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Properties of polyphenol oxidase from Anamur banana (*Musa cavendishii*)

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Abstract

Polyphenol oxidase (PPO) was extracted from Anamur banana, grown in Turkey, and its characteristics were studied. The optimum temperature for banana PPO activity was found to be 30 °C. The pH-activity optimum was 7.0. From the thermal inactivation studies, in the range 60–75 °C, the half-life values of the enzyme ranged from 7.3 to 85.6 min. The activation energy (E_a) and Z values were calculated to be 155 kJ mol^{-1} and 14.2 °C, respectively. K_m and V_{max} values were 8.5 mM and 0.754 OD_{410} min^{-1}, respectively. Of the inhibitors tested, ascorbic acid and sodium metabisulphite were the most effective.
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1. Introduction

Polyphenol oxidase (PPO) (EC 1.14.18.1), which is widely distributed in the plant and animal kingdoms, is a copper-containing enzyme and is responsible for the enzymatic browning reaction occurring in many plants and vegetables damaged by improper handling, resulting in bruising, compression or indentations (Zawitowski, Bilideris, & Eskin, 1991). In the presence of molecular oxygen, PPO catalyzes the *o*-hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and oxidation of the *o*-diphenols to *o*-quinones (diphenolase activity) (Chararra, Carcia-Carmona, & Cabanes, 2001). PPOs are a very important enzymes in the food industry, due to their involvement in the enzymatic browning of edible plants, which is highly undesirable. Enzymatic browning impairs the sensory properties and marketability of the product and also lowers the nutritional value (Gauillard & Richard-Forget, 1997; Ramirez, Whitaker, & Virador, 2004).

PPO has been investigated in numerous sources, e.g. in apple (Rocha, Cano, Galeazzi, & Morais, 1998), artichoke head (Espin, Tudela, & Garcia-Canovas, 1997), grape (Sanchez-Ferrer, Bru, Cabanes, & Carcia-Carmona, 1988), aubergine (Dogan, Arslan, & Dogan, 2002), mulberry (Arslan, Erzengin, Sinan, & Ozensoy, 2004), lychee (Jiang, 2000), banana (Cano, Ancos, Lobo, & Santos, 1997), prawn (Montero, Avalos, & Perez-Mateos, 2001), and lobster (Williams, Davidson, & Mamo, 2003). No such research has been carried out on the Anamur banana, which is a dwarf variety cultivated in Turkey. The present work was undertaken to determine some of the properties of Anamur banana PPO.

2. Materials and methods

2.1. Materials

Anamur bananas used in this study were purchased from a local market. Catechol was obtained from Sigma–Aldrich (St. Louis, USA). Acetone was purchased from Merck, (Darmstadt, Germany). All other chemicals were of analytical grade.

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2.2. Methods

2.2.1. Preparation of crude enzyme extract

About 200 g of peeled and sliced bananas was homogenized in 400 ml of cold acetone (−25 °C), using a prechilled Waring blender for 2 min at maximum speed. The slurry was filtered and the residue was extracted with 200 ml of cold acetone. This procedure was repeated until a white powder was obtained. The resultant acetone powder was dried overnight at room temperature and stored at −25 °C (Coseteng & Lee, 1987).

In order to obtain enzyme extract, 0.5 g of acetone powder was suspended in 37.5 ml of prechilled 0.1 M phosphate buffer, pH 6.8, and then stirred for 1 h at 4 °C. The suspension was centrifuged at 7500*g* for 30 min at 4 °C. The supernatant was used as crude PPO.

2.2.2. Assay of enzyme activity

PPO activity was assayed in triplicate and the results expressed as means. PPO activity was determined in a spectrophotometer (Shimadzu UV-1201), by measuring the increase in absorbance at 410 nm at 30 °C. The initial rate was calculated from the slope of the absorbance–time curve. The reaction mixture contained 0.25 ml of freshly prepared enzyme solution, 0.25 ml of 50 mM catechol and 1 ml of 0.1 M phosphate buffer, pH 7.0.

