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Use of Kinetic and Thermodynamic Parameters for the Prevention of Enzymatic Browning of Edible Yam *Dioscorea cayenensis-rotundata* cv. "Zrèzrou"

J. Kouamé, S.N. Gnangui, F.M.T. Koné* and L.P. Kouamé

Department of Food Science and Technology, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire
**Corresponding author*

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A B S T R A C T

The effect of heat treatment on edible yam (*Dioscorea cayenensis-rotundata* cv. "Zrèzrou") polyphenol oxidase activity was studied over a range of 35 to 75°C. Under all conditions investigated, a first-order kinetic model could describe the thermal inactivation data with k-values between 0.004 and 0.108 min^{-1}. The *D*- and *k*-values decreased and increased, respectively, with increasing temperature, indicating faster polyphenol oxidase inactivation at higher temperatures. Results suggested that polyphenol oxidase of *D. cayenensis-rotundata* cv "Zrèzrou" (PPOZ) is a relatively thermostable enzyme with a *Z*-value of 26.32°C and activation energy (*Ea*) of 78.93 $kJ.mol^{-1}$. The average values of enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$) and Gibbs free energy ($\Delta G^{\#}$) were respectively 76.20 $kJ.mol^{-1}$, -36.41 $J.mol^{-1}K^{-1}$ and 88.15 $kJ.mol^{-1}$at 308-348 K. 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 ($\Delta S^{\#}<0$).

Introduction

Yams belong to the genus Dioscorea in the family of Dioscoreacea and are monocotyledonous. They are an important source of carbohydrate for many people of the sub-Sahara region, especially in the yam zone of West Africa (Akissoe *et al.,* 2003). In Côted'Ivoire, due to traditions, yam plays a vital role in feeding the population, despite the strong expansion of cassava, bananas and rice(Amani *et al.,* 2008; Kone *et al.,* 2016). Its popular taste and high nutritional value and dietary allow him to enjoy a picture prestigious product, and support competition from other starchy foods such as cereals and cassava. Indeed, yam tubers, rich in starch are consumed almost in tropical regions in different forms (Amani *et al.,* 2008; Dabonne *et al.,* 2010; Kone *et al.,* 2016). Despite its strong contribution to the nutritional well-being and economic of populations, yam tubers are perishable and seasonal products. The loss is however higher in early tubers. Losses are due to rots caused by bacteria, fungi, damage during harvesting, transport and the germination, but also to oxidation reactions catalyzed endogenous phenolic compounds by polyphenol (Treche, 1989). When they were pelled, the pulp colour

ranges from creamy white to dark brown. This browning process leads to a change in flavour and a reduction in nutritional quality, especially ascorbic acid (Golan-Goldhirsh and Whitaker, 1984). The discoloration phenomenon has long been studied on fresh tubers and has mainly been associated with enzymatic browning, due to the action of polyphenol oxidase, peroxidase and to the production of polyphenols and derived products (Adams and Brown, 2007).

Polyphenoloxidase (PPO) is present in most fruits and vegetables. It is a copper containing oxidoreductase which catalyzes two distinct reactions involving phenolic compounds and molecular oxygen, namely a) the *o*-hydroxylation of monophenols to *o*-diphenols, or cresolase activity (monophenol, mono-oxigenase, EC 1.14.18.1); and b) the subsequent oxidation of *o*-diphenols to *o*-quinones, or catecholase activity (diphenol oxygen oxidoreductase, EC 1.10.3.1)**.** These quinones are highly reactive, electrophilic molecules that covalently modify one cross-link to a variety of cellular constituents (Abbattista Gentile *et al.,* 1988).

