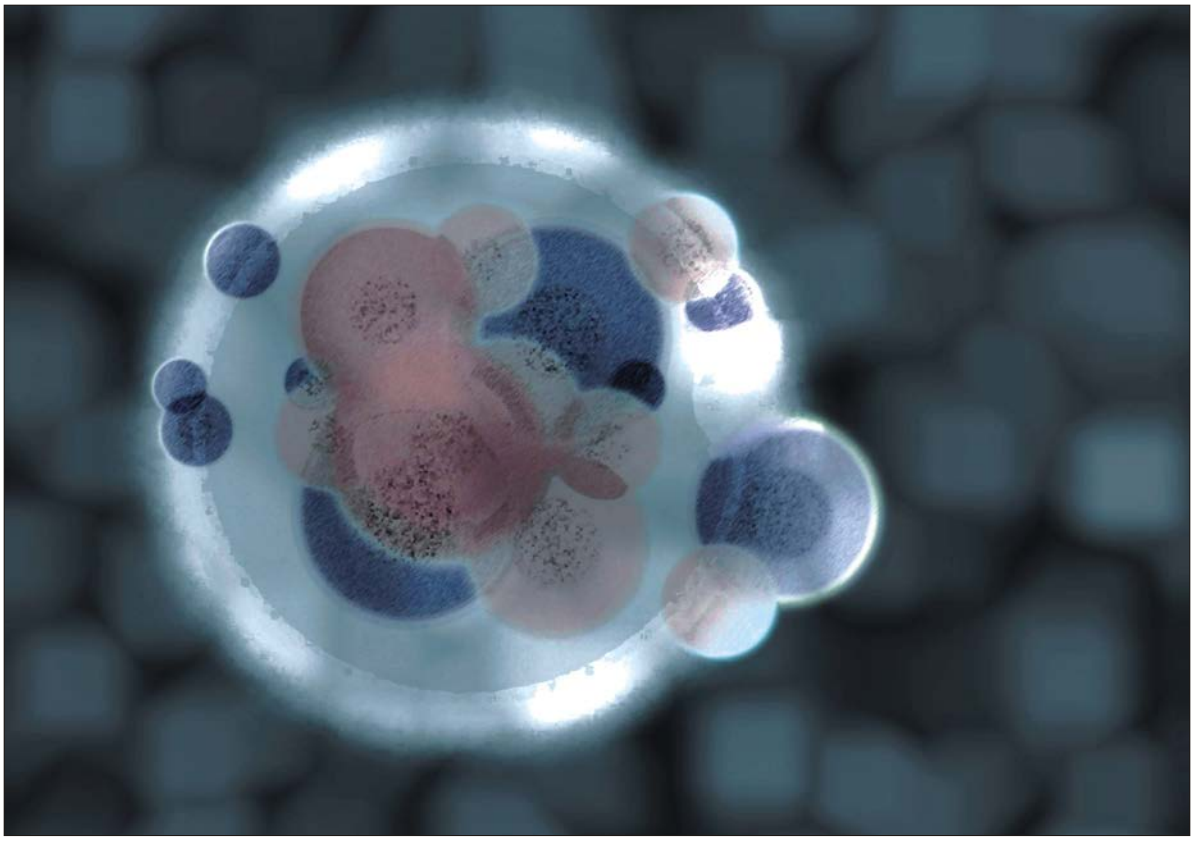

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The Banana Pulp Polyphenol Oxidase is a Tyrosinase

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Abstract: The best extraction result for the polyphenol oxidase (PPO) from the *Musa cavendishii* pulp was obtained using a phosphate buffer (0.02 M, pH 7) containing PVP (2%), triton X-100 (1%) and ascorbic acid (0.01%). Two active PPO isozymes were obtained applying a procedure involving dialysis, stepwise precipitation, DEAE and Sephadex column chromatography. The most active isozyme cresolase and catecholase activities in the presence of 4-[(4-Methylphenyl)azo]-phenol and caffeic acid showed an 11.67 and 13.3 fold recovery, respectively, after isolation. It exhibited a single band PAGE and had a MW of 58.2 kD and pI of 5.28 with the kinetic parameters of (K_m = 18.6 μM and V_{max} = 2.8 μM min^{-1}) for caffeic acid and (K_m = 0.94 mM and V_{max} = 14.8 μM min^{-1}) for dopamine. The presence of dopamine in banana pulp and peel was established and the results were discussed in terms of the identity and physiological role of the banana PPO in a collective and conclusive manner.

Key words: *Musa cavandishii*, banana pulp, polyphenol oxidase, tyrosinase

INTRODUCTION

Banana is a nutritious fruit with a pleasant flavor that is widely consumed throughout the world. It is a commercially important fruit crop in the world trade. In countries such as Costa Rica and Honduras, bananas account for more than 25% of the total export (Ortiz *et al*., 1998). Bananas are prone to rapid browning during handling, peeling and slicing operations and even storage, if ripening is not adequately controlled. This phenomenon lowers the fruit quality and decreases its marketability (Lozano, 2007).