2.2.3. pH optima

The PPO activity was determined in a pH range of 4.0–5.5 in 0.1 M acetate buffer and in a pH range of 6.0–8.0 in 0.1 M phosphate buffer. Catechol (50 mM) was used as substrate. PPO activity was assayed, using the standard reaction mixture but changing the buffer. PPO activity was calculated in the form of percent residual PPO activity at the optimum pH. The optimum pH obtained for this enzyme was used in all other studies.

2.2.4. Temperature optima

The activity of Anamur banana PPO, at temperatures ranging from 20 to 70 °C, was determined. The standard reaction mixture, without the enzyme, was heated to the appropriate temperature in a water bath. After equilibration of the reaction mixture at the selected temperature, the enzyme was added and the enzyme activity was measured. PPO activity was calculated in the form of percent residual PPO activity at the optimum temperature.

2.2.5. Enzyme kinetics

In order to determine Michaelis constant (K_m) and maximum velocity (V_{max}), PPO activities were measured using catechol as substrate at various concentrations. K_m and V_{max} values of the enzyme were calculated from a plot of $1/V$ vs. $1/S$ by the method of Lineweaver and Burk.

2.2.6. Heat inactivation

Thermal inactivation of crude PPO was studied at the selected temperatures (60, 65, 70 and 75 °C) for various times (10, 20 and 30 min) using screw-cap tubes. A 1 ml aliquot of enzyme solution was added to 4 ml of 0.1 M phosphate buffer (pH 7.0), that was previously preheated to the selected temperature. The enzyme samples were removed from the water bath after pre-set times and were immediately transferred to an ice bath to stop thermal inactivation. After the sample was cooled in an ice bath and brought to room temperature, 1.25 ml of the heated enzyme solution were mixed 0.25 ml of 50 mM catechol, and the residual activity (A) was determined spectrophotometrically. A non-heated enzyme sample was used as blank (A_0). The percentage residual activity was calculated by comparison with the unheated sample. First-order inactivation constant (k) was calculated from the slope of the natural logarithm (ln) of A/A_0 vs. time graph. The half-life of the enzyme ($t_{1/2}$) calculated by using the following equation: $t_{1/2} = 0.693/k$.

Decimal reduction time (D value) was estimated from the relationship between k and D value: $D = \ln(10)/k$. The Z value, which is the temperature increase required for a one-log10 reduction (90% decrease) in D value was determined from a plot of $\log_{10}D$ vs. temperature. The slope of the graph is equal to the $1/Z$ value. The energy of activation of denaturation (E_a) was calculated by multiplying the slope of the Arrhenius plot (i.e. natural logarithm of k values vs. reciprocal of absolute temperatures ($1/T$)) by the universal gas constant, R (kJ mol^{-1} K^{-1}).

2.2.7. Effects of inhibitors

Sodium chloride (50 and 100 mM), ascorbic acid (0.02 and 0.08 mM), citric acid (10 and 20 mM) and sodium metabisulphite (0.01 and 0.05 mM) were used as PPO inhibitors and the effects of inhibitors on banana PPO were determined by using catechol as substrate. The reaction mixture contained 0.9 ml of 0.1 M phosphate buffer, 0.1 ml of inhibitor, 0.25 ml of enzyme extract and 0.25 ml of 50 mM catechol. Percentage inhibition was calculated using the following equation: Inhibition (%) = $(A_0 - A_i/A_0)100$, where

A_0: initial PPO activity (without inhibitor)
A_i: PPO activity with inhibitor.

3. Results and discussion

3.1. pH optima

Assay of Anamur banana PPO activity, between pH 4.0 and 8.0, using catechol as substrate, showed two activity peaks, one at 5.5 and the other at 7.0 (Fig. 1). However, the drop in enzyme activity at pH 6.0 coincides with the pH where change of buffer was introduced. Since the activities of some enzymes are affected by the buffer used (Tipton, 2002), the first peak could be due to an artefact caused by changing buffers. However, two pH optima have been reported by other researchers, e.g. 4.5–5.0 and 7.5–7.6 for

