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Physicochemical Characterization and Effect of Thermal Treatments on the Enzymatic Browning Inhibition of Violet Eggplant (*Solanum melongena* L.) Cultivated in Daloa (Côte d'Ivoire)

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Authors' contributions

This work was carried out in collaboration among all authors. Author YJC designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors DZAB and FJB 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

Background: The polyphenol oxidase (PPO) responsible for the enzymatic browning of fruits and vegetables, has been involved in the undesirable brown discolouration of food products that resulted in negative effects on colour, taste, and nutritional value. This is a generally undesired process and needs to be prevented in food technology.
Objective: The present work was carried out to evaluate the effect of chemical and thermal treatments on browning inhibition of eggplant fruit (*Solanum melongena* L.).

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Materials and Methods: A screening of PPOs activities from eggplant was carried out. The physicochemical characteristics and thermal stability of main PPOs activities were determined in order to develop methods of anti-browning.
Results: Dopamine oxidase and pyrocatechol oxidase activities were the most active main eggplant fruit PPO activities. Maximal PPO activity was found at 30°C, pH 7.0 for dopamine and 25°C, pH 6.6 for pyrocatechol. The enzymes were stable and retained almost all of their catalytic activity at their optimum temperature (30 and 25°C) for 120 min and their pH stability was in the range of 5.0 - 7.0. Polyphenol oxidases (dopamine oxidase and pyrocatechol oxidase) remained their full activity in the presence of ion Na^{+}, Cu^{2+}, Pb^{2+} (1 mM) but were inhibited strongly by the ion Fe^{2+} and Pb^{2+} (5 mM). On the other hand, the ion K^{+}, Ba^{2+} and chemical agents, EDTA, citric acid have virtually no effect on dopamine oxidase and pyrocatechol oxidase activities. Energy for inactivation (E_a) obtained using dopamine and pyrocatechol were 30.8 kJ/mol and 7.1 kJ/mol from respective substrates.
Conclusion: Ascorbic acid was a better inhibitor where 82.32% of PPOs inhibition was achieved. At 65°C, their D-values ranged from 44.72 to 72.72 min. Hence, heat treatment at 65°C for 30 min reduced browning of eggplant fruit. These data regarding the properties of PPO should enhance understanding of the browning reaction in eggplant and lead to the development of techniques for controlling this undesirable process.

Keywords: Browning; inhibition; polyphenoloxidase; eggplant; Solanum melongena L.

1. INTRODUCTION

Eggplants belong to the family of *Solanaceae* and the plant genus *Solanum*. It is a climacteric fruit originally from India and China. They are an important food both from the economic and nutritional points of view and are cultivated and consumed world-wide [1]. According to FAO statistic, it was produced 54.07 million tons in 2018 [2]. Both cultivated and wild types of this vegetable are consumed in Côte d'Ivoire. They include *S. aethiopicum*, *S. melongena*, and *S. macrocarpon*, with *S. torvum* and *S. anguivi* representing the wild forms. Moreover, various parts of these plants are used in indigenous medicine by virtue of their high levels of phytochemical components [3,4]. Eggplants are rich in essential nutrients needed for the normal growth of humans [3]. They contain the pro-vitamin A, thiamine, riboflavin, niacin, ascorbic acid, especially polyphenols that retard the process of oxidation to keep the optimum level of reactive oxygen and nitrogen species and prevent a large number of chronic diseases [5].

In-vitro studies have shown that eggplant extracts suppress the development of blood vessels required for tumor growth and metastasis [6], inhibit protein-activated receptor 2 inflammation that has been implicated in atherosclerosis [7]. Nevertheless, one drawback of increasing the concentration of the polyphenolic antioxidants in eggplant is that their oxidation causes browning of the fruit flesh after exposure to the air, which may reduce the apparent quality of the eggplant in consumers' eyes.

Despite the high antioxidant capacity of eggplant attributable to its high content of phenolic compounds, the strong tendency of eggplant fruit to undergo browning remains a major problem during storage and processing. Preserving the quality and nutritional value of fruits and vegetables between harvest and consumption is widely agreed to be very important. Eggplant quality and shelf-life are markedly reduced by the development of skin and tissue browning [8]. The unattractive appearance and concomitant off-flavour development associated with browning adversely affects consumer acceptability and palatability [9]. Furthermore, the brown pigments may change the biochemical and nutritional characteristics of eggplant. In general, browning of foods is caused by the enzyme-catalyzed oxidation of naturally occurring phenolic compounds by polyphenol oxidase (PPO).

