Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 1047–1053
Food Chemistry
www.elsevier.com/locate/foodchem

Characterization of polyphenol oxidase from broccoli (*Brassica oleracea* var. *botrytis italica*) florets

Urszula Gawlik-Dziki *, Urszula Szymanowska, Barbara Baraniak

Department of Biochemistry and Food Chemistry, Agricultural University, ul. Skromna 8, 20-704 Lublin, Poland

Received 3 February 2006; received in revised form 27 April 2007; accepted 1 May 2007

Abstract

Polyphenol oxidase (PPO) from broccoli florets was extracted and purified through $(NH_4)_2SO_4$ precipitation, ion-exchange and gel filtration chromatography. The molecular weight was estimated to lie between 51.3 and 57 kDa by sodium dodecyl sulphate-polyacrylamide gel electophoresis (SDS-PAGE) and gel filtration. The effects of substrate specificity, pH, and sensitivity to various inhibitors: citric acid, ascorbic acid, sodium sulphate and EDTA (sodium salt of ethylenediaminetetraacetic acid) of partially purified PPO were investigated. Polyphenol oxidase showed the best activity toward catechol ($K_M = 12.34 \pm 0.057$ mM, $V_{max} = 2000 \pm 8736$ U/ml/min) and 4-methyl catechol ($K_M = 21 \pm 0.087$ mM, $V_{max} = 28.20 \pm 0.525$ U/ml/min). The optimum pH for broccoli PPO was 5.7 with catechol and 4-methylcatechol as substrates. The most effective inhibitor was sodium sulphate.

Keywords: Polyphenol oxidase; Broccoli; Inhibitors

1. Introduction

Polyphenol oxidase (PPO) is a common copper-containing enzyme which is responsible for melanization in animals and browning in plants. The enzyme catalyzes two distinct reactions: the o-hydroxylation of monophenols to *o*-diphenols (acts like cresolase (E.C. 1.14.18.1.)) and the oxidation of *o*-diphenols to *o*-quinones (acts like catecholase (E.C. 1.10.3.2.)) (Rodríguez-López et al., 2001; van Gelder, Flurkey, & Wichers, 1997). The role of PPO in plants is not yet clear. It is suggested that it may be involved in immunity reactions and in biosynthesis of plant components, and it also may play the role of a scavenger of free radicals in photo-synthesizing tissues (Heimdal, Larsen, & Poll, 1994). Quinones are highly reactive electrophilic molecules that can polymerise, leading to the formation of brown or black pigments. 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 (Prota, 1988). For this reason, the activity of PPO has been studied in apples (*Malus* sp.) (Espin, Morales, Varon, Tudela, & Garcia-Carnovas, 1995), pears (*Pyrus* sp.) (Hwang, Yoon, & Kim, 1996), potatoes (*Solanum tumerosum* L.) (Chen et al., 1992), artichokes (*Cynara scolymus* L.) (Leoni & Palmeri, 1990), broad beans (*Vicia faba* L.) (Ganesa, Fox, & Flurkey, 1992), lettuce (*Lectuca sativa* L.) (Heimdal et al., 1994), banana (*Musa cavendishii* L.) (Galleazi, Sgarbieri, & Constantinides, 1981), plums (*Prunus* sp.) (Siddiq, Sinha, & Cash, 1992), peppermint (*Mentha piperita* L.) (Kavrayan & Aydemir, 2001), coffee (*Coffea arabica* L.) (Mazzafera & Robinson, 2000) and seeds of field bean (*Dolichos lablab*) (Paul & Gowda, 2000). The literature on the subjects contains no data on purification and characterization of PPO from broccoli. The main objective of this investigation was to characterize PPO from broccoli florets and to elucidate the mechanism of its inhibition by selected chemical compounds.

* Corresponding author. Tel.: +48 81 4623327; fax: +48 81 4623324.
E-mail addresses: urszula.gawlik@ar.lublin.pl (U. Gawlik-Dziki), barbara.baraniak@ar.lublin.pl (B. Baraniak).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.05.012

2. Materials and methods

2.1. Materials

Frozen broccoli florets/Hortex Company/were used. Catechol, 4-methylcatechol, polyvinylpyrrolidone (PVP40), DEAE-Sephadex A-50, Sephadex G50, Sephadex-G100, Bradford reagent, vaniline, ferulic acid, phloroglucinol (1,3,5-trihydroxybenzene), chlorogenic acid, caffeic acid, protein markers, ascorbic acid, citric acid, sodium sulphate, EDTA (sodium salt of ethylenediaminetetraacetic acid) were purchased from Sigma–Aldrich, USA. All others chemicals were of analytical grade.

