International Journal of Biochemistry Research & Review

24(2): 1-11, 2018; Article no.IJBCRR.43643
ISSN: 2231-086X, NLM ID: 101654445

Thermal Inactivation of Crude Polyphenol Oxidases and Peroxidase from Three Tissues of Yellow-fleshed Cassava (*Manihot esculenta* Crantz) Root cv. Bocou 2

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2018/43643
Editor(s):
(1) Dr. Noureddine Benkeblia, Professor, Department of Life Sciences, The University of the West Indies, Jamaica.
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Reviewers:
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Complete Peer review History: http://www.sciencedomain.org/review-history/27552

Original Research Article

Received 31 July 2018
Accepted 16 November 2018
Published 03 December 2018

ABSTRACT

Aims: The objective of this work was to study the thermal inactivation of the polyphenol oxidase (PPO) and peroxidase (POD) activities of cassava (*Manihot esculenta* Crantz) root cv. Bocou 2 in order to prevent enzymatic browning.
Study Design: Crude PPO and POD from yellow-fleshed cassava root were subjected to heat treatment and their thermal inactivation characteristics were examined.
Place and Duration of the Study: The study was conducted at Biocatalysis and Bioprocesses Laboratory, Food Science and Technology Unit, Nangui Abrogoua University Abidjan, Côte d'Ivoire, between January and December 2015.

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Methodology: The crude PPO and POD were extracted from three tissues (cortex, cambium and central pith) of yellow-fleshed cassava root cv. Bocou 2. The thermal inactivation of these enzymatic activities was evaluated between 50 and 70 °C. The kinetic data of thermal inactivation and thermodynamics were analysed.
Results: The $t_{1/2}$- and D-values decreased with increasing temperature, indicating a faster inactivation of PPO and POD at higher temperatures. Z-values ranged from 16.10 to 27.70 °C and activation energy (Ea) from 73.37 to 129.66 kJ mol-1. Thermodynamic investigations indicated that the oxidation reactions involving these enzyme activities were: not spontaneous ($\Delta G > 0$), slightly endothermic ($\Delta H > 0$) and reversible ($\Delta S < 0$).
Conclusion: The PPO and POD activities from yellow-fleshed cassava root decreased due to heat denaturation with increasing temperature from 60 to 70 °C. These kinetic data can be used to prevent enzymatic browning in cassava roots.

Keywords: Cassava; Manihot esculenta Crantz; post-harvest; polyphenol oxidases; peroxidase; thermal inactivation; thermodynamics.

1. INTRODUCTION

Cassava root, *Manihot esculenta* Crantz*,* is reported to be one of the most important of root and tuber crops in the tropics [1]. It is one of the most important food products by the volume of its production and its consumption in Africa, Latin America, Europe and Asia [2,3]. The main food products from cassava root are “gari”, “fufu” and “Attieke” [4]. In addition, the International Institute of Tropical Agriculture (IITA) is promoting the incorporation of high quality cassava flour into wheat flour for bakery bread making (up to 20% incorporation) and confectionery. To produce high quality cassava flour, the cassava roots must be well preserved in order to prevent the browning of the pulp which may affect the colour of the flour. The producers believe that cassava cannot be stored after harvest. They do not have adequate methods for preserving cassava roots. Thus, several factors influence cassava's ability to meet growing market demands. Indeed, this foodstuff has a very short self-life because of a physiological process called post-harvest physiological deterioration (PPD). This post-harvest deterioration quickly renders the taste and colour of the pulp unpleasant and the roots unmarketable [5]. As a result, cassava roots must be consumed soon after harvest [6]. The processes involved in PPD are a cascade of biochemical reactions involving reactive oxygen species occurring as a result of shocks, as in the case of the plant's response to injury. Specific genes involved in PPD have been identified, characterised, and their expression evaluated [7]. The damages due to mechanical injuries during the harvest lead to a contact between PPO and POD with phenolic compounds and oxygen in the air. These PPO and POD catalyse the oxidation of phenolic compounds thereby producing undesirable pigments [8]. Indeed, enzymes such as phenylalanine ammonia-lyase, polyphenol oxidases and peroxidases are involved in the PPD process of the cassava root [9]. Thus, treatments aimed at inhibiting the activity of these enzymes could slow the PPD.

