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Biochemical characterization and process stability of polyphenoloxidase extracted from Victoria grape (*Vitis vinifera* ssp. *Sativa*)

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Abstract

Polyphenol oxidase (PPO) was isolated from Victoria grapes (*Vitis vinifera* ssp. *Sativa*) grown in South Africa and its biochemical characteristics were studied. Optimum pH and temperature for grape PPO activity were pH 5.0 and $T = 25$ °C with 10 mM catechol in McIlvaine buffer as substrate. PPO showed activity using the following substances: catechol, 4 methyl catechol, D, L-DOPA, (+) catechin and chlorogenic acid. K_m and V_{max} values were 52.6 ± 0.00436 mM and 653 ± 24.0 $OD_{400\ nm}$/min in the case of 10 mM catechol as a substrate. Eight inhibitors were tested in this study and the most effective inhibitors were found to be ascorbic acid, L-cysteine and sodium metabisulfite. Kinetic studies showed that the thermal inactivation of Victoria grape PPO followed first-order kinetics, with an activation energy, $E_a = 225 \pm 13.5$ of kJ/mol. Both in semipurified extract and in grape juice, PPO showed a pronounced high pressure stability.

Keywords: Grape; *Vitis vinifera* ssp. *Sativa*; Polyphenoloxidase; Characterisation; Thermal and high pressure stability

1. Introduction

Polyphenoloxidase (PPO) is a copper-containing oxidoreductase which catalyzes two distinct reactions involving phenolic compounds and molecular oxygen, namely: (a) the *o*-hydroxylation of monophenols to *o*-diphenols, or cresolase activity (monophenol, monooxygenase, E.C. 1.14.18.1) and (b) the subsequent oxidation of *o*-diphenols to *o*-quinones, or catecholase activity (diphenol oxygen oxidoreductase, E.C. 1.10.3.1). The main step in enzymatic browning is the oxidation of phenolic compounds (to corresponding quinone intermediates that polymerize to form undesirable pigments) by PPO in the presence of oxygen. PPO has been studied in several plant tissues, such as apples (Murata, Tsurutani, Tomita, Homma, & Kaneko, 1995; Oktay, Küfrevioğlu, Kocaçalişkan, & şakiroğlu, 1995; Rocha & Morais, 2001), pears and peaches (Carbonaro & Mattera, 2001; Halim & Montgomery, 1978; Jen & Kahler, 1974), bananas (Galeazzi, Scarbieri, & Constantinides, 1981; Yang, Fujita, Ashrafuzzaman, Nakamura, & Hayashi, 2000), plums (Siddig, Sinha, & Cash, 1992), avocado (Weemaes, Ludikhuyze, Van den Broeck, & Hendrickx, 1998; Weemaes, Ludikhuyze, Van den Broeck, Hendrickx, Tobback, 1998; Gómez-López, 2002), litchi fruit (Yue-Ming, Zauberman, & Fuchs, 1997), longan fruit (Yue-Ming, 1999), egg plant (Concellón, Añón, & Chaves, 2004; Doğan, Arslan, & Doğan, 2002), Jerusalem artichoke (Aydemir, 2004; Ziyan & Pekyardimci, 2003), herbs (Arslan, Temur, & Tozlu, 1997), field beans (Paul & Gowda,

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2000), taro and potatoes (Duangmal & Owusu Apenten, 1999), peppermint (Kavrayan & Aydemir, 2001) and tea leaves (Halder, Tamuli, & Phaduri, 1998). Browning reactions in grape products, such as fresh fruits, juices and wines, during processing and storage, are well known and are an economic problem for producers and consumers. Investigations to determine the characteristics of grape PPO and the conditions under which PPO is most active have been widely reported for some grape cultivars (Cash, Sistrunk, & Stutte, 1976; Lamikanra, Kirby, & Musingo, 1992; Lee, Smith, & Pennesi, 1983; Sànchez-Ferrer, Bru, Cabanes, & Garcia-Carmona, 1988; Valero, Varón, & Garcia-Carmona, 1988; Wissemann & Lee, 1981; Weemaes et al., 1998; Weemaes, Ludikhuyze, Van den Broeck, Hendrickx, Tobback, 1998). Our objective was to isolate PPO from Victoria white grapes and to study its activity, inhibition and process stability.