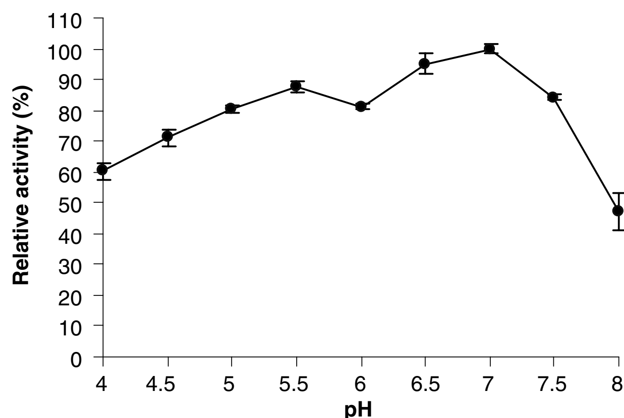

Fig. 1. Effect of pH on PPO activity.

two varieties of avocado PPO (Gomez-Lopez, 2002) and 5.0 and 7.5 for Jonagored apple PPO (Rocha & Morais, 2001). As the pH was increased from acidic to neutral, the enzyme activity increased and started to decline after pH 7.0. The pH optimum for PPO is found to be dependent on the enzyme source and substrate used. Yang, Fujita, Ashrafuzzaman, Nakamura, and Hayashi (2000) reported an optimum pH of 6.5 for banana, which compares well with the pH optimum obtained in this study. Other reported optimum pH values include 4.6 for taro PPO and 6.8 for potato PPO (Duangmal & Apenten, 1999), and 5.0 for what bran PPO (Soysal & Soylemez, 2004).

3.2. Temperature optima

Effect of temperature on PPO activity is depicted in Fig. 2. Temperature optimum of Anamur banana PPO is 30 °C. The enzyme activity declined rapidly on increasing temperature above 40 °C. Cash, Sistrunk, and Stutte (1976) reported an optimum temperature of 20 °C for Stanley plum PPO. In a study carried out by Dogan et al. (2002) on different aubergine cultivars, the temperature optima varied between 20 and 30 °C, using catechol and 4-methylcatechol as substrates. Wissemann and Lee (1981) reported an optimum temperature of 25 °C for Ravat and Niagara grape PPOs. The temperature optimum of 30 °C obtained in this study compares well with these values. A higher optimum temperature (50 °C) for strawberry PPO was reported by Serradell et al. (2000).