Polyphenoloxidase (PPO; EC 1.14.18.1) is a key enzyme in this degradation. In the presence of molecular oxygen, PPO catalyzes the o-hydroxylation of mono-phenols to *o*-diphenols (cresolase activity), and further oxidation of o-diphenols to o-quinones (catecholase activity). *O*-quinones are very unstable and rapidly react with amino acids or proteins, generating brown pigments by polymerization [10], and these reactions are more important in fruits with high phenol contents such as eggplants [11].

The inhibition of PPO enzyme and thus prevention of browning is a big challenge for the industry of fruit and vegetable [12]. The present work was carried out to evaluate the effect of chemical and thermal treatments on browning inhibition of violet eggplant (*Solanum melongena* L.) in sight to the develop of techniques for controlling this undesirable process.

2. MATERIALS AND METHODS

2.1 Plant Material and Chemicals

Fresh violet eggplant (*Solanum melongena* L.) was purchased from the tall market, of Lobia (Daloa, Côte d'Ivoire). 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)

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 activity assays [13].

2.2.2 Enzyme assay

The PPO activities were determined using various phenolic compounds consisting of dopamine, pyrocatechol, pyrogallol, guaïacol, Naphtol and L-Phenylalanine as substrates. The reaction mixture adjusted to 2 mL with extraction buffer at appropriate pH, contained 0.1 mL of crude enzymatic extract and 0.8 mL of substrate at 10 mM. This reaction mixture was incubated at 25°C for 10 min. *O*-quinones content starting from the dopamine and guaïacol was estimated at 480 nm and those resulting from the others phenolic compounds at 420 nm. 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 (standard test conditions) [14,15].

2.2.3 Optimal pH and stability

The effect of pH on the crude PPO activities was determined by measuring the oxidation of phenolic substrates in different buffers at various pH values ranging from pH 2.6 to 8.0. The buffers (100 mM concentration) used were sodium acetate from pH 3.6 to 5.6, sodium phosphate from 5.6 to 8.0 and citrate phosphate from pH 2.6 to 7.0. The pH stability of each polyphenol oxidase was studied at a pH range of 2.6-8.0 with 100mM buffers. Buffers used were the same as in the pH study. After 2 hours preincubation at 25°C (room temperature), residual polyphenol oxidase activities were measured at 25°C for 10 min by adding substrate dopamine or pyrocatechol. Experiments were performed in triplicate, and the results expressed as percentage activity of zero-time control of untreated enzyme.

2.2.4 Temperature optimal and thermostability

The effect of temperature on PPO activities was performed in 100 mM sodium phosphate buffer pH 7.0 (for dopamine oxidase) pH 6.6 (of pyrocatechol oxidase), after 10 min incubation at temperatures ranging from 10 to 80°C under standard test conditions. The thermal inactivation was determined at 37°C and at each enzyme optimum temperature (30°C for dopamine oxidase and 25°C for Pyrocatechol oxidase). Enzymes in appropriate buffers were exposed to each temperature for up to 120 min. Then, aliquots were withdrawn at intervals (15 min) and immediately cooled. In the thermal denaturation tests, aliquots of each enzyme solution were preheated at different temperatures at a range of 10-90°C for 15min. Residual activities, determined at 25°C under the enzyme assay conditions were expressed as a percentage of activity of zero-time control of untreated enzymes [16].

The effect of temperature and the rate constant in an activation process was related according to the Arrhenius equation [17]:

$$k = Ae^{(-\frac{Ea}{RT})}$$

Where;

k is the reaction rate constant value,
A is the Arrhenius constant,

Ea is the activation energy (energy required for the activation to occur), R is the gas constant (8.31 $Jmol^{-1}K^{-1}$),

T is the absolute temperature in Kelvin.