2. Materials and methods

2.1. Materials

White grapes (Victoria variety, South of Africa) were purchased at commercial maturity from a local store. Catechol and insoluble PVP were obtained from Sigma–Aldrich, USA. Triton X-100 was purchased from Fluka Chemicals Co. (Switzerland). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Extraction and partial purification procedure

Extraction of the enzyme from grapes was carried out using a modification of the method of Valero et al. (1988). Grapes (250 g) were suspended in 125 ml McIlvaine buffer (pH 5) and mixed for 15 s in a Waring commercial blender (New Hartford, USA). The resulting homogenate was filtered through four layers of cheese cloth, the filtrate centrifuged for 15 min (4 °C; 4000*g*) in a Beckman J2-HS centrifuge. The resulting precipitate was extracted for 1 h/4 °C with 1.5% Triton X-100 and 2.0% insoluble PVP in 100 ml McIlvaine buffer (pH 5) and centrifuged for 1 h (4 °C; 15,000*g*). The resulting supernatant was subjected to ammonium sulphate (UCB) precipitation. The fraction precipitating between 30% and 90% saturation was redissolved in the same buffer and dialysed for 24 h (cellulose membrane, Medicell International Ltd., 6–27/32) to remove excess ammonium sulphate ions. The dialyzed samples were kept in tubes at −80 °C and were used as the PPO enzyme source in further experiments.

2.2.2. Grape juice preparation

Grapes (1 kg) were crushed and filtered through four layers of cheese cloth. The filtrate juice was clarified at 4 °C for 24 h and then kept in tubes at −80 °C and used as the PPO enzyme source in the subsequent experiments. The white must had 13.34° Brix and pH 4.03.

2.2.3. PPO activity assay

PPO activity was assayed by a spectrophotometric procedure. The increase in absorbance at 400 nm at 25 °C was recorded automatically for 30 min (Ultrospec 2100 pro, UV–Vis spectrophotometer). The sample cuvette contained 1.0 ml substrate (catechol 10 mM in McIlvaine buffer pH 5 or 0.1 M catechol in McIlvaine buffer, pH 5 in the case of grape juice) and 100 μl undiluted enzyme extract or grape juice. The blank sample contained only 1.0 ml substrate solution in McIlvaine buffer, pH 5. Enzyme activity was calculated from the linear part of the curve of OD at 400 nm vs. incubation time. The activity values reported are the mean of three determinations and the relative standard deviations are less than ±1%. The pH-dependence of the PPO activity was determined under standard assay conditions using McIlvaine buffer and phosphate buffer of pH values ranging from 2.5 to 9.0. The effect of temperature on the activity of grape PPO was investigated by equilibrating the substrate in a water bath (10–80 °C) for 10 min before introduction of the enzyme. PPO activity was measured at different temperatures in the range of 10–80 °C using 10 mM catechol in McIlvaine buffer (pH 5.0) as substrate.

2.2.4. Protein determination

Protein concentration was determinated using Sigma Procedure No. TPRO-562 (for Kit No. BCA-1) and product No. B-9643. Bovine serum albumin was used as a standard.

2.2.5. Enzyme kinetics

The activity of PPO extract as a function of the concentration of catechol, 4-methyl catechol, (+) catechin, chlorogenic acid and D, L-DOPA was investigated. The kinetic parameters, Michaelis Menten constant (K_m) and maximum rate (V_{max}) values for grape PPO activities were estimated by non-linear regression analysis using the following equation.

$$v = \frac{V_{max} \cdot [S]}{K_M + [S]}, \quad (1)$$

where [S] corresponds to the substrate concentration, while K_M is the Michaelis Menten constant and V_{max} is the apparent maximum rate for the enzymatic reaction.

2.2.6. Substrate specificity

The specificity of grape PPO extract was investigated for nine different commercial grade substrates (catechol, 4-methyl catechol, D, L-DOPA, (+) catechin, chlorogenic

acid, L-tyrosine, *p*-cresol, pyrogallol and gallic acid) at concentrations of 10 mM, except for L-tyrosine (2.5 mM), due to its limited solubility. The increase in absorbance at the optimum wavelength for each substrate was measured per time unit.

