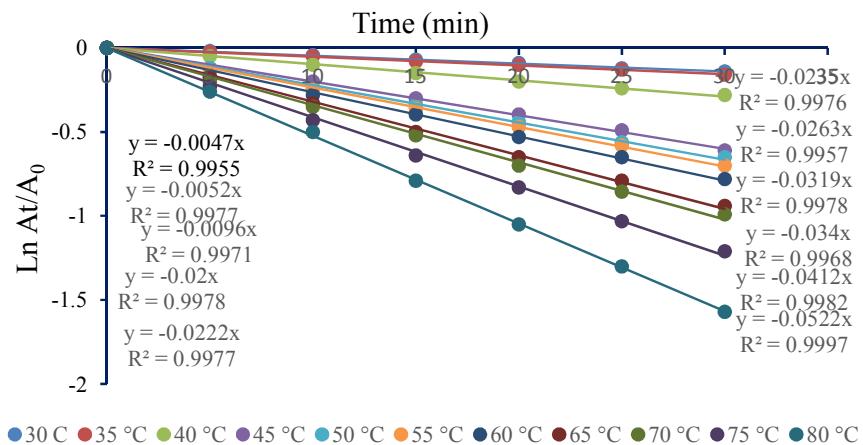


Fig. 1. Thermal inactivation curves of polyphenol oxidase from violet eggplant (*Solanum melongena* L.) in phosphate buffer (pH 6.6) in the temperature range 30-80 °C. A_0 is the initial enzymatic activity and A_t the activity at each holding time. Each data point is the mean of three determinations

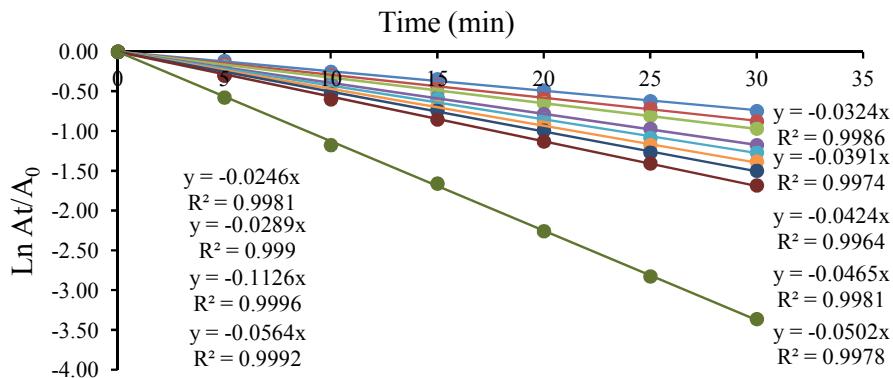


Fig. 2. Thermal inactivation curves of peroxidase from violet eggplant (*Solanum melongena* L.) in citrate buffer (pH 6.0) in the temperature range 40-80 °C. A_0 is the initial enzymatic activity and A_t the activity at each holding time. Each data point is the mean of three determinations

A large value of E_a is indicative that more energy is required to inactivate the enzyme [33]. They further stated that high E_a is indication that the process is strongly temperature dependent, at lower temperature, this rate becomes insignificant [33]. $\Delta E^\#$ and $\Delta H^\#$ for POD were lower than those for PPO. Then considering the lower values of $\Delta E^\#$ and $\Delta H^\#$ for POD in comparison with those for PPO, it is possible to state that violet eggplant POD is less stable than PPO (Table 1). This suggests that the denaturation process requires a high energy input to the enzyme substrate complex to initiate denaturation probably due to a possible compact structure of enzymes and the strength of the thiol groups (SH) or disulfide bond at the active site

[34]. The results are similar to those for strawberry fruit, showing that PPO is more thermostable than POD [35], but different from those for melon [36].