The Q_{10} temperature coefficient is a measure of the reaction rate of temperature increase of 10°C the Q_{10} is calculated as:

$$Q10 = \frac{X2}{XI}$$

Where;

X1 represents the lower absorbance (D.O at 10°C); X2 represents the higher absorbance (D.O at 20°C)

2.2.5 Effect of metal ions and chemical agents on enzyme activity

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

2.2.6 Thermal inactivation and kinetic parameters ($t_{1/2}$ and D-values) determination

The thermal inactivation of PPO activities was determined at temperatures ranging from 30 to 80°C. The crude enzymatic extract in appropriate buffers [100 mM sodium phosphate pH 7.0 (for dopamine oxidase) and pH 6.6 (for pyrocatechol oxidase) was preincubated at different temperatures. Aliquots were withdrawn at intervals and cooled at room temperature for 10 min. The enzymatic activities of the aliquots were measured under standard conditions. The Kinetic data analysis of thermal inactivation of the PPO activities was done from the equation 1 (1). This equation is derived from the equation of first-order reactions [18].

$$\ln [At/A0] = -kt \quad (1)$$

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

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

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

$$t1/2 = \ln (2)/kt \quad (2)$$

The D-value, defined as the treatment time (min) needed to reduce the initial activity of 90% was calculated according to the equation 3 (3) [19].

$$D = 2{,}303/k \quad (3)$$

2.3 Statistical Analysis

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

3. RESULTS AND DISCUSSION

Fig. 1 presents the results of the screening of PPO activities, the diphenolic compounds were suitable substrates for PPOs from the fruit of eggplant (*Solanum melongena* L.). Our results were in agreement with those of Adeosun [20], who reported that PPOs from ripe and unripe plantain (Musa paradisiaca cultivars-Agbagba, Cardaba and plantain hybrid) were able to oxidize diphenolic substrates more than monophenolic substrates. The fact that pyrocatechol and dopamine were the most suitable diphenolic substrates suggested that pyrocatechol oxidase and dopamine oxidase activities were the main enzymatic activities involved in the enzymatic browning of eggplant pulp (*Solanum melongena* L.). To this end, browning prevention strategies during the processing of eggplant pulp should focus on these enzymatic activities. Hence, their physicochemical characterization has been considered.

The effect of pH on PPO activities from eggplant pulp was examined at different pH values, ranging from 2.6 to 8.0 (Fig. 2). The optimal activities were found at pH 6.6 for pyrocatechol oxidase and pH 7.0 for dopamine oxidase activities. Similar results about pH profile were observed by several authors for PPOs from various plants [21]. Indeed, Chaisakdanugull and Theerakulkait [21] reported an optimal pH (7.0)

for dopamine oxidase activity from banana pulp [Musa (AAA Group) 'Gros Michel']. The work carried out by Barthet [22] on isozymes isolated from roots of Manihot esculenta, indicated optimal pH values of 6.5, 6.8 and 7.5 using dopamine and pyrocatechol as substrates. Since PPO activities from violet eggplant were optimal at pH ranging from 6.6 to 7.0, their activity could be inhibited when exposed to an acidic environment. According to Yoruk and Marshall [23], the activity of PPOs decreases in an acidic environment by protonation of the catalytic site, thereby preventing the conformation of the active site, the binding of the substrates and/or the catalysis of the reaction.

The PPO activities showed the best stability over pH values ranging from 5.0 to 7.0 (Fig. 3), conserving at least more than 80% of total activities. Therefore, pH 5.0 and 7.0 is a good compromise between the activity and stability of these enzymes to perform oxidation of phenolic substrates over a long time.

As concerned the effect of temperature on PPOs from violet eggplant, the optimal temperature, half-life ($t_{1/2}$-value) and D-value which are important parameters for enzyme thermal stability evaluation were determined as shown in Table 1. The optimal temperatures of these enzymatic activities ranging from to 25 to 30°C indicated their mesophilic character (Fig. 4). Arogba et al. [24], note that mesophilic enzymes are relatively sensitive to elevated temperatures. The increase in temperature from 30 to 80°C resulted in a decrease in PPO activities, hence the decrease of $t_{1/2}$-values and D-values. At 65°C, the $t_{1/2}$-values of the studied enzymatic activities decreased to values between 13.46 and 21.72 min. These values were lower than those obtained by Ünal [25] who reported a $t_{1/2}$-value of 54.6 min at 65°C for the PPO of Anamur banana (Musa cavendishii). Thus, the low $t_{1/2}$-values obtained suggested that PPOs from violet eggplant are strongly inactivated at temperatures from 65°C. For D-values, a 70% reduction in the activity of PPOs from purple eggplant was observed at times ranging from 44.72 to 72.72 min at 65°C. Similar results were obtained by Gouzi et al. [26] for the PPO of Agaricus bisporus at 60°C ($t_{1/2}$-value = 11.8 min, D-value = 39.0 min) (Table 1).