2.2.7. Effect of inhibitors on PPO activity

The inhibitory effects of L-ascorbic acid, benzoic acid, citric acid, EDTA, glutathione, L-cysteine, sodium chloride and sodium metabisulfite on PPO activity were determined at different concentrations. Each inhibitor was prepared in McIlvaine buffer (pH 5.0). Inhibition of PPO activity was investigated in a reaction mixture (1.2 ml) consisting of 1.0 ml 10 mM catechol, 100 μl inhibitor solution and 100 μl enzyme solution.

2.2.8. Thermal stability of grape PPO

Thermal stability of grape PPO extract was investigated at pH 5.0, at various constant temperatures from 25 to 90 °C using an incubation time of 10 min, whereas detailed thermal inactivation kinetics of Victoria grape PPO extract at pH 5 were determined in a temperature range from 55 to 65 °C. The samples were filled in to capillaries (200 μl) (Blaubrand, Germany), thermally treated, cooled in ice water and the residual activity was measured within 60 min of storage at 0 °C.

2.2.9. Pressure stability of grape PPO

Pressure stability of grape PPO extract was investigated at pH optimum (pH 5), at pH 4 (similar to that of grape juice) and in grape juice (pH 4.03). In order to adjust the pH, partially purified Victoria grape PPO extract was dialyzed for 10 h (cellulose membrane, Medicell International Ltd., 6-27/32), against McIlvaine buffer with pH 4. The pH values mentioned are those prevailing at atmospheric pressure and room temperature.

Pressure treatments were conducted in a multivessel high-pressure apparatus (eight vessels of 8 ml) (Resato, Roden, The Netherlands). The pressure medium was a glycol–oil mixture (TR-15, Resato). Enzyme samples were enclosed in 0.3 ml flexible microtubes (Elkay, Leuven, Belgium). The microtubes were placed in the pressure vessels, already equilibrated at 10, 25, 40 and 60 °C. Pressure was build up slowly (100 MPa/min) to minimize temperature increase due to adiabatic heating. After pressure build-up, an equilibration period of 2 min, to allow temperature to evolve to its desired value, was taken into account. After an additional 15 min, the pressure was released, the samples were immediately cooled in ice water and the residual activity was measured within 60 min of storage time in ice water.

2.2.10. Kinetic data analysis

Often, thermal inactivation of enzymes can be described by a first-order reaction:

$$\frac{dA}{dt} = -k_{obs}A, \tag{2}$$

where A is the enzyme at treatment time t and k_{obs} is the reaction rate constant at the temperature studied. For constant extrinsic/intrinsic factors, in the case of a first-order reaction, the kinetics can be described by the following equations:

$$\log A = \log A_0 - \frac{t}{D} \tag{3}$$

or

$$\ln A = \ln A_0 - kt, \tag{4}$$

where A_0 is the initial activity, A the activity at time t, and D the decimal reduction time (i.e. time needed to reduce the initial activity by one log unit at a constant temperature). At each temperature, the decimal reduction time D and the rate constant k were estimated using linear regression analysis on, respectively, Eqs. (3) and (4). The temperature-dependence of the D-value is characterized by the z_T value, which is the temperature-increase necessary to induce a 10-fold reduction in D,

$$\log D = \log D_0 - \frac{T - T_0}{z_T}. \tag{5}$$

The z_T value was estimated using linear regression analysis of Eq. (5). The temperature-dependence of the inactivation rate constants can be estimated using the Arrhenius model (6)

$$\ln(k) = \ln(k_0) + \left[\frac{E_a}{R} \cdot \left(\frac{1}{T_0} - \frac{1}{T}\right)\right], \tag{6}$$

where T and T_0 are the absolute and the reference temperatures (K), k_0 is the rate constant at T_0, E_a is the activation energy (kJ/mol) and R is the universal gas constant (8.314 J/mol/K). The activation energy was estimated using linear regression analysis of Eq. (6).

3. Results and discussion

3.1. Extraction and partial purification of PPO

The purification achieved was 135-fold with 8.1% recovery of Victoria grape PPO activity (Table 1), similar to the 126-fold purification of Monastrell grape PPO activity reported by Sànchez-Ferrer et al. (1988).