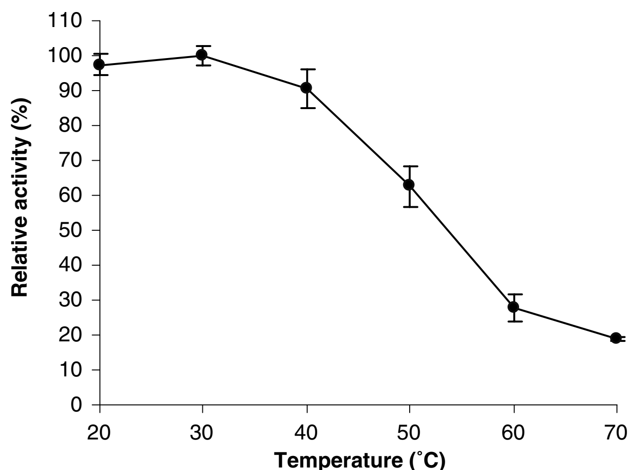

Fig. 2. Effect of temperature on PPO activity

3.3. Thermal inactivation

The heat-inactivation of Anamur banana PPO between 60 and 75 °C followed first-order kinetics and the first-order inactivation constants (k values) are presented in Table 1. From the table, it is clear that the enzyme is less thermostable at higher temperatures since a higher rate constant means that the enzyme is less thermostable (Marangoni, 2003). The half-life ($t_{1/2}$) is another important parameter used in the characterization of enzyme stability. Increasing the temperature from 60 to 75 °C resulted in a decrease in $t_{1/2}$ values (Table 1). PPO is generally considered to be an enzyme of low thermostability. Heat-stability was reported to differ among cultivars and multiple forms of PPO from the same source, as well as between fruit tissue homogenates and their respective juices (Zawitowski et al., 1991). Some of the reported PPO half-life values include between 25.6–91.2 min at 68 °C and 2.4–4.3 min at 78 °C for PPO of various apple cultivars (Yemenicioglu, Ozkan, & Cemeroglu, 1997), 18.8 min at 60 °C and 8.5 min at 70 °C for mango kernel PPO (Arogba, Ajiboye, Ugboko, Essienette, & Afolabi, 1998), 4.5 and 31.6 min at 75 °C for Ravat and Niagara grapes (Wissemann & Lee, 1981), respectively.

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 ranged between 286 and 24.3 min at the temperatures studied (Table 1). Yemenicioglu et al. (1997) reported D values of 30.3–56.6 min at 73 °C and 8.1–14.4 min at 78 °C for various apple cultivars. E_a and Z values for thermal inactivation of Anamur banana PPO are 155 kJ mol^{-1} and 14.2 °C ($r = 0.942$), respectively. Some of the reported E_a values are 87.8 kJ mol^{-1} for taro PPO (Yemenicioglu, Ozkan, & Cemeroglu, 1999), and 37.8–49.2 kJ mol^{-1} for two cherry laurel cultivars (Colak, Ozen, Dincer, Guner, & Ayaz, 2005). The Z value found in this study compares well with the Z value of 13.02 °C for grapes, reported by Weemaes, Ludikhuyze, Broeck, Hendricks, and Tobback (1998).

Table 1
Inactivation parameters of Anamur banana PPO

Temperature (°C)	k (10^{-2} min^{-1})	r^2	$t_{1/2}$ (min)	D (min)
60	0.81	0.6018	85.6	286
65	1.27	0.9157	54.6	181
70	2.50	0.8892	27.7	92
75	9.48	0.9421	7.3	24

Table 2
Effects of inhibitors on Anamur PPO activity

Inhibitor	Concentration (mM)	Inhibition (%)
NaCl	50	50.6 ± 0.63
	100	61.7 ± 3.82
Citric acid	10	61.6 ± 0.26
	20	69.7 ± 1.48
Ascorbic acid	0.2	99.8 ± 0.24
	0.8	100 ± 0.00
Sodium metabisulfite	0.01	100 ± 0.00
	0.05	100 ± 0.00

3.4. Kinetic parameters

The K_m value for Anamur banana PPO was determined to be 8.5 mM. K_m value is a measure of affinity of the enzyme for the substrate, with smaller values representing greater affinity (Siddiq, Sinha, & Cash, 1992). The affinity of Anamur banana PPO is low, as evidenced by a high K_m value. Affinity of plant PPO for the phenolic substrates is relatively low. The K_m is high, usually around 1 mM (Zawitowski et al., 1991). The literature provides information on a number of different K_m values, e.g. 18 mM for banana PPO (Galeazzi & Sgarbierii, 1981), 12.4 mM for persimmon fruit PPO (Ozen, Colak, Dincer, & Guner, 2004), 4.99 mM for marula fruit (Mdluli, 2005), and 4.54 mM for quince (Yagar & Sagiroglu, 2002). The K_m value obtained in this study is within the range of the reported values. The maximum reaction velocity (V_m) was found to be 0.754 Abs/min.

3.5. Effects of inhibitors

Effects of inhibitors on banana PPO activity were studied at two concentrations, using catechol as the substrate, and the results are reported as percentage inhibition (Table 2). Of the inhibitors tested, ascorbic acid and sodium metabisulphite were the most effective. Both inhibitors, at both concentrations tested, inhibited the enzyme completely. Yagar and Sagiroglu (2002) demonstrated that the use of ascorbic acid, at 2 and 20 mM concentrations, resulted in 98% and 100% of inhibition of quince PPO, respectively. They also observed 52% and 98% inhibition with sodium metabisulphite at concentrations of 2 and 20 mM, respectively. Total inhibition of the enzyme with NaCl and citric acid, at the concentrations tested, was not achieved. Yemenicioglu et al. (1999) found that NaCl, only at a high concentration (5 M), inhibited taro PPO by 19%.