2.2. Enzyme extraction and purification

Ten grams of material were homogenized in 80 ml of 0.1 M sodium phosphate buffer pH 6.8 containing 10 mM ascorbic acid and 0.5% polyvinylpyrrolidone (PVP40) and extracted with the aid of a magnetic stirrer for 1 h. The crude extract samples were centrifuged at 32,000*g* for 20 min. The process was conducted at the temperature of 4 °C. Solid $(NH_4)_2SO_4$ was added to the supernatant to obtain 80% saturation. After an hour, the precipitated proteins were separated by centrifugation at 32,000*g* for 30 min. The precipitate was dissolved in a small amount of 5 mM phosphate buffer (pH 6.8) and dialyzed at 4 °C in the same buffer for 24 h (MW cut-off >12,000) with four changes of the buffer during dialysis. In order to conduct further purification, the dialysate was transferred to a column (2.5 × 100 cm) filled with DEAE-Sephadex A-50 gel, balanced with 5 mM phosphate buffer, pH 6.8. The column was eluted with the same eluent at the flow rate of 30 ml/h and linear gradient of NaCl concentration from 0 to 1.0 M. Five millilitre fractions were collected in which the protein level and PPO activity towards catechol as substrate were monitored. The fractions which showed PPO activity were collected and concentrated and then dissolved in 3 ml of phosphate buffer, pH 6.8. The combined fractions were transferred to a glass column (2.5 × 100 cm) filled with Sephadex G50 gel. The column was eluted with the same buffer solution. 3-ml fractions were collected and the protein content and PPO towards catechol was monitored in them spectrophotometrically. The fractions showing PPO activity were combined, concentrated and transferred to a column (2.5 × 100 cm) filled with Sephadex-G100 gel. The column was eluted as described above.

2.3. Enzyme assay

PPO activity was determined by measuring the initial rate of quinone formation, as indicated by an increase in absorbance at 420 nm (Perkin–Elmer Lambda 40 UV–Vis spectrophotometer was used) (Wisserman & Lee, 1980). An increase in absorbance of 0.001 min^{-1} was taken as one unit of enzyme activity (Ho, 1999). The increase in absorbance was linear with time for the first 120 s. The sample cuvette contained 2.95 ml of substrate solution (10 mM catechol or 4-methylcatechol) 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.

2.4. Protein estimation

Protein content was determined according to the dye-binding method of Bradford (1976) using bovine serum albumin as standard.

2.5. Determination of molecular weight

The molecular weight of the purified enzyme was estimated by SDS-PAGE and gel permeation. SDS-PAGE was performed according to the method of Laemmli (1970). Proteins were dissolved in a 12.5% polyacrylamide gel and visualized with colloidal Coomassie staining.

The molecular weight of PPO was also estimated on a Sephadex G 100 with protein markers of cytochrome C (12.40 kDa), albumin (66.00 kDa), carbonic anhydrase (29.00 kDa), aprotinin (6.50 kDa) and aldolase (158.00 k kDa). Blue dextran 2000 was used to determine the void volume (V_0). The protein markers were chromatographed and the elution volume (V_e) was measured. The distribution coefficient (K_{av}) was given by equation:

$$K_{av} = (V_e - V_0)(V_t - V_0)^{-1}$$

where V_t stands for the total bed volume. The calibration curve was obtained by plotting log MW versus K_{av}.

2.6. Characterization of PPO

2.6.1. Effect of pH on enzyme activity

PPO activity, as a function of pH, was determined under standard conditions using various buffers in a pH range 2.0–12.0. The buffer systems were prepared according to Britton–Robinson (Kłyszejko-Stefanowicz, 2003). The optimum pH for the PPO was obtained using two substrates: 10 mM catechol and 10 mM 4-methyl catechol. The pH value corresponding to the highest enzyme activity was taken as the optimal pH.

2.6.2. Kinetic data analysis and substrate specificity

The specificity of broccoli PPO extract was investigated for seven commercial grade substrates: vaniline, ferulic acid, phloroglucinol (1,3,5-trihydroxybenzene), chlorogenic acid, caffeic acid, catechol and (4-methylcatechol) 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-methyl catechol was investigated. Michaelis constant (K_m) and V_{max} of the PPO was determined by Lineweaver–Burk's method.

2.6.3. Effect of inhibitors on PPO

The inhibitory effects of ascorbic acid, citric acid, sodium sulphate, EDTA (sodium salt of ethylenediaminetetraacetic acid) on PPO activity were determined. Different concentrations (0.5–5 mM) of these compounds were added to the above-described reactions. The corresponding control contained the same concentration of enzyme, in the absence of inhibitor.