Browning is mainly attributed to the oxidation of phenolic compounds by polyphenol oxidases (PPO). These enzymes catalyze the oxidation of different phenols to the corresponding quinines; highly reactive compounds that finally polymerize to melamins. The relationship between the phenolic content, PPO activities, pH, temperature, oxygen availability within the tissue and the browning rate has been examined for various fruits (Marshall *et al*., 2000; Yoruk and Marshall, 2003). Griffiths (1961) presented evidence indicating that the browning of banana fruit resulted from the enzymatic oxidation of 3,4-dihydroxyphenylethylamine (dopamine). The presence of a large quantity of dopamine in the different tissues of bananas was confirmed in later reports (Tono *et al*., 1999).

In spite of some valuable studies on the features and function of the banana PPO (Table 1), there are still ambiguities regarding the identity and, more seriously, the physiological role of the banana PPO (both pulp and peel tissues). A clear understanding of banana PPO not only leads to more efficient control of the enzyme, but it also sheds light on its physiological role. Literature survey reflects the ambiguity encircling the identity of the banana pulp PPO (BP-PPO) as both the enzyme numbers of (EC 1.10.3.2) for catechol oxidase and (EC 1.14.18.1) for phenolases are being used (Yang *et al*., 2004; Wuyts *et al*., 2006; Unal, 2007). To address this controversy, the BP-PPO was purified to a single isozyme level, then, the activities and some important biochemical properties of the enzyme were investigated. The assay methods and the results are compared with previous reports and finally discussed in terms of assumptions regarding the physiological roles of the BP-PPO.

MATERIALS AND METHODS

Chemicals and solutions: Bananas (*Musa cavendishii*) in the yellow to light brown spot stage of ripening were purchased from the local market. Sephadex G-100 was purchased from Pharmacia and diethylaminoethyl cellulose (DE-52) from Whatman™ (Maidstone, UK).

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Bovine serum albumin (BSA), ovalbumin, 3,4-Dihydroxy cinnamic acid (caffeic acid), 3,4-dihydroxyphenyl ethylamine (Dopamine) and 4-aminoantipyrine were purchased from Sigma Chemical Company. Triton X-100, insoluble polyvinylpyrrolidone and ascorbic acid were purchased from Merck Chemical Company. 4-[(4-Methylphenyl)azo]-phenol (MePAPh) was prepared as described earlier (Haghbeen and Tan, 1998). Mushroom tyrosinase (MT) was prepared according to Haghbeen *et al.* (2004). All the other chemicals and reagents used in this research were taken from the authentic samples. Double-distilled water was used for preparing the desired solutions. The applied extraction solution in this research was a phosphate buffer solution (PBS, 0.02 M, pH 7.0±1) containing triton X-100 (1%), ascorbic acid (0.01%) and water-insoluble polyvinylpyrrolidone (2%). PBS 0.01 M at pH 6.8±1 was used for the purification and assaying the enzyme.

Protein extraction: The diced fresh pulp was frozen in liquid N_2, then, 50 g of that was powdered in a pre-chilled porcelain mortar. Extra liquid N_2 was applied intermittently during grinding to prevent the tissues from thawing. Next, 100 mL of the extraction medium was added to the frozen powder and macerated for 20 min. The macerate was passed through a cotton cloth. The filtrate centrifuged at 4°C and 20000xg for 20 min. The supernatant was collected for further processing.

Enzyme purification: The obtained extract was subjected to a two-stage protein precipitation. Ammonium sulfate powder was added to the collected supernatant from the former step to make a 30% saturated solution. The resulting solution was stirred in an ice bucket for 30 min, then, centrifuged for 30 min at 20000xg and 4°C. After removing the precipitate at this stage, the supernatant was saturated to 65% of ammonium sulfate. The solution was left stirring on ice for 2 h, followed by centrifugation at 20000xg and 4°C for 30 min. After discarding the supernatant, the resulting precipitate was dissolved in PBS and dialyzed against similar PBS at 4°C overnight.

The dialyzed protein solution was loaded onto an ion exchange (DE-52) column, 2.5×45 cm. The proteins were eluted from the column by a gradient of NaCl solution, 0 to 200 mM, with a flow rate of approximately 1 mL min^{-1} controlled by a peristaltic pump. The output of the column was monitored spectrophotometrically at 280 nm. The collected fractions were also checked for the catecholase activity in the presence of caffeic acid at each step of elution. According to the monitoring results, the collected protein fractions at 150 mM salt eluent were pooled and lyophilized.

The final stage of purification was carried out by dissolving the lyophilized sample in the desired volume of PBS and loading it onto a Sephadex G-100 column (1.6×100 cm Pharmacia). The column was washed by PBS using a peristaltic pump at a flow rate of 3 mL min^{-1}. The ensuing fractions were collected and checked for the presence of the protein and enzyme. Native polyacrylamide gel electrophoresis (PAGE) of the collected fractions was performed according to the conventional method introduced by Davis and the protein bands were visualized through staining with Coomassi brilliant blue G250. The protein concentration of the fractions was determined by the Bradford method.

Molecular weight estimation and determination of isoelectric pH (PI): Molecular weight of the purified enzyme was estimated from PAGE observations using MT (128 kD), BSA (65 kD), ovalbumin (45 kD) as standards. The isoelectric pH was determined by isoelectric focusing on polyacrylamide gel in glass tubes (150×1.2 mm) following the technique introduced primarily by O'Farrell (Sojo *et al.*, 1998a). The ampholine (LKB, Stockholm, Sweden) was in a pH range of 3.5-10. The electrodes were connected to a Hoefer power supply and the current was maintained at 600 V for 12 h then, raised to 1000 V for 1 h. The protein bands were revealed by Coomassie blue R-250 (0.01%). The pH gradient was determined by cutting the unstained gel into 0.5 cm fragments which eluted by distilled water for 2 h. The pH of the eluent was recorded finally.

