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Ciencia y Tecnología Alimentaria, vol. 4, núm. 4, diciembre, 2004, pp. 267-273,
Sociedad Mexicana de Nutrición y Tecnología de Alimentos
México

Ciencia y Tecnología Alimentaria,
ISSN (Printed Version): 1135-8122
somenta@gmail.com
Sociedad Mexicana de Nutrición y Tecnología de Alimentos
México

Cienc. Tecnol. Aliment. Vol. 4, No. 4, pp 267-273, 2004 www.altaga.org/cyta
 ISSN 1135-8122

CHARACTERIZATION OF POLYPHENOL OXIDASE OF SOURSOP (*Annona muricata* L.) FRUIT AND A COMPARATIVE STUDY OF ITS INHIBITION IN ENZYME EXTRACT AND IN PULP

CARACTERIZACIÓN DE POLIFENOLOXIDASA DE FRUTOS DE GUANÁBANA (*Annona muricata L.*) Y ESTUDIO COMPARATIVO DE SU INHIBICIÓN EN EXTRACTOS DE ENZIMA Y EN PULPA

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Recibido: 21 de Abril de 2004; recibida versión revisada: 5 de Julio de 2004; aceptado: 5 de Julio de 2004
Received: 21 April 2004; revised version received: 5 July 2004; accepted: 5 July 2004

Abstract

Crude enzyme extract containing polyphenoloxidase (PPO) was obtained from ripe soursop (*Annona muricata L.*) fruit by extraction with 0.2 M phosphate buffer (pH 7.5) containing 0.5 M NaCl, followed by precipitation with 20, 40, 60 and 80% saturation of ammonium sulfate and dialysis against distilled water at 4ºC. The maximum PPO activity was observed in the protein fraction obtained with 60% ammonium sulphate saturation. The effect of pH (3 to 8.8), temperature (20 to 60ºC) and chemical inhibitors (ascorbic acid, EDTA and SO_2) at several concentrations on the PPO activity was studied. The optimum pH and temperature for PPO activity were 7.5 and 32ºC, respectively. Among chemical inhibitors, ascorbic acid and SO_2 were almost equally effective, but EDTA showed very small inhibitory effect. 61.9 and 71.5% inhibition of PPO was achieved using 0.28 mM and 0.84 mM concentrations of ascorbic acid, while for 55.4 and 70.2% inhibition 0.78 mM and 2.34 mM concentrations of SO_2 were needed. Heating the enzyme extract at 70, 80, 90 and 95ºC for 6 s reduced 15.2, 49.5, 84.8 and 95.1% PPO activity, respectively. At the natural pH of pulp (4.3), SO_2 at concentration of 3.9 mM reduced specific activity of PPO from about 60 to about 8.4 U/mg protein in partially-purified enzyme extract (PPEE), while the specific activity of 22.5 U/mg protein was retained in the pulp. Heating PPEE at 80ºC at pH 4.3 (natural pH of pulp) for about 14 s decreased specific enzyme activity from 60.0 to about 11.0 U/mg and heating during 8 min decreased to 9.0 U/mg protein.

Keywords: Soursop, *Annona muricata,* Polyphenol oxidase, Enzyme inhibition

Resumen

Se obtuvo un extracto crudo de enzima conteniendo polifenoloxidasas (PPO) de frutos maduros de Guanábana (*Annona muricata L.*) por extracción con 0,2 M tampón fosfato (pH 7,5) conteniendo 0,5 M NaCl, seguido de precipitación con 20, 40, 60 y 80% de saturación de sulfato amónico y diálisis con agua destilada a 4ºC. El máximo de actividad de PPO se observó en la fracción de proteína obtenida con 60% de saturación sulfato amónico. Se estudió el efecto del pH (3 a 8,8), temperatura (20 a 60ºC) y inhibidores químicos (ácido ascórbico, EDTA y SO_2) a varias concentraciones, sobre la actividad de PPO. Los óptimos de pH y temperatura para la actividad de PPO fueron 7,5 y 32ºC, respectivamente. Mientras que para los inhibidores químicos, ácido ascórbico y SO_2 fueron casi igual de efectivos, pero EDTA mostró un bajo efecto inhibidor.Se obtuvo un 61,9 y 71,5% de inhibición de PPO con 0,28 mM y 0,84 mM de concentración de ácido ascórbico, mientras que con 0,78 mM y 2,34 mM de concentración de SO_2 obtuvo un 55,4 y 70,2% de inhibición, respectivamente. Calentando el extracto enzimático a 70, 80, 90 y 95ºC durante 6 s, se redujo a 15,2, 49,5, 84,8 y 95,1% la actividad de PPO, respectivamente. Al pH natural de la pulpa (4,3), SO_2 a la concentración del extracto enzimático 3,9 mM redujo la actividad específica de PPO desde 60 a 8,4 U/mg proteína en extracto enzimático parcialmente purificado (PPEE), mientras que en la pulpa la actividad específica se retuvieron 22,5 U/mg de proteína. Calentando PPEE a 80ºC a pH 4,3 (ph natural de la pulpa) por 14 s se redujo la actividad enzimática específica de 60,0 a 11,0 U/mg y calentando 8 min a 9,0 U/mg de proteina.