3. Results and discussion

3.1. Enzyme extraction and purification

The extraction and purification of the enzyme was described in Section 2. The protein obtained after precipitation with ammonium sulphate (2 ml; 0.586 mg protein) was separated by ion-exchange chromatography using Sephadex DEAE A50 gel. The results of the operation are shown in Fig. 1. Two protein peaks containing PPO activity were recovered (Peak I, fraction 3–6; Peak II, fraction 9–14). The peaks were collected and concentrated and further purified by gel filtration using Sephadex G50 bed separately. Peak I was separated into two while Peak II into four or more protein peaks (Fig. 2). However, the PPO activity was recovered in the early fractions of the gel column for both DEAE peak materials. The enzyme fractions from the Sephadex G50 chromatography were combined and further purified by another gel filtration step using Sephadex G100 bed (Fig. 3). Once again, the enzyme activity was recovered in the early fractions but well resolved from the major protein peak. The results of PPO purification at various stages of the process are presented in Table 1. The ammonium sulfate precipitation, ion-exchange and gel chromatography was found to yield 47.4-fold purification of the enzyme, with protein recovery amounting to 34.69%. The technique of gel filtration is widely used in enzyme preparation. Selles-Marchart, Casado-Vela, and Bru-Martinez (2006) purified polyphenol oxidase obtained from *Eriobotrya japonica* Lindl. and

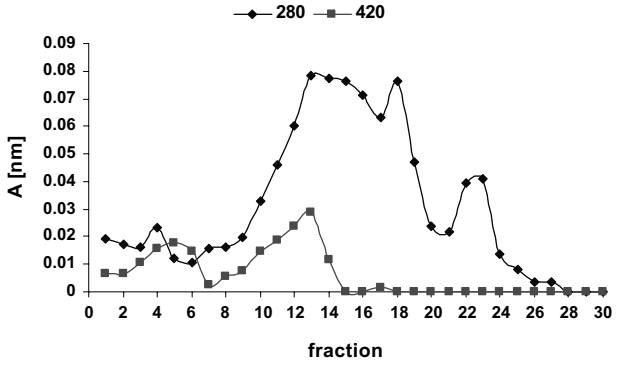

Fig. 1. Purification of broccoli PPO by ion exchange chromatography on DEAE Sephadex A 50 column. Proteins were monitored by absorbance at ⟨lambda⟩ 280 nm and polyphenol oxidase activity by absorbance at ⟨lambda⟩ 420 nm as described in methods.

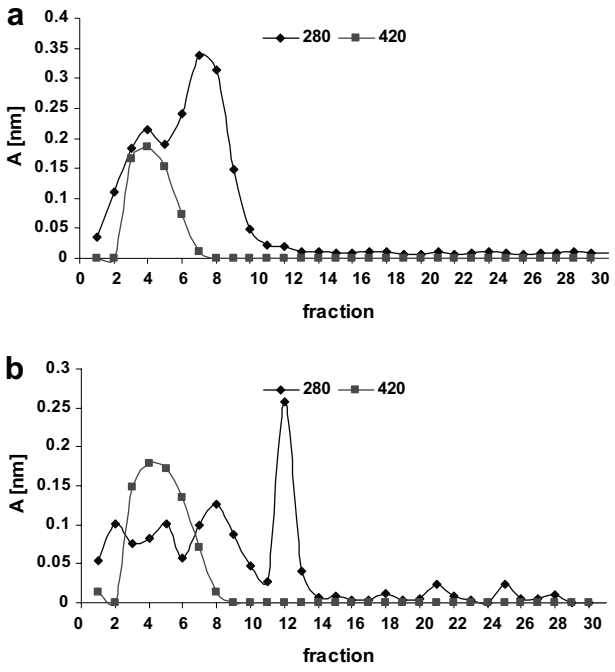

Fig. 2. Purification of broccoli PPO by gel filtration chromatography on Sephadex G-50 column A – peak I, B – peak II.

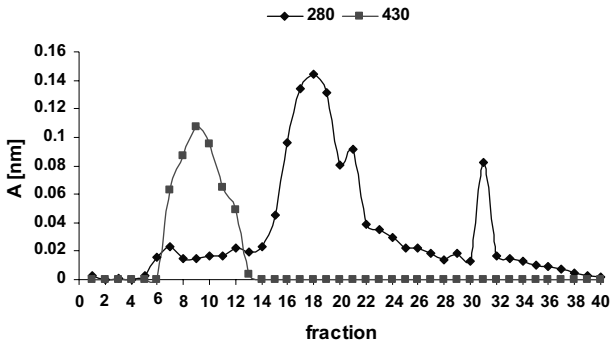

Fig. 3. Eluent profile of broccoli PPO on Sephadex G-100.

achieved the purification fold of 39.9 with the protein recovery of 15%. Erat, Sakiroglu, and Kufrevioglu (2006) employed the technique of gel chromatography on Sephadex G100 in purification of PPO from leaves and stalks of *Ferula* sp. As a result, they achieved a purification fold of 43.33 with protein recovery of 80.6% from the leaves and purification fold of 25.28 with protein recovery of 77.3% from the stalks of the plant. Xu, Zheng, Meguro, and Kawachi (2004) purified PPO from Henry chestnuts (*Castanea henryi*) and achieved purification fold of 36.87 with protein recovery of 12.94%.