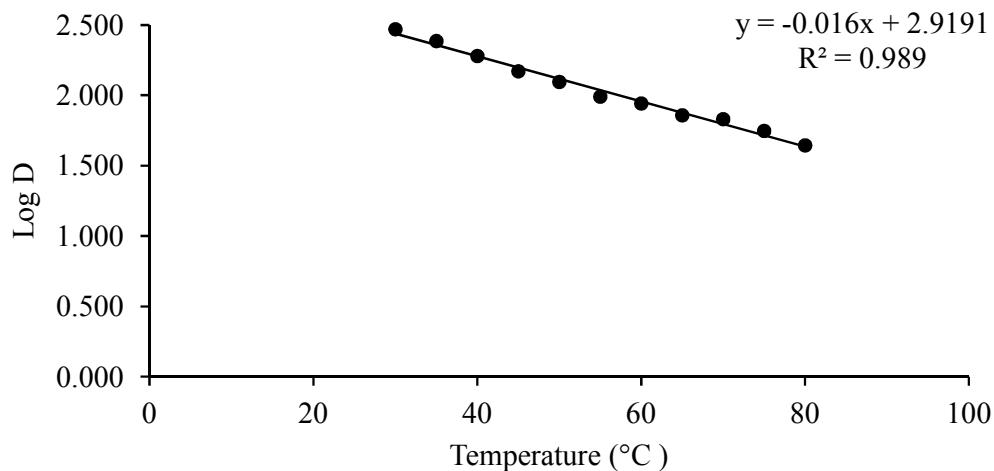
The values of the kinetic parameters, D and the inactivation rate constant k, were determined using Equations 1 and 2 (Eq. 1 and Eq. 2). The obtained D-values showed that successive increase as the temperature was increasing in all the enzymes assayed. Eze et al. [33] reported that corresponding decrease with increasing temperature is indicative of fast enzyme inactivation at higher temperature. The D-values were increasing as the temperature (K) was increasing this implies faster enzyme inactivation at higher temperature.

Table 2. D-, Z- and Ea-values for thermal inactivation of polyphenol oxidase (PPO) and peroxidase from violet eggplant (*Solanum melongena L.*) at temperature range (30-80 °C)

Kinetic parameters	Polyphenol oxidase	Peroxidase
D-values (min)		
D30	490.00 ± 1.98 ^a	
D35	442.88 ± 2.25	
D40	239.89 ± 1.05	93.49 ± 1.43
D45	115.15 ± 1.74	79.58 ± 2.67
D50	103.73 ± 1.07	70.98 ± 0.8
D55	98.00 ± 2.65	58.82 ± 1.76
D60	87.56 ± 1.03	54.24 ± 1.43
D65	21.72 ± 1.05	49.46 ± 1.32
D70	67.73 ± 0.9	45.81 ± 2.12
D75	55.89 ± 1.03	40.78 ± 2.23
D80	44.11 ± 1.05	20.42 ± 1.78
Z-value (°C)	50.25 ± 0.71	41.21 ± 0.68
Ea (kJ.mol ⁻¹)	41.21 ± 0.62	27.78 ± 1.23

^aMean (±SD) for triplicate experiments

The effect of temperature on D-values is shown in Figs. 3 and 4 respectively for PPO and POD, and from this representation, the Z-values were calculated and found to be 50.25 °C at 30 - 80 °C for the PPO and 88.33 at 40 - 80 °C for the POD (Table 2). High Z-value indicates that high amount of energy was required to initiate denaturation and vice versa [33], low Z-values were obtained for the enzyme. High Z-values indicate more sensitivity to the duration of heat treatment, while low Z-value implies more sensitivity to increase in temperature [37].

**Fig. 3. Variation of decimal reduction times with temperature for polyphenol oxidase from violet eggplant (*Solanum melongena L.*). Each data point is the mean of three determinations**

The thermodynamic parameters for thermal inactivation of the PPO and POD from violet eggplant are shown in Table 3. At temperatures of 30 – 80 °C, the average values of ΔH , ΔS and ΔG were respectively 38.48 (kJ/mol), -152.39 (J mol⁻¹ K⁻¹) and 48.87 (kJ/mol) for PPO. While at temperatures of 40 - 80 °C these average values were respectively of 25.02 ± 0.01 (kJ/mol), -

188.17 ± 0.02 (J mol⁻¹ K⁻¹) et 87.68 ± 0.01 (kJ/mol). The high values of change in enthalpy obtained for the different treatment temperatures indicate that enzyme undergoes a considerable change in conformation during denaturation. Positive values of ΔH of the enzymes indicate the endothermic nature of the oxidation reactions.