Palabras clave: Guanábana, *Annona muricata*, Polifenoloxidasa, Inhibición enzimática

Resumo

Obtívose un extracto cru de enzima contendo polifenoloxidasas (PPO) de froitos maduros de Guanábana (*Annona muricata L.*) por extracción con 0,2 M tampón fosfato (pH 7,5) contendo 0,5 M NaCl, seguido de precipitación con 20, 40, 60 y 80% de saturación de sulfato amónico e diálise con auga destilada a 4ºC. O máximo de actividade de PPO observouse na fracción de proteína obtida con 60% de saturación sulfato amónico. Estudiouse o efecto do pH (3 a 8,8), temperatura (20 a 60ºC) e inhibidores químicos (ácido ascórbico, EDTA e SO_2) a varias concentracións, sobre a actividade de PPO. Os óptimos de pH e temperatura para a actividade de PPO foron 7,5 e 32ºC, respectivamente. Mentras que para os inhibidores químicos, ácido ascórbico y SO_2 foron case igual de efectivos, pero EDTA mostrou un baixo efecto inhibidor. Obtívose un 61,9 e 71,5% de inhibición de PPO con 0,28 mM e 0,84 mM de concentración de ácido ascórbico, mentras que con 0,78 mM e 2,34 mM de concentración de SO_2 se obtivo un 55,4 e 70,2% de inhibición, respectivamente. Quentando o extracto enzimático a 70, 80, 90 e 95ºC durante 6 s, reduciuse a 15,2, 49,5, 84,8 e 95,1% a actividade de PPO, respectivamente. O pH natural da pulpa (4,3), SO_2 á concentración do extracto enzimático 3,9 mM reduciu a actividade específica de PPO dende 60 a 8,4 U/mg de proteína en extracto enzimático parcialmente purificado (PPEE), mentras que na polpa a actividade específica retivéronse 22,5 U/mg de proteína. Quentando PPEE a 80ºC a pH 4,3 (pH natural de la polpa) por 14 s reduciuse a actividade enzimática específica de 60,0 a 11,0 U/mg e quentando 8 min a 9,0 U/mg de proteina.

INTRODUCTION

Fruits and vegetables frequently undergo browning as a consequence of mechanical injury suffered during harvest, transport, storage and processing. Browning is attributed to the oxidation of the phenolic substances by the enzyme polyphenol oxidase.

Soursop (*Annona muricata* L.) one of the most cherished fruits of the family of *annonaceae* and genus *annona*, is cultivated in the tropical regions of Central and South America, Florida and tropical Africa (Gomes, 1975). Some work on the growth, maturation and ripening (Warrel *et al*., 1994), preservation (Maciel *et al*., 1994) of the fruit and characterization of the volatile constituents (Iwaoka *et al*., 1993; Wong and Khoo, 1993; Pelissier *et al*., 1994) has been published. The fruit is susceptible to browning catalyzed by the enzyme polyphenol oxidase (PPO) affecting its sensory and nutritional qualities. In spite of this very little attention has been directed towards the characterization of PPO in soursop fruit and consequently to minimize the enzymatic browning. In an only report available so far, Lima *et al*. (1994) studied PPO activity in soursop fruit as a function of pH and polyphenol concentration during maturation, and observed that the pH of optimum activity was at 7.0 for fully ripe and 7.5 for immature, mature and ripe fruits. Important to note that inhibition studies of PPO are carried out in enzyme extract at its optimum pH but not in the plant tissue at its natural pH. This fact invalidates the application of inhibition conditions to the fruits and vegetable tissues that may possess a different pH. Therefore, the present work was carried out with the objectives: (a) to characterize soursop fruit PPO in semipurified enzyme extracts and (b) to compare the efficiency of thermal and chemical inhibition conditions on PPO activity in semi purified enzyme extract and in soursop pulp, both at the natural pH of pulp.

