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Thermal inactivation of the dimeric dopamine oxidase from edible tuber yam *Dioscorea cayenensis rotundata* cv "Kponan": kinetic and thermodynamic analysis

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Abstract The edible yam tuber *Dioscorea cayenensis rotundata* cv "Kponan" contained two polyphenol oxidases. They were purified to homogeneity and designated PPO1 and PPO2. PPO1 had monomeric structure whereas PPO2 was dimeric with native molecular weights of approximately 115.65±1.78 and 113.7±0.34 kDa, respectively. In addition, PPO2 exhibited high dopamine oxidase activity compared to PPO1. In this study, thermal inactivation of PPO2 was examined in more detail between 40 and 70 °C and in relation to exposure time. Denaturation of this enzyme, measured by loss in activity, could be described as a first-order model, with *k*-values between 0.005 and 0.079 min^{-1}. The *D*- and *k*-values decreased and increased respectively, with increasing temperature, indicating faster polyphenol oxidase (PPO2) inactivation at higher temperatures. Results suggested that PPO2 is a relatively thermostable enzyme with a *Z*-value of 27.03 °C and *Ea* of 76.23 $kJ.mol^{-1}$. Thermodynamic parameters were also calculated. The Gibbs free energy ($\Delta G^{\#}$) values range from 88.75 to 90.21 $kJ.mol^{-1}$. The results of the thermodynamic investigations indicated that the oxidation reactions were: (1) not spontaneous ($\Delta G^{\#}>0$), (2) slightly endothermic ($\Delta H^{\#}>0$) and (3) reversible ($\Delta S^{\#}<0$). These kinetic data can be used to predict prevention of browning in the tuber yam by thermal inactivation of enzyme.

Keywords Yam, Polyphenol oxidase, Enzymatic browning, Thermal inactivation, Kinetics and thermodynamic analysis

1. Introduction

Yam is one of the most important tubers produced in tropical regions and subtropical areas particularly in West Africa, south Asia and the Caribbean, where it also has a social and cultural importance [1]. Recognized as an excellent source of starch, yam is an energy-rich tuber and provides protein three times more superior than cassava and sweet potato [2]. Furthermore, the proteins, lipids, vitamins and minerals have been found in yam [3]. Yam is unfortunately hampered by a phenomenon of enzymatic browning during postharvest storage or processing [4]. These browning reactions have been linked to mechanical damage during handling and processing, abrasions, washing, senescence, and bacterial infestations. In yam tuber *Dioscorea cayenensis rotundata* cv "Kponan", polyphenol oxidase (PPO, monophenol, dihydroxy-L-phenylalanine/oxygen oxidoreductase, EC 1.14.18.1) is considered as the first enzyme responsible for browning [5]. PPO is a widely distributed copper-containing protein which catalyzes two distinct reactions, both of which involve molecular oxygen: the hydroxylation of monophenols to *O*-diphenols (monophenolase activity) and the oxidation of *O*-diphenols to *O*-quinones (diphenolase activity) [6]. These quinones undergo polymerization reactions leading to the formation of black, brown or red pigments. Apart from this color change, enzymatic browning results in development of off-flavors and reduction of nutritional and market value. Hence, the possibility to inhibit enzymatic browning is a great challenge in the food industry [7]. It is currently accepted that the PPO

inactivation by heat treatment is the most effective method to control enzymatic browning but it is limited by alteration in sensory characteristics and the nutrient losses, so that optimization of time and temperature must be achieved [8]. Indeed, PPO enzyme does not belong to an "extremely heat-stable enzyme" group, and short exposures of product to temperatures between 70 and 90 °C are sufficient to inactivate the enzyme [9].
Recently, two polyphenol oxidases (PPO1 and PPO2) have been purified to homogeneity from edible yam (*D. cayenensis-rotundata* cv "Kponan") cultivated in Côte d'Ivoire [5]. Due to the importance of colour preservation of the raw material before any processing, the inactivation of naturally-occurring enzymes including PPO is necessary. The aim of this study was to inactivate the second isoform of the PPO from yam *D. cayenensis-rotundata* cv "Kponan", using the effect of heat treatment over a range of temperatures from 40 to 70 °C on this enzyme, permitting to determine accurate calculations of kinetic and thermodynamic parameters. These results can indicate adequate temperature conditions to inactivate yam enzymes and enzyme-based biotechnological devices.