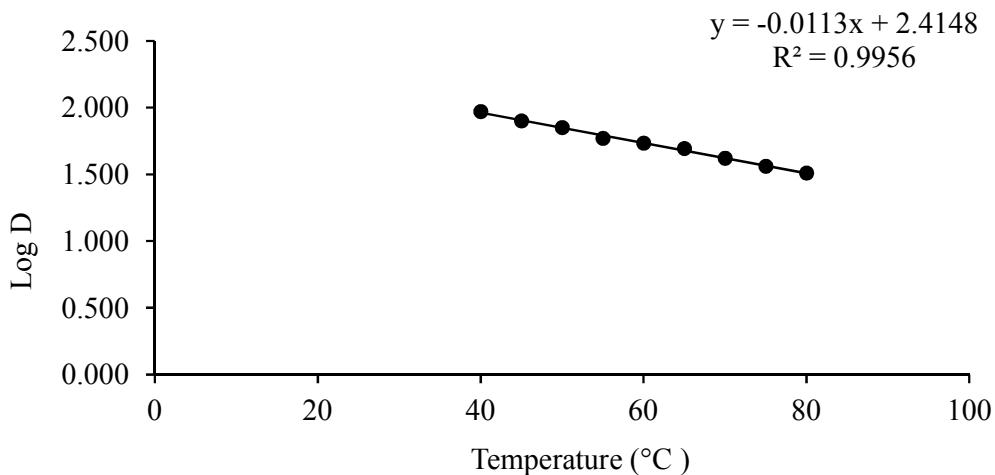


Fig. 4. Variation of decimal reduction times with temperature for of peroxidase from violet eggplant (*Solanum melongena L.*). Each data point is the mean of three determination

Free energy (ΔG) is defined as the maximum amount of energy available to a thermodynamic process that can be converted into useful work [38]. The change in free energy (ΔG) can be used to predict the direction of a reaction at constant temperature and pressure [39]. If ΔG has a negative value, there is a net loss of energy, and the reaction occurs spontaneously, if ΔG has a positive value, there is a net gain of energy, and the reaction does not go spontaneously, if ΔG value is equal to zero, the reactants are in equilibrium [38]. Enzyme molecule with high or positive ΔG is considered to be stable [40]. This indicates that values obtained for violet eggplant polyphenol oxidase and peroxidase were stable at 303 and 343 K, which suggested that these enzymes would not disintegrate non-spontaneously, hence, the enzymes could be said to be stable. Also, these positive values at all temperatures for the enzymes indicate that the inactivation processes were not spontaneous.

Entropy (ΔS) is a measure of degree of randomness or disorder of a system; it increases (ΔS is positive) when the system becomes more disordered [38]. The rate of any chemical reaction is a function of the temperature and energy difference between the reactants and the activation energy E_a [41].

Eze [40] stated that a negative ΔS show there is an aggregation process in which a few inter or intra molecular bonds are formed. The negative values observed for the variation in entropy indicate that there are no significant processes of

aggregation, since, if this would happen, the values of entropy would be positive [42].

4. CONCLUSION

Thermal inactivation of polyphenol oxidase (PPO) and peroxidase (POD) could be described by a first-order kinetic model for each enzyme. The efficient and effective heat treatment to reduce PPO and POD and enzymatic browning in violet eggplant (*Solanum melongena*) can be derived from any of the D-, z-, Ea- or k-values. The D-, Z-, k-values and the high values obtained for activation energy and change in enthalpy indicate that a high amount of energy is required to initiate denaturation of these enzymes, most likely due to its stable molecular conformation. POD was much more thermolabile than PPO, losing more than 50 % of the activity after 30 min of heating at 40 °C. The results obtained in this study indicate that PPO and POD characteristics are variable and dependent on eggplant fruit, and that PPO of eggplant fruit plays an important role in browning, which is an item of knowledge that will significantly help to control the enzymatic browning taking place during processing and storage of violet eggplant (*Solanum melongena*)

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not

intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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