3.2. Determination of molecular weight

The enzyme purified by three chromatographic separations was analyzed electrophoretically under denaturating conditions. Only one protein band was detected under

Table 1
Purification of PPO from broccoli florets

Purification step	Activity (Uml^{-1})	Total activity	Protein content (mg/ml)	Total protein (mg)	Specific activity ($Umin^{-1}\ mg^{-1}_{protein}$)	Recovery (%)	Purification fold
Crude	53.37	8150	0.067	10.23	800	100	1
Ammonium sulfate precipitation	896	5734	0.293	1.87	3058	70.35	3.8
DEAE	559	3356	0.027	0.16	20,425	41.18	25.5
G-50	472	4725	0.015	0.15	31,430	57.97	39.3
G-100	377	2827	0.010	0.08	37,953	34.69	47.4

the staining condition specified. The analysis revealed a single band on the SDS-PAGE which corresponded to a molecular weight of 51.3 kDa (Fig. 4). The protein size of the purified enzyme in native conditions as determined by gel filtration chromatography was found to be 57 kDa, thus providing strong evidence that the enzyme is a monomer. The results obtained in the present study correspond well to those obtained by other researchers, indicating that the molecular weight of polyphenol oxidase ranges from 45 to 67 kDa. For the PPO obtained from apples the value is 57 kDa, from beans – 58 kDa, from potato – 57–60 kDa, from tomato (*Lycopersicon* sp.) – 57–62 kDa, from coffee – 45–67 kDa, from avocado pear – 58.5–66.5 kDa (de Fatima Pereira, Alves, Magalhaes, de Oliveira Lima, & Meyer, 2003; van Gelder et al., 1997; Weemaes, Ludikhuyze, Van den Broeck, & Hendrickx, 1998).

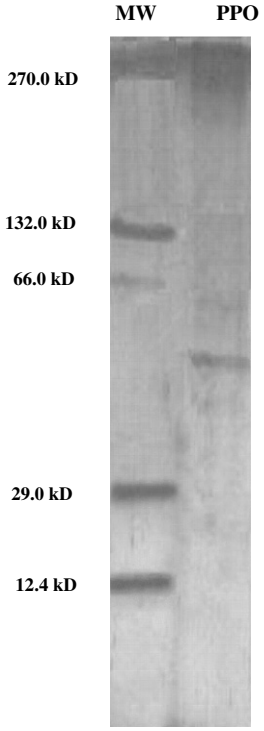

Fig. 4. SDS-PAGE of purified PPO from broccoli florets (detected under the staining condition specified). The lane marked MW contained the molecular weight markers, lane marked PPO contained the purified enzyme.

3.3. Characterization of PPO

3.3.1. Effect of pH on enzyme activity

The pH optimal of phenol oxidase from broccoli was found to be pH 5.72 for both catechol and methyl catechol substrates (Fig. 5). This result corresponds well with the results obtained by McCallum and Walker (1990) for phenol oxidase determined in wheat flour against 4-methylcatechol (pH 5.6) and with results obtained by Robert, Cadet, Rouch, and Pabion (1995b), for phenol oxidase from edible parts of palm (pH 5.2). On the other hand, phenol oxidase isolated from apple peel and separated by column chromatography on phenylo-Sepharose CL-4B by Zhou, Smith, and Lee (1993) showed maximum activity at pH 5.0 when catechol was the substrate, and for 4-methylcatechol the enzyme reached peak efficiency at pH 4.6. The optimum action of PPO isolated from aubergine and determined against chlorogenic acid was at pH 4.0 (Fujita & Tono, 1988), and when it was obtained from potato and analyzed against catechol – at pH about 6.7 (Cho & Ahn, 1998). Similar maximum activity (determined against 3,4-dihydroxyphenylalanine) was shown by PPO from pears (Hwang et al., 1996), whereas monophenolase from apples was most active at pH 4.6 (Espin et al., 1995). In addition, Ho (1999) obtained four isomorphic forms of PPO from aerial roots of orchid that showed maximum activity determined against 4-methylcatechol at pH 7.0.