Assay of enzyme activities: All the enzymatic reactions were run in PBS at constant temperature, 25±0.1°C. The final volume of all the reaction mixtures was 3 mL filling up three quarters of the conventional, 1 cm width, UV-Vis cuvette. Freshly prepared BP-PPO solution (1 mg mL^{-1}) was used for both the cresolase and catecholase reactions (Fig. 1). Using a CECIL CE9500 spectrophotometer, the rate of the enzymatic reaction of BP-PPO was monitored as described previously (Haghbeen and Tan, 2003). Therefore, the cresolase and catecholase activities were assayed through the depletion of MePAPh (λ_{max} = 352 nm, ε = 20800 M^{-1} cm^{-1}) and caffeic acid (λ_{max} = 311 nm ε = 12000 M^{-1} cm^{-1}), respectively. Using this method, the Michaelis-Menten constants for the desired substrates were obtained from the corresponding kinetic data of their reactions in the presence of a constant amount of the purified enzyme (150 μL) which analyzed by the Lineweaver and Burk (1934) method. All the results presented in this article are the averages of, at least, triplicate measurements. Catecholase activity of BP-PPO in the presence of dopamine was monitored at 470 nm

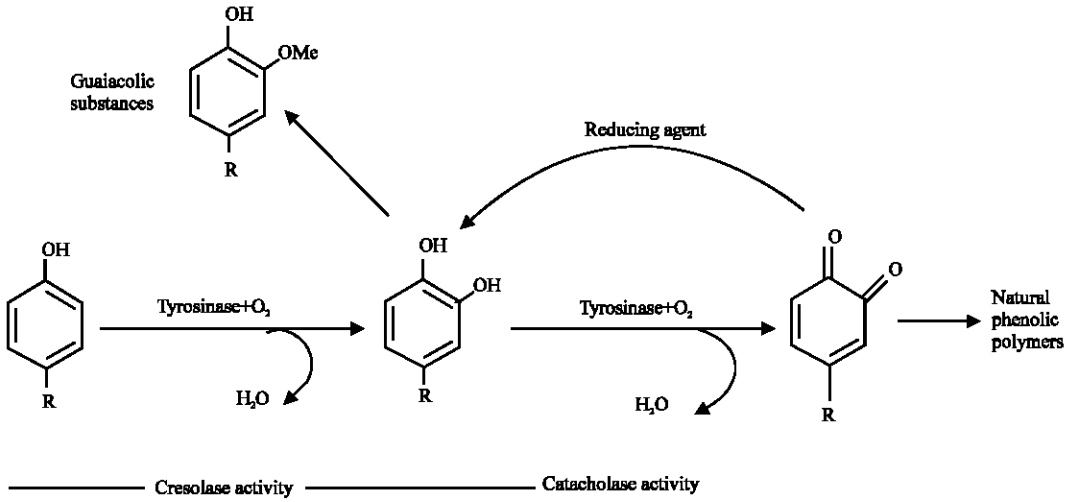

Fig. 1: Tyrosinase reactions and possible modification of the *ortho*-quinonic product (R = could be any type of substitute)

using Palmer (1963) method. Peroxidase activity of the desired solutions was also checked according to the Gallati (1977) method.

Dopamine extraction and analysis: Twenty gram of the diced pulp (or peel) was frozen in liquid N_2 and powdered in a prechilled porcelain mortar. To this frozen powder, 50 mL of degassed 2-propanol was added and stirred for 20 min. Then, it was passed through Whatman paper and centrifuged at 8000xg for 10 min. The filtrate, containing phenolic compounds, was dried at low pressure. Dopamine was separated form the dried extract by applying an acidic solution at pH 3.

The extracted dopamine from the banana pulp (or peel) was dissolved in a mixture of acetonitrile and water (20:80, v/v) and subjected to High Performance Liquid Chromatography by a Beckman HPLC, 125 Solvent module with Beckman C18 silica column (4.6×250 mm) and 168 Detector. A mobile phase of acetonitrile-water (20:80, v/v) at a flow rate of 0.8 mL min^{-1} was applied. The injection volume was 50 μL and detector was set at 288 nm.

RESULTS AND DISCUSSION

Extraction of enzyme: Some scientists have reported extraction of BP-PPO using a buffer system containing no detergent (Padron *et al*., 1975; Yang *et al*., 2000; Unal, 2007). In contrast, some others applied extraction medium containing insoluble PVP and Triton X-100 (Thomas and Janave, 1986; Jayaraman *et al*., 1987; Ngalani *et al*., 1993). Wuyts *et al*. (2006) reported the optimized amount of these substances in the extraction medium for the PPO from the *Musa acuminate* root. Results of our experiments on the BP-PPO were generally in agreement with the later case. Apparently, detergents increase the extractability of PPO while the removal of phenols in the presence of PVP is sufficient to avoid browning of the enzyme solution. However, it seems necessary to dialyze the extract against PBS since it improves the stability and activity of the enzyme (Table 2). This effect could be due to the renaturation of PPO in the presence of phosphate ions and the removal of inhibitory substances and detergents during dialysis (Galeazzi *et al*., 1981).