2. Materials and Methods

2.1. Enzyme Sources

Mature tubers of *D. cayenensis rotundata* cv "Kponan" were harvested from the Biological Garden University of Nangui Abrogoua (Abidjan, Côte d'Ivoire) and stored at -20°C until used. The PPO2 substrate dopamine was obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). All other chemicals and reagents were of analytical grade.

2.2. Enzyme Extraction and Purification Procedures

Yams (150 g) were ground using a blender in 200 mL NaCl 0.9% (w/v). The homogenate was subjected to sonication (4°C) at 50 - 60 Hz frequency using a TRANSSONIC T420 for 10 min and then centrifuged at 10,000 g for 30 min at 4°C. The supernatant filtered through cotton wool was kept refrigerated and used as the crude extract.
The purification procedure was carried out in the cold room (4°C). The crude extract of yam tuber *D. cayenensis-rotundata* cv "Kponan" was loaded onto an anion-exchange chromatography using a DEAE-Sepharose Fast Flow column (2.5 cm x 4.5 cm), equilibrated with 20 mM sodium phosphate buffer (pH 6.6). The column was washed at a flow rate of 3 mL/min with two bed volumes of equilibration buffer to remove unbound proteins. Bound proteins were then eluted with a stepwise salt gradient (0.3, 0.5, 0.7 and 1 M) of NaCl in 20 mM sodium phosphate buffer (pH 6.6), and fractions of 3 mL were collected. Two peaks (PPO1 and PPO2) of polyphenol oxidase activity were obtained. The bound polyphenol oxidase activity (Peak 2: PPO2) eluted was also subjected to 80 % saturation with ammonium sulphate. The precipitate obtained after centrifugation (10 000 g) was dissolved in 1 mL of 20 mM sodium phosphate buffer (pH 6.6) and loaded onto the Sephacryl S-100 HR column (1,6 x 64 cm), equilibrated with 20 mM sodium phosphate buffer (pH 6.6). Polyphenol oxidase activity was eluted with the same buffer at a flow rate of 0.25 mL/min. Fractions of 1 mL were collected and, to the pooled active fractions, solid ammonium sulphate was slowly added to give a final concentration of 1.7 M. The resulting enzyme solution was subsequently applied on a Phenyl Sepharose 6 Fast Flow column (1.5 cm × 3.2 cm) previously equilibrated with 20 mM sodium phosphate buffer (pH 6.6) containing 1.7 M of ammonium sulphate salt. The column was washed with a reverse stepwise gradient of ammonium sulphate concentrations (from 0 to 1.7 M) dissolved in the same sodium phosphate buffer at a flow rate of 1 mL/min and fractions of 1 mL were collected. The pooled active fractions were dialyzed overnight against 20 mM sodium phosphate buffer (pH 6.6) and constituted the purified enzyme solution.

2.3. Enzyme Assay and Protein Determination

Under the standard test conditions, the PPO2 activity was measured with dopamine as a substrate using a modification of the previous method [10]. An assay mixture (2 mL) containing 100 mM phosphate buffer pH 6.6, 8 mM dopamine and enzyme solution was incubated at 30°C for 10 min. After incubation, the activity was determined by measuring the absorbance at 480 nm. Experiments were performed in triplicate, and the results expressed as units (U) of enzymatic activity per mg of protein. One unit of enzymatic activity was defined as an

increase in absorbance of 0.001 per min [11]. Protein was determined according to the Lowry method using bovine serum albumin as standard [12].