The main step in enzymatic browning is the oxidation of phenolic compounds by PPO in the presence of oxygen to corresponding quinone intermediates that polymerize to form undesirable pigments. Browning reactions in tubers such as fresh fruits, juices, and wines during processing and storage are well known and are an economic problem for producers and consumers. Several routes are planned to delay or block this physiological phenomenon (Cheriot, 2007). Currently, in addition to traditional technological processes (bleaching, freezing) or innovative (pulsed electric field, controlled atmosphere packaging), synthetic antioxidants are used to prevent these alterations. Natural additives, such as vitamin C, citric acid is also used against enzymatic browning, but the quantities used to effectively prevent the oxidation and the cost of these two compounds are expensive treatments for products low value added(Cheriot, 2007). This is why many current research aims to discover or invent ways to prevent these oxidations, which are effective, easy to implement, requiring little investment and inexpensive to use while being devoid of adverse effects on the organoleptic properties of food products.

Thus, the search for better methods of struggle against enzymatic browning through the mastery and control of PPO activity in foods today still arouses considerable interest in researchers.Several methods were used to prevent enzymatic browning but inactivation of PPO by thermal processing is considered the most effective method to inhibit enzymatic browning(Weemaes *et al.,* 1998). Therefore, the aim of this study is to prevent enzymatic browning by kinetic and thermodynamic parameters of polyphenoloxidase (PPOZ) from edible yam *Dioscorea cayenensis-rotundata* cv "Zrèzrou".

Materials and Methods

Enzyme source

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

Preparation of polyphenol oxidase

Freshly peeled tubers (150 g) were homogenized in 300 ml of cold NaCl 0.9% (w/v) for 10 min. The resulting homogenate

was centrifuged at 20000g for 10 min at 4°C. The supernatant represented the crude extract. This enzymatic solution (20 ml) was loaded onto aDEAE-Sepharose CL-6B gel (2.4 cm x 6.5 cm) that had been equilibrated previously with 100 mM phosphate buffer pH 6.0. The unbound proteins were removed from the column by washing with two column volumes of the same buffer pH 6.0. Proteins were eluted using a stepwise gradient with 0, 0.3, 0.5and 1 M NaCl in 100 mM phosphate buffer pH 6.0. Fractions (3 ml each) were collected at a flow rate of 180 ml/h and assayed for enzyme activity. The active fractions were pooled and saturated overnight by 80 % ammonium sulphate in a cold room. The precipitated pellet was then separated by centrifugation at 20000 g for 30 min and dissolved in 1 ml of 100 mM phosphate buffer pH 6.0.

The enzyme solution was loaded directly into a Sephacryl S-100 HR (1.6 cm x 64 cm), which was pre-equilibrated with the same buffer pH 6.0. Proteins were eluted at a flow rate of 20 ml/h using 100 mM phosphate buffer pH 6.0. Fractions (1 ml) were collected and active fractions were pooled together. The pooled fraction from the previous step was saturated to a final concentration of 1.7 M ammonium sulphate and applied on a Phenyl-Sepharose CL-6B column (1.4 cm x 7.5 cm) previously equilibrated with 100 mM phosphate buffer pH 6.0 containing 1.7 M ammonium sulphate.

The column was washed with equilibration buffer and the proteins retained were then eluted using a stepwise gradient with 1.7, 1, 0.7, 0.3 and 0 M ammonium sulphate in 100 mM phosphate buffer pH 6.0. Fractions of 1 ml were collected at a flow rate of 15 ml/h and active fractions were pooled together. The pooled fraction was dialyzed against 100 mM phosphate buffer pH 6.0 overnight in a cold room.

Enzyme assay

The PPOZ activity was assayed by a spectrophotometric procedure. The increase of absorbance at 480 nm at 30°C was recorded automatically for 10 min. The sample cuvette contained 0.8 ml substrate dopamine 10 mM in 100 mM phosphate buffer (pH = 6.0) and 100 µl undiluted enzyme extract. The blank sample contained only 0.8 ml substrate solution in 100 mM phosphate buffer pH 6.0. Experiments were performed in triplicate, and the results expressed as units (U) of enzymatic activity. One unit of enzymatic activity was defined as an increase in absorbance of 0.001 per min(Bartolo and Birk, 1998).