The aim of this work was therefore to study the effect of thermal treatments on the activities of the crude PPO and POD from different tissues (cortex, cambium and central pith) of the yellow-fleshed cassava root cv. Bocou 2. The kinetic and thermodynamic parameters of thermal inactivation were determined in order to provide helpful information for preventing of enzymatic browning of cassava roots.

2. MATERIALS AND METHODS

2.1 Plant Materials

The variety of yellow-fleshed cassava named Bocou 2 was use for this study. This variety was chosen because it is a high yield variety being popularise in Côte d'Ivoire. It was grown at an experimental garden of Soumalékro (Bonoua, Côte d’Ivoire). The tuberous roots were randomly harvested twelve months after planting. These were immediately transported to the Laboratory of Biocatalysis and Bioprocess, Food Science and Technology Unit, at Nangui Abrogoua University and stored at -20°C until used.

2.2 *In situ* Description of Enzymatic Browning

The *in situ* description of enzymatic browning of cassava slices was determined according to the method of Lee et al. [10]. Thin slices (about 19.62 cm) were cut from the cassava root

transversally with a stainless steel kitchen knife. Each slice placed in an individual Petri dish was immersed for 2 min in 100 mM citrate-phosphate, phosphate, Tris or glycine buffer in pH range of 6.6-9.6. Then, 1.0 mL of 5 mM substrate (pyrogallol, dopamine, pyrocatechol, 4-methylcatechol and gaïacol), was spread over the whole surface of each slice. A lid was placed on each Petri dish to minimise evaporation. All the slices were then incubated at 25°C for 10 min. From this profile the total area and the percentage of brown area were measured immediately after cutting by image analysis.

2.3 Enzymes Extraction

An aliquot of each fresh tissue (cortex, cambium and central pith) of cassava roots (150 g) was ground using a crusher (Binatone Blender, model BLG 555, China) in 300 mL NaCl solution 0.9% (w/v). The homogenate was centrifuged at 4ºC for 30 min at 8 000 g. The supernatant was collected and used as the crude extract.

2.4 Polyphenol Oxidases (PPO) Assay

The PPO activity was determined using pyrogallol, dopamine, pyrocatechol and 4-methylcatechol as the substrates [11]. An assay mixture (2 mL) consisting of 100 mM citrate phosphate, Tris and glycine buffer (1.1 mL) in pH range of 6.6-9.6, 10 mM substrate (0.8 mL) and enzymatic extract (0.1 mL) was incubated at 25ºC for 10 min. After incubation, the activity was determined by measuring the absorbance of the reaction mixture at 480 for dopamine oxidase and pyrogallol oxidase activities and 410 nm pyrocatechol oxidase and 4-methylcatechol oxidase activities. Experiments were performed in triplicate, and the results expressed as units of enzymatic activity per mg of protein (standard test conditions). One unit of enzymatic activity (U) was defined as an increase in absorbance of 0.001 per min [12].

2.5 Peroxidase (POD) Assay

The POD activity was assayed using hydrogen peroxide (H_2O_2) as the oxidising substrate and guaiacol as the organic substrate [13]. An assay mixture (2 mL) consisting of 100 mM citrate-phosphate, phosphate, Tris and glycine buffer (1.9 mL) in pH range of 6.6-9.6, 10 mM substrate (1 mL of H_2O_2 and 0.2 mL of gaïacol) and enzyme solution (0.1 mL) was incubated at 25ºC for 10 min. After incubation, the activity was determined by measuring the absorbance of the reaction mixture at 480 nm. Experiments were performed in triplicate, and the results expressed as units of enzymatic activity per mg of protein (standard test conditions). One unit of enzymatic activity was defined as an increase in absorbance of 0.001 per min [12].