MATERIAL AND METHODS

Plant Material

Ripe soursop (*Annona muricata* L.) fruits were obtained from an orchard in the municipality of Texeira, situated in the tropical semi-arid region of the State of Paraíba, Brazil. The fruits were washed with running water, dried with a towel and stored at room temperature (~ 28º C). Healthy fruits were cut with a stainless steel knife and the pulp and seeds were separated manually. The pulp (pH 4.3) was homogenized in a domestic mixer and stored at -15º C.

Extraction and partial purification of crude enzyme extract

The pulp was mixed with 0.2 M phosphate-phosphate buffer containing 0.5 M NaCl (pH 7.5) in the ratio of 1:5 and homogenized in a domestic mixer (Walita S.A.) at top speed for 3 min. The mixture was centrifuged at 4.300 g for 20 min at 5ºC. The fractionation of proteins from crude enzyme extract was carried out by precipitation with 20, 40, 60 and 80% ammonium sulfate saturation, successively. The required quantity of ammonium sulfate for 20% saturation was added to the crude enzyme extract and the solution was kept for 24 h in a refrigerator (~ 4ºC). The precipitate was recovered by centrifugation at 4.300 g for 20 min at 5ºC. Ammonium sulfate was added to the supernatant to increase the saturation to 40%, kept for another 24 h in the refrigerator and centrifuged. The process was repeated to obtain enzyme fractions with 60 and 80% ammonium sulfate saturation. The precipitate from each fraction was dissolved in extraction buffer and purified by dialysis against distilled water in a ratio of 1 to 20 volumes at about 4ºC for 24 h with two changes of water.

Enzyme assay and protein determination

PPO activity in the partially purified enzyme extract (PPEE) was measured spectrophotometrically using catechol as a substrate. The reaction mixture contained 1 ml catechol (0.01 M), 0.9 ml buffer (pH 7.5) and 0.1 ml of dialyzed enzyme extract. The absorbance was measured immediately after the addition of enzyme at 420 nm with an interval of 10 s during 5 min. One unit of polyphenol oxidase was considered as the quantity of enzyme, which caused an increase of 0.001 unit of absorbance per min (Oktay *et al*., 1995).

The protein determination was carried out according to the method of Lowry *et al*. (1951) using bovine serum albumin (Sigma) as standard.

Determination of optimum pH

The optimum pH for the PPO activity of PPEE was determined at room temperature (28±2º C) by varying the pH of the reaction mixture from pH 3.0 to 8.8. The pH in the range from 3.0 to 7.0 was maintained by using 0.2 M citrate-phosphate buffer and from 7.5 to 8.8 by 0.2 M phosphate-phosphate buffer both containing 0.5 M NaCl.

Determination of optimum temperature

The optimum temperature for the PPO activity of PPEE was determined by incubating the substrate and the buffer of the assay mixture (pH 7.5) at temperatures varying from 10 to 60ºC. 0.1 ml of enzyme solution was then added to initiate the reaction. The PPO activity was measured as described earlier.

Inhibition of PPO in PPEE at pH 7.5

Thermal inactivation

Thermal inactivation of PPO in PPEE was studied at temperatures 70, 80, 90 and 95ºC for time intervals varying from 0 to 14 s after attaining the desired temperature. The procedure used was as follows:

(1) A test tube containing 5 ml of enzyme solution in 0.2 M phosphate buffer, 0.5 M NaCl (pH 7.5) was immersed in a boiling water bath and the enzyme solution was stirred continuously to ensure a uniform heating. A thermometer was inserted in the enzyme solution to monitor the temperature and the contents of the tube were brought to the desired temperature.

(2) After attaining the desired temperature, the enzyme solution was kept in a second water bath

maintained at the same temperature and holding the solution for the required time period.

After this treatment, the enzyme solution was immediately cooled in an ice bath and the PPO activity was determined.