2.4. Thermal Inactivation

The thermal inactivation of the enzyme was investigated at various constant temperatures between 40 and 70°C after exposure to each temperature for a period of 5 to 30 min. The enzyme was incubated in 100 mM phosphate buffer pH 6.6. Aliquots were drawn at intervals and immediately cooled in ice-cold water. Experiments were performed in triplicate. The residual enzymatic activity, determined at 40°C under the standard test conditions, was expressed as percentage activity of zero-time control of the untreated enzyme.

2.5. 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 [13]:

$$ln(At/A0) = -kt \quad \text{(Eq.1)}$$

where, *At* is the residual enzyme activity at time t (min), *Ao* is the initial enzyme activity, *k* (min^{-1}) is the inactivation rate constant at a given condition. The *k*-values were obtained from the regression line of *Ln(At/Ao)* versus time as slope.

The *D*-value is defined as the time needed, at a constant temperature, to reduce the initial enzyme activity (*Ao*) by 90 %. The *D*-values (D_t) were calculated by regression analysis of the lines obtained by plotting the logarithm of the activity expressed as the percentage of initial activity against time. The *D*-values correspond to the reciprocal of the slope of those lines. The decimal reduction time (*D*) was calculated as follows [14]:

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

The *Z*-value (°C) is the temperature increase needed to induce a 10-fold reduction in *D*-value [14]. This *Z*-value follows the equation:

$$log(D1/D2) = (T2 - T1)/Z \quad \text{(Eq.3)}$$

where, *T1* and *T2* are the lower and higher temperatures in °C or K. Then, *D1* and *D2* 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).

2.6. Thermodynamic Parameters

The treatment temperature and the rate constant in a denaturation process are related according to the Arrhenius equation:

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

where, *k* is the reaction rate constant value, *A* the Arrhenius constant, *Ea* ($kJ.mol^{-1}$) the activation energy, *R* ($8.31\ J.mol^{-1}K^{-1}$) the universal gas constant and *T* (K) the absolute temperature.

Equation 4 (Eq. 4) can be transformed to:

$$lnk = lnA - (Ea/RT) \quad \text{(Eq.5)}$$

when *lnk* is plotted versus 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 *Ea* and the ordinate intercept corresponds to *lnA* [15].

The values of the activation energy (*Ea*) and Arrhenius constant (*A*) allowed the determination of different thermodynamic parameters such as variations in enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$) and Gibbs free energy ($\Delta G^{\#}$) according to the following expressions:

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

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

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

where, K_B ($1.38 \times 10^{-23}\ J.K^{-1}$) is the Boltzmann's constant, h_P the Planck's constant (6.626×10^{-34} J.s) and *T* the absolute temperature.

2.7. Statistical Analyses

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

3. Results and Discussion

3.1. Kinetic Analysis of Thermal Denaturation

The optimum temperature of second isoform of polyphenol oxidase (PPO2) purified from edible yam (*D. cayenensis-rotundata* cv. "Kponan") was 35°C [5]. In this study, thermal inactivation of yam tuber PPO2 was screened at different temperatures ranged from 40 to 70°C by determining the residual enzymatic activity (Table 1). The PPO2 activity was decreased with increasing heating time (5 - 60 min) and temperature (40 - 70°C). Indeed, at temperatures between 40 and 70°C, heat-denaturation of PPO2 occurred after 5 min of incubation (98.18 to 70.36 %). Although heating at 60°C for 40 min resulted in partial (52.48%) inactivation. However, PPO2 is almost completely inactivated at 70°C after 35 min of thermal treatment (5.96 %). 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 [16]. It has been noted that heat stability of the enzyme may be related to ripeness of the plant and molecular forms of enzyme [17].

