International Journal of Food Science and Technology 2008, **43**, 1316–1322

Original article

Inhibition kinetic and mechanism of polyphenol oxidase from various sources by diethyldithiocarbamic acid

Serap Doğan,[1] Pınar Turan,[2] Mehmet Doğan,[2]* Mahir Alkan[2] & Oktay Arslan[2]

1 Balikesir University Faculty of Science and Literature, Department of Biology, 10145 Balıkesir/Turkey
2 Balikesir University Faculty of Science and Literature, Department of Chemistry, 10145 Balıkesir/Turkey

(Received 18 September 2006; Accepted in revised form 23 April 2007)

Summary Inhibition kinetics and mechanism of polyphenol oxidases (PPO) partially purified from various sources such as *Thymbra spicata* L. var. *spicata* and *Ocimum basilicum* L., and of mushroom PPO bought from Sigma by diethyldithiocarbamic acid have been described using catechol, 4-methylcatechol and pyrogallol as substrates. The inhibition type was competitive for *O. basilicum* L. PPO using catechol and 4-methylcatechol as substrates, for mushroom PPO using catechol, 4-methylcatechol and pyrogallol as substrates, and for *T. spicata* L. var. *spicata* PPO using 4-methylcatechol as a substrate; uncompetitive inhibition for *T. spicata* L. var. *spicata* PPO using pyrogallol as a substrate; and non-competitive inhibition for *O. basilicum* L. and *T. spicata* L. var. *spicata* PPO using pyrogallol and catechol as substrates, respectively. The inhibition effect of diethyldithiocarbamic acid on enzymatic browning varied greatly from one phenol to another and from one enzyme to another. Hence, no general rule can easily be established with regard to the type of inhibition observed.

Keywords Diethyldithiocarbamic acid, inhibition, inhibitors, polyphenol oxidase, substrates, type of inhibition.

Introduction

Polyphenol oxidase (monophenol, dihydroxyphenylalanine: oxygen oxidoreductase, EC 1.14.18.1; PPO) is a bifunctional, copper-containing enzyme widely distributed in the phylogenetic scale which, in the presence of molecular oxygen, catalyses both the *o*-hydroxylation of monophenols to give *o*-diphenols (cresolase activity) and the further oxidation of *o*-diphenols to *o*-quinones (catecholase activity). The *o*-quinones thus generated are very unstable and rapidly react with themselves and with amino acids or proteins, polymerising to the brown or black pigments (Mason & Peterson, 1965; Matheis & Whitaker, 1984; Garcia-Carmona *et al.*, 1988) that are responsible for melanisation in animals and browning in plants. This browning phenomenon is generally undesirable in food technology because of the unpleasant appearance and the concomitant development of off flavour. Owing to its technological importance, therefore, numerous studies have been devoted to the inhibition of the enzyme from different sources by different chemical compounds (Ferrar & Walker, 1996).

*Correspondent: E-mail: sdogan@balikesir.edu.tr

The prevention or inhibition of enzymatic browning is a major concern of the food industry all over the world, and attempts have been made to eliminate from the reaction one or more of its essential components: oxygen, enzyme, copper, or (poly)phenols (Richardson & Hyslop, 1985). Oxygen can be excluded from the reaction by immersing the fruits and vegetables in water, syrup, and brine or by exposure to vacuum or modified atmospheric packing (Langdon, 1987). However, this treatment is not definitive, because when the package is opened, oxygen is reintroduced and browning will restart. The enzyme can be effectively heat-denatured by steam blanching before freezing or carrying food (McEvily *et al.*, 1992) at the expense of adverse off flavours and texture changes in fresh material (Langdon, 1987). Thermal processing is generally considered as the most effective method to inactivate PPO and, consequently, to inhibit enzymatic browning (McEvily *et al.*, 1992). Thermal treatments can, however, be responsible for considerable sensorial and nutritional quality losses in fruits and vegetables (Lund, 1977; Sapers, 1993). In this regard, molecular biology is starting to produce antisense PPO mRNA in transgenic plants, to inhibit enzymatic browning in fresh fruits. However, development of this technology will depend on a better knowledge of PPO genes in different plant sources

doi:10.1111/j.1365-2621.2007.01610.x
© 2008 The Authors. Journal compilation © 2008 Institute of Food Science and Technology Trust Fund

(Bachem *et al.*, 1994) and must at the same time overcome the consumer's refusal of transgenic food.