Purification of the BP-PPO: To avoid the possible risk of the denaturating effect of acetone (Thomas and Janave, 1986), it was preferred to use ammonium sulfate for precipitating proteins from the pulp extract. The partially purified enzyme was obtained after removing the heavy proteins from the pulp extract at 30% ammonium sulfate saturation. Precipitate collected at this stage showed little activity while the proteins collected at 65% ammonium sulfate saturation showed both catecholase and cresolase activities (Fig. 2, Table 2). Interestingly, the BP-PPO showed the characteristic lag time observed during the cresolase activity of tyrosinases (Sojo *et al*., 1998b).

To purify the BP-PPO, the precipitate collected at 65% ammonium sulfate saturation was first chromatographed on an ion-exchange column (DE-52). Similar to that observed during the MT purification (Haghbeen *et al*., 2004), the enzymatic assays revealed the highest PPO activity in the fraction eluted by 150 mM NaCl while the other fractions were either inert or showed low activity (Fig. 3A). The native PAGE of the separated fractions is shown in Fig. 3B.

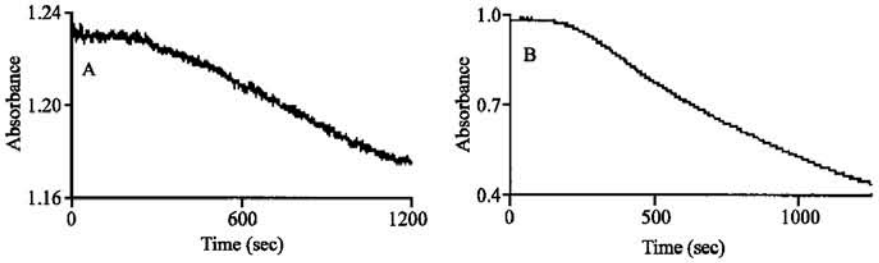

Fig. 2: Progress of the cresolase reactions of the A) crude extract and B) purified isozyme of the BP-PPO in the presence of MePAPh. Refer to the materials and method section for the experimental details

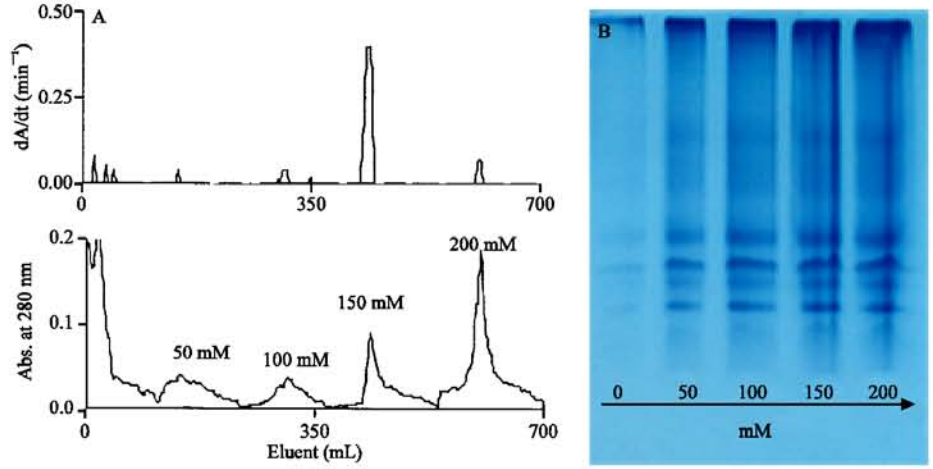

Fig. 3: The resulting chromatogram of the BP-PPO elution from the DE-52 column (A-bottom). The results of the catecholase assay of each fraction in the presence of caffeic acid (A-top) and their corresponding PAGE (B). Refer to the materials and method section for the experimental details

Table 1: The information collected from some important reports on the BP-PPO. The numbers in parentheses show the extinction coefficients (cm^{-1} M^{-1}) of the *ortho*-quinonic intermediate formed after the oxidation of a phenolic compound by a PPO. The numbers with astric show the K_m value of caffeic acid. R^2 is the regression coefficient of the kinetic plot. Blank cell and minus sign are for the "lack of any information and not identified, correspondingly

Banana variety	No. of Isozymes	EC No.	MW kD	pI	pH	°C	Assay λ_{max} nm	K_m M	R^2	References
Musa acuminata	-				7	25	470 (2512)	0.00063		Palmer (1963)
Musa cavendishii	-	1.10.3.1			-	30	415	-		Montgomery and Sgarbieri (1975)
Musa cavendishii	-		60							Padron *et al.* (1975)
Musa cavendishii	-	1.10.3.1	60-65	5.2						Galeazzi *et al.* (1981)
Musa cavendishii	4-5	1.10.3.1				-	480	0.00017		Galeazzi *et al.* (1981)
Musa cavendishii	14			4-5.5			Oximetric	-		Thomas and Janave (1986)
Musa paradisiaca	2	1.14.18.1			6.8	27	462	0.0005	+	Oba *et al.* (1992)
							420	0.005*		
Plantain	2		30±5 and 70		6.5	25	460			Ngalani *et al.* (1993)
							420	-		
Musa acuminata	Single	1.14.18.1		6.2	6.5	25	480 (3300)	0.00057		Sojo *et al.* (1998a)
Musa sapientum	Single	1.10.3.1	41-42		7	30	420	0.0028	+	Yang *et al.* (2000)
Musa acuminata	-	1.14.18.1				25	480	0.0006		Wuyts *et al.* (2006)
							400	0.0095*		
Musa cavendishii	-	1.14.18.1				30	410	-		Unal (2007)

