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Characterization of Polyphenol Oxidase from Feijoa Fruit

Li-Zhang Fan, Meng Zhang, Dan Wang and Shao-Xiong Ren
College of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, 621010 Sichuan, China

Abstract: The activity of feijoa fruit Polyphenol Oxidase (PPO) was determined by spectrophotometer at 420 nm using catechol as substrate.The effects of the pH, temperature, substrate concentration and inhibitors on PPO were studied. A range of pH 3.0-8.0 was tested and the highest enzyme activity was at pH 5.5. The optimum temperature was determined by measuring the enzyme activity at various temperatures over the range of 10-70°C with 10°C increments. The optimum temperatures were found to be 30°C. The V_{max} and K_m value of the reaction were determinedand found to be 137 U min^{-1} protein and 0.38 mM. The three inhibitors as ascorbic acid, $NaHSO_3$ and EDTA had different effects on inhibiting enzymatic browning. Inhibitors used for investigation in this study were placed in relative order of inhibition: $NaHSO_3$>ascorbic acid>EDTA.

Key words: Polyphenoloxidase, *Feijoa sellowiana* berg, enzyme, characterization, inhibitors, China

INTRODUCTION

Feijoa sellowiana is a subtropical species belonging to the Myrtaceae family. It is native to South Brazil with a secondary dispersion in Uruguay and is mainly consumed as juice, jellies, confectionery and liquor (Thorp and Bieleski, 2002). The fresh fruit is enjoyed for its characteristic flavour and aroma which are similar to pineapple. For this reason, it is also called pineapple guava. As a newly rising species for its edible fruits, ornamental and medicinal properties, feijoa shows great potential in foods (Kolesnik *et al*., 1991) drug (Nakashima, 2001) and cosmetics (Hardy and Micbapl, 1970). However, it has a very short shelf-life under normal ambient conditions due to skin colour loss (browning) and deterioration during storage and transportation. The colour loss reduces its commercial value and has been considered a main postharvest problem. Browning has been attributed to oxidation of phenolics by polyphenol oxidase producing brown-coloured byproducts. Polyphenol Oxidase (PPO) is a common copper-containing enzyme which is responsible for melanization in animals and browning in plants. The role of PPO in plants is not yet clear. Heimdal *et al*. (1994) have suggested that it may be involved in immunity reactions and in bio synthesis of plant components and it also may play the role of a scavenger of free radicals in photo-synthesizing tissues. Quinones are highly reactive electrophilic molecules that can polymerise, leading to the formation of brown or black pigments. Prota (1988) have showed that the phenomenon of enzymatic browning often occurs in fruits and vegetables and is the cause of a decrease in their sensory properties and nutritional value. PPO has been widely studied in various fruits such as apples (Espin *et al*., 1995) pears (Hwang *et al*., 1996) banana (Galleazi and Sgarbieri, 1981) plums (Siddiq *et al*., 1992) grape (Harel and Mayer, 1971) but little has been known about feijoa fruit PPO. The objective of this study was to achieve purification and a better understanding of the properties of the feijoa fruit PPO that catalyses the browning reaction during fruit storage and transportation.

MATERIALS AND METHODS

Mature fruit of a major cultivar, unique, from a commercial orchard in Mianyang, P.R. China was obtained on the day of harvest and stored at 4°C. All chemicals and reagents used were analytical grade. Catechol, 4-methylcatechol, Polyvinylpyrrolidone (PVP40), DEAE-SephadexA-50, SephadexG100, chlorogenic acid, caffeic acid, ferulic acid, tyrosine, vanilin, sodium salt of Ethylene Diamine Tetraacetic Acid (EDTA) were obtained from Sigma-Aldrich, USA.