The most common method for controlling browning is to use sulphites in any of their forms (sulphur dioxide, sodium or potassium metabisulphite and sodium or potassium bisulphite) (Sapers, 1993). Sulphites are unique and multifaceted compounds as they are economical and can control both enzymatic and non-enzymatic browning while functioning as antimicrobials, bleaching agents, reducing agents, and antioxidants (Lambrecht, 1995). However, because of adverse health effects, the Food and Drug Administration (FDA) has prohibited their use in fruits and vegetables served or sold raw to consumers (Taylor, 1986). Other chemical compounds such as acidifiers (citric, malic, and phosphoric acids), chelators (EDTA), and reducing agents (ascorbic acid, alone or in combination) have been used (Fayad *et al.*, 1997). However, recent changes in lifestyle and an awareness of the relationship between food and health have led to a demand for more natural foods and safer and fewer added chemicals in processed foods. Diethyldithiocarbamic acid, stable under normal temperature and pressure, does not have any toxicity effect in the food industry and has been used as inhibitor (Deübowska & Podstolski, 2001).

The family Lamiaceae (Labiatae) includes between 200 and 250 genera and between 3200 and 6500 species. Distribution is throughout the world but is particularly well represented in tropical and temperate areas such as the Mediterranean region and tropical upland savannas (Dorman *et al.*, 2004). The members of this family are added to foods for their organoleptic properties and are often consumed as herbal teas in Turkey (Kurkcuoglu *et al.*, 2001). Lamiaceae species possess a variety of activities, including anti-inflammatory, antioxidant, antibacterial, antifungal, and antiviral properties (Pizzale *et al.*, 2002). *Thymbra* is a genus belonging to the Lamiaceae family. An important food product in the east Anatolian part of Turkey is herb cheese. A number of herbs such as *Thymus* sp., *Allium* sp., and *Ferule* sp. are used in making herb cheese (Doğan *et al.*, 2003a). In addition, *Thymbra* and *Ocimum* use the most widely. *Thymbra* is known and used as thyme or 'kekik' which is the name given to those species with a thymol-/carvacrol-type odour in Turkey (Baydar *et al.*, 2004). *Ocimum basilicum* L. is an important medicinal plant and culinary herb. It is called as 'Fesleğen' in Turkey. Fesleğen is widely cultivated for the production of essential oils, and is also marketed as an herb – fresh, dried, or frozen. These herbs are added into the vat to get the desired flavour for the cheese. The pickled herb is also sold in markets, so they can be found throughout the year. Furthermore, the dried leaves of these plants have been used as a spice and as a herbal tea (Doğan *et al.*, 2005a). One other important point is that this plant contains an enzyme called PPO. In our previous studies, we investigated the characterisation of PPO from different *Thymus* species (Doğan & Doğan, 2004; Doğan *et al.*, 2003a,b) and *Origanum* (Doğan *et al.*, 2005b); some kinetic properties of PPO and peroxidase from *Thymbra* (Doğan *et al.,* 2006; Doğan *et al*, in press) and *Salvia* sp. (Gündoğmaz *et al.*, 2003; Doğan *et al.*, 2007); the inhibition of PPO from different sources by 2,3-diaminopropionic acid (Arslan & Doğan, 2005) and glutamic acid (Doğan *et al.*, in press).