The collected fraction at 150 mM NaCl was dialyzed, concentrated and transferred onto a Sephadex G-100 column for final purification. Accordingly, it seems that two active isoforms of the BP-PPO can be separated applying this method (Fig. 4A). The result of the native electrophoresis of the most active isoform of BP-PPO in comparison with MT, ovalbumin and BSA is shown in Fig. 4B. Considering these results and shown in Fig. 3A

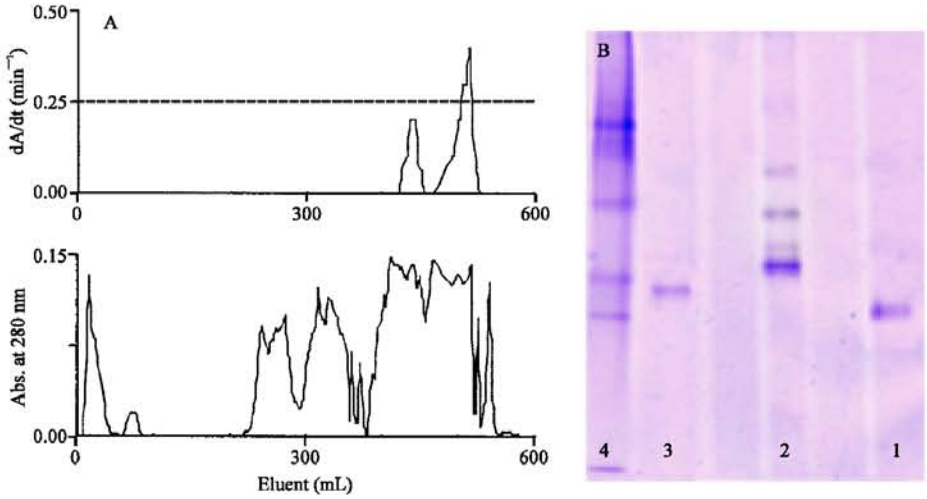

Fig. 4: The resulting chromatogram of the BP-PPO elution from the Sephadex G-100 column (A-bottom). The results of the catecholase assay of each fraction in the presence of caffeic acid (A-top). The PAGE (B) of the purified isozyme of the BP-PPO with the highest activity (3) in comparison with ovalbumine (1), BSA (2) and MT (4). Refer to the materials and method section for the experimental details

as well as the high similarity between the PAGE patterns of the collected fractions shown in Fig. 3B indicate the existence of several isoforms for the BP-PPO, however, the fraction eluted by 150 mM NaCl contained the most active ones. It means that the other isoforms of the BP-PPO had lost a great deal of their activities either prior or during the extraction process.

A molecular weight of 58.2 kD can be estimated from Fig. 4B for the purified BP-PPO. Yang *et al.* (2000) and Sojo *et al.* (1998a) had also attained a single band PAGE for the *Musa sapientum* L. and *Musa acuminate* PPO, respectively. The former had estimated a molecular weight of 41-42 kD for the separated PPO while the latter reported no molecular weight. But the MW(s) reported for the *Musa Cavendishii* PPO in previous works, 60 kD (Padron *et al.*, 1975) and 65 kD (Galeazzi *et al.*, 1981) are very close to the MW estimated in this research.

The isoelectic focusing of the purified isoform with the highest activity was performed in polyacrylamide gel containing ampholines in the range of pH 3.5-10. The corresponding zymogram (Fig. 5) indicates the existence of only one band with a pI value of 5.28. This result clearly supports the success of the applied method for purification of a single isoform of the *Musa cavendishii* PPO. Galeazzi *et al.* (1981) had reported a similar pI for the *Musa cavendishii* PPO and Thomas and Janave (1986) had reported a range of 4 to 5.5 for the pI values of a group of *Musa cavendishii* PPO isozymes.

BP-PPO is a tyrosinase: Determining the presence of both monophenolase and catecholase activities for an

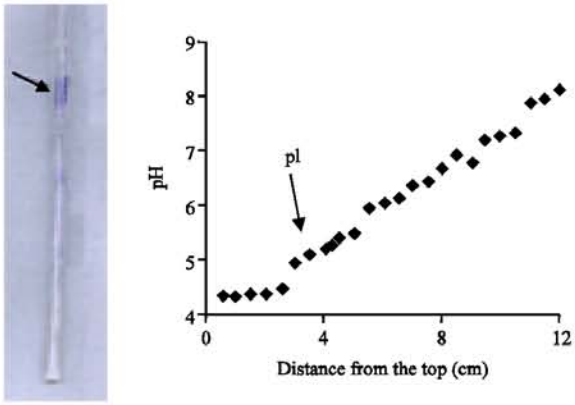

Fig. 5: (Left) The isoelectic focusing of the purified isoform of BP-PPO on the polyacrylamide gel containing ampholines and (Right) the corresponding zymogram. The arrows refer to the stained enzyme and its pI on the left and right figures, respectively

active PPO present in a plant material is a definitive way to categorize that enzyme as a tyrosinase (Sa´nchez-Ferrer *et al.*, 1988). Tyrosinases usually show a more pronounced catecholase activity in comparison with the cresolase activity. Besides, catecholase is much faster than the cresolase. This is why most researchers prefer to follow the PPO through its *ortho*-diphenolase activity. However, ignoring the monophenolase activity of the desired PPO might bring about confusion regarding the true identity of the PPO. The identity of BP-PPO was a matter of disagreement until Sojo *et al.* (1998b)