From Arrhenius plot, values of 30.8 ± 2.04 and 7.1 ± 1.12 kJ/mol were obtained as the activation energy of dopamine oxidase and pyrocatechol oxidase activities, respectively. These activation energies are lower than that obtained (82.8 kJ/mol) for the polyphenol oxidases from pineapple puree [27]. Values of activation energy indicate the relative tendency of a failure mechanism to be accelerated by temperature. In this respect, the studied polyphenol oxidases should be top-grade tools for various catalyzing reactions since it is well known that enzymes are biocatalysts that speed up chemical reactions by lowering the required activation energy [28]. The thermal denaturation (Fig. 5) showed that the enzymes retained 100% of their activities to temperatures up to their optima. Above, their activities declined progressively as the temperature increased. The activities polyphenol oxidases were completely inactivated at 90°C (Fig. 5).

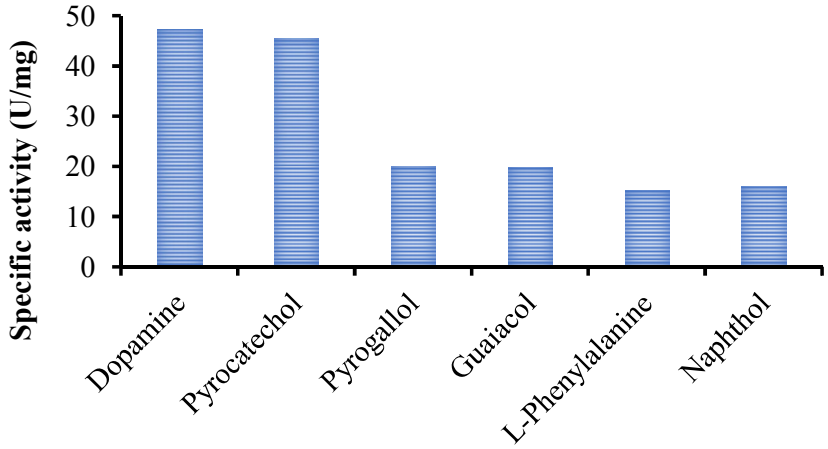

Fig. 1. Screening of polyphenol oxidase (PPO) activities of the violet eggplant (*Solanum melongena* L.)

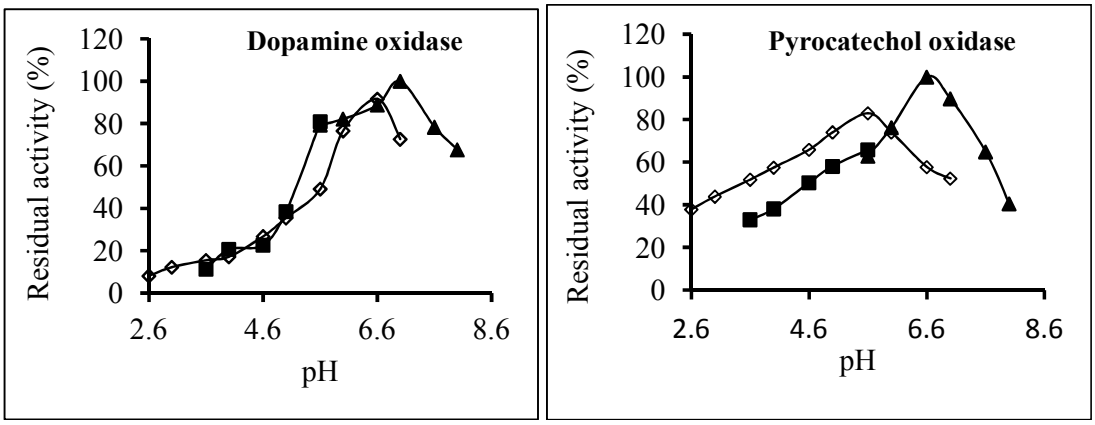

Fig. 2. Effect of pH on the activities of polyphenol oxidases from violet eggplant (*Solanum melongena* L.). Citrate buffer (◊), acetate buffer (■), phosphate buffer (▲)