Protein determination

Protein was determined according to the method of Lowry *et al.*,(1951)using bovine serum albumin as standard.

Thermal inactivation

Thermal inactivation of the enzyme was investigated at pH 6.0 at various constant temperatures from 25to 75°C after exposure to each temperature for a period of 5 to 60 min. Aliquots were withdrawn at intervals and immediately cooled in ice bath, in order to stop heat inactivation.

Experiments were performed in triplicate. The residual enzymatic activity, determined at 30°C under the standard test conditions, was expressed as percentage activity of zero-time control of the untreated enzyme.

Kinetic analysis

The temperature dependence of the reaction rate constant for the studied enzyme served as the basis for fitting to the Arrhenius equation(Arrhenius, 1889):

$Ln\ (At/A0) = -\ kt$ (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 according to Stumbo(1973) as:

$D = 2.303/k$ (Eq.2)

The *Z*-value (°C) is the temperature increase needed to induce a 10-fold reduction in *D*-value(Stumbo, 1973). This *Z*-value follows the equation:

$log(D1/D2) = (T2 - T1)/Z$ (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).

Thermodynamic parameters

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

$k = Ae^{(-Ea/RT)}$ (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:

$lnk = lnA - (Ea/RT)$ (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*(Dogan *et al.*, 2002). 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:

$\Delta H^{\#} = Ea - RT$ (Eq.6)
$\Delta S^{\#} = R\ (ln\ A - ln\ K_B/\ h_P - ln\ T)$ (Eq.7)
$\Delta G^{\#} = \Delta H^{\#} - T\ \Delta S^{\#}$ (Eq.8)

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

Statistical analyses

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

Results and Discussion

Influence of temperature and time of pre-incubation

The profile of thermal stability of polyphenoloxidase from edible yam *Dioscorea cayenensis-rotundata* cv "Zrèzrou" (PPOZ) is showed in Table 1. For temperatures between 35-75°C, the

denaturation of the enzyme occurred after 5 min of pre-incubation in the phosphate buffer 100 mM (pH 6.0). The enzyme inactivation is total from 75°C after 25 min of pre-incubation. The logarithmic linear relationship between PPOZ activity and treatment time for the temperature range of 35-75°C (Fig. 1) followed first-order kinetics and was consistent with the relationships found in earlier studies on fruits and vegetables (Dimick *et al.,* 1951; Mc Cord and Kilara, 1983; Dogan *et al.,* 2005; Ditchfield *et al.,* 2006; Rapeanu *et al.,* 2006; Gnangui *et al.,* 2009). This result suggests that PPOZ is the only enzyme which is present in the reaction mixture of oxidizing the dopamine in the presence of molecular oxygen environment. This also reflected the only phase obtained for graphs *ln(At/Ao)* based on pre-incubation time. Indeed, the presence of isoforms usually results in a curve with several phases. The increasing temperature from 30 to 75°C results in a decrease of enzyme activities.

Rate constants of the reaction and half-life

The rate constants of the first order (*k*-value) of the catalyzed reaction of PPOZ during the thermal inactivation and the half-life are shown in Table 2. The half-life ($t_{1/2}$) of the catalyzed reactions by the PPOZ decrease as the temperature increases. At 60°C, it is equal to 27.72 min. Therefore the half-life decreases with increasing temperature. The rate constants of the enzyme protein increase with the temperature of pre-incubation. This observation reflects that this biocatalyst is sensitive to temperature change.