Table 2: Results of the catecholase assay of BP-PPO in the presence of caffeic acid (50 μM) at 25 °C in PBS (0.01 M) at each stage of the purification procedure introduced in this work. The number in parenthesis shows the specific unit of activity [(μM min^{-1})/mg]

Purification stages	dAbs/dt (min^{-1})
Pulp extract	0.0036
Precipitate at 65% $(NH_4)_2SO_4$ saturation	0.0042
Dialysis	0.018
Selected fraction from DE-52 column	0.036
Purified isozyme from Sephadex column	0.048 (26.7)

presented the details of a study on the phenolase activity of *Musa acuminate* pulp PPO. Yang *et al.* (2000) also reported the phenolase activity of *Musa sapientum* L. pulp PPO in the presence of p-cresol. Now, results of this research confirm the cresolase activity of the purified isozyme of BP-PPO from *Musa cavendishii* in the presence of a synthetic phenolic substrate. The cresolase and catecholase activities of the BP-PPO showed an 11.67 and 13.3 fold recovery, respectively, upon applying the introduced purification procedure in this paper (Fig. 2, Table 1).

The extract and the isolated isozyme were checked for the peroxidase activity too. Results were negative. The outcome of this research and similar reports on different varieties of banana indicate that BP-PPO is a tyrosinase. However, the cresolase activity of the enzyme is very susceptible to the conditions of the purification procedure. In fact, the lability of the banana pulp tyrosinase decreases the reproducibility of the observed results which is a setback observed in similar works (Sanchez-Ferrer *et al.*, 1993).

Kinetics parameters: The kinetic parameters of BP-PPO are usually determined in the presence of dopamine. As Table 1 shows, except one report, a spectrophotometric method based on the formation of the *ortho*-quinonic intermediate during the dopamine oxidation by the BP-PPO is commonly used for assaying the enzyme activity (Yoruk and Marshall, 2003). The UV-Vis spectrum of the intermediate overlaps with the absorption spectrum of the phenolic substrate at wavelengths lower than 300 nm. Therefore, the formation of this intermediate is followed at its other λ_{max} (s) about 420 or 470 nm. Because of the broad absorption band of the intermediate at 470 nm region, different λ_{max} (s) of 462, 465, 470 and 480 nm have been selected (Table 1). There are also two extinction coefficients for the *ortho*-quinonic intermediate at this region in the literature. It was preferred to use the extinction coefficient used by Palmer (1963), 2512 M^{-1}•cm^{-1}, in this research because it is very close to that obtained by Waite (1976). Consequently, the K_m and V_{max} values of 0.94 mM and 14.8 mM•min^{-1} were extracted from the fitted equation ($y = 6.355x + 0.6779$, $R^2 = 0.995$) for the Lineweaver-Burk plot of dopamine shown in Fig. 6.

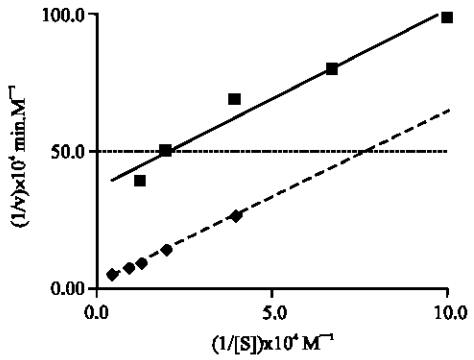

Fig. 6: The Lineweaver-Burk plots of the kinetics results of dopamine (♦) and caffeic acid (■) oxidation by the purified isozyme of BP-PPO in this work. Refer to the materials and methods for the experimental details

Caffeic acid is also a natural phenolic acid usually found in plants. The Michaelis-Menton constants of this substrate in the presence of the BP-PPO have been reported in some papers using the spectrophotometric method for following the quinonic intermediate formation (Table 1). However, Haghbeen and Tan (2003) showed that it is possible to follow the enzymatic oxidation of caffeic acid at its λ_{max} of 311 nm since the absorption spectra of the substrate and its *ortho*-quinonic intermediate do not show large overlap at this wavelength. Therefore, it is possible to study the kinetics of the enzymatic reaction directly through the depletion of the substrate. Using this method, the K_m and V_{max} values of 18.6 μM and 2.8 μM min^{-1} were extracted from the fitted equation ($y = 6.6387x + 356880$, $R^2 = 0.96$) for the Lineweaver-Burk plot of caffeic acid shown in Fig. 6. It is clear that these data are outstandingly smaller than the previously reported kinetic parameters for caffeic acid or even dopamine mainly because of the setbacks associated with the assay method at 420 or 470 nm which has been discussed earlier (Haghbeen and Tan, 2003).