References

Arogba, S. S., Ajiboye, O. L., Ugboko, L. A., Essienette, S. Y., & Afolabi, P. O. (1998). Properties of polyphenol oxidase in Mango (*Mangifera indica*) kernel. *Journal of the Science of Food and Agriculture, 77*, 459–462.

Arslan, O., Erzengin, M., Sinan, S., & Ozensoy, O. (2004). Purification of mulberry (*Morus alba* L.) polyphenol oxidase by affinity chromatography and investigation of its kinetic and electrophoretic properties. *Food Chemistry, 88*, 479–484.

Cano, M. P., Ancos, B., Lobo, M. G., & Santos, M. (1997). Improvement of frozen banana (*Musa cavendishii*, cv. Enana) colour by blanching: relationship between browning, phenols ad polyphenol oxidase and peroxidase activities. *Zeitschrift für Lebensmitteluntersuchung und Forschung A, 204*, 60–65.

Cash, J. N., Sistrunk, W. A., & Stutte, C. A. (1976). Characteristics of concord grape polyphenoloxidase involved in juice color loss. *Journal of Food Science, 41*, 1398–1402.

Chararra, S., Carcia-Carmona, F., & Cabanes, J. (2001). Hysteresis and positive cooperativity of iceberg lettuce PPO. *Biochemical and Biophysical Research Communications, 289*, 769–775.

Colak, A., Ozen, A., Dincer, B., Guner, S., & Ayaz, F. A. (2005). Diphenolases from two cultivars of cherry laurel (*Laurocerasus officinalis* Roem.) fruits at an early stage of maturation. *Food Chemistry, 90*, 801–807.

Coseteng, M. Y., & Lee, C. Y. (1987). Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. *Journal of Food Science, 52*, 985–989.

Dogan, M., Arslan, O., & Dogan, S. (2002). Substrate specificity, heat inactivation and inhibition of polyphenol oxidase from different aubergine cultivars. *International Journal of Food Science and Technology, 37*, 415–423.

Duangmal, K., & Apenten, R. K. O. (1999). A comparative study of polyphenoloxidases from taro (*Colocasia esculenta*) and potato (*Solanum tuberosum* var. *Romano*). *Food Chemistry, 64*, 351–359.

Espin, J. C., Tudela, J., & Garcia-Canovas, F. (1997). Monophenolase activity of polyphenol oxidase from artichoke heads (*Cynara scolymus* L.). *Lebensmittel-Wissenschaft und-Technologie, 30*, 819–825.

Galeazzi, M. A. M., & Sgarbierii, V. C. (1981). Substrate specificity and inhibition of polyphenoloxidase (PPO) from a dwarf variety of banana (*Musa cavendishii*, L.). *Journal of Food Science, 46*, 1404–1406.

Gauillard, F., & Richard-Forget, F. (1997). PPOs from Williams pear (*Pyrus communis* L. cv Williams): activation, purification and some properties. *Journal of the Science of Food and Agriculture, 74*, 49–56.

Gomez-Lopez, V. M. (2002). Some biochemical properties of polyphenol oxidase from two varieties of avocado. *Food Chemistry, 77*, 163–169.

Jiang, Y. (2000). Role of anthocyanins, polyphenol oxidase and phenols in lychee pericarp browning. *Journal of the Science of Food and Agriculture, 80*, 305–310.

Marangoni, A. G. (2003). *Enzyme kinetics: a modern approach*. New Jersey: John Wiley & Sons (pp. 140–157).

Mdluli, K. M. (2005). Partial purification and characterisation of polyphenol oxidase and peroxidase from marula fruit (*Sclerocarya birrea* subsp. Caffra). *Food Chemistry, 92*, 311–323.

Montero, P., Avalos, A., & Perez-Mateos, M. (2001). Characterization of polyphenoloxidase of prawns (*Penaeus japonicus*). Alternatives to inhibition: additives and high-pressure treatment. *Food Chemistry, 75*, 317–324.