3.2. Effect of pH on PPO activity

Fig. 1 shows the effect of pH on the activity of Victoria grape PPO. Maximal activity of grape PPO extract occurred at 5.0. the common pH range for optimal grape PPO activity, as well as other fruits, is known to be pH 5.0–7.0. At acid pH (2.5–4), grape PPO still remained active (70% at pH 3.5). The enzyme activity decreased

Table 1
Extraction and partial purification of Victoria grape PPO extract

Purification step	Volume (ml)	Protein (mg/ml)	Specific activity ($OD_{400\ nm}$/min/mg protein)	Purification fold	Recovery (%)
Crude extract	310	165	5.4	1	100
PVP and Triton X-100 extract	81	18.7	161	29.8	26.2
30–90% $(NH_4)_2SO_4$	25	5.18	729	135	8.1

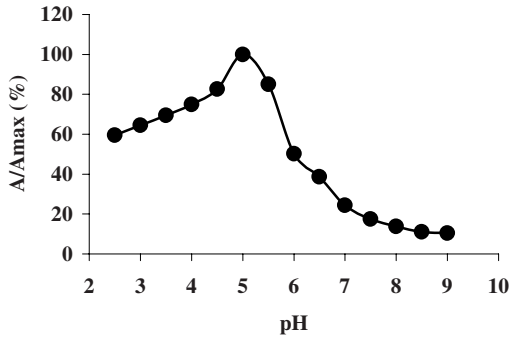

Fig. 1. Activity of Victoria grape PPO extract as a function of pH. Assay conditions: catechol 10 mM, 25 °C.

rapidly at the alkaline pHs (7–9). These results are in agreement with an optimal pH value of 5.5 for Ravat 51 and Niagara grapes (Wissemann & Lee, 1981) and 5.5 for Noble muscadine grapes (Lamikanra et al., 1992). The pH of grape juice and wine is around 3–4 and PPO activity is relatively high. At pH 3.0 and 4.0, Victoria grape PPO activities were, respectively, about 65% and 75% of the maximum activities.

3.3. Effect of temperature on PPO activity

The optimum temperature for maximum PPO activity was at 25 °C (Fig. 2). Below and above 25 °C the enzyme activity decreased gradually. This was consistent with reported temperatures for PPO activities in Concord (25–30 °C) (Cash et al., 1976) and Ravat 51 and Niagara grapes (25 °C) (Wissemann & Lee, 1981) but higher than those of the DeChaumac (20 °C) (Lee et al., 1983) and lower than those of Monastrell (25–40 °C) (Sànchez-Ferrer et al., 1988), Airen (25–45 °C) (Valero et al., 1988) and muscadine (30 °C) (Lamikanra et al., 1992) grapes.

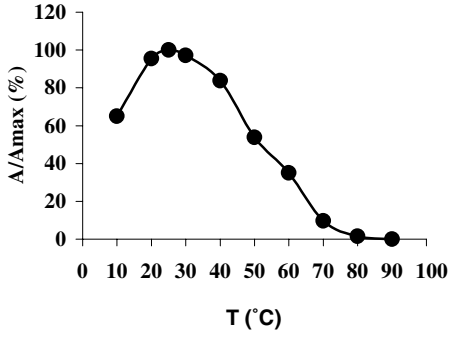

Fig. 2. Activity of Victoria grape PPO extract as a function of temperature. Assay conditions: catechol 10 mM, pH 5.0.

Table 2
K_m and V_{max} values of Victoria grape PPO extract

Substrate	K_m (mM)	V_{max} (OD/min)	Wavelength (nm)
Catechol	52.6 ± 0.00436[a]	653 ± 24.0	400
4-Methyl catechol	7.51 ± 0.00058	584 ± 15.1	400
(+) Catechin	4.34 ± 0.00087	464 ± 4.69	387
Chlorogenic acid	3.23 ± 0.00039	562 ± 2.29	400
D, L-DOPA	68.2 ± 0.00232	362 ± 6.07	476

[a] Standard error of regression.

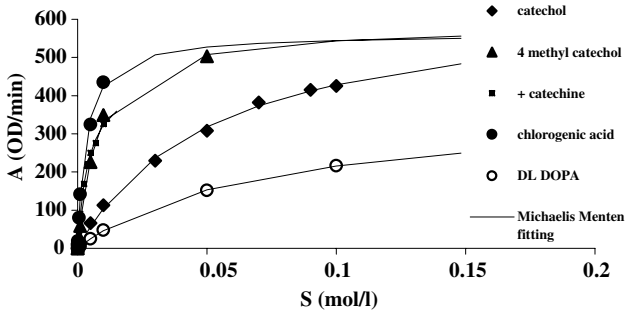

Fig. 3. Activity of Victoria grape PPO extract as a function of substrate concentration. Assay conditions: pH 5.0, 25 °C.