2.6 Protein Determination

Protein contents of the crude extract were determined by the method of Lowry et al. [14] using bovine serum albumin (BSA) as a standard.

2.7 Thermal Inactivation and Kinetic Data Analysis

For thermal inactivation, the crude enzyme extract was preincubated at temperatures ranging from 50 to 70°C. Aliquots were withdrawn at time intervals and cooled at room temperature for 10 min. The enzymatic activities of the aliquots were measured under standard conditions.

The temperature dependence of the reaction rate constant for the studied enzymatic activities served as the basis for fitting to the Arrhenius equation [15]:

$$\ln [At/A\circ] = -kt \quad (1)$$

Where A_t is the residual enzyme activity at time t, A_0 is the initial enzyme activity.

The temperature of treatment and the rate constant in a denaturation process was related according to the Arrhenius equation.

$$k = A.e^{(-Ea/RT)} \quad (2)$$

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. Equation 2 can be transformed to:

$$\ln k = \ln A - Ea/R \times T. \quad (3)$$

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 [16,17].

The D-value is the time needed to reduce the initial activity to 90%. It is related to k value by (Equation 4) and the mathematical expression [18]:

$$D = 2.303/k. \quad (4)$$

The Z-value is the temperature increase needed for a 90% reduction in D-value (temperature sensitivity parameter), and follows the equation:

$$\log [D_1/D_2] = [T_2-T_1]/Z_T \quad (5)$$

Where T_1 and T_2 represent the lower and higher temperatures (°C or °K), respectively; D_1 and D_2 are D-values at the lower and higher temperatures in minutes. The Z-values were estimated from the linear regression of log D and temperature (T).

2.8 Thermodynamic Analysis

The values of the activation energy (Ea) and Arrhenius constant (A) allowed the determination of different thermodynamic parameters such as variations in enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG) according to the following expressions [18,19]:

$$\Delta H = Ea - RT \quad (6)$$

$$\Delta S = R (\ln A - \ln K_B/h_P - \ln T) \quad (7)$$

$$\Delta G = \Delta H - T \Delta S \quad (8)$$

Where K_B is the Boltzmann constant (1.3806 x 10^{-23} J/K), h_P is the Planck constant (6.626 x 10^{-34} J.s), T is the absolute temperature.

2.9 Statistical Analysis

Data obtained from the chemical analyses were recorded in triplicate. The statistical treatment of enzymatic activities consisted of an analysis of variance (ANOVA) with a classification criterion using STATISTICA software (STATISTICA version 7.1). The means were compared by the Duncan test at the 5% significance level.

3. RESULTS

3.1 *In situ* Browning

Fig. 1 shows that various phenolic compounds (pyrogallol, dopamine, pyrocatechol, 4-methylcatechol and gaïacol) were oxidised on contact with the tissues of the yellow-fleshed cassava root cv. Bocou 2. Compared to the control (Fig. 1A), the slices tested were discoloured respectively in yellow-orange (Fig. 1B), brown (Fig. 1C) and pink (Fig. 1D, 1E and 1F) when pyrogallol, dopamine, pyrocatechol, 4-methylcatechol and guaiacol were used as a substrate. The browning was intense for pyrogallol and dopamine.