Diethyldithiocarbamic acid is a strong reducing agent and is also the inhibitor of some enzymes such as proteinase, lipoxygenase, and polyphenolase (Fried, 1976). The inhibition potency of diethyldithiocarbamic acid is different from one enzyme to another. For example, diethyldithiocarbamic acid strongly inhibited the proteinase activity in tomato leaves when it only weakly inhibited tomato lipoxygenase activity. It is the purpose of the present paper to describe an inhibition kinetic study of diethyldithiocarbamic acid on the catecholase activity of PPO extracted from *Thymbra spicata* L. var. *spicata* and *O. basilicum* L., and of mushroom PPO bought from Sigma (Deisenhofen, Germany). Morever, in this study, we present data demonstrating that diethyldithiocarbamic acid rapidly and efficiently causes the chemical reduction of the product of oxidation of phenolic compunds by PPO. The efficient reduction of oxidation products prevents the enzymatic browning of vegetables, fruits, and plants. This effect has not been previously reported.

Materials and methods

Plant material

Plants of *T. spicata* L. var. *spicata* and *O. basilicum* L., and mushroom have been used as research materials in this study. The original activity of mushroom PPO activity specified by Sigma is 1000 EU mg^{-1}. *Thymbra spicata* L. var. *spicata* was collected in spring from a field near Balikesir in Turkey and stored at −70 °C until used in the study. *Ocimum basilicum* L. was harvested directly from a local garden in İzmir and Aydin cities in Turkey and stored at −70 °C until processed. Mushroom PPO was purchased from Sigma Chem Co. All chemicals used were the best grade available and were used without further purification as they were obtained from Sigma. Enzyme assays were measured with the aid of a Cary |1E|g UV-Visible Spectrophotometer (Varian, Australia).

Extraction and partial purification of PPO

Thymbra spicata L. var. *spicata* and *O. basilicum* L. were placed in a Dewar flask under liquid nitrogen for 10 min in order to decompose cell membranes. For preparing the crude extract, the sample (10 g) of plants was

© 2008 The Authors. Journal compilation © 2008 Institute of Food Science and Technology Trust Fund

homogenised in 100 mL of 0.1 M phosphate buffer (pH 6.5). The homogenate was filtered and kept at 4 °C for 60 min before being centrifuged at 17 000 ***g*** for 30 min at 4 °C. The supernatant was brought to 80% $(NH_4)_2SO_4$ saturation with solid $(NH_4)_2SO_4$. The precipitated PPO was separated by centrifugation at 20 000 ***g*** for 30 min. The precipitate was dissolved in a small amount of 5 mM phosphate buffer (pH 6.5) and dialysed at 4 °C in the same buffer for 24 h with three changes of the buffer during dialysis. The dialysed sample was used as the PPO enzyme source in the following experiments (Arslan & Doğan, 2005).

Assay of PPO activity

PPO activity was assayed by measuring the rate of increase in absorbance at a given wavelength using a double beam model of a Cary |1E|g UV-Visible Spectrophotometer, as described previously (Doğan *et al.*, 2005c). The activity was determined using different substrates by measuring the increase in absorbance at 420 nm for 4-methylcatechol and catechol substrates and 320 nm for pyrogallol substrate. Total reaction volume was always maintained at 3.0 mL. The sample cuvette contained 0.1 mL of the enzyme, 2.3 mL of 0.1 M buffer solution and 0.6 mL of 0.1 M substrate solution. The blank sample contained only 0.6 mL of 0.1 M substrate and 2.4 mL of 0.1 M buffer solution. The 0.1 M concentration was chosen to avoid the influence of enzymatic extract ionic strength on PPO activity, described by Angleton & Flurkey (1984). The reaction was carried out in a 1-cm light path quartz cuvette. The temperature was kept constant at 25 °C using a Beckmann Peltier temperature controller attached to the cell holder of the spectrophotometer. The linear portion of the absorbance vs. time curve was used to determine the initial rates. It was found that: (i) PPO activity of *O. basilicium* L. was 5330 EU mg^{-1} (Doğan *et al.*, 2005c); and V_{max} and K_m values were 6941 EU mL^{-1} and 2.72 mM; 11 586 EU mL^{-1} and 1.62 mM; 10 369 EU mL^{-1} and 3.42 mM for *O. basilicium* L. PPO (Doğan *et al.*, 2005c) and, 6813 EU mL^{-1} and 20 mM; 2516 EU mL^{-1} and 3 mM; and 5123 EU mL^{-1} and 39.4 mM for *T. spicata* L. var. *spicata* PPO (Doğan *et al.*, 2006) using catechol, 4-methylcatechol and pyrogallol as substrates in our previous studies under the same conditions, respectively. One unit of PPO activity was defined as the amount of enzyme causing 0.001 increase of absorbance per minute (Doğan *et al.*, 2005b).