Enzyme extraction and partially purification: All steps were carried out at 4°C. The feijoa fruit was homogenised with 0.1 M sodium phosphate buffer (pH 6.8). After filtration of the homogenate through muslin, the filtrate was centrifuged at 16,000 g for 20 min and the supernatant was collected. Solid $(NH_4)2SO_4$ was added to the

Corresponding Author: Li-Zhang Fan, College of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, 621010 Sichuan, China

supernatant to obtain 80% saturation. After an hour, the precipitated proteins were separated by centrifugation at 16,000 g for 30 min. The precipitate was dissolved in a small amount of 5 mM phosphate buffer (pH 6.8) and dialyzed in the cellulose bag (MW cut off >12,000) at 4°C in the same buffer for 24 h with four changes of the buffer during dialysis. In order to conduct further purification, the dialysate was transferred to a column filled with DEAE-SephadexA-50 gel, balanced with 5 mM phosphate buffer, pH 6.8. The column was eluted with the same buffer at the flow rate of 30 mL h^{-1} and linear gradient of NaCl concentration from 0-1.0 M. About 3 mL fractions were collected in which the protein level and PPO activity towards catecholas substrate were monitored. The fractions which showed PPO activity were collected, concentrated and then dissolved in 2 mL of phosphate buffer, pH 6.8. The combined fractions were transferred to a glass column filled with SephadexG100 gel. The column was eluted with the same buffer solution. About 3 mL fractions were collected and the protein content and PPO activity towards catechol was monitored in them spectrophotometrically. The fractions showing PPO activity were combined and concentrated.

Enzyme assay: PPO activity was determined by measuring the initial rate of quinone formation, as indicated by an increase in absorbance at 420 nm. An increase in absorbance of 0.001 min^{-1} was taken as one unit of enzyme activity. The increase in absorbance was linear with time for the Ist 120 sec. The sample cuvette contained 2.95 mL of substrate solution in 50 mM phosphate buffer (pH = 6.8) and 0.05 mL of the enzyme solution. The blank sample contained 2.95 mL of substrate solution and 0.05 mL of phosphate buffer. Information of substrates used is provided in the adequate sections.

Kinetic data analysis and substrate specificity: The specificity of feijoa fruit PPO extract was investigated for seven commercial grade substrates (Catechol, 4-methylcatechol, chlorogenic acid, caffeic acid, ferulic acid, tyrosine and vanilin) at concentrations 10 mM. PPO activity was assayed in triplicate. The activity of PPO extract as a function of the concentration of catechol and 4-methylcatecho 1 was investigated. Michaelis constant (K_m) of the PPO was determined by Lineweaver-Burk's Method.

Effect of pH on enzyme activity: To determine the effect of pH on PPO stability 0.01 mL of enzyme solution was incubated in 1.49 mL of various buffer solutions ranging from pH 3.0-8.0 for 24 h at 4°C. The enzyme activity was performed according to the method described before and expressed a percentage of the maximum activity.

Effect of temperature on PPO: The optimum temperature for PPO activity was determined by measuring the enzyme activity at various temperatures over range 10-70°C with 10°C increments using a circulation water bath. The substrate and buffer were incubated for 15 min at various temperatures as indicated before prior to the addition of the enzyme solution. For each stage all assays were performed in triplicate using the separate three extractions.

Effect of inhibitors on PPO: The inhibitory effects of ascorbic acid EDTA (sodium salt of ethylenedia minetetra acetic acid) and sodium metabisulfiteon PPO activity were determined. Three different concentrations of these inhibitors (0.1, 1.0 and 10 mM) were tested using 10 mM ofcatecholsubstrate. The corresponding control contained the same concentration of enzyme in the absence of inhibitor.

RESULTS AND DISCUSSION

Curve of reaction course of PPO: From Fig. 1, the OD value changed quickly at the Ist 6 min and it was basically stable linear increasement which showed that it reached the peak value at 1.43 in 35 min and browning reaction occurred obviously. There were slowly declining trends for OD value after the peak value.

Effect of pH on enzyme activity: The activity of PPO was measured at different pHs using catechol as substrates. As seen in Fig. 2, the optimum pHs of the enzyme were found to be 6.0 using catecholas substrate. In general, most plants show maximum PPO activity at or near neutral pH values. Different optimum pHs for PPO obtained from various sources are reported in the literature. For example, it is reported that optimum pH values are 5.5 for