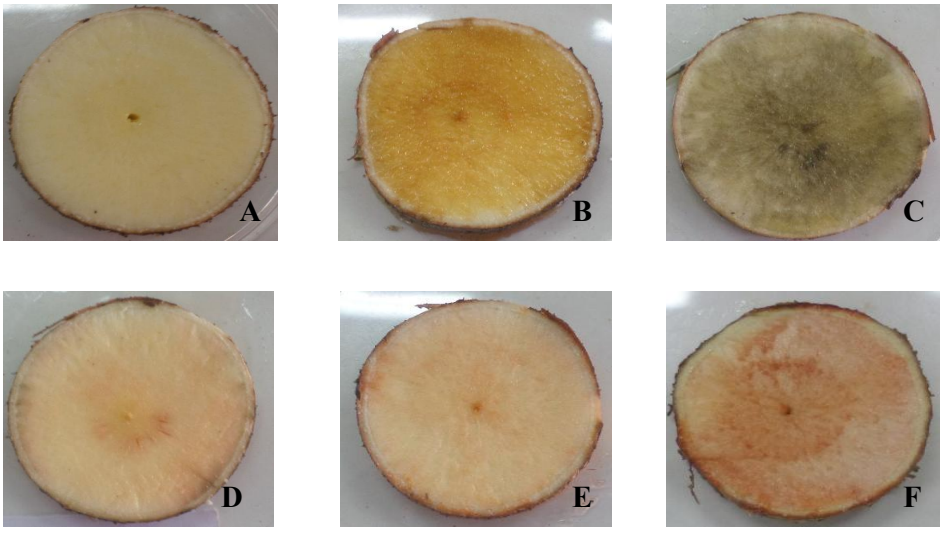

Fig. 1. Enzymatic browning of yellow-fleshed cassava (*Manihot esculenta* Crantz) roots cv. Bocou 2 after adding various phenolic substrates on root slices

(A) Control: No phenolic substrate. (B) Addition of pyrogallol. (C) Addition of dopamine. (D) Addition of pyrocatechol. (E) Addition of 4-methylcatechol. (F) Addition of guaiacol

3.2 Optimum Temperature and Thermostability

The specific activities of the enzymes studied are shown in Table 1. The activities of PPO and POD ranging from 6.14 ± 0.40 to 19.97 ± 0.34 U mg $^{-1}$, were significantly higher ($P < 0.05$) in the crude extract from the cambium and the central pith compared to those of the crude extract from the cortex of the yellow-fleshed cassava root (Table 1).

3.3 Thermal Inactivation Kinetics of PPO and POD Activities from Three Tissues of Yellow-fleshed Cassava Root cv. Bocou 2

The results of thermal inactivation of PPO and POD activities from yellow-fleshed cassava root, measured over temperature range of 50-70°C, are shown in Tables 4 and 5. Increasing the incubation temperature from 50 to 70°C, resulted in a decrease in $t_{1/2}$- (time where the residual activity reaches 50% defined as half-life) and D-values (Tables 2 and 3). From 65°C, $t_{1/2}$- and D-values were less than or equal to 5.09 min and 14.39 min, respectively, for all of these enzyme activities whatever the tissue considered. The Z-values were found to be ranged between 16.10 and 27.70°C for all enzyme activities (Table 4). From the Arrhenius plot, the activation energy values of these enzyme activities ranged from 73.37 to 129.66 kJ/mol (Table 5).

3.4 Thermodynamic Analysis of Thermal Denaturation

The determination of the thermodynamic parameters of denaturation provides information on the enzyme thermal stability for each step of the heat-induced denaturation process. The average values of ΔH, ΔS and ΔG ranged from 126.97 to 70.52 (kJ/mol), -61.21 to 3.22 (J mol^{-1} K^{-1}) and 83.06 to 147.74 (kJ/mol), respectively (Tables 6, 7 and 8).