3.4. Enzyme kinetics

K_m and V_{max} values for different substrates are listed in Table 2. [1] As seen in Fig. 3, K_m value was 52.6 ± 0.00436 mM for Victoria grape PPO with catechol as substrate. Cash et al. (1976) have reported a K_m of 67 mM for Concord grape PPO with catechol as substrate. Victoria grape PPO activity on 4 methyl catechol was characterized by a K_m of 7.51 ± 0.00058 (Table 2). Sànchez-Ferrer et al. (1988) and Valero et al. (1988) also found K_m values of 9.0 and 9.5 mM for Monastrell and Concord grapes with 4 methyl catechol as substrate, while Lee et al. (1983) found K_m values of 15.9 and 24.6 mM, respectively, for DeChaumac

[1] Due to problems of cloudness, it was not possible to evaluate PPO activity at higher substrate concentrations.

grape with caffeic acid and 4 methyl catechol as substrates. The maximum reaction rate (V_{max}) value was 653 ± 24.0 $OD_{400\ nm}$/min for grape PPO with catechol as substrate.

3.5. Substrate specificity

A number of monohydroxy, dihydroxy and trihydroxy phenols were used to test substrate specificity (Table 3). The enzyme showed activity towards the *ortho*-diphenols (catecholase activity). A low activity for monophenols, such as *p*-cresol, and trihydroxy phenols, such as gallic acid and pyrogallol, was detected. The number of hydroxyl groups and their position in the benzene ring of the substrate affected oxidase activity. Cash et al. (1976), Wissemann and Lee (1981) and Lee et al. (1983) found only catecholase activity with Concord, Ravat 51, Niagara and DeChaumac grape PPO and no activity when monohydroxy phenols were used as substrates, while Sànchez-Ferrer et al. (1988) and Valero et al. (1988) found both catecholase and cresolase activity with Monastrell and Airen grape PPO.

3.6. Effect of inhibitors on PPO activity

The inhibitory study (Table 4) showed that the most potent inhibitors for grape PPO were ascorbic acid, L-cysteine and sodium metabisulfite, since these compounds induced a high degree of inhibition, even at the lowest concentration used. These results are in line with results obtained by Cash et al. (1976) and Lee et al. (1983). During the measurements of PPO activity, in the presence of ascorbic acid and sodium metabisulfite, an initial lag phase of, respectively, 2 and 6 min was noticed. This was also observed by Wissemann and Lee (1981) for L-cysteine, sodium diethyldithiocarbamate, glutathione, ascorbic acid, dithiothreithol and sodium metabisulfite. The inhibitor reaction mechanism differs, depending on the reducing agent employed. Inhibition by thiol compounds is attributed to either the stable colourless products formed by an addition reaction with *o*-quinones (Ikediobi & Obasuyi, 1982) or binding to the active centre of PPO, like metabisulfite (Valero & Garcia-Carmona, 1992). Ascorbate 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. Ascorbic acid has also been reported to cause irreversible enzyme inhibition (Golan, Goldhirsh, & Whitaker, 1984).

Table 3
Substrate specificity of Victoria grape PPO extract

Substrate	Concentration (mM)	Relative activity (%)	Wavelength (nm)
o-Dihydroxyphenols			
Catechol	10	100 ± 0.25[a]	400
4-Methyl catechol	10	338 ± 0.64	400
(+) Catechin	10	69 ± 0.37	387
Chlorogenic acid	10	378 ± 0.22	400
D,L-DOPA	10	85 ± 0.78	476
Monohydroxyphenols			
p-Cresol	10	1.4 ± 0.59	400
D,L-Tyrosine	2.5	0	400
Trihydroxyphenols			
Gallic acid	10	0.75 ± 0.36	400
Pyrogallol	10	0.2 ± 0.07	400

[a] Standard deviation.