Effect of inhibitor

The inhibition kinetic analysis of *T. spicata* L. var. *spicata, O. basilicum* L., and mushroom PPO was determined for diethyldithiocarbamic acid in the absence of and in the presence of inhibitors at two or three different concentrations. Inhibition of PPO by diethyldithiocarbamic acid was measured at 420 nm for catechol and 4-methylcatechol substrates, and 320 nm for pyrogallol substrate at pH 6.5 and 25 °C. Three millilitres of the reaction mixture contained the substrate solutions at various concentrations in 100 mM phosphate buffer (pH 6.5), 0.1 mL enzyme solution and the inhibitor solution at fixed concentrations. Inhibition constants (K_i and K_i') were deduced from the Lineweaver–Burk plots for each inhibitor (Gündoğmaz *et al.*, 2003).

Results and discussion

Inhibitory properties of partially purified PPO preparation from various sources using diethyldithiocarbamic acid were first investigated with catechol, 4-methylcatechol, and pyrogallol as substrates. Increasing amounts of diethyldithiocarbamic acid were studied. In enzyme inhibition, a molecule (inhibitor) binds to an enzyme forming an unreactive complex. Different types of inhibitors have different effects on enzyme kinetics. The prevention of enzymatic browning by a specific inhibitor may involve a single mechanism or be the result of an interplay of two or more mechanisms of inhibitor action. There are various mechanism through which enzyme inhibitors can act (Arslan & Doğan, 2005).

Competitive inhibition

A *competitive inhibitor* has a chemical similarity to the substrate and competes with the substrate for binding to the active site of the enzyme. The Lineweaver–Burk equation for competitive inhibition is:

$$\frac{1}{v_0} = \left(\frac{\alpha K_m}{V_{max}}\right) \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \quad (1)$$

where

$$\alpha = \left(1 + \frac{[I]}{K_i}\right) \quad (2)$$

In this equation, $[S]$ is substrate concentration (mol L^{-1}); $[I]$ is inhibitor concentration (mol L^{-1}); K_i is the dissociation constant of the enzyme–inhibitor complex; V_{max} is the maximum velocity at saturating concentration of the substrate (EU mL^{-1} min^{-1}); v_0 is the enzyme activity value (EU mL^{-1} min^{-1}); and K_m is the Michaelis constant (M) (Voet & Voet, 2003). K_i values were obtained by plotting $1/v_0$ vs. $1/[S]$. A plot of $1/v_0$ vs. $1/[S]$ for competitive inhibition of mushroom PPO using 4-methylcatechol as a substrate has been given in Fig. 1. Lineweaver–Burk plots for PPO using five or six concentrations of substrates in the absence and presence of two or three different concentrations of diethyldithiocarbamic acid were typical of competitive

 © 2008 The Authors. Journal compilation © 2008 Institute of Food Science and Technology Trust Fund