Table 1. Specific activities of crude polyphenol oxidase and peroxidase from three tissues of yellow-fleshed cassava root (*Manihot esculenta* Crantz) cv. Bocou 2

	Specific activity (U. mg $^{-1}$)				
Tissues	**Pyrogallol oxidase**	**Dopamine oxidase**	**Pyrocatechol oxidase**	**4-Methylcatechol oxidase**	**Peroxidase**
Cortex	12.50±0.22^{h}	6.14±0.40^{a}	6.32±0.22^{a}	7.33±0.17^{c}	7.73±0.26^{bc}
Cambium	15.74±0.31^{i}	6.81±0.05^{b}	6.23±0.69^{a}	10.29±0.12^{f}	9.62±0.34^{e}
Central pith	19.97±0.34^{j}	7.24±0.49^{c}	8.16±01.44^{d}	11.71±0.34^{g}	11.53±0.39^{g}

The values are means ± standard deviation of triplicate determinations. On the lines or in columns, the means affected of same letters are not significantly different ($p<0.05$) according to the test of Duncan. "U" was defined as an increase in absorbance of 0.001 per min

Table 2. $t_{1/2}$-values for thermal inactivation of activities of crude polyphenol oxidase and peroxidase from three tissues of yellow-fleshed cassava root (*Manihot esculenta* Crantz) cv. Bocou 2 at temperature range of 50-70°C

Enzyme activities	**Tissues**	**$t_{1/2}$ (min)**				
		50	**55**	**60**	**65**	**70**
Pyrogallol oxidase activity	Cortex	33.77	15.85	5.27	3.18	2.45
	Cambium	23.55	12.07	6.25	4.09	3.42
	Central pith	29.45	11.75	5.33	3.36	2.78
Dopamine oxidase activity	Cortex	54.5	22.87	11.77	5.09	3.17
	Cambium	35.85	27.77	10.08	4.33	3.55
	Central pith	47.54	19.13	8.45	3.84	2.77
Pyrocatechol oxidase activity	Cortex	28.74	15.61	9.17	4.33	2.95
	Cambium	33.56	23.45	15.77	4.08	3.27
	Central pith	14.33	11.27	6.3	3.71	2.31
4-methylcatechol oxidase activity	Cortex	8.37	5.33	4.87	3.25	2.7
	Cambium	10	6.33	5.26	4.21	3.72
	Central pith	8.5	5.97	4.55	3.08	2.21
Peroxidase activity	Cortex	9.36	6.51	4.38	3.07	2.14
	Cambium	9.86	7.16	4.36	2.97	2.08
	Central pith	10.45	8.29	4.55	3.24	2.2

Table 3. D-values for thermal inactivation of activities of crude polyphenol oxidase and peroxidase from three tissues of yellow-fleshed cassava root (*Manihot esculenta* Crantz) cv. Bocou 2 at temperature range of 50-70°C

Enzyme activities	Tissues	D-values (min)				
		50	55	60	65	70
Pyrogallol oxidase activity	Cortex	112.23	52.67	17.51	10.57	8.14
	Cambium	78.26	40.11	20.77	13.59	11.37
	Central pith	97.87	39.05	17.71	11.17	9.24
Dopamine oxidase activity	Cortex	181.12	76.00	39.11	16.92	10.53
	Cambium	119.14	92.29	33.50	14.39	11.80
	Central pith	157.99	63.57	28.08	12.76	9.21
Pyrocatechol oxidase activity	Cortex	95.51	51.88	30.47	14.39	9.80
	Cambium	111.53	77.93	52.41	13.56	10.87
	Central pith	47.62	37.45	20.94	12.33	7.68
4-methylcatechol oxidase activity	Cortex	27.82	17.71	16.18	10.80	8.97
	Cambium	33.23	21.04	17.48	13.99	12.36
	Central pith	28.25	19.84	15.12	10.24	7.34
Peroxidase activity	Cortex	31.11	21.63	14.56	10.20	7.11
	Cambium	32.77	23.79	14.49	9.87	6.91
	Central pith	34.73	27.55	15.12	10.77	7.31

4. DISCUSSION

The browning observed in slices of the yellow-fleshed cassava root cv Bocou 2 by exogenic contribution of phenolic compounds could be due to the presence of PPO and POD activities in this biological environment. Indeed, the availability of PPO and POD substrates, and the presence of the enzyme activities in the cells lead to a browning initiation [20].