Table 4
Effect of various inhibitors on the activity of Victoria grape PPO extract

Inhibitor	Concentration (mM)	Inhibition (%)
Ascorbic acid	0.05	25 ± 0.05[a]
	0.5	46 ± 0.11
	5	99 ± 0.14
Benzoic acid	0.05	18 ± 0.35
	0.5	23 ± 0.47
	5	56 ± 0.21
Citric acid	0.05	16 ± 0.04
	0.5	22 ± 0.52
	5	26 ± 0.22
L-Cysteine	0.05	43 ± 0.07
	0.5	99 ± 0.61
	5	100 ± 0.43
EDTA	0.05	12 ± 0.09
	0.5	17 ± 0.17
	5	24 ± 0.47
Glutatione	0.05	12 ± 0.34
	0.5	17 ± 0.29
	5	27 ± 0.18
NaCl	0.05	15 ± 0.11
	0.5	21 ± 0.27
	5	24 ± 0.14
Na metabisulfite	0.05	21 ± 0.41
	0.5	99 ± 0.49
	5	100 ± 0.09

[a] Standard deviation.

3.7. Effect of storage temperature on enzyme stability

The effect of different storage temperatures on partially purified grape PPO activity was evaluated over a 60 day period at pH 5.0 (Fig. 4). At 25 °C, the enzyme completely lost its activity after 45 days. At 4 °C, 25% loss in grape PPO activity was observed during the first 10 days. However, more than 50% of its activity was lost after 50 days storage at 4 °C. The enzyme was very stable at −80 and −35 °C. Cabbage PPO completely lost its activity after 14 days at 4 °C (Nagai & Suzuki, 2001), while for artichoke PPO, at the end of 21 days, more than 87% of its activity was lost (Aydemir, 2004). On

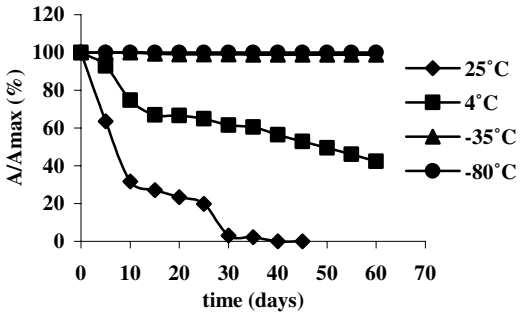

Fig. 4. Stability of Victoria grape PPO extract during storage at −80, −35, 4, 25 °C.

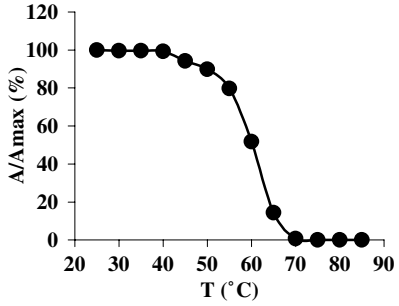

Fig. 5. Thermal stability of Victoria grape PPO extract. Residual activity was measured after a 10-min treatment at different temperatures. Assay conditions: catechol 10 mM, pH 5.0, 25 °C.

the other hand, Kavrayan and Aydemir (2001) reported that peppermint PPO was relatively stable at −15 °C for 6 days, but was completely inactivated after 21 days at −15 °C.

3.8. Temperature stability of PPO

In Fig. 5, relative residual PPO activity is plotted as a function of inactivation temperature after a 10-min treatment time. At 60 °C, 50% of PPO activity was lost after 10 min of treatment whereas, at 65 °C, more than 85% of the PPO activity was lost after 10 min and, at 70 °C, grape PPO extract was completely inactivated after 10 min of heating. Lamikanra et al. (1992) reported a loss of about 70% of PPO activity for Welder and Noble grapes at 60 °C for 30 min, while Wissemann and Lee (1981) found a 50% reduction of PPO activity after 15 min at 68.1 °C for Ravat and 76.1 °C for Niagara grapes.

3.9. Pressure stability of PPO

Pressure stability, at 10, 25, 40 and 60 °C, of PPO in semipurified extract at pH 4 and 5 and grape juice was screened by pressuring samples for 15 min in the pressure range 400–800 MPa (Figs. 6–8).