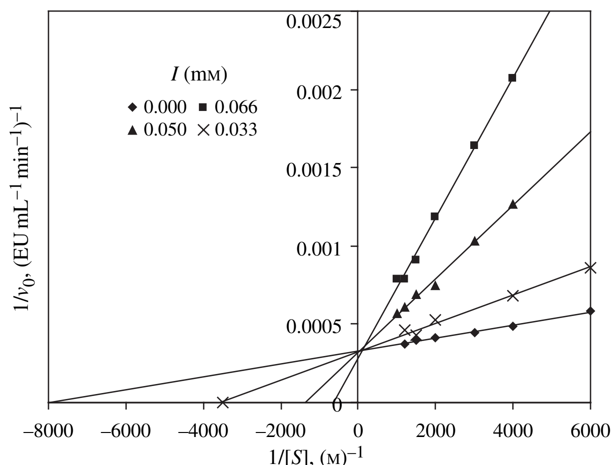

Figure 1 Lineweaver–Burk double reciprocal plots showing inhibition of mushroom polyphenol oxidase by diethyldithiocarbamic acid using 4-methylcatechol as a substrate at pH 6.5 and 25 °C.

inhibition, as illustrated in Fig. 1, in which V_{max} values have not changed when K_m values have increased. This result was shown to be the competitive inhibition of reaction between inhibitor and substrate catalysed by PPOs in this study. The dependencies obtained justified this type of inhibition. Table 1 also shows the types of inhibition and K_i and $K_i^{'}$ values for inhibition by diethyldithiocarbamic acid of *T. spicata* L. var. *spicata, O. basilicum* L., and mushroom PPO using catechol, 4-methylcatechol, and pyrogallol as substrates. As seen from Table 1, competitive inhibition was obtained for *O. basilicum* L. PPO using 4-methylcatechol and catechol as substrates; for mushroom PPO using 4-methylcatechol, catechol, and pyrogallol as substrates; and for *T. spicata* L. var. *spicata* PPO using 4-methylcatechol as substrate. A similar result was obtained with agaritine on mushroom PPO when L-tyrosine was used as a substrate (Espin *et al.*, 1998). Janovitz-Klapp *et al.* (1990) found that the inhibition effect of some carboxylic acids on PPO using 4-methylcatechol as a substrate was competitive. In the presence of the competitive inhibitor, a complex forms with the enzyme when the inhibitor binds, the EI complex. This is a dead-end complex and cannot go on to form product. However, the inhibitor is bound reversibly to the enzyme and when more substrate is added, the inhibition is overcome by pulling the enzyme free via the breakdown of the EI complex, which is in equilibrium with free enzyme and free inhibitor. Another way to think about this is when lots of substrate is added, the concentration of free enzyme (E) falls to such a low level, that some of the EI

Table 1 Inhibition types and average K_i values of polyphenol oxidase (PPO) obtained from different sources using catechol, 4-methylcatechol, and pyrogallol as substrates by diethyldithiocarbamic acid

PPO sources	Substrates	[*I*] (mM)	Average K_i (mM)	Type of inhibition	R^2
Ocimum basilicum L.	4-methylcatechol	0.033	0.033	Competitive	0.9962
		0.050			0.9927
		0.066			0.9888
	Catechol	0.066	0.04	Competitive	0.9950
		0.083			0.9941
		0.100			0.9904
	Pyrogallol	0.016	0.031	Non-competitive	0.9915
		0.033			0.9926
		0.050			0.9949
Mushroom	4-methylcatechol	0.033	0.015	Competitive	0.9928
		0.050			0.9928
		0.066			0.9954
	Catechol	0.003	0.105	Competitive	0.9747
		0.033			0.9933
		0.066			0.9962
	Pyrogallol	0.003	0.064	Competitive	0.9908
		0.007			0.9993
		0.010			0.9966
Thymbra spicata L. var. *spicata*	4-methylcatechol	0.033	0.120	Competitive	0.9962
		0.066			0.9984
		0.100			0.9981
	Catechol	0.016	0.069	Non-competitive	0.9945
		0.033			0.9962
		0.050			0.9962
	Pyrogallol	0.033	0.031	Uncompetitive	0.9969
		0.066			0.9965
		0.100			0.9971

© 2008 The Authors. Journal compilation © 2008 Institute of Food Science and Technology Trust Fund

complex must breakdown to replenish the free enzyme demanded by the equilibrium between enzyme and inhibitor.