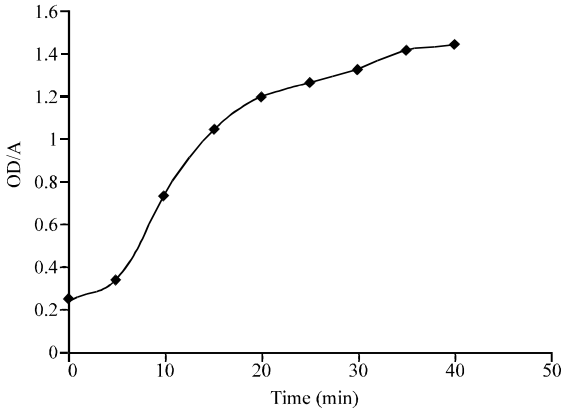

Fig. 1: The curve of reaction course of PPO

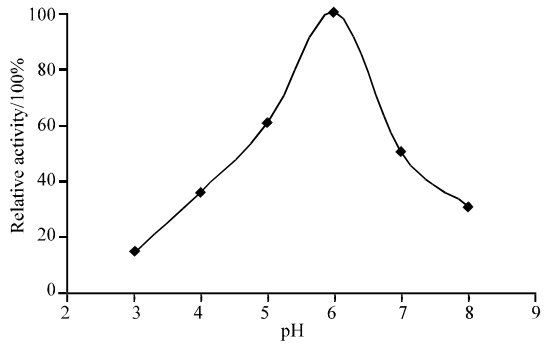

Fig. 2: Optimum pH of feijoa fruit PPO activity

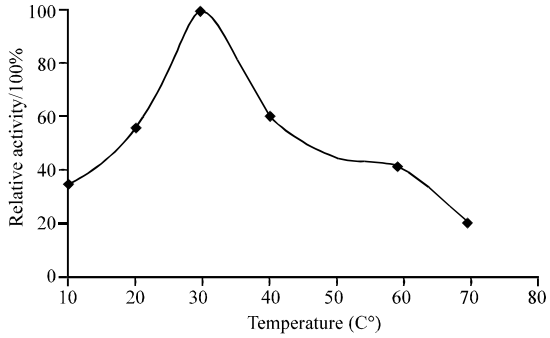

Fig. 3: Optimum temprature of feijoa fruit PPO activity

strawberry, 6.0 for De Chaunac apple, 7.0 for aubergine, 7.5 for Allium and 8.5 for Dog rose using catechol as substrate and 4.5 for strawberry, 6.0 for aubergine, 8.5 for Dog rose and 9.0 for Amasaya apple using 4-metchylcatechol as substrate. The optimum pH for PPO activity in fruits was also highly dependent on the enzyme source and the nature of substrate used.

Effect of temperature on enzyme activity: The effects of assay temperature between 10 and 70°C were measured using catecholas a substrate (Fig. 3). It is clear that the optimum temperature is 30°C. These values were considered as 100% of the relative activity. Enzymatic activity began to decrease more strongly >40°C which showed that it was weak high-temperature tolerant of feijoa fruit and blanching could prevent fruit from browning in processing. Variations in optimal temperature for fruit PPO activity ranging between 18 and 37°C have also reported by some other researchers (Ding *et al*.,1998; Oktay *et al*., 1995).

Effect of inhibitors on PPO: Enzymatic browning of plants may be reduced by using appropriate inhibitors. In this study, three inhibitors (ascorbic acid, $NaHSO_3$ and EDTA) were used to monitor prevention of enzymatic browning (Fig. 4). Ascorbic acid was the most effective

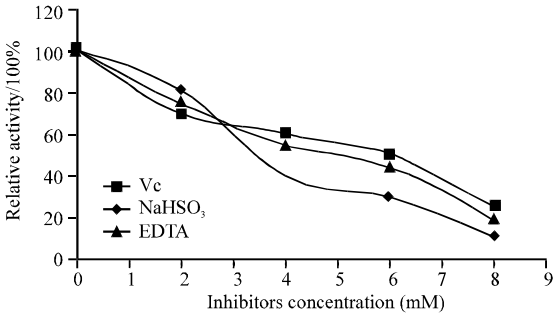

Fig. 4: Effect of inhibitors on feijoa fruit PPO activity

inhibitor at all stages and followed by EDTA and $NaHSO_3$. Inhibitors used for investigation in this study were placed in relative order of inhibition ascorbic acid>EDTA> $NaHSO_3$. Ascorbic acid acts more as an antioxidant than as an enzyme inhibitor because it reduces the initial quinone formed by the enzyme to the original diphenol before it undergoes secondary reactions which lead to browning.

CONCLUSION

In this study, ascorbic acid as compounds of fruits had a obvious inhibition effect, therefore it has found a wide application in production.

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