The values of specific activities of crude PPO and POD from yellow-fleshed cassava ranged from 6.14±0.40 to 19.97±0.34 U mg $^{-1}$, depending on tissue type. These activities were higher in the central pith than the cambium and cortex. Accordingly, the central pith would be the main site of the enzymatic browning of the cassava root. Moreover, pyrogallol oxidase activity would be the most implicated in the browning process. Specific activities of crude PPO and POD from yellow-fleshed cassava root were found higher than those of potatoes root. Indeed, variation in PPO and POD activities depends on species and plant sources [21].

During inactivation process, PPO and POD activities decreased with increasing temperatures. At higher temperatures, the enzymatic proteins most likely underwent denaturation and lost their activity. Note that, thermal denaturation is the heat induced spontaneous, irreversible breakdown of the secondary and tertiary structure of the enzyme protein such that the enzyme will no longer function and cannot re-activate.

The half-life ($t_{1/2}$) is another parameter that plays an important role in the characterisation of enzyme stability [22]. Increasing temperature from 50°C to 70°C resulted in a decrease in $t_{1/2}$-values of crude PPO and POD activities tested. It is worth noting that from 65°C, $t_{1/2}$-values of these enzyme activities were less than 5.09 min.

The decimal reduction time (D-value) is the time, at a given temperature and pressure, needed for 90% reduction of the initial activity. D-values of crude PPO and POD activities from yellow-fleshed cassava root ranged from 181.12 min to 6.91 min for temperatures between 50°C and 70°C. At 65°C, recorded values ranged from 9.87 to 16.92 min. These D-value ranges were lower and narrower than those recorded for PPO in tubers of Sweet Potato (*Ipomoea Batatas (L.)*) which ranged from 305.7 to 35 min between 60 and 75°C [23]. Comparing these D- and $t_{1/2}$-values at 65°C, yellow-fleshed cassava root PPO and POD activities ($t_{1/2}$ = 5.09-2.97 min; D = 16.92-9.87 min) were less thermostable.

Table 4. Z-values for thermal inactivation of activities of crude polyphenol oxidase and peroxidase from three tissues of yellow-fleshed cassava root (*Manihot esculenta* Crantz) cv. Bocou 2 at temperature range of 50-70°C

	Z-Values (°C)				
Tissues	**Pyrogallol oxidase activity**	**Dopamine oxidase activity**	**Pyrocatechol oxidase activity**	**4- methylcatechol oxidase activity**	**Peroxidase activity**
Cortex	16.00	16.80	19.72	31.05	41.32
Cambium	23.31	17.76	17.98	48.30	28.81
Central pith	19.26	15.79	24.21	34.24	28.40

Table 5. Activation energy (Ea) values for thermal inactivation of activities of crude polyphenol oxidase and peroxidase from three tissues of yellow-fleshed cassava root (*Manihot esculenta* Crantz) cv. Bocou 2 at temperature range from 50-70°C

Tissues	Ea Values (kJ/mol)				
	Pyrogallol activity	**Dopamine oxidase activity**	**Pyrocatechol oxidase activity**	**4-methylcatechol oxidase activity**	**Peroxidase activity**
Cortex	105.51	129.24	90.68	81.63	76.62
Cambium	103.33	119.42	112.11	80.1	84.73
Central pith	112.22	129.66	88.62	73.37	75.43

Table 6. Enthalpy variation values of activities of crude polyphenol oxidase and peroxidase from three tissues of yellow-fleshed cassava root (*Manihot esculenta* Crantz) cv. Bocou 2 under heat treatment between 50 to 70°C