Chemical inhibition

Ascorbic acid, EDTA and SO_2 (as sodium metabisulphite) at concentrations varying from 0.28, 0.56, 0.84 , 1.12, 1.68 , 2.34 and 3.12 mM; 0.16, 0.32, 0.48, 0.64, 0.80, 1.60, and 3.20 mM, and, 0.78, 1.56, 2.34 and 3.12 mM were evaluated for their inhibitory effect on PPO activity at room temperature (28±2ºC), respectively. In this case the assay mixture consisted of 0.1 ml enzyme extract, 0.1ml of inhibitor solution, 0.8 ml of 0.5 M NaCl, 0.2 M phosphate buffer (pH 7.5) and 1 ml of 0.01 M catechol solution.

Inhibition of PPO in the soursop pulp at its natural pH 4.3

Thermal Inactivation

300 g lot of soursop pulp (pH 4.3) was put in a beaker and immersed in a boiling water bath. Immediately after the pulp attained a temperature of 80°C, the beaker was put in a second water bath maintained at 80°C. 50 g samples of pulp were taken out at different time periods and immediately cooled to room temperature (28±2°C) in an ice bath. To each sample, 250 ml of extraction buffer (pH 7.5) was added and the mixture was homogenized in a domestic homogenizer at the maximum speed for 3 min. The mixture was centrifuged at 4.300 g for 30 min at 5ºC and the precipitate obtained with 40% ammonium sulfate saturation in supernatant was discarded. The PPO activity was determined in the fraction precipitated with 60% ammonium sulfate saturation and dissolved in 10 ml 0.2 M citrate-phosphate buffer containing 0.5 M NaCl (pH 4.3). The reaction mixture contained 1.0 ml catechol (0.01 M), 0.9 ml of 0.2 M citrate-phosphate buffer (pH 4.3) containing 0.5 M NaCl and 0.1 ml of the supernatant.

Chemical inhibition

In 50 g portions of soursop pulp, SO_2 as sodium metabisulphite at concentrations of 0.78, 1.56 2.34, 3.12 and 3.90 mM was added. The same procedure, as described above, was utilized for the extraction of PPO and determination of its activity.

Inhibition of PPO in PPEE at pH 4.3

Thermal Inactivation

The protein fraction obtained with 60% saturation (fraction obtained with 40% saturation was discarded) was dissolved in 0.2 M citrate- phosphate buffer containing 0.5 NaCl, pH 4.3. The enzyme extract was subjected to a temperature of 80ºC for different time periods after which the samples were immediately cooled in an ice bath until attained the room temperature (~ 28ºC). The PPO activity in the heated PPEE was determined as described above.

Chemical inhibition

The same procedure as in 2.6.2 was adopted for evaluating the inhibitory effect of SO_2 at concentrations of 0.78, 1.56, 2.34, 3.12 and 3.90 mM on polyphenol oxidase activity except that the enzyme extract was prepared in 0.2 M citrate-phosphate, 0.5 M NaCl (pH 4.3) In this case the assay mixture consisted of 0.1 ml enzyme extract, 0.1ml of inhibitor solution, 0.8 ml of 0.2 M citrate-phosphate buffer (pH 4.3) containing 0.5 M NaCl and 1 ml of 0.01M catechol solution.

All above experiments were carried out in triplicate.

Statistical Analysis

Statistical analysis of the results was done with Statistics for Windows 5.0 (1995) and are presented as mean value ± standard deviation.

RESULTS AND DISCUSSION

Extraction and partial purification of enzyme extract

The PPO activity, specific activity, yield and purification fold of the protein fractions obtained by successive precipitation with 20, 40, 60 and 80% saturation of ammonium sulfate in crude enzyme extract followed by dialysis, is shown in Table 1. It was observed that the protein fraction obtained with 60% saturation of ammonium sulfate contained maximum PPO activity (43.3 units). In comparison to crude enzyme extract, a five-fold increase in the specific activity (167.8 units/mg protein) was observed in this fraction. For this reason, further studies on PPO characterization and inhibition were carried out with partially purified enzyme fraction obtained with 60% saturation of ammonium sulfate.