Uncompetitive inhibition

Lineweaver–Burk equation for *uncompetitive inhibition* is given by the following equation:

$$\frac{1}{v_0} = \left(\frac{K_M}{V_{\max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{\max}} \tag{3}$$

where

$$\alpha' = 1 + \frac{[I]}{Ki'} \tag{4}$$

Figure 2 shows a plot of $1/v_0$ vs. $1/[S]$ for uncompetitive inhibition of *T. spicata* L. var. *spicata* PPO using pyrogallol as a substrate. Arslan *et al.* (2004) found that inhibition type for mulberry PPO was uncompetitive for *p*-aminobenzenesulphonamide and sulphosalicilylic acid inhibitors using catechol as a substrate. Furthermore, agartine for mushroom PPO showed uncomptetive inhibition with L-DOPA L-3,4-dihydroxyphenylalanine and L-tyrosine as substrates (Espin *et al.*, 1998). 2,3-diaminopropionic acid for mushroom PPO showed a simple linear uncompetitive inhibition when catechol and pyrogallol were used as substrates (Arslan & Doğan, 2005). The binding of the uncompetitive inhibitor, which needs to resemble the substrate, is envisaged to cause structural distortion of the active site, thereby rendering the enzyme catalytically inactive. Uncompetitive inhibition requires the inhibition to affect the catalytic function of the enzyme but not its substrate binding (Voet & Voet, 2003).

Non-competitive inhibition

Lineweaver–Burk equation for *non-competitive inhibition* is given by the following equation:

$$\frac{1}{v_0} = \left(\frac{\alpha K_m}{V_{\max}}\right)\cdot\frac{1}{[S]} + \frac{\alpha'}{V_{\max}} \tag{5}$$

In classic example of pure non-competitive inhibition, the uninhibited reaction and the enzyme in the presence of the inhibitor will yield the same K_m value. The plot of this equation consists of lines that have the slope $(1 + ([I]/K_i))(K_m/V_{\max})$ and intercept $(1 + ([I]/K_i))(1/V_{\max})$ (Arslan & Doğan, 2005). As is seen from Fig. 3, it was found that the type of inhibition for *T. spicata* L. var. *spicata* and *O. basilicum* L. PPO was non-competitive inhibition using pyrogallol and catechol as substrates, respectively. Again, K_i values obtained are given in Table 1. Non-competitive inhibition was found for palmito PPO with *p*-coumaric acid and sinapic acid inhibitors using 4-methylcatechol as a substrate (Robert *et al.*, 1997).

Again, as seen from results, the inhibition type was competitive for majority of the substrates used. The molecular weights of both substrates and inhibitors are similar and are of small volume. Therefore, it can be said that inhibitor can compete with the substrates for binding to the active site of the enzyme. As seen before, the inhibitory effect of diethyldithiocarbamic acid on enzymatic browning has varied greatly from one phenol to another and from one enzyme to another. Hence, no general rule can easily be established with regard to the type of inhibition observed. The inhibition mechanism of diethyldithiocarbamic acid can be explained as follows: *o*-diphenol is oxidised by PPO, in the absence