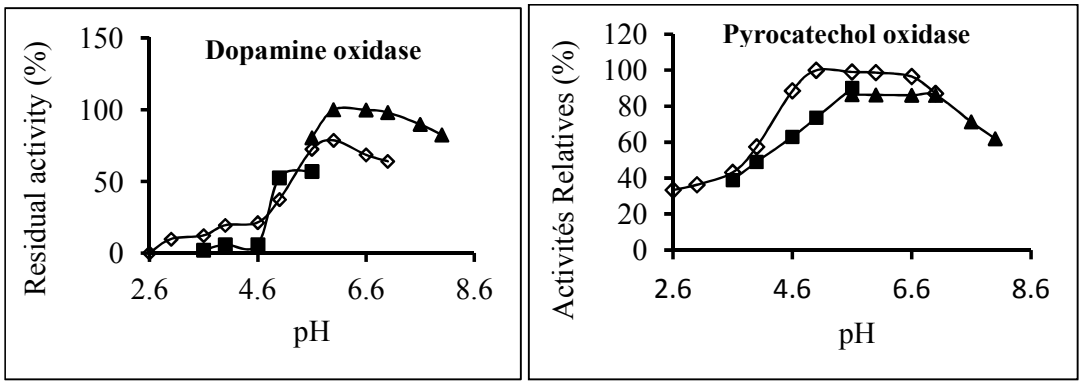

Fig. 3. Stability of pH on the activities of polyphenol oxidases from violet eggplant Solanum melongena L. Citrate buffer (◊), acetate buffer (■), phosphate buffer (▲)

Table 1. Kinetic parameters of polyphenol oxidases (PPO) activities of the violet eggplant (*Solanum melongena* L.)

T (°C)	Enzymatic activities			
	Pyrocatechol oxidase		Dopamine oxidase	
	$t_{1/2}$ (min)	D (min)	$t_{1/2}$ (min)	D (min)
30	147.47 ± 1.9	490.00 ± 1.98		
35	133.29 ± 2.01	442.88 ± 2.25	68.63 ± 1.0	228.02 ± 1.7
40	72.20 ± 1.5	239.89 ± 1.05	39.38 ± 1.3	130.85 ± 1.5
45	34.65 ± 0.9	115.15 ± 1.74	33.81 ± 1.1	112.34 ± 1.3
50	31.22 ± 1.5	103.73 ± 1.07	24.32 ± 1.6	80.80 ± 1.1
55	29.49 ± 1.9	98.00 ± 2.65	18.05 ± 1.5	59.97 ± 1.5
60	26.35 ± 0.9	87.56 ± 1.03	15.40 ± 1.1	51.18 ± 1.0
65	21.72 ± 1.5	72.72 ± 1.05	13.46 ± 1.0	44.72 ± 1.2
70	20.38 ± 0.9	67.73 ± 0.90	11.16 ± 1.3	37.09 ± 1.6
75	16.82 ± 1.9	55.89 ± 1.03	9.31 ± 1.2	30.95 ± 1.1
80	13.27 ± 0.04	44.11 ± 1.05	8.01 ± 1.1	26.62 ± 1.0