Table 1: Effect of treatment temperature and time on the inactivation of polyphenol oxidase PPO2 from edible yam (*Dioscorea cayenensis-rotundata* cv "Kponan")

Temp	**Relative activity (%) at each treatment time (min)**											
(°C)	**5***	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**
40	98.18 ±0.8[a]	94.18 ±1.9	90.94 ±1.0	88.51 ±0.9	85.98 ±0.6	83.61 ±1.1	81.30 ±0.0	79.61 ±0.4	76.80 ±0.1	74.58 ±0.2	72.54 ±0.4	70.36 ±0.3
45	95.53 ±0.6	92.22 ±0.9	89.49 ±0.4	86.76 ±0.4	83.03 ±0.2	81.30 ±0.6	78.54 ±0.3	75.13 ±0.7	73.39 ±0.7	71.56 ±0.2	68.45 ±0.7	65.84 ±0.2
50	95.42 ±0.7	91.30 ±1.0	89.12 ±0.4	85.45 ±0.8	81.46 ±1.0	79.06 ±0.4	75.66 ±0.4	73.33 ±0.7	70.33 ±1.1	68.25 ±0.3	65.53 ±0.5	62.62 ±0.3
55	95.21 ±0.8	90.29 ±1.6	87.11 ±0.2	83.44 ±1.3	80.33 ±0.5	76.86 ±0.2	73.71 ±0.2	71.52 ±0.5	68.59 ±1.0	65.57 ±0.6	63.19 ±0.9	60.56 ±0.6
60	90.39 ±0.6	84.79 ±0.5	79.09 ±0.4	73.06 ±0.8	68.94 ±0.7	65.62 ±0.4	60.71 ±0.3	52.48 ±0.9	50.62 ±1.0	48.33 ±0.8	45.48 ±0.9	41.55 ±0.7
65	90.21 ±0.4	69.00 ±0.8	65.64 ±1.6	53.42 ±0.3	47.95 ±1.2	41.51 ±0.5	36.06 ±0.3	30.12 ±0.9	26.77 ±0.0	21.37 ±1.0	18.08 ±0.2	16.53 ±0.2
70	66.17 ±1.3	48.77 ±0.9	30.12 ±0.5	19.79 ±0.3	14.52 ±0.6	8.77± 0.6	5.96± 0.1	4.50± 0.3	2.90± 0.1	1.71± 0.4	1.39± 0.2	0.77± 1.1

The logarithmic linear relationship between the PPO2 activity and heat treatment time for the temperature ranged from 40 to 70°C followed first-order kinetics (Figure 1). This result agrees with those reported for PPO from *Agaricus bisporus* [18] and from Jubileu clingstone peach [19].

From the slopes of these lines, the inactivation rate constants (*k*) were calculated and are given in Table 2. The rate constant increased with the heating temperature, indicating that PPO2 from edible yam (*D. cayenensis-rotundata* cv. "Kponan") is less thermostable at higher temperatures (60-70 °C). The dependence of the *k*-values with temperature was adequately fitted by the Arrhenius equation (R^2 = 0.990) (Figure 2). This linearity is an indication that the inactivation in edible yam PPO2 occurs through a unique mechanism dependent on temperature, such as protein unfolding [20,21].