BP-PPO and MT: Haghbeen and Tan (2003) obtained the kinetic parameters of caffeic acid in the presence of MT under conditions similar to those applied in this work (K_m = 27.5 μM and V_{max} = 2.2 μM min^{-1}). Theses parameters are very close to those obtained for caffeic acid in this report. On the other hand, if no stabilizer is added to the purely lyophilized MT, its single-band PAGE pattern (Haghbeen *et al.*, 2004) changes upon storage at 4°C and shows extra bands (Fig. 4). It is assumed that these extra bands belong to the dissociated mers of the tetrameric MT. Interestingly, one of these dissociations stands right beside the purified BP-PPO isozyme (Fig. 4).

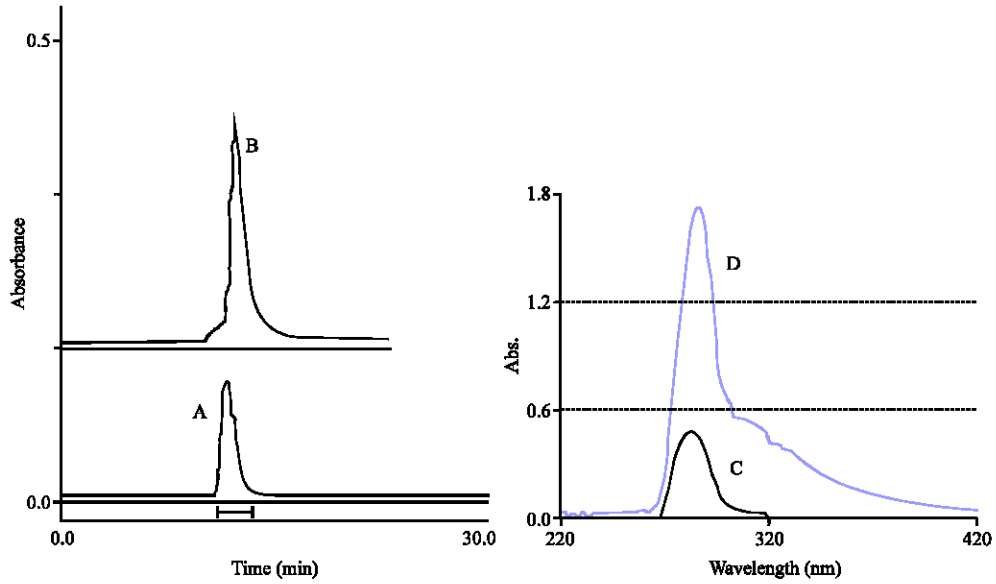

Fig. 7: HPLC results of (A) the commercial dopamine and (B) the 2-propanol extract of the *Musa cavandishii* peel and their corresponding UV-Vis spectra (C and D), respectively

The kinetic and PAGE evidence suggests significant similarity between BP-PPO and MT. It seems the most stable quaternary structure of MT is a tetramer composed from two dimmers while the banana tyrosinase is a dimmer similar to that suggested by Galeazzi *et al.* (1981).

Dopamine and physiological role of the banana tyrosinase: As mentioned earlier in this paper, dopamine presence in banana and its participation in the browning phenomenon have already been shown (Griffiths, 1961). The analysis of the 2-propanol extracts of the pulp and peel of the used banana in this work also confirms the presence of high quantity of dopamine in these tissues, however, it seems that it is dominant in peel (Fig. 7). It is known that different compartmentalization of the enzyme and substrate in cells of healthy fruits avoids the occurrence of browning. But, if direct contact does not occur between these two, what is the real physiological role of banana tyrosinase in healthy fruit and skin.

All the proposed answers to this question are based on the chemistry of the final product of BP-PPO reactions. *ortho*-quinones are highly reactive substances which can easily polymerized to melanins. They are prone to nucleophilic attacks and hence, are readily associated with amino acids or proteins. Besides, *ortho*-quinones can take part in interamolecular cyclization. All these options have been taken into account to describe the role of PPO in the protection of plants against diseases and invading pathogens or biosynthesis of natural products like betalains (Yoruk and Marshall, 2003). But, *ortho*-quinones can also be reduced back to *ortho*-dihydroxy compounds which can be used in redox systems or further enzymatic reaction such as the one proposed in Fig. 1. Considering the PPO location in plant cells, some suggested that PPO is important mostly for dark processes in thylakoid lumen (Sheptovitsky and Brudvig 1996), but there is little evidence for participation of this enzyme in biosynthesis of guaicolic substances.

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REFERENCES

Galeazzi, M.A., V.C. Sgarbieri and S.M. Constantinides, 1981. Isolation, purification and physicochemical characterization of polyphenol oxidase (PPO) from a Dwarf Variety of banana (*Musa cauendishii* L.). J. Food Sci., 46 (1): 150-155.

Gallati, H., 1977. Enzyme immunological tests: Determination of the activity of peroxidase with the aid of the Trinder reagent. J. Clin. Chem. Clin. Biochem., 15 (12): 699-703.

Griffiths, L.A., 1961. Relationship between 3,4-dihydroxyphenylethylamine content and the genome of *Musa acuminate*. Nature, 192 (479): 84-85.