The *D*-, *Z*- and *Ea*-values of the polyphenol oxidase during thermal inactivation

The effects of temperature on *D*-, *Z*- and *Ea*-values for thermal inactivation of purified PPOZ are presented in Table 3. As expected the decimal reduction time decreases with temperature increase. At 75°C the *D*-value is almost 12.72 min. The *D*-values obtained at pre-incubation temperatures of 35 to 75°C, decreased linearlyfrom 767.67 to 8.63 min (Table 3, Fig. 2). The *log(D)*representation according to the pre-incubation temperature of PPOZ was described by an affine line. The equation of this representation is: $log(D) = -0.038T + 14.66$ ($R = 0.965$). It was determined that the *Z*-values are 26.32 °C. The graph of *lnk* as a function of the inverse of the temperature in *k* also gave an affine negative slope (Fig. 3). The kinetic is described by the equation: $k = ln\,[-9498.2x(1/T) + 25.173]$ ($R^2 = 0.965$) where *T* is the absolute temperature. The activation energy (*Ea*) of the polyphenol oxidase is positive and is 78.93kJ.mol^{-1}. The kinetic parameters *D*, *Z* and *Ea* permit to know the degree of enzyme stability to temperature variations. It is well to define these terms to better understand their involvement in the process of destabilization of the enzyme. The decimal reduction time (*D*) reflects the time required to reduce the enzyme activity by 90%. A high *D*-value indicates that the enzyme is thermostable. The thermal resistance (*Z*) is the elevation of the temperature necessary to reduce the *D*-value of 90 % and the activation energy (*Ea*) is the amount of energy required to keep the enzyme-substrate complex its activated form. The *Z*-value (25°C) obtained for PPOZ is lower than that obtained by Gnangui *et al.*,(2009) with PPO of yam tuber *D. cayenensis-rotundata*cv “Longbo". This result shows that PPO of this cultivar is more thermostable than that of the cultivar “Zrèzrou" from *D. cayenensis-rotundata*. According to Barrett *et al.*,(1999), low *Z*-values(3.1-20 °C) indicate a high sensitivity to heat, so that high *Z*-values indicate a high resistance of the enzyme against heat during heat treatments. But PPOZ is more resistant against heat than that reported by Vamos-Vigyazo(1981) on fruits and vegetables

whose values are between 8.5 and 10.1°C.Results obtained for the activation energy showed that PPOZ (83.96 kJ.mol^{-1}) is more sensitive to heat than PPO from wild rice (23.3 kJ.mol^{-1}, Aguilera *et al.*, 1987),plantain (18 kJ.mol^{-1}, Ngalani *et al.*, 1993)and yam *D.cayenensis-rotunda*ta cv "Longbo" (67.67 kJ.mol^{-1}, Gnangui *et al.*, 2009). However, it is less sensitive than PPO from banana (413 kJ.mol^{-1}, Dimick *et al.*, 1951) and apple (241-323 kJ.mol^{-1}, Yemenicioglu *et al.*, 1999).

Fig.1 Thermal inactivation curves of polyphenol oxidase from edible yam (*D. cayenensis-rotundata* cv "Zrèzrou") in sodium phosphate buffer (pH 6.0) in temperature range 35-75°C. $A_0$is the initial enzymatic activity and *At* the activity at each holding time