Enzymes activities	Tissues	ΔH (kJ/mol)				
		50	55	60	65	70
Pyrogallol oxidase activity	Cortex	102.82	102.78	102.74	102.7	102.66
	Cambium	100.65	100.6	100.56	100.52	100.48
	Central pith	109.54	109.5	109.45	109.41	109.37
Dopamine oxidase activity	Cortex	126.56	126.51	126.47	126.43	126.39
	Cambium	116.73	116.69	116.65	116.61	116.57
	Central pith	126.97	126.93	126.89	126.85	126.81
Pyrocatechol oxidase activity	Cortex	88.00	87.96	87.91	87.87	87.83
	Cambium	109.43	109.39	109.35	109.30	109.26
	Central pith	85.94	85.90	85.85	85.81	85.77
4-methylcatechol oxidase activity	Cortex	78.94	78.90	78.86	78.82	78.78
	Cambium	77.41	77.37	77.33	77.29	77.25
	Central pith	70.68	70.64	70.60	70.56	70.52
Peroxidase activity	Cortex	73.94	73.90	73.86	73.82	73.77
	Cambium	82.05	82.01	81.96	81.92	81.88
	Central pith	72.75	72.71	72.67	72.63	72.58

Table 7. Entropy change values of activities of crude polyphenol oxidase and peroxidase from three tissues of yellow-fleshed cassava root (*Manihot esculenta* Crantz) cv. Bocou 2 under heat treatment between 50 to 70°C

Enzymes activities	Tissues	ΔS (J mol^{-1} k^{-1})				
		50	55	60	65	70
Pyrogallol oxidase activity	Cortex	-22.66	-22.79	-22.92	-23.04	-23.16
	Cambium	-13.44	-13.57	-13.69	-13.82	-13.94
	Central pith	-10.70	-10.83	-10.96	-11.08	-11.20
Dopamine oxidase activity	Cortex	-60.90	-61.02	-61.15	-61.27	-61.40
	Cambium	-0.97	-1.10	-1.23	-1.35	-1.47
	Central pith	-61.21	-61.34	-61.47	-61.59	-61.71
Pyrocatechol oxidase activity	Cortex	-10.52	-10.65	-10.78	-10.90	-11.02
	Cambium	-6.26	-6.39	-6.51	-6.64	-6.76
	Central pith	2.82	2.70	2.57	2.45	2.32
4-methylcatechol oxidase activity	Cortex	-16.26	-16.38	-16.51	-16.63	-16.76
	Cambium	-22.68	-22.81	-22.93	-23.06	-23.18
	Central pith	-40.17	-40.30	-40.43	-40.55	-40.67
Peroxidase activity	Cortex	-30.81	-30.94	-31.07	-31.19	-31.31
	Cambium	-6.81	-6.93	-7.06	-7.18	-7.31
	Central pith	-35.15	-35.28	-35.41	-35.53	-35.65

Table 8. Gibbs free energy change values of activities of crude polyphenol oxidase and peroxidase from three tissues of yellow-fleshed cassava root (*Manihot esculenta* Crantz) cv. Bocou 2 under heat treatment between 50 to 70°C

Enzymes activities	Tissues	ΔG (kJ/mol)				
		50	55	60	65	70
Pyrogallol oxidase activity	Cortex	102.16	102.22	102.33	102.35	102.34
	Cambium	100.03	100.08	100.12	100.14	100.12
	Central pith	113.00	113.05	113.10	113.16	113.21
Dopamine oxidase activity	Cortex	146.23	146.53	146.84	147.14	147.45
	Cambium	117.05	117.05	117.06	117.07	117.07
	Central pith	146.74	147.05	147.36	147.66	147.97

Enzymes activities	Tissues	ΔG (kJ/mol)				
		50	55	60	65	70
Pyrocatechol oxidase activity	Cortex	91.40	91.45	91.5	91.56	91.61
	Cambium	111.45	111.48	111.51	111.55	111.58
	Central pith	85.02	85.01	85.00	84.99	84.97
4-methylcatechol oxidase activity	Cortex	84.19	84.28	84.36	84.44	84.52
	Cambium	84.74	84.85	84.97	85.08	85.20
	Central pith	83.66	83.86	84.06	84.26	84.47
Peroxidase activity	Cortex	83.89	84.05	84.20	84.36	84.51
	Cambium	84.25	84.28	84.32	84.35	84.39
	Central pith	84.10	84.28	84.46	84.63	84.81