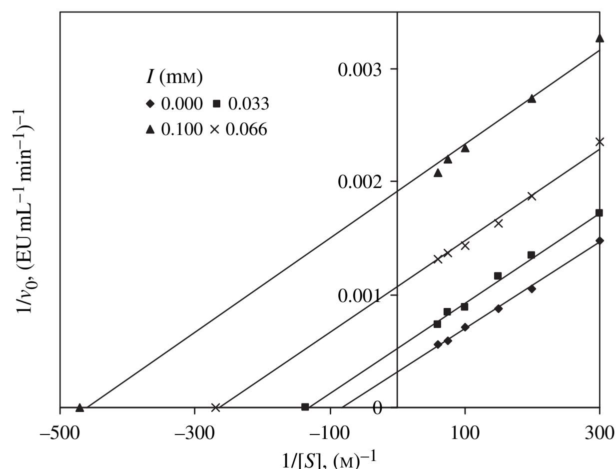

Figure 2 Lineweaver–Burk double reciprocal plots showing inhibition of *Thymbra spicata* L. var. *spicata* polyphenol oxidase by diethyldithiocarbamic acid using pyrogallol as a substrate at pH 6.5 and 25 °C.

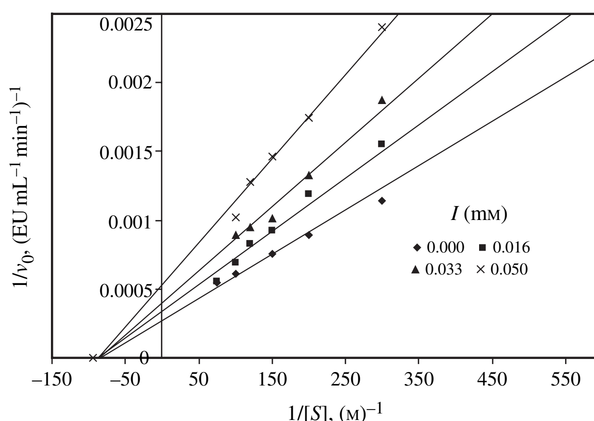

Figure 3 Lineweaver–Burk double reciprocal plots showing inhibition of *Thymbra spicata* L. var. *spicata* polyphenol oxidase by diethyldithiocarbamic acid using catechol as a substrate at pH 6.5 and 25 °C.

 © 2008 The Authors. Journal compilation © 2008 Institute of Food Science and Technology Trust Fund

Table 2 K_i values for the competitive inhibition of artichoke polyphenol oxidase (PPO) (Doğan *et al.*, 2005c)

	4-methylcatechol		Catechol		Pyrogallol	
Inhibitors	**[I] (M)**	**K_i (M)**	**[I] (M)**	**K_i (M)**	**[I] (M)**	**K_i (M)**
L-cysteine	1.00×10^{-4}	1.1×10^{-4}	6.66×10^{-5}	1.1×10^{-4}	1.00×10^{-4}	1.2×10^{-4}
	1.33×10^{-4}	8.3×10^{-5}	1.33×10^{-4}	6.2×10^{-5}	3.33×10^{-4}	1.6×10^{-4}
D,L-dithiothreitol	1.00×10^{-4}	2.2×10^{-4}	6.66×10^{-5}	1.1×10^{-4}	1.66×10^{-5}	2.4×10^{-5}
	1.33×10^{-4}	1.8×10^{-4}	1.00×10^{-4}	7.3×10^{-5}	2.33×10^{-5}	7.9×10^{-6}
Sodium azide	3.33×10^{-4}	6.3×10^{-4}	1.33×10^{-3}*	3.6×10^{-3}	3.33×10^{-4}	2.1×10^{-4}
	1.00×10^{-3}	7.3×10^{-4}	1.66×10^{-3}*	2.8×10^{-3}	6.66×10^{-3}	2.3×10^{-3}
	1.66×10^{-3}	5.8×10^{-4}	2.50×10^{-3}*	2.7×10^{-3}	1.00×10^{-2}	2.0×10^{-3}

*Non-competitive inhibition.