Ozen, A., Colak, A., Dincer, B., & Guner, S. (2004). A diphenolase from persimmon fruits. *Food Chemistry, 85*, 431–437.

Ramirez, E. C., Whitaker, J. R., & Virador, V. M. (2004). Polyphenol oxidase. In J. R. Whitaker, A. G. J. Voragen, & D. W. S. Wong (Eds.), *Handbook of food enzymology*. New York: Marcel Dekker, pp. 509–522.

Rocha, A. M. C. N., Cano, M. P., Galeazzi, A. M., & Morais, A. M. M. B. (1998). Characterisation of 'Starking' apple polyphenoloxidase. *Journal of the Science of Food and Agriculture, 77*, 527–534.

Rocha, A. M. C. N., & Morais, A. M. M. B. (2001). Characterization of polyphenoloxidase (PPO) extracted from 'Jonagored' apple. *Food Control, 12*, 85–90.

Sanchez-Ferrer, A., Bru, R., Cabanes, J., & Carcia-Carmona, F. (1988). Characterization of catecholase and cresolase activities of Monastrell grape polyphenol oxidase. *Phytochemistry, 27*, 319–321.

Serradell, M. A., Rozenfeld, P. A., Martinez, G. A., Civello, P. M., Chaves, A. V., & Anon, M. C. (2000). Polyphenoloxidase activity from strawberry fruit (*Fragaria x ananassa*, Duch., cv Selva): characterisa-

tion and partial purification. *Journal of the Science of Food and Agriculture, 80*, 1421–1427.

Siddiq, M., Sinha, N. K., & Cash, J. N. (1992). Characterization of polyphenoloxidase from Stanley plums. *Journal of Food Science, 57*, 1177–1179.

Soysal, C., & Soylemez, Z. (2004). Properties of wheat bran polyphenol oxidase. *Nahrung/Food, 48*, 5–8.

Tipton, K. F. (2002). Principles of enzyme assay and kinetic studies. In R. Eisenthal & M. J. Danson (Eds.), *Enzyme assays*. Oxford: Oxford University Press, p. 20.

Weemaes, C. A., Ludikhuyze, L. R., Broeck, L., Hendricks, M. E., & Tobback, P. P. (1998). Activity, electrophoretic characteristics and heat inactivation of polyphenoloxidases from apples, avocados, grapes, pears and plums. *Lebensmittel-Wissenschaft und-Technologie, 31*, 44–49.

Williams, H. G., Davidson, G. W., & Mamo, J. C. (2003). Heat-induced activation of polyphenoloxidase in western rock lobster (*Panulirus cygnus*) Hemolymph: implications for heat processing. *Journal of Food Science, 68*, 1928–1932.

Wissemann, K. W., & Lee, C. Y. (1981). Characterization of polyphenoloxidase from Ravat 51 and Niagara grapes. *Journal of Food Science, 46*, 506–508.

Yagar, H., & Sagiroglu, A. (2002). Partially purification and characterization of polyphenol oxidase of quince. *Turkish Journal of Chemistry, 26*, 97–103.

Yang, C. P., Fujita, S., Ashrafuzzaman, M., Nakamura, N., & Hayashi, N. (2000). Purification and characterization of polyphenol oxidase from banana (*Musa sapientum* L.) pulp. *Journal of Agricultural and Food Chemistry, 48*, 2732–2735.

Yemenicioglu, A., Ozkan, M., & Cemeroglu, B. (1997). Heat inactivation kinetics of apple polyphenoloxidase and activation of its latent form. *Journal of Food Science, 62*, 508–510.

Yemenicioglu, A., Ozkan, M., & Cemeroglu, B. (1999). Some characteristics of polyphenol oxidase and peroxidase from taro (*Colocasia antiquorum*). *Turkish Journal of Agriculture and Forestry, 23*, 425–430.

Zawitowski, J., Bilideris, C. G., & Eskin, N. A. M. (1991). Polyphenol Oxidase. In D. S. Robinson & N. A. M. Eskin (Eds.), *Oxidative enzymes in foods*. New York: Elsevier, pp. 217–273.