Most of Z-values recorded for crude PPO and POD of yellow-fleshed cassava root (16.00 – 48.30) were lower than Z-values of PPO from edible yams (*Dioscorea cayenensis-rotundata* cv. Longbô (35-75°C) and similar to that of PPO from *Dioscorea cayenensis-rotundata* cv. Kponan (38.48°C) [24,25]. In general, low Z-values indicate greater sensitivity to heat [26]. The activation energy (*Ea*) values of crude PPO and POD activities from yellow-fleshed cassava root ranged from 73.37 to 129.66 kJ mol^{-1}. The *Ea* values from this study suggest that PPO and POD from yellow-fleshed cassava root can be classified as heat-sensitive enzymes. The values of ΔH, ΔS and ΔG of PPO and POD activities studied ranged from 126.97 to 70.52 kJ mol^{-1}, -61.21 to 3.22 J mol^{-1} K^{-1} and 146.74 to 83.06 kJ mol^{-1}, respectively. The high values of change in enthalpy (ΔH) obtained for the different treatment temperatures indicate that the crude enzymes underwent a considerable change in conformation during denaturation. Positive values of ΔH of the PPO and the POD indicate the endothermic nature of the oxidation reactions. This energy was lower than that of potato PPO **(**98.02 kJ mol^{-1}) and similar to those of PPO and POD of the different parts of the white yam (Dioscorea rotundata) [27,28]. When the enzyme molecule folds/unfolds from its stable native structure, the randomness of the system, entropy, changes [29]. The entropy (ΔS) change indicates the net enzyme and solvent disorder [30]. The possible reason for negative ΔS of these data is an increase in order of the system through an aggregation process in which a few inter- and/intramolecular bonds are formed [31]. The negative entropy changes during inactivation are consistent with the compaction of the enzyme molecule [29]. ΔH, the enthalpy change, is a measure of the number of bonds broken during inactivation [30,32]. The positive values of ΔH indicate that denaturation of PPO and POD activities from yellow-fleshed cassava root is an endothermic reaction. Positive values of ΔH have also been reported for peroxidase from strawberry and kinnow [33,34]. The ΔG value is directly related to protein stability: the higher the ΔG is, the higher will be the enzyme stability. When the incubation temperature was elevated from 30 to 70°C, there was a significant reduction of ΔG values for PPO and POD activities tested (147.97 to 83.06 kJ mol^{-1}). All ΔG values were in agreement with the relative constant value of 100 kJ/mol characteristic of the protein denaturation reaction as already found for other PPOs [35,36].

5. CONCLUSION

The *In situ* browning description showed that tissues of yellow cassava root (*Manihot esculenta* Crantz) cv. Bocou 2 were susceptible to browning. Since enzymatic activities were higher in the central pith than the cambium and cortex, the central pith would be the main site of the enzymatic browning of the cassava root. Of all activities of PPOs measured, pyrogallol oxidase activity appeared as the most enzymatic activity implicated in the browning process. For thermal inactivation study, the enzymatic activities decreased with increasing temperature from 60°C to 70°C. Thus, these enzymes were sensitive to relative high temperature. These thermal characteristics can be used to prevent enzymatic browning in cassava roots. For the future studies, the blanching of the fresh cassava root should be done and also the studies should be related to methods of the conservation of the cassava root.

ACKNOWLEDGEMENTS

This research was funded by the PAES UEMOA programme N°P- Z1 – IAD – 002, and the West Africa Agricultural Productivity Programme (WAAPP).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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