Optimum pH

A wide range of pH optima for the activity of PPO ranging from 4.0 (Paul and Gowda, 2000) in field bean seed to 8.5 (Seol *et al*., 1999) in ginkgo biloba leaves has been reported in literature. The soursop PPO activity as a function of pH is shown in Figure 1. The enzyme had

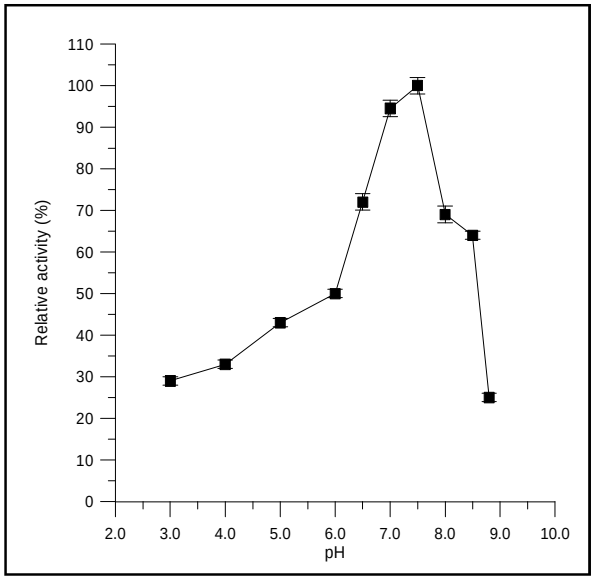

Figure 1. PPO activity (~28ºC) of the partially purified enzyme extract

Table 1.- PPO activity and PPO specific activity in different ammonium sulphate precipitated fractions dissolved in 0.2M phosphate – phosphate (pH 7.5) buffer. The table shows the results (mean value ± standard deviation) of three experiments.

Fraction	Volume (ml)	Protein (mg/ml)	Enzyme activity (u)	Total enzyme activity (total units)	Specific enzyme activity (u/mg prot.)	Yield (%)	Purification fold
Crude enzyme extract	103.0 ± 3.6	3.32 ± 0.15	10.03 ± 1.32	1.030 ± 99.3	30.36 ± 2.67	100.00	1.0
Ammonium sulphate saturation (%)							
20.0	10	5.52 ± 0.5	8.30 ± 1.70	83.0 ± 5.0	15.23 ± 0.56	8.43 ± 0.4	0.50 ± 0.7
40.0	10	4.79 ± 0.23	21.66 ± 1.9	216.6 ± 19.0	45.22 ± 1.84	21.03 ± 0.19	1.49 ± 0.07
60.0	10	2.58 ± 0.32	43.33 ± 3.69	433.3 ± 36.9	168.23 ± 6.25	42.10 ± 0.48	5.58 ± 0.7
80.0	10	3.55 ± 0.27	11.66 ± 0.38	116.6 ± 3.8	32.87 ± 0.49	11.37 ± 0.73	1.09 ± 0.11

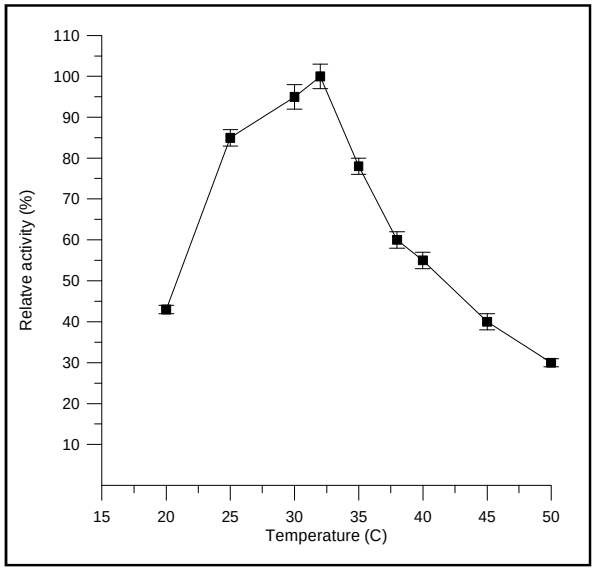

Figure 2. PPO activity (pH 7.5) of the partially purified enzyme extract of soursop fruit pulp as a function of temperature.

maximum activity at pH 7.5. Besides this, the activity curve also showed a shoulder at pH 8.5. In some species such as egg plant (Fujita and Tono, 1988) and Hale Haven peaches (Yemenicioglu and Cemeroglu, 1998), blueberry fruit (Kader *et al.*, 1997) etc. two pH optima have been reported. The soursop PPO presented very low activity below pH 4.0 and above pH 8.8. Lima *et al.* (1994) reported the optimum soursop PPO pH optimum at 7.5 for fully developed immature, mature unripe, and ripe fruits and, 7.0 for fully ripe fruits. Gomez-Lopez (2002), Dogan *et al.* (2002), and Gomes *et al.* (2001) have also reported pH optima of 7.5 for avocado, 7.0 for aubergine, and 7.2 for beans PPO respectively.