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

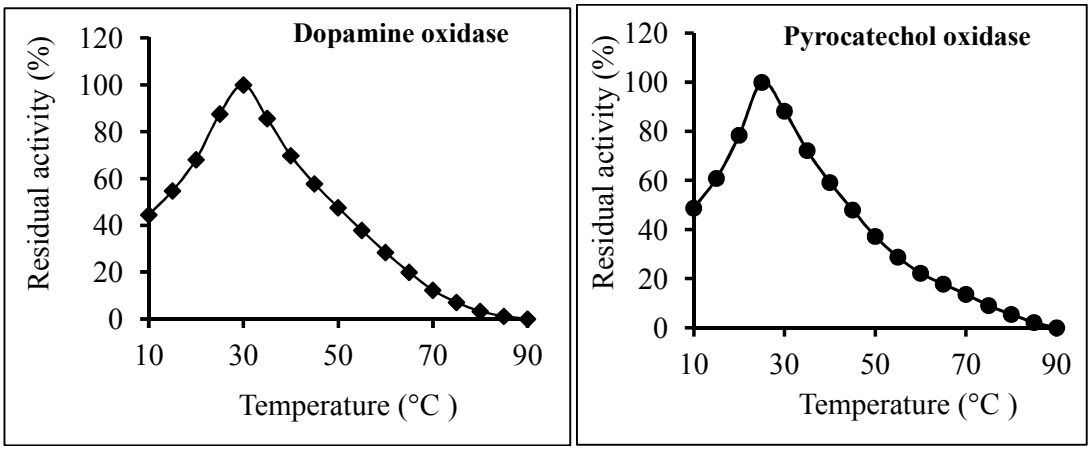

Fig. 4. Effect of temperature on the activities of polyphenol oxidases from violet eggplant (*Solanum melongena* L.)

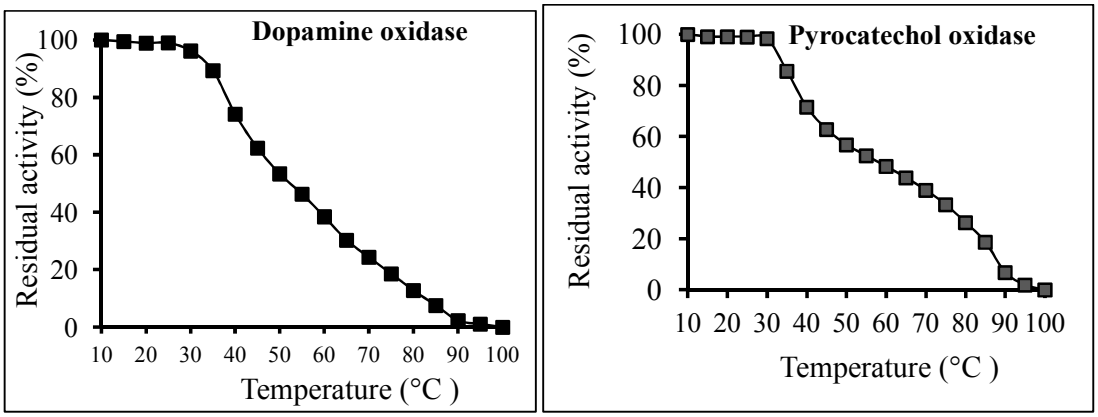

Fig. 5. Thermal denaturation of the activities of polyphenol oxidases from violet eggplant (*Solanum melongena* L.)

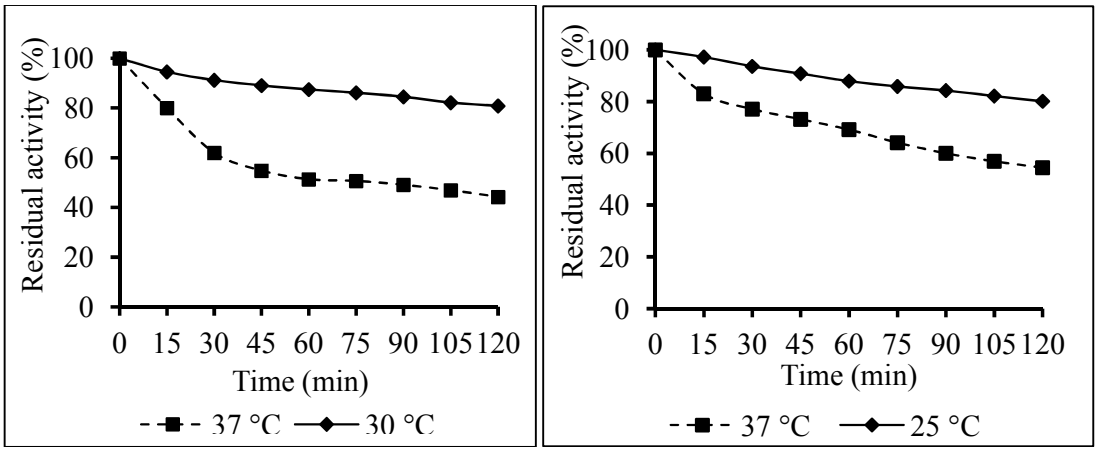

Fig. 6. Thermal inactivation of the activities of polyphenol oxidases from violet eggplant (*Solanum melongena* L.)