At 25 °C, there was a slight decrease of 22% in the relative residual activity after 15 min of treatment at 800

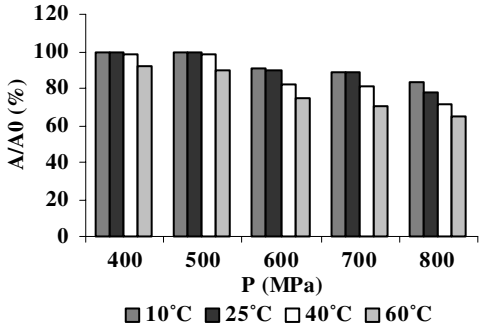

Fig. 6. Pressure stability of Victoria grape PPO extract (pH optimum 5) at 10, 25, 40 and 60 °C. Residual activity was measured after 15 min of treatment. Assay conditions: catechol 10 mM, pH 5.0, 25 °C.

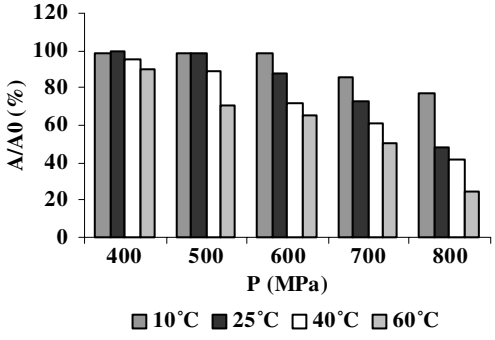

Fig. 7. Pressure-stability of Victoria grape PPO extract (pH 4) at 10, 25, 40 and 60 °C. Residual activity was measured after 15 min of treatment. Assay conditions: catechol 10 mM, pH 5.0, 25 °C.

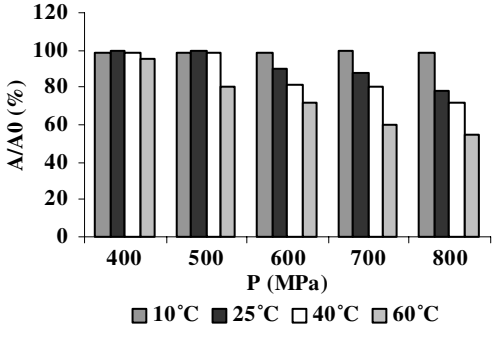

Fig. 8. Pressure stability of PPO in grape juice (pH 4.03) at 10, 25, 40 and 60 °C. Residual activity was measured after 15 min of treatment. Assay conditions: catechol 0.1 M, pH 5.0, 25 °C.

MPa, whereas, at 40 or 60 °C PPO in semipurified extract at optimum pH 5, is quite stable upto 600 MPa. The PPO activity in semipurified extract at pH 4 was less pressure/temperature-stable. At 800 MPa and 25 °C, after 15 min of treatment, there was a decrease of 52% in the relative residual activity while, for grape juice under the same conditions, the decrease was only 14% in the relative residual activity. The degree of decrease in the relative residual activity increased with increasing pressure/temperature conditions. After 15 min of treat-

ment at 60 °C and 800 MPa the relative residual activity was 36%, 75% and 55% for PPO, respectively, in semi-purified extract at pH 4 and 5 and in grape juice. When comparing all systems, PPO in grape juice seems to be more pressure/temperature stable than PPO in McIlvaine buffer at pH 4 and 5. The difference between inactivations in all these systems could be due to the different characteristics of the medium (pH, initial PPO activity, presence of colloid components), which influence the effect of the temperature and high pressure treatment, as already reported by other authors (Estiaghi & Knorr, 1993; Castellari, Matricardi, Arfelli, Rovere, & Amati, 1997).