Optimum Temperature

In general, enzyme activity is very low at subzero temperatures, and increases with increase in temperature to about 45°C. The majority of enzymes exhibit optimum temperature in the range of 30 to 40°C. However, temperature optima as low as 20ºC for bartlet pear and as high as 45°C for berry fruit PPO has been observed by Siddiq and Cash

The results of the PPO activity in partially purified enzyme extract in the temperature range of 10 to 50°C are shown in Figure 2. The enzyme showed maximum activity between 30 to 32°C, the later being the temperature of optimum activity. Though soursop PPO started losing its activity above 32^0C, yet at 40^0C it still retained almost 48% of its maximum activity. Various other fruits and vegetables such as banana (Chang *et al.*, 2000), potato (Zang and Huang, 2002), medlar (Dincer *et al.*, 2002) PPO also showed 30^0C as an optimum temperature.

Thermal Inactivation

The effect of various temperatures: 70, 80, 90 and 95°C for different time periods on the inactivation of soursop PPO is shown in Figure 3. To attain these temperatures, 5 ml of the partially purified enzyme extract took 68, 86, 130 and 190 s. After attaining the temperature of 70°C, the PPO still retained 98% of its activity for further 2 sec, after that started losing its activity at a slight faster rate but even after 10 s exposure the enzyme retained almost 70% of its activity. At temperatures of 80, 90 and 95°C, for the first 2 s the PPO inactivation was steep (27.1, 67.4 and 79.8%, respectively) but after that inactivation was more gradual retaining about 42.6, 9.5 and 4.4% activity after an interval of 8 s, respectively. PPO from the different sources exhibited different heat resistance. For example Welsche-Ebeling and Montgomery (1990) reported that to inhibit 99% strawberry PPO activity 4, 5 and 6 min heating was necessary at 95, 90 and 80°C respectively, while at 70°C, 7 min heating was required to inhibit about 80% activity. Wakayama (1994) also observed about 1.4 min to 2.4 min were required for 90% inhibition of the PPO from the core of six Japanese apple varieties. However, PPO from some other fruits such as banana (Chang *et al.*, 2000), lichi (Ming *et al.*, 1997) and mango sap (Robinson *et al.*, 1993) offered higher resistance to thermal inactivation.

Chemical Inhibition

The results of the effect of chemical inhibitors such as ascorbic acid, EDTA and SO_2 on the PPO activity are

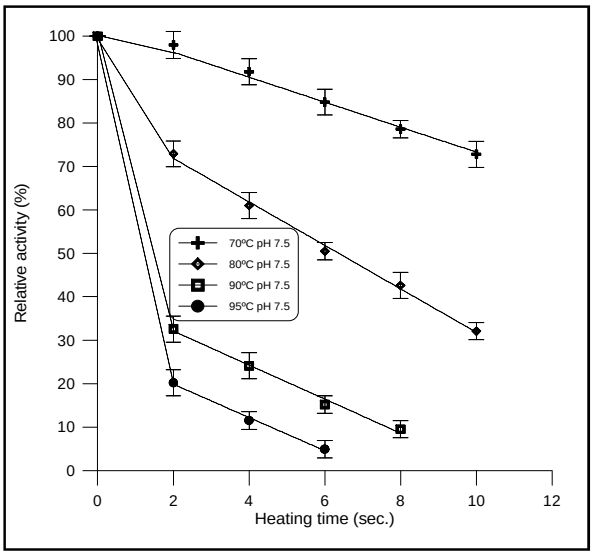

Figure 3. Thermal inactivation of PPO activity (pH 7.5) of the partially purified enzyme extract of soursop fruit pulp at different temperature and time periods.