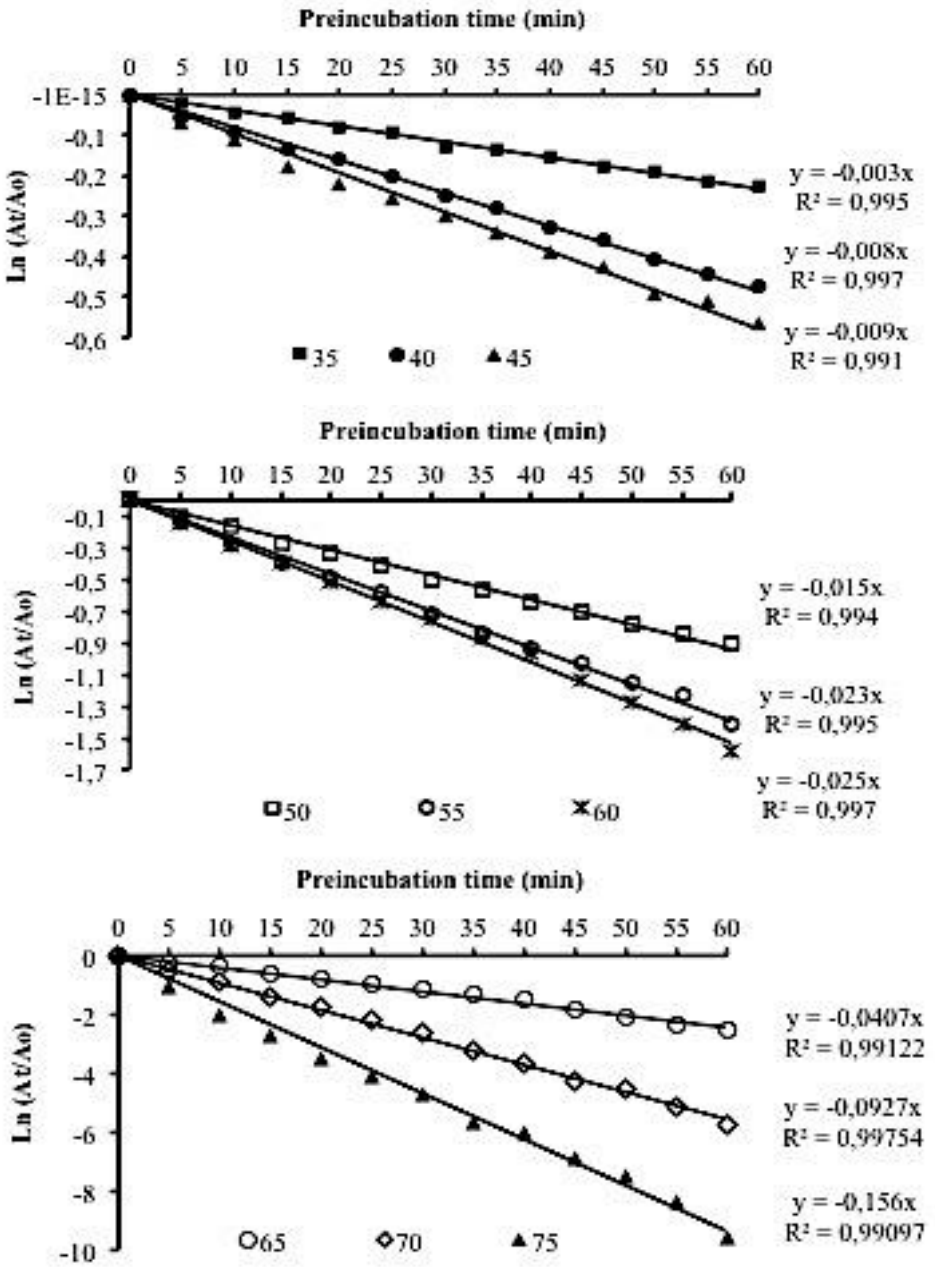

Fig.2 Effect of temperature on *D*-values for inactivation of edible yam (*D. cayenensis-rotundata* cv "Zrèzrou") polyphenol oxidase activity