3.3.2. Kinetic data analysis and substrate specificity

Relative enzyme activity with different substrates was determined with the use monohydroxy-, dihydroxy- and

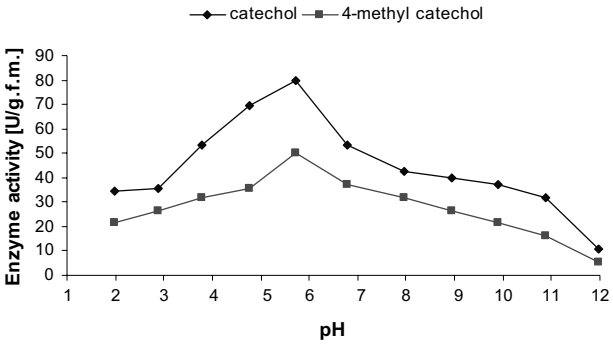

Fig. 5. Activity of broccoli PPO as function of pH.

Table 2
Relative enzyme activity at 10 mM substrate concentration

Substrate (10 mM)	Relative activity (%)
Catechol	100.00 ± 2.39
4-Methyl catechol	62.76 ± 3.36
Caffeic acid	5.92 ± 0.024
Ferulic acid	1.10 ± 0.03
Chlorogenic acid	1.89 ± 0.027
Vaniline	0.45 ± 0.008
Phloroglucinol	0.16 ± 0.002

trihydroxyphenol at 10 mM substrate concentration. Polyphenol oxidase showed the highest activity towards *o*-dihydroxyphenols (catecholase activity). The enzyme had the highest affinity to catechol and 4-methylcatechol. No activity was found towards monophenols (tyrosine) and low activity – towards trihydroxyphenol–phloroglucinol (Table 2). Polyphenol oxidase isolated from fruits and vegetables is most active towards mono- and diphenols. 4-Methylcatechol and catechol are substrates that are often chosen for determining the activity of polyphenol oxidase isolated from food of plant origin. McCallum and Walker (1990) determined polarographically the activity of *o*-diphenoloxidase in wheat flour using 4-methylcatechol; Janovitz-Klapp, Richard, Goupy, and Nocolas (1990) compared the activity of polyphenol oxidase in apples against several substrates, Lourenco, Neves, and da Silva (1992) tested the activity of polyphenol oxidase using catechol and 4-methylcatechol as standard; Zhou et al. (1993) used catechol and 4-methylcatechol along with other compounds as substrates of polyphenol oxidase isolated from apple peel; Wesche-Ebeling and Montgomery (1990) compared the activity of polyphenol oxidase isoenzymes isolated from strawberries towards 4-methycaltechol; 4-methylcatechol and catechol were tested, along with other phenols, as substrates of PPO from lettuce by Heimdal et al. (1994); Cho and Ahn (1998) used catechol in the studies of kinetic properties of PPO from potatoes.

The analysis of the Lineweaver–Burke curves revealed that PPO from broccoli showed a higher affinity to catechol than to 4-methylcatechol (Fig. 6). The K_M and V_{max} values were: 12.34 ± 0.057 mM and 2000 ± 8736 U/min/ml for catechol and 21 ± 0.087 mM and 28.20 ± 0.525 U/min/ml for 4-methyl catechol. These results correspond well to those reported in the available literature. The K_M values obtained towards catechol are: 12.52 mM for PPO from tea leaves, 3.13 mM for PPO from spinach, 10.5 mM for PPO from beans, 4 mM from PPO from Jerusalem artichoke (*Helianthus tuberosus*) and 18 mM for PPO from thyme. When 4-methylcatechol was used as the substrate for PPO, the following K_M values were obtained: 9.8 mM for PPO from thyme, 10 mM for PPO from strawberry, 3.1 mM for PPO from Amasya apples, 94.3 mM for PPO from beans (Dogan & Dogan, 2004).

3.3.3. Effect of inhibitors on PPO

When tested as inhibitors, the compounds analyzed in the present study acted as noncompetitive inhibitors: sodium sulphate, citric acid and ascorbic acid, or acompetitive ones: EDTA (Fig. 7). The K_i values and the inhibition mechanisms are shown in Table 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 (Rapeanu, van Loey, Smout, & Hendrickx (2006). Two other mechanisms of inhibition involving direct interaction with the enzyme have been reported: chelation of the copper at the active site and reduction of Cu^{++} to Cu^{+} (Gomez-Lopez, 2002).