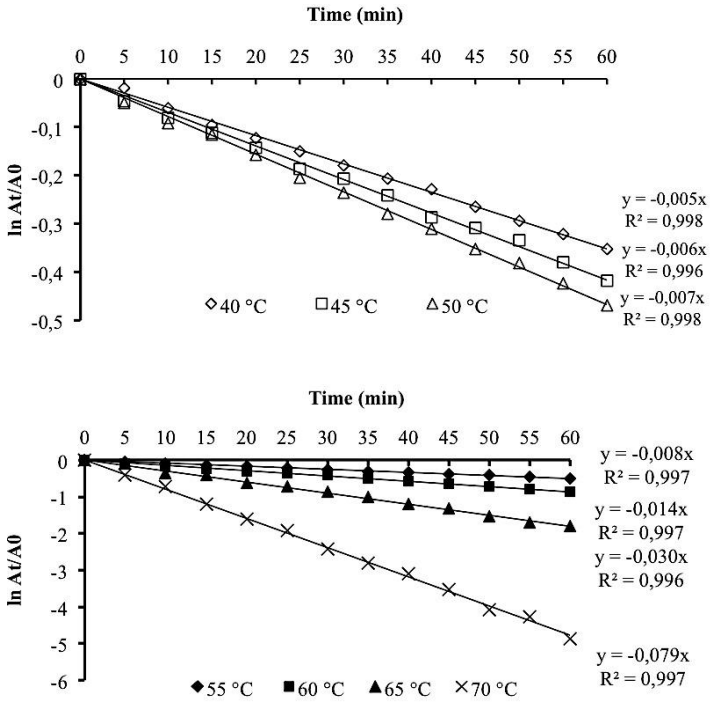

Figure 1: Thermal inactivation curves of polyphenol oxidase (PPO2) from edible yam (Dioscorea cayenensis-rotundata cv "Kponan") in sodium phosphate buffer (pH 6.6) in the temperature range 40-70°C. A_0 is the initial enzymatic activity and A_t the activity at each holding time. Each data point is the mean of three determinations

Table 2: k, D-, $t_{1/2}$, Z- and Ea-values for thermal inactivation of edible yam (*Dioscorea cayenensis-rotundata* cv "Kponan") polyphenol oxidase PPO2 in the 40-70°C temperature range

Temperature (°C)	Kinetic parameters					
	K (min^{-1})		D (min)	$t_{1/2}$ (min)	Z (°C)	Ea ($kJ.mol^{-1}$)
	Value	R^2				
40	0.005 ± 0.001[a]	0.992	460.60 ± 25	138.60 ± 8		
45	0.006 ± 0.002	0.994	383.83 ± 17	115.50 ± 5		
50	0.007 ± 0.001	0.995	329.00 ± 15	99.00 ± 3		
55	0.008 ± 0.001	0.998	287.88 ± 17	86.63 ± 5	27.03 ± 0.1	76.23 ± 0.16
60	0.014 ± 0.002	0.997	164.50 ± 8.5	49.50 ± 2.3		
65	0.03 ± 0.01	0.992	76.77 ± 0.7	23.10 ± 1.1		
70	0.079 ± 0.02	0.922	29.15 ± 0.2	8.77 ± 0.9		

[a] Mean (±SD) for triplicate experiments

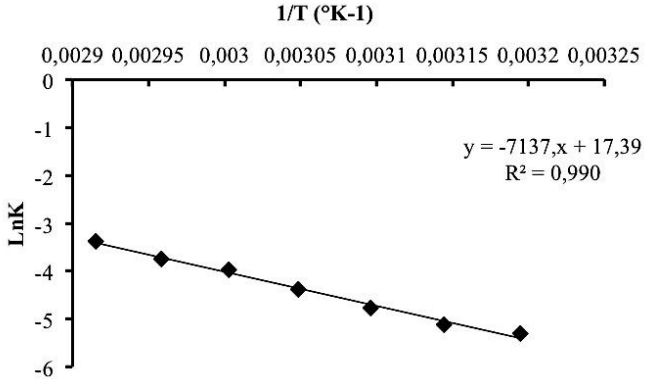

Figure 2: Arrhenius plot showing the effect of temperature on the rate constant for the thermal inactivation of edible yam (Dioscorea cayenensis-rotundata cv "Kponan") polyphenol oxidase (PPO2). 1/T represents the reciprocal of the absolute temperature. Each data point is the mean of three determinations

The half-life ($t_{1/2}$) and the decimal reduction time (*D*-value) are other important parameters commonly used in the characterization of enzyme stability. Increasing the temperature from 40 to 70 °C resulted in a decrease in $t_{1/2}$ and *D*-values (Table 2). At the same temperature (70 °C), PPO2 from yam tuber "Kponan" ($t_{1/2}$ = 8.77 min; *D* = 29.15 min) was less thermostable than those of other vegetable sources such as Emir grape ($t_{1/2}$ = 15.9 min; *D* = 52.7 min) [22] and Anamur banana ($t_{1/2}$ = 27 min; *D* = 92 min) [23].