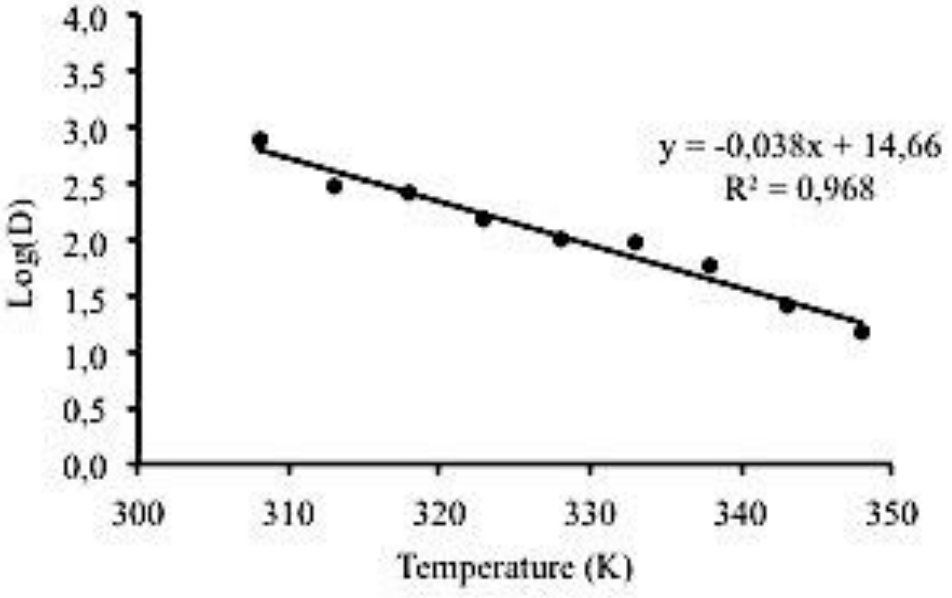

Fig.3 Temperature dependence of inactivation rate constant for thermal inactivation of edible yam (*D. cayenensis-rotundata* cv "Zrèzrou") polyphenol oxidase. 1/T represents the reciprocal of the absolute temperature

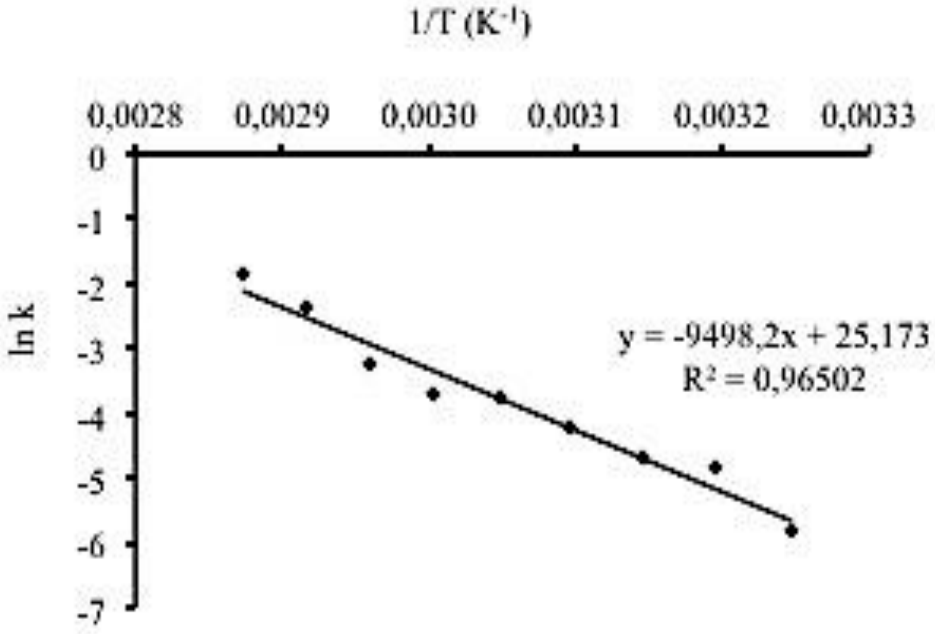

Table.1 Effect of treatment temperature and time on the polyphenol oxidase inactivation from edible yam (*D. cayenensis-rotundata* cv "Zrèzrou")