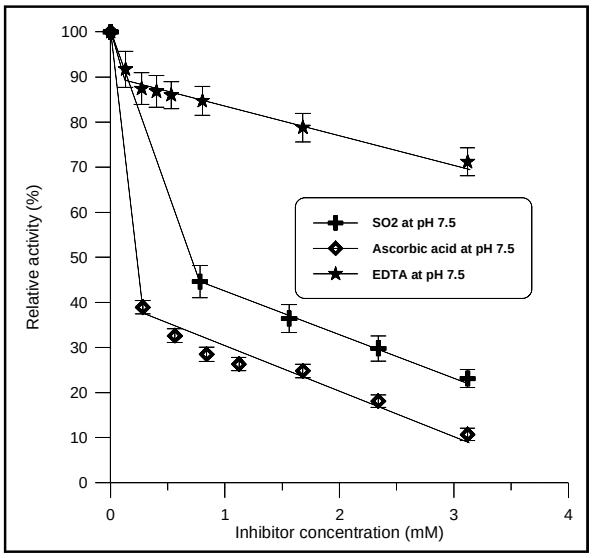

Figure 4. Inhibition of PPO activity (pH 7.5, ~28^{0}C) of the partially purified enzyme extract of soursop fruit pulp by different concentrations of chemical inhibitors.

Ascorbic acid

Ascorbic acid is known to inhibit browning due to its capacity of reducing o-quinones to corresponding o-diphenols. In recent reports Zang and Huang (2002), Gomez-Lopez (2002), Nagai and Suzuki (2001) Chang *et al*. (2001) to mention a few, have observed strong inhibitory effect of ascorbic acid against potato, avocado, Chinese cabbage and banana PPO. In our study, ascorbic acid at concentrations of 0.28, 0.56, 0.84 , 1.12, 1.68, 2.34 and 3.12 mM reduced the residual PPO activity to 38.9, 32.6, 28.5, 26.3, 24.8, 18.1 and 10.7% respectively, of the original activity in the partially purified enzyme extract of soursop fruit at pH 7.5. Beyond this concentration, there was almost no further inhibition of soursop PPO by ascorbic acid. In a similar study for Fuji apple PPO Wakayama (1994) reported 64.0, 44.0 19.5 and 14.10% retention at pH 5.0 and, 87.5, 62.6, 9.9 and 2.4% retention in PPO activity at pH 3.0 with 0.028, 0.085, 0.142 and 0.179 mM concentrations of ascorbic acid.. Ming *et al.* (1997) also reported 69% inhibition of lichi PPO with 1mM ascorbic acid.

EDTA

EDTA was utilized with an objective to bind Cu^{2+} of the PPO to block the prosthetic group and consequently to inhibit its activity. Contrary to expectations, only about 30 % of the PPO activity in partially purified enzyme extract of soursop at pH 7.5 was inhibited with 3.2 mM EDTA. Earlier, Ming *et al*. (1997) also observed very little inhibitory effect of EDTA on lichi fruit PPO.

Metabisulphite

Metabisulphite (SO_2) possibly inhibits the enzyme or can react directly with the quinones to reduce them to original phenols. In the earlier studies Kavrayan and Aydemir (2001), Siddiq and Cash (2000), Paul and metabisulfite as the most effective inhibitor against peppermint, pears, field bean and pineapple fruit PPO, respectively. A strong inhibitory effect of SO_2 on soursop PPO at pH 7.5 was also observed in our study. With 0.78, 1.56, 2.34 and 3.12 mM concentrations the residual PPO activity was reduced to 44.6, 36.4, 29.8 and 23.1% of the original in PPEE.

The thermal and chemical inactivation curves for soursop PPO consisted initially of a steep line followed by another straight line with a relatively shallow slope. As the optimum pH curve showed the presence of a peak and a shoulder, there is a possibility that two PPO isoenzymes with different resistance are present in soursop, the first and second straight lines representing less resistant and more resistant isoenzyme, respectively. Similar observation was also made by Yemenicioglu and Cemeroglu (1998) for hale heaven peache PPO.

Inactivation/ Inhibition of PPO in the Soursop Pulp

In this study, the PPO in the pulp and in partially purified enzyme extract was subjected to heat/chemical treatment at the natural pH of the fruit (4.3) and the PPO activity was also determined at pH 4.3.