From 40 to 70°C, the activation energy (*Ea*) value for thermal inactivation of PPO2 was calculated to be 76.23 kJ.mol^{-1} (Table 2). High activation energy reflects a greater sensitivity of PPO2 to temperature change [24]. 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 [25]. The *Ea*-value from yam PPO2 was much higher than those reported for edible yam (68 kJ.mol^{-1}) [20] and cassava *(Manihot esculenta*) (13.99 kJ.mol^{-1}) [26], but lower than that for taro (87.8 kJ.mol^{-1}) [27], sweet potato (*Ipomoea batatas*) (95 kJ.mol^{-1}) [27] and Anamur banana (*Musa cavendishi*) (155 kJ.mol^{-1}) [23]. On this basis, PPO in yam tuber can be classified as heat-sensitive PPO in terms of inactivation kinetics.

Figure 3 presents the relationship between decimal reduction time and temperature, where the slope of the curve represents -1/Z_T. The estimated value of yam tuber PPO was 27.03 °C (R^2 = 0.994). This result is in good agreement previous reports indicating that Z_T values of PPO from various fruits were 8.5-10.1°C [9]. Some investigators reported significantly higher Z_T values, up to 30 °C, for inactivation of PPO prepared from different foods [21]. In general, low Z_T values are thought to indicate greater sensitivity to heat [21]. This confirms that the PPO2 studied can be considered as a heat-sensitive enzyme in this family. This *Z*-value was much higher than that reported for polyphenol oxidase *Agaricus bisporus* (9.3°C) [18].

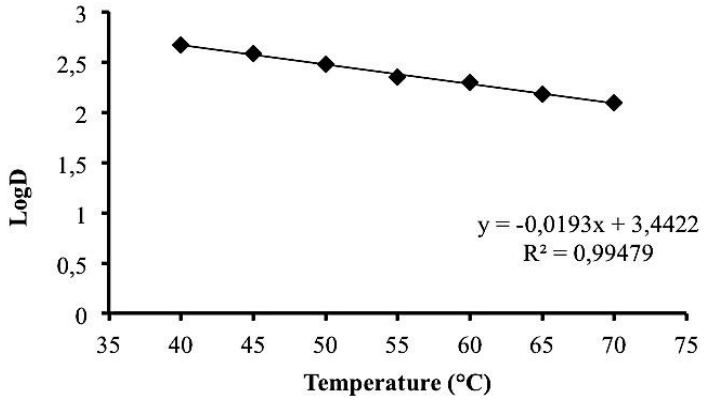

Figure 3: Variation of decimal reduction times with temperature for the edible yam (Dioscorea cayenensis-rotundata cv "Kponan") polyphenol oxidase (PPO2). Each data point is the mean of three determinations

3.2. Thermodynamic Analysis of Thermal Denaturation

Table 3: Thermodynamic parameters for the thermal inactivation of edible yam (*Dioscorea cayenensis-rotundata* cv "Kponan") PPO2 at different temperatures

Temperature	Thermodynamic parameters		
(°C)	$\Delta H^{\#}$ (kJ.mol^{-1})	$\Delta S^{\#}$ (J.mol^{-1}.K^{-1})	$\Delta G^{\#}$ (kJ.mol^{-1})
40	73.62 ± 0.6[a]	-48.32 ± 1.6	88.75 ± 0.3
45	73.59 ± 0.5	-48.46 ± 1.7	88.99 ± 0.4
50	73.54 ± 0.6	-48.58 ± 0.3	89.23 ± 0.1
55	73.50 ± 0.6	-48.71 ± 0.3	89.48 ± 0.1
60	73.46 ± 0.4	-48.84 ± 0.6	89.72 ± 0.2
65	73.41 ± 0.6	-48.96 ± 0.1	89.97 ± 0.3
70	73.37 ± 0.3	-49.08 ± 0.4	90.21 ± 0.6
Mean	73.50 ± 0.5	-48.71 ± 0.2	89.48 ± 0.3

[a] Mean (±SD) for triplicate experiments

The thermodynamic parameters provide information on the enzyme thermal stability for each step of the heat-induced denaturation process. This could help in detecting any secondary stabilization or destabilization effects that would go unnoticed if only the half-life times were considered [28]. These parameters include the Gibbs free energy ($\Delta G^{\#}$) change considered as the energy barrier for enzyme inactivation, the enthalpy ($\Delta H^{\#}$) change measuring of the number of bonds broken during inactivation, and the entropy ($\Delta S^{\#}$) change that indicates the net enzyme and solvent disorder. In this study, they were calculated in the temperature range from 40 to 70 °C (Table 3).