Cho & Ahn (1998) proved non-competitive action of sodium azide, sodium chloride and L-cysteine, whereas for potassium cyanide, similar to ascorbic acid, they obtained competitive action in inhibiting the activity of phenol oxidase from potatoes. Tropolon proved to be a competitive inhibitor for polyphenol oxidase from grape-

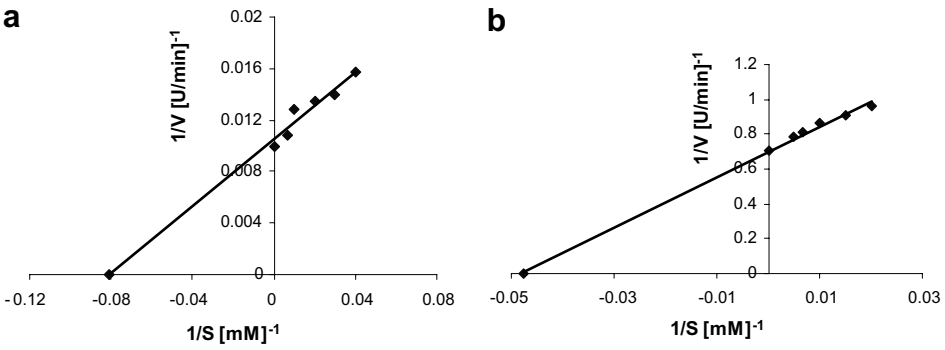

Fig. 6. The Lineweaver–Burk plots for catechol (a) and 4-methylcatechol (b).

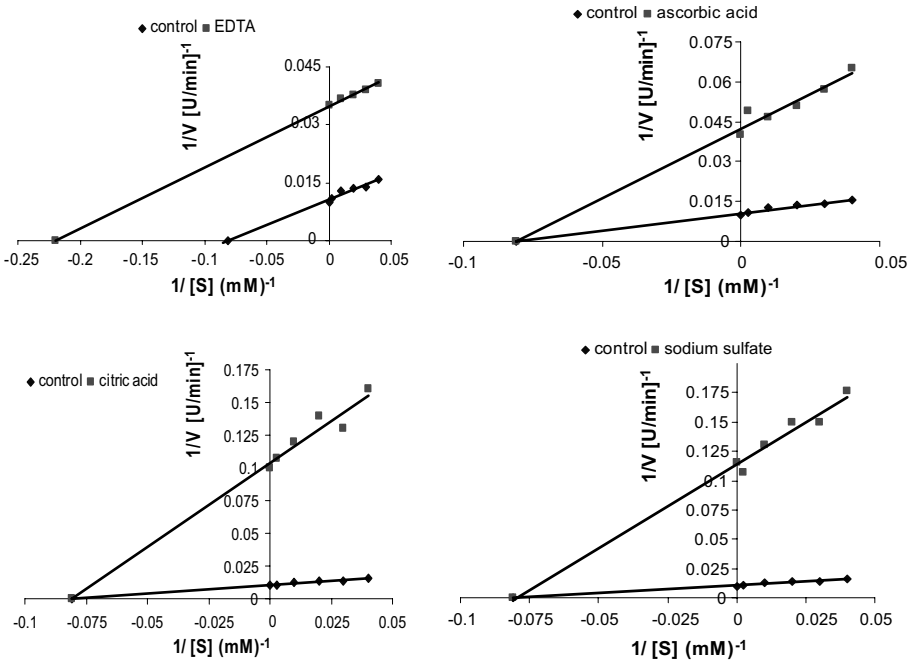

Fig. 7. Lineweaver–Burk plots for broccoli PPO activity with catechol as substrate in the presence of inhibitors.

fruit (Valero, Varon, & Garcia-Carmona, 1990), and for oxidase from apples it was benzoic acid (Janovitz-Klapp et al., 1990), whereas 1-butanol showed a mixed character of inhibition towards PPO from grapefruit (Valero et al., 1990). Benzoic acid, *p*-hydroxybenzoic acid, vanillic acid, cinnamic acid, ferulic acid, phenyloacetic acid, 3-*p*-hydroxyphenyloacetic acid and sorbic acid proved to be competitive inhibitors of PPO isolated from edible parts of palm (Robert, Rouch, Payet, Pabion, & Cadet, 1995a). The same authors showed an uncompetitive character of actions when testing the effect of cinnamic acid and synapinic acid. The compounds tested in the present study (EDTA, ascorbic acid, sodium sulphate and citric acid) also inhibited the action of the enzyme isolated from pears in the studies by Hwang et al. (1996) and from aubergine, which was proved by studies by Fujita & Tono (1988).