Haghbeen, K. and E.W. Tan, 1998. Facile synthesis of catechol azo dyes. J. Org. Chem., 63 (13): 4503-4505.

Haghbeen, K. and E.W. Tan, 2003. Direct spectrophotometric assay of monooxygenase and oxidase activities of mushroom tyrosinase in the presence of synthetic and natural substrates. Anal. Biochem., 312 (1): 23-32.

Haghbeen, K., F. Rastgar Jazii, A.A. Karkhane and S. Shareefi Borojerdi, 2004. Purification of tyrosinase from edible mushroom. Iranian J. Biotechnol., 2 (3): 189-194.

Jayaraman, K.S., M.N. Ramanuja and P.K. Vijayaraghavan, 1987. Studies on the purification of banana polyphenol oxidase. Food Chem., 24 (3): 203-217.

Lineweaver, H. and D. Burk, 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc., 56: 658-666.

Lozano, J.E., 2007. Fruit Products. Deterioration by Browning, Springer US.

Marshall, M.R., J. Kim and C. Wei, 2000. Enzymatic browning in fruits, vegetables and seafoods. FAO report at http://www.fao.org/ag/ags/agsi/ENZYMEFINAL/.

Montgomery, M.W. and V.C. Sgarbieri, 1975. Isoenzymes of banana polyphenol oxidase. Phytochemistry, 14 (5-6): 1245-1249.

Ngalani, J.A., A. Signoret and J. Crouzet, 1993. Partial purification and properties of plantain polyphenol oxidase. Food Chem., 48 (4): 341-347.

Oba, K., N. Iwatsuki, I. Uritaki, A..M. Alvarez and V.V. Garcia, 1992. Partial purification and characterization of polyphenol oxidase isozymes in banana. Biosci. Biotech. Biochem., 56 (7): 1027-1030.

Ortiz, R., S. Madsen and D. Vuylsteke, 1998. Classification of African plantain landraces and banana cultivars using a phenotypic distance index of quantitative descriptors. Theor. Appl. Genet., 96 (6-7): 904-911.

Padron, M.P., A. Lozano and G. Gonzalez, 1975. Properties of *o*-diphenol, O_2 oxidoreductase from *Musa cavendishii*. Phytochemistry, 14 (9): 1959-1963.

Palmer, J.K., 1963. Banana polyphenoloxidase. Purification and properties. Plant Physiol., 38 (5): 508-513.

Sa´nchez-Ferrer, A., R. Bru, J. Cabanes and F. Garcia-Carmona, 1988. Characterization of cresolase and catecholase activities of Monastrell grape polyphenol oxidase. Phytochemistry, 27 (2): 319-321.

Sanchez-Ferrer, A., F. Laveda and F. Garcia-Carmona, 1993. Cresolase activity of potato tuber partially purified in a two-phase partition system. J. Agric. Food Chem., 41 (8): 1225-1228.

Sheptovitsky, Y.G. and G.W. Brudvig, 1996. Isolation and characterization of spinach photosystem II membrane-associated catalase and polyphenol oxidase. Biochemistry, 35 (50): 16255-16263.

Sojo, M., E. Nunez-Delicado, F. Garcia-Carmona and A. Sanchez-Ferrer, 1998a. Partial purification of a banana polyphenol oxidase using triton X-114 and PEG 8000 for removal of polyphenols. J. Agric. Food Chem., 46 (12): 4924-4930.

Sojo, M., E. Nunez-Delicado, A. Sanchez-Ferrer and F. Garcia-Carmona, 1998b. Monophenolase activity of latent banana pulp polyphenol oxidase. J. Agric. Food Chem., 46 (12): 4931-4936.

Thomas, P. and M.T. Janave, 1986. Isoelectric focusing evidence for banana isoenzymes with mono and diphenolase activity. J. Food Sci., 51 (2): 384-387.

Tono, T., K. Takedomi, T. Ikeda, C. Oka and H. Kishi, 1999. Difference spectrophotometric analysis of catecholamine using polyphenol oxidase from edible *Yam* (*Dioscorea*). J. Nishikyushu Univ. Saga Junior College, 29: 1-5.

Unal, U., 2007. Properties of polyphenol oxidase from Anamur banana (*Musa cavendishii*). Food Chem., 100 (3): 909-913.

Waite, J.H., 1976. Calculating extinction coefficients for enzymatically produced o-quinones. Anal. Biochem., 75 (1): 211-218.

Wuyts, N., D. De Waele and R. Swennen, 2006. Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* Grande naine) roots. Plant Physiol. Biochem., 44 (5-6): 308-314.

Yang, C., S. Fujita, M.D. Ashrafuzzaman, N. Nakamura and N. Hayashi, 2000. Purification and characterization of polyphenol oxidase from Banana (*Musa sapientum* L.) pulp. J. Agric. Food Chem., 48 (7): 2732-2735.

Yang, C., Z. Nong, J. Lu, L. Lu, J. Xu, Y. Han, Y. Li and S. Fujita, 2004. Banana polyphenol oxidase: Occurrence and change of polyphenol oxidase activity in some banana cultivars during fruit development. Food Sci. Technol. Res., 10 (1): 75-78.

Yoruk, R. and M.R. Marshall, 2003. Physicochemical properties and function of polyphenol oxidase: A review. J. Food Biochem., 27 (5): 361-422.