Table 2. Activity of polyphenol oxidases from violet eggplant (*Solanum melongena* L.) preincubated with some cations and chemical agents

Reagent	Concentration (mM)	Relative activity (%)	
		Dopamine oxidase	Pyrocatechol oxidase
Control	**0**	**100**	**100**
K^+	1	93.25 ± 3.1	94.86 ± 2.21
	5	96.28 ± 2.2	79.13 ± 0.6
Na^+	1	103.25 ± 4.5	103.70 ± 1.36
	5	110.92 ± 2.9	102.69 ± 2.05
Cu^{2+}	1	106.33 ± 2.1	101.78 ± 1.42
	5	104.95 ± 1.5	103.10 ± 4.20
Pb^{2+}	1	122.82 ± 2.9	102.88 ± 1.89
	5	39.52 ± 1.1	42.13 ± 4.5
Ba^{2+}	1	91.02 ± 1.2	93.65 ± 3.9
	5	96.78 ± 1.1	93.80 ± 2,5
Fe^{2+}	1	76.35 ± 2.5	47.86 ± 3.1
	5	31.30 ± 3.1	12.07 ± 2.8
Mg^{2+}	1	92.73 ± 1.8	93.85 ± 3.1
	5	100.19 ± 4.5	92.88 ± 2.8
Chemical agents			
EDTA	1	95.67 ± 3.9	81.44 ± 1.1
	5	107.21 ± 2.3	91.58 ± 1.8
Ascorbic acid	1	17.68 ± 1.2	16.82 ± 1.2
	5	16.29 ± 1.1	15.06 ± 2.3
Citric acid	1	95.15 ± 2.7	94.09 ± 2.7
	5	80.74 ± 4.3	82.59 ± 1.1
Sodium thiosulfate	1	17.87 ± 1.20	20.05 ± 2.3
	5	15.26 ± 2.2	20.63 ± 2.5

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

The PPOs activities of eggplant were fully active for more than 2 hours at their optimal temperature in sodium phosphate buffer, indicating a thermal stability at this temperature (Fig. 6). The enzymes held these properties in common with the majority of plant polyphenol oxidases [16,29]. In this context, running biotechnological processes at moderate temperatures would be advantageous for the application of these enzymes.

The activators of the two polyphenol oxidases activity were Na^+ and Cu^{2+}. The PPO activities are inhibited by the ions Pb^{2+} and Fe^{2+} (Table 2). The inhibitor effect of polyphenol oxidase activities by Fe^{2+} had also been reported for eggplant fruit during three ripening stages [30]. These cations should not be included in the two enzymes preparations. The inhibitory study showed that the most potent inhibitors for violet eggplant (*Solanum melongena* L.) PPO was ascorbic acid and sodium thiosulfate (Table 2), since these compounds induced a high degree of inhibition, even at the lowest concentration used. Oms-Oliu et al. [31] and Jiang and Fu [32] have also reported a similar effect of these chemical agents on inhibition of polyphenol oxidase and the browning control of fresh-cut pear wedges and litchi fruit, respectively. Ascorbic acid has the ability to be strongly reducing. It acts on oxygen by an oxide reduction reaction thanks to its enediol function and it is converted into dehydroascorbic acid which has the same biological activity as the ascorbic acid [15].

4. CONCLUSION

The screening of PPO activities has shown that pyrocatechol oxidase and dopamine oxidase were enzymatic activities mainly involved in the browning of violet eggplant. Moreover, the PPO activities is sensitive to the heat and some of general inhibitors, especially to ascorbic acid and sodium thiosulfate. Ascorbic acid was a better inhibitor where 82.32 % of PPOs inhibition was achieved and heat treatment at 65 °C for 30 min reduced browning of eggplant fruit. Based on the physicochemical properties and thermal stability of these PPOs activities, treatments carried out on the eggplant pulp led to reducing browning of eggplant (*Solanum melongena* L.).

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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