Temperature (°C)	Relative activity (%) at each treatment time (min)											
	5	10	15	20	25	30	35	40	45	50	55	60
35	97.73 ±0.002	95.43 ±0.001	94.41 ±0.002	92.10 ±0.002	91.28 ±0.003	87.99 ±0.001	87.19 ±0.003	85.56 ±0.001	83.86 ±0.001	82.56 ±0.003	80.82 ±0.001	79.61 ±0.003
40	94.47 ±0.003	91.30 ±0.002	87.46 ±0.002	85.21 ±0.002	81.79 ±0.002	77.72 ±0.002	75.50 ±0.002	72.18 ±0.002	69.70 ±0.002	66.34 ±0.002	64.15 ±0.003	62.09 ±0.002
45	93.52 ±0.003	89.67 ±0.002	83.86 ±0.001	80.36 ±0.003	77.34 ±0.001	74.20 ±0.003	70.96 ±0.003	67.64 ±0.001	65.22 ±0.001	61.26 ±0.003	60.05 ±0.002	56.89 ±0.001
50	90.39 ±0.001	84.74 ±0.002	76.72 ±0.002	71.46 ±0.001	66.38 ±0.002	60.23 ±0.001	57.06 ±0.001	52.83 ±0.001	49.25 ±0.002	45.83 ±0.002	42.74 ±0.001	40.78 ±0.002
55	87.41 ±0.002	75.69 ±0.003	67.64 ±0.003	61.82 ±0.002	56.05 ±0.002	48.82 ±0.001	43.04 ±0.002	39.46 ±0.001	36.06 ±0.002	31.63 ±0.002	29.23 ±0.002	24.17 ±0.003
60	85.83 ±0.001	75.20 ±0.001	67.71 ±0.002	59.67 ±0.003	52.62 ±0.003	46.90 ±0.002	41.77 ±0.003	37.95 ±0.002	32.30 ±0.002	28.06 ±0.003	24.22 ±0.002	20.47 ±0.001
65	77.03 ±0.003	67.98 ±0.001	53.96 ±0.003	46.63 ±0.001	39.65 ±0.002	31.44 ±0.001	26.47 ±0.001	21.98 ±0.002	16.69 ±0.002	12.14 ±0.001	9.33 ±0.003	8.17 ±0.002
70	60.59 ±0.002	40.62 ±0.003	25.13 ±0.002	17.20 ±0.002	10.94 ±0.003	7.17 ±0.001	3.95 ±0.001	2.47 ±0.002	1.37 ±0.001	1.09 ±0.001	0.60 ±0.002	0.33 ±0.002
75	34.23 ±0.001	13.53 ±0.001	6.72 ±0.002	2.99 ±0.001	1.61 ±0.002	0.89 ±0.003	0.36 ±0.001	0.25 ±0.001	0.10 ±0.003	0.06 ±0.001	0.02 ±0.002	0.01 ±0.001

Table.2 Reaction rate constant (*k*) and half-life ($t_{1/2}$) for heat inactivation of polyphenol oxidase from edible yam (*D. cayenensis-rotundata* cv "Zrèzrou") between 35 to 75°C

Temperature (°C)	*k*-value (min^{-1})	$t_{1/2}$ (min)	R^2
35	0.003 ± 0.001	231.00 ± 18.230	0.995 ± 0.002
40	0.008 ± 0.001	86.63 ± 11.030	0.997 ± 0.001
45	0.009 ± 0.002	77.00 ± 18.150	0.991 ± 0.003
50	0.015 ± 0.001	46.20 ± 3.100	0.994 ± 0.002
55	0.023 ± 0.003	30.13 ± 4.010	0.995 ± 0.002
60	0.025 ± 0.001	27.72 ± 1.110	0.997 ± 0.003
65	0.040 ± 0.00	17.33 ± 0.001	0.991 ± 0.002
70	0.092 ± 0.003	7.53 ± 0.246	0.997 ± 0.002
75	0.181 ± 0.001	4.44 ± 0.030	0.994 ± 0.001

Table.3 *D*-, *Z*- and *Ea*-values for thermal inactivation of polyphenol oxidase from edible yam (*D. cayenensis-rotundata* cv "Zrèzrou") in sodium phosphate buffer (pH 6.0) in the temperature range 35-75°C