Unlike in the results obtained in this study, ascorbic acid has been found to show competitive activity towards polyphenol oxidase isolated from peppermint (Kavrayan & Aydemir, 2001) and potato (Cho & Ahn, 1998). According to Golan-Goldhirsh & Whitaker (1984), ascorbic acid completely and irreversibly inhibited the activity of polyphenol oxidase from fungi. Aydemir (2004) examined the effect of inhibitors on the polyphenol oxidase from artichoke and found ascorbic acid to act as a competitive inhibitor, whereas according to Erat et al. (2006), the compound inhibits the activity of polyphenol oxidase from *Ferula* sp. in a non-competitive manner.

It can be concluded that PPO isolated from broccoli florets have an activity very similar to that of other plants. The enzyme is a catecholase, active toward diphenols, and has greatest substrate specificity towards catechol and 4-methyl catechol among the substrates tested. The pH optimum for the enzyme was 5.7. Moreover, the broccoli PPO activity was sensitive to some of general PPO inhibitors, especially to sodium sulphate.

Table 3
The K_i values and inhibition modes of the PPO with different inhibitors

Inhibitor	4-Methyl catechol	Catechol	Type of inhibition
	K_i (M)	K_i (M)	
EDTA	18.8×10^{-5}	6.8×10^{-5}	Uncompetitive
Ascorbic acid	8.8×10^{-5}	5.6×10^{-5}	Noncompetitive
Citric acid	7.4×10^{-5}	8.2×10^{-5}	Noncompetitive
Sodium sulphate	3.17×10^{-5}	8.2×10^{-5}	Noncompetitive

References

Aydemir, T. (2004). Partial purification and characterization of polyphenol oxidase from artichoke (*Cynara scolymus* L.) heads. *Food Chemistry, 87*, 59–67.

Bradford, M. M. (1976). A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Chen, Y. K., Preston, J. F., Cheng-I, Wei., Hooshar, P., Gleenson, R. A., & Marshall, M. R. (1992). Structural comparison of crustacean, potato, mushroom polyphenol oxidase. *Journal Agricultural and Food Chemistry, 40*(8), 1326–1330.

Cho, Y. K., & Ahn, H. K. (1998). Purification and characterization of polyphenol oxidase from potato: II. Inhibition and catalytic mechanism. *Journal of Food Biochemistry, 23*, 593–605.

de Fatima Pereira, Goulart, Alves, J. D., Magalhaes, M. M., de Oliveira Lima, L. C., & Meyer, L. E. (2003). Purification of polyphenoloxidase from coffee fruits. *Food Chemistry, 83*, 7–11.

Dogan, S., & Dogan, M. (2004). Determination of kinetic properties of polyphenol oxidase from *Thymus* (*Thymus longicaulis* subsp. *chaubardii* var. *chaubardii*). *Food Chemistry, 88*, 69–77.

Erat, M., Sakiroglu, H., & Kufrevioglu, O. I. (2006). Purification and characterization of polyphenol oxidase from *Ferula* sp. *Food Chemistry, 95*, 503–508.

Espin, J. C., Morales, M., Varon, R., Tudela, J., & Garcia-Carnovas, F. (1995). Monophenolase activity of polyphenol oxidase from verdedoncella apple. *Journal Agricultural and Food Chemistry, 43*(11), 2807–2812.

Fujita, S., & Tono, T. (1988). Purification and some properties of polyphenoloxidase in eggplant (*Solanum melongema*). *Journal of Science and Food Agricultural, 46*, 115–123.

Galleazi, M. A., Sgarbieri, V. C., & Constantinides, S. M. (1981). Isolation, purification and physicochemical characterization of PPO from a dwarf variety of banana (*Musa cavensdishii*, L.). *Journal of Food Science, 46*, 150–155.

Ganesa, C., Fox, M. T., & Flurkey, W. H. (1992). Microheterogenity in purified broad bean polyphenol oxidase. *Plant Physiology, 98*, 472–479.

Golan-Goldhirsh, A., & Whitaker, J. R. (1984). Effect of ascorbic acid, sodium bisulfite, and thiol compounds on mushroom polyphenol oxidase. *Journal Agricultural and Food Chemistry, 32*, 1003–1009.

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

Heimdal, H., Larsen, M. L., & Poll, L. (1994). Characterization of polyphenol oxidase from photosynthetic and vascular lettuce tissues (*Lectuca sativa*). *Journal Agricultural and Food Chemistry, 42*, 1428–1433.

Ho, K. (1999). Characterization of polyphenol oxidase from aerial roots of an orchid, Aranda "Christine 130". *Plant Pysiology et Biochemistry, 37*(11), 841–848.

Hwang, I. G., Yoon, K. R., & Kim, W. Y. (1996). Rapid measurement of the enzymatic browning of pear juice by the addition of l-DOPA. *Food Biotechnology, 5*(2), 152–155.