Thermal Inactivation

The effect of thermal treatment (80°C for different time) on the activity of PPO in soursop pulp as well as in PPEE at pH 4.3 is shown in Figure 5. It can be observed that 90% inactivation of PPO in the pulp was achieved after 8 min of treatment. Comparing with the results of the inactivation of the PPO in the partially purified enzyme extract at 80°C, which took nearly 15 s, the PPO in the pulp required a more rigorous heat treatment. Possibly, high viscosity and the presence of some chemical constituents exert a protective effect on the pulp PPO,

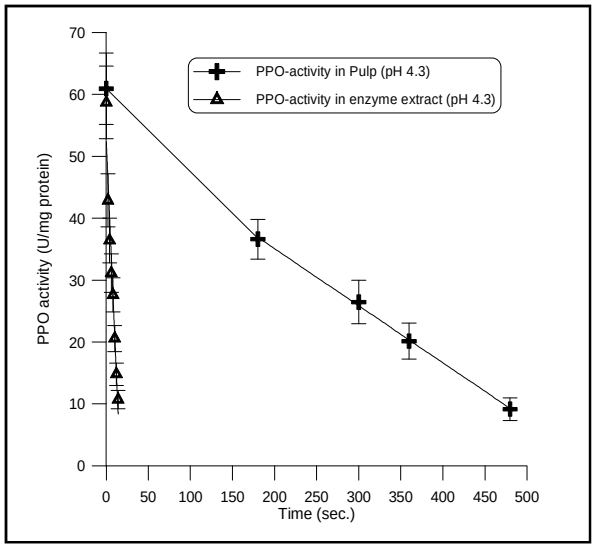

Figure 5. Comparative inhibition of PPO in the partially purified enzyme extract and in soursop pulp at 80^0 C at the natural pH (4.3) of pulp.

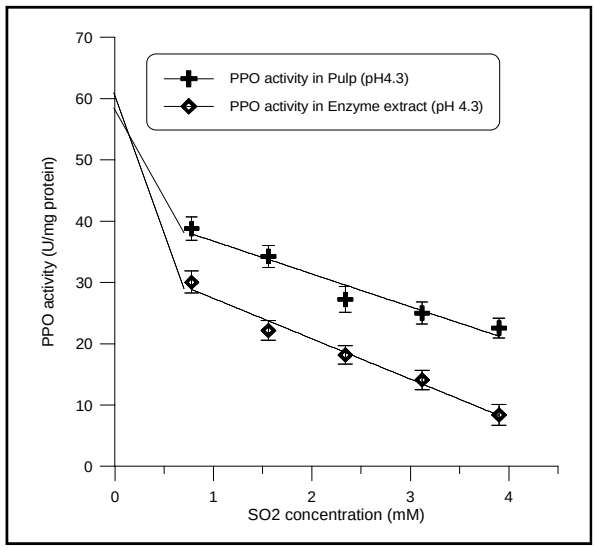

Figure 6. Comparative inhibition of PPO in partially purified enzyme extract and in soursop pulp by SO_2 at the natural pH (4.3) of pulp.

Inhibition with SO_2

The results of the effect of SO_2 on the PPO activity of pulp and PPEE are shown in Figure 6. Similar to thermal treatment, higher concentration of SO_2 was required for the inhibition of the PPO in the pulp when compared with the concentration of SO_2 used to inhibit the same percentage of PPO in PPEE. To bring down the PPO activity from about 59 units/mg to about 25 units/mg protein, 3.9 mM of SO_2 were required in soursop pulp against 1.56 mM in PPEE.

CONCLUSIONS

The PPO of the soursop fruit exhibited optimum pH and temperature at 7.5 and 32°C, respectively. Its behavior with respect to inhibition in the partially purified enzyme extract was different than in the fruit pulp. At the natural pH of pulp, to achieve a similar degree of PPO inhibition higher SO_2 concentrations as well as heating time at 80°C were required for the pulp pH than for partially purified enzyme extract. The results shows that the conditions for enzyme inhibition obtained with enzyme extract are not sufficient to avoid browning in food products. The difference in inhibition behavior may be attributed to the possible protection offered by certain pulp constituents as well as to the slow heat transfer in viscous pulp. In further studies, we plan to investigate the role of the viscosity of fruit pulp with respect to thermal and chemical inhibition of PPO.

ACKNOWLEDGEMENT

The author thank the National Council for the Development of Science and Technology (CNPQ), Brazil for financing this project (no. 403619/92-4/EA/FV/PQ).

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