Kinetic parameters	Values
D-values (min)	
D_{35}	767.67 ± 293.16
D_{40}	287.88 ± 36.65
D_{45}	255.89 ± 60.31
D_{50}	153.53 ± 10.29
D_{55}	100.13 ± 13.32
D_{60}	92.12 ± 3,69
D_{65}	57.58 ± 0.00
D_{70}	25.03 ± 0.82
D_{75}	14.76 ± 0.09
Z-value (°C)	26.32 ± 1.03
Ea ($kJ.mol^{-1}$)	78.93 ± 3.78

D-, *Z*- and *Ea* were respectively the decimal reduction time, the constant thermal resistance and the activation energy of polyphenol oxidase

Table.4 Thermodynamic parameters for edible yam (*D. cayenensis-rotundata* cv "Zrèzrou") polyphenol oxidase under heat treatment between 35 to 75°C (assuming a 1st-order kinetic model)

Temperature (°C)	Thermodynamic parameters		
	$\Delta H^{\#}$ ($kJ.mol^{-1}$)	$\Delta S^{\#}$ ($J.mol^{-1}.K^{-1}$)	$\Delta G^{\#}$ ($kJ.mol^{-1}$)
35	76.37 ± 6.66	-35.90 ± 10.94	87.42 ± 3.78
40	76.33 ± 6.81	-36.03 ± 11.05	87.60 ± 2.52
45	76.29 ± 6.97	-36.16 ± 10.88	87,78 ± 3.12
50	76.24 ± 7.12	-36.29 ± 11.12	87.97 ± 4.02
55	76.20 ± 7.27	-36.42 ± 11.02	88.15 ± 3.78
60	76.16 ± 7.42	-36.54 ± 10.99	88.33 ± 3.81
65	76.12 ± 7.58	-36.67 ± 11.08	88,51 ± 3.69
70	76.08 ± 7.73	-36.79 ± 11.21	88.70 ± 2.95
75	76.04 ± 7.89	-36.91 ± 11.33	88.88 ± 3.28
Mean	76.20 ± 7.27	-36.41 ± 11.06	88.15 ± 3.44

$\Delta H^{\#}$,$\Delta S^{\#}$ and $\Delta G^{\#}$ were respectively variations in enthalpy, entropy and Gibbs free energy

Thermodynamic analysis of thermal inactivation of polyphenoloxidase

Enzymes become inactive at temperatures above a critical level due to unfolding of the molecules (Lapanje, 1978). This process is usually reversible for most enzymes but prolonged heating results in irreversible loss of catalytic activity involving destruction of various covalent and no covalent interactions (Ghosh and Nanda, 1993).

Thermodynamic values of variation in enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$) and Gibbs free energy ($\Delta G^{\#}$) of PPOZ from edible yam calculated at different temperatures are shown in Table 4. At temperatures range from 35 to 75°C,the average values of $\Delta H^{\#}$, $\Delta S^{\#}$ and $\Delta G^{\#}$ were respectively 76.20, -36.41and 88.15kJ.mol^{-1}.The high enthalpy ($\Delta H^{\#}$) change obtained at these different temperaturesfrom edible yam *D. cayenensis-rotundata* cv "Zrèzrou" indicates that PPOZ 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(Marin *et al.*, 2003). Positive values of $\Delta H^{\#}$ indicate the endothermic nature of the oxidation reaction.

According to Anema and Mckenna(1996), the negative values found for entropy ($\Delta S^{\#}$)indicates that there are significant processes of aggregation. Indeed $\Delta S^{\#}$ is a physical parameter related to the concentration of the reagents, to the steric hindrance and to the orientation of functional groups. It can be interpreted as measuring the degree of disorder of a system at a microscopic level. Thus most of the system is high $\Delta S^{\#}$ least these elements are ordered, linked together, to produce effects and the higher the proportion of energy used to obtain a work where high values of free energies. Low $\Delta S^{\#}$ values of PPOZ obtained reflect a low state of disorder during the transition phase following the thermal inactivation, which naturally leads to reduced the $\Delta H^{\#}$ values.

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