Janovitz-Klapp, A. H., Richard, F. C., Goupy, P. M., & Nocolas, J. J. (1990). Kinetic studies on apple polyphenol oxidase. *Journal Agricultural and Food Chemistry, 38*(7), 1437–1441.

Kavrayan, D., & Aydemir, T. (2001). Partial purification and characterization of polyphenoloxidase from peppermint (*Mentha pipetita*). *Food Chemistry, 74*, 147–154.

Kłyszejko-Stefanowicz, L. (2003). (red) Ćwiczenia z Biochemii, wydawnictwo Naukowe PWN, Warszawa (in Polish).

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 68012–68015.

Leoni, O., & Palmeri, S. (1990). Polyphenol oxidase from architoke (*Cynara scolymus* L.). *Food Chemistry*, 3827–3839.

Lourenco, E. J., Neves, V. A., & da Silva, M. (1992). Inhibition of heart-of-palm polyphenoloxidase (PPO) by some compounds. *Ciencia y Technologia Alimentaria, 12*(1), 83–90.

Mazzafera, P., & Robinson, S. P. (2000). Characterization of polyphenol oxidase in coffee. *Phytochemistry, 55*, 285–296.

McCallum, J. A., & Walker, J. R. L. (1990). *O*-Diphenol oxidase activity, phenolic content and colour of New Zealand wheats, flours and milling streams. *Journal of Cereal Science, 12*, 83–96.

Paul, B., & Gowda, L. (2000). Purification and characterization of a polyphenol oxidase from the seeds of the field bean (*Dolichos lablab*). *Journal Agricultural and Food Chemistry, 48*, 3839–3846.

Prota, G. (1988). Progress in the chemistry of melanins and related metabolites. *Medicinal Research Reviews, 8*, 525–556.

Rapeanu, G., van Loey, A., Smout, C., & Hendrickx, M. (2006). Biochemical characterization and process stability of polyphenoloxidase extracted from Victoria grape (*Vitis vinifera* ssp *Sativa*). *Food Chemistry, 94*, 253–261.

Robert, C., Cadet, F., Rouch, C., & Pabion, M. (1995b). Information on the active site of palmito polyphenol oxidase. *C. R. Acad. Sci. Paris, Science de la vie/Life sciences, 318*, 141–144.

Robert, C., Rouch, C., Payet, J. M., Pabion, M., & Cadet, F. (1995a). L'acide kojique inhibiteur de l'activite de la polyphenoloxydase de palmiste. *Journal of Nature, 7*(1), 3–9.

Rodríguez-López, J. N., Fenoll, L. G., Peñalver, M. J., García-Ruiz, P. A., Varón, R., Martínez-Ortíz, F., et al. (2001). Tyrosinase action on monophenols; evidence for direct enzymatic release of *o*-diphenol. *Biochimica and Biophysica Acta, 1548*, 238–256.

Selles-Marchart, S., Casado-Vela, J., & Bru-Martinez, R. (2006). Isolation of a latent polyphenol oxidase from loquat fruit (*Eriobotya Japonica* Lindl): Kinetic characterization and comparison with the active form. *Archives of Biochemistry and Biophysics, 446*, 175–185.

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

Valero, E., Varon, R., & Garcia-Carmona, F. (1990). Inhibition of grape polyphenol oxidase by several natural aliphatic alcohols. *Journal Agricultural and Food Chemistry, 38*(94), 1097–1100.

van Gelder, C. W. G., Flurkey, W. H., & Wichers, H. (1997). Sequence and structural features of plant and fungal tyrosinases. *Phytochemistry, 45*(7), 1309–1323.

Weemaes, C., Ludikhuyze, L. R., Van den Broeck, I., & Hendrickx, M. (1998). High pressure inactivation of polyphenoloxidase. *Journal of Food Science, 63*(5), 873–877.

Wesche-Ebeling, P., & Montgomery, M. W. (1990). Strawberry polyphenoloxidase: Its role in anthocyanin degradation. *Journal of Food Science, 55*(3), 731–734.

Wisserman, K. W., & Lee, C. Y. (1980). Purification of grape polyphenoloxidase with hydrophobic chromatography. *Journal of Chromatography, 192*, 232–235.

Xu, J., Zheng, T., Meguro, S., & Kawachi, S. (2004). Purification and characterization of polyphenol oxidase from Henry chestnuts (*Castanea henryi*). *The Japan Wood Research Society, 50*, 260–265.

Zhou, P., Smith, N. L., & Lee, Ch. Y. (1993). Potential purification and some properties of monroe apple peel polyphenol oxidase. *Journal Agricultural and Food Chemistry, 41*(4), 532–536.