When considering the adiabatic effect in the pressure range studied, the residual PPO activity in semipurified extract at optimum pH 5, after the 2 min of equilibration for 10, 25, 40 and 60 °C is already around 99–84%, 98–79%, 96–74% and 91–68%, respectively, compared to the control at 25 °C and at atmospheric pressure (data not shown). Increased enzyme activity after pressure treatment, which has been reported for pear (Asaka & Hayashi, 1991), onion (Butz, Koller, Tauscher, & Wolf, 1994) and mushroom (Gomes & Ledward, 1996) PPO, was not noticed for Victoria grape PPO. On the other hand, there are examples of PPO inhibition when pressurization was used on vegetable extracts (Anese, Nicoli, Dall'Aglio, & Lerici, 1995; Gomes & Ledward, 1996; Weemaes et al., 1998; Weemaes, Ludikhuyze, Van den Broeck, Hendrickx, Tobback, 1998) or on products such as juices and purees (Cano, Hernandez, & de Ancos, 1997; Hernàndez & Cano, 1998; Palou, López-Malo, Barbosa-Cánova, Welti-Chanes, & Swanson, 1999). PPO enzymes from different origins display different pressure stabilities. Pressure inactivation behaviour of PPO is dependent on the enzyme source, immersion medium, pH, temperature and time. Moreover, there are differences depending on the species of origin (Weemaes et al., 1998; Weemaes, Ludikhuyze, Van den Broeck, Hendrickx, Tobback, 1998). Generally, enzyme inactivation was more pronounced at high pressures (700–900 MPa) than at to lower pressures, but the results vary with treatment conditions.

3.10. Thermal inactivation kinetics of grape PPO

Based on the results of thermal stability, a detailed kinetic study of thermal inactivation of grape PPO was performed in the range 55–65 °C at atmospheric pressure. From the log linear plots of residual grape PPO activity and inactivation time at constant temperature, it can be concluded that the thermal inactivation of grape PPO can be adequately described by a first-order model in the temperature range 55–65 °C (Fig. 9). As expected, at higher inactivation temperatures the inactivation proceeds faster. *D*-values and rate constant for thermal inactivation of grape PPO were estimated using

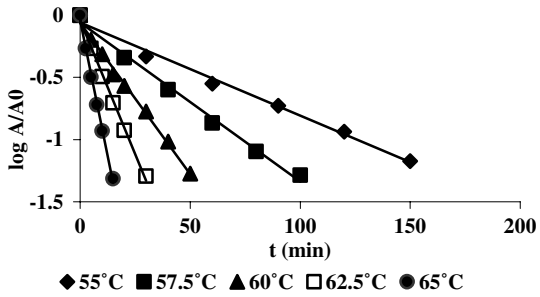

Fig. 9. Thermal inactivation curves of Victoria grape PPO extract in the temperature range 55–65 °C. Assay conditions: catechol 10 mM, pH 5.0.

Table 5
D, *k*, z_T and E_a values of inactivation of Victoria grape PPO extract

T (°C)	*D*-values (min)	*k* (min^{-1})
55	133 ± 2.66[a]	0.0172 ± 0.0021
57.5	78.1 ± 3.72	0.0294 ± 0.0350
60	41.2 ± 0.61	0.0559 ± 0.0125
62.5	23.3 ± 0.39	0.0987 ± 0.0176
65	11.5 ± 0.19	0.2000 ± 0.0239
	z_T (°C)	E_a (kJ/mol)
	9.41 ± 0.63	225 ± 13.47

[a] Standard error of regression.

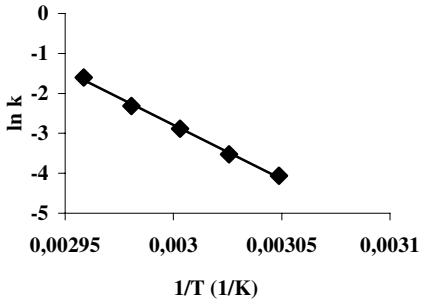

Fig. 10. Temperature-dependence of inactivation rate constant for thermal inactivation of Victoria grape PPO extract.

Eqs. (3) and (4) and are shown in Table 5. As expected, the decimal reduction time decreases with temperature increase. At 65 °C, the *D*-value is almost 12 min. The temperature-dependence of the rate constants for thermal inactivation of PPO in the grape extract is depicted in Fig. 10. Fig. 10 showed no obvious deviation from linearity ($r^2 = 0.9972$), with an activation energy of 225 kJ/mol. An activation energy in the same range (219–276 kJ/mol) was found for DeChaunac grape PPO (Lee et al., 1983), and Ravat 51 PPO (Wissemann & Lee, 1981), but these were higher than that found for Niagara (Wissemann & Lee, 1981) grape PPO. A larger value of (E_a) indicates a greater influence of temperature on thermal inactivation (Wissemann & Lee, 1981; Weemaes et al., 1998; Weemaes, Ludikhuyze, Van den Broeck, Hendrickx, Tobback, 1998).

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