Within the error range of our measurements, results showed that the enthalpy ($\Delta H^{\#}$) is independent of temperature; thus, there is no change in enzyme heat capacity. The $\Delta H^{\#}$ value of PPO2 (73.50 kJ.mol^{-1}) was highly than those reported for other PPOs: 13 kJ.mol^{-1} for *Lepista nuda* mushroom [29] and 36 kJ.mol^{-1} for *Hypholoma fasciculare* [30]. The high $\Delta H^{\#}$ change obtained indicates that PPO2 undergoes a significant conformational change in order to find in its activated during heat treatment and high amount of energy was required to initiate denaturation, probably due to the molecular conformation of polyphenol oxidase [31]. Positive $\Delta H^{\#}$ values indicate the endothermic nature of the oxidation reaction. Our results would therefore suggest that PPO2 from edible yam (*D. cayenensis-rotundata* cv. "Kponan") is more structurally robust than the others enzymes.

In contrast, the Gibbs free energy ($\Delta G^{\#}$) value is directly related to protein stability: the higher the ΔG is, the higher will be the enzyme stability. Results showed that the $\Delta G^{\#}$ values pf the yam PPO2 slightly increased from 88 kJ.mol^{-1} to 90 kJ.mol^{-1}, when the incubation temperature was elevated (40 - 70 °C) indicating that the inactivation processes were not spontaneous. Since $\Delta G^{\#}$ decreases with increasing temperature whereas $\Delta H^{\#}$ is overall constant, one could expect a significant contribution of entropy changes to the thermodynamics of the considered system. Indeed, it was already demonstrated that activation entropy has a dominant role in thermal inactivation of proteins in aqueous solutions [32].

As indicated in Table 3, the entropy ($\Delta S^{\#}$) values of PPO2 from edible yam (*D. cayenensis-rotundata* cv. "Kponan") are negative at temperature range from 40 to 70 °C. The negative $\Delta S^{\#}$ values found indicates that there are significant processes of aggregation [33]. Low $\Delta S^{\#}$ values of PPO2 obtained reflect a low state of disorder during the transition phase following the thermal inactivation, which naturally leads to reduced the $\Delta H^{\#}$ values. The most common cause of the heat inactivation of enzymes is the loss of the native conformation (unfolding of the active tertiary protein structure to a disordered polypeptide), a process identified as thermodenaturation, which takes place as a result of increased molecular mobility at elevated temperature [34].

The second isoform of the PPO from edible yam (*D. cayenensis-rotundata* cv "Kponan") studied here therefore involves a structurally robust but temperature-sensitive enzymatic system, whose thermal denaturation is mainly under entropic control. In the context of browning inhibition in edible yam, it suggests that high temperature should be preferred to long heating time to achieve efficient deactivation of PPO2.

4. Conclusion

The results of this study reveals that thermal inactivation of second isoform of polyphenol oxidase (PPO2) from edible yam (D. cayenensis-rotundata cv "Kponan") could be described by a first-order kinetic model. The thermodynamic parameters (*D*-, Z_T-, *k*-, high activation energy and change in enthalpy) indicated that a high amount of energy was needed to initiate the PPO2 denaturation, most likely due to its stable molecular conformation. Therefore, PPO2 involves a structurally robust but temperature-sensitive enzymatic system, whose thermal denaturation is mainly under entropic control. In the context of browning inhibition in edible yam, high temperature should be preferred to long heating time to achieve efficient deactivation of PPO2.

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