of diethyldithiocarbamic acid, whence the quinone radicals polymerise by oxidative coupling reaction, resulting in the browning phenomenon. As diethyldithiocarbamic acid is a strong reductant, it reduces part of the *o*-quinone back to its original structure, *o*-dihydroxyphenol. On the other hand, radicals from diethyldithiocarbamic acid or other thiol compounds immediately react with *o*-quinone radical to form addition compounds, consequently, the browning by PPO will be inhibited. Thiol compounds and amino acids (L-cysteine) inhibit polymerisation of quinones by reducing quinones back to phenols or by forming complexes with quinones and preventing polymerisation, while substrate continues to be consumed (Wuyts *et al.*, 2006). L-cysteine and reduced glutathione are also the effective inhibitors of PPO. These compounds prevent enzymatic browning by reacting with *o*-quinone to produce stable, colourless adducts instead of the brown pigments (McEvily *et al.*, 1992). Again, it was found that L-cysteine formed a product with catechol and these products inhibited the enzyme activity (Dudley & Hotchkiss, 1989). The mechanism of inhibition of enzymatic browning leads to the identification of another role for thiol compounds, in light of the reports by Weaver *et al.* (1970, 1972), which describe enzyme inhibition by γ-L-glutaminyl-3,4-benzoquinone. It was observed that thiol compounds underwent addition reactions during the extraction of the enzyme and inhibited the browning of the solution. These results suggest that an excess of thiol compounds including reductants may prevent inhibition of sulphhydryl enzymes such as dedhydrogenases by quinone compounds, i.e. the reaction which couples SH groups in the active site of the enzymes with quinones produced by PPO. Furthermore from a technological point of view, it would be conceivable to use this inhibitor in processed fruits provided that their safety is assessed and their commercial feasibility is demonstrated.

Table 2 has shown K_i values for the competitive inhibition of artichoke PPO. Comparing Tables 1 and 2, it can be said that the inhibition power of diethyldithiocarbamic acid is generally higher than those of L-cysteine, D,L-dithiothreitol, and sodium azide (e.g. for D,L-dithiothreitol, $K_i = 7.9 \times 10^{-6}$).

References

Angleton, E.L. & Flurkey, W.H. (1984). Activation and alteration of plant and fungal polyphenol oxidase isoenzymes in sodium dodecylsulfate electrophoresis. *Phytochemistry*, **23**, 2723–2725.

Arslan, O. & Doğan, S. (2005). Inhibition of polyphenol oxidase obtained from various sources by 2,3-diaminopropionic acid. *Journal of the Science of Food and Agriculture*, **85**, 1499–1504.

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

Bachem, C.W.B., Speckmann, G.F., van der Linde, P.C.G., *et al.* (1994). Antisense expression of polyphenol oxidase genes inhibits enzymatic browning in potato tubers. *Bio/Technology*, **12**, 1101–1105.

Baydar, H., Sagdic, O., Ozkan, G. & Karadogan, T. (2004). Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control*, **15**, 169–172.

Deübowska, R. & Podstolski, A. (2001). Properties of diphenolase from *Vanilla planifolia* (Andr.) shoot primordia cultured *in vitro*. *Journal of Agriculture and Food Chemistry*, **49**, 3432–3437.

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

Doğan, S., Doğan, M. & Arslan, O. (2003a). Characterization of polyphenol oxidase from *Thymus* (*Thymus longicaulis* var. *subisophyllus*). *Advances in Food Science*, **25**, 56–64.

Doğan, S., Doğan, M. & Arslan, O. (2003b). Determination of some kinetic properties of polyphenol oxidase from *Thymus* (*Thymus zygioides Griseb.* var. *lycaonicus* (Čelak) Ronninger). *Advances in Food Science*, **25**, 130–136.

Doğan, S., Arslan, O. & Ozen, F. (2005b). Polyphenol oxidase activity of oregano at different stages. *Food Chemistry*, **91**, 341–345.

Doğan, S., Turan, Y., Ertürk, H. & Arslan, O. (2005c). Characterization and purification of polyphenol oxidase from artichoke (*Cynara scolymus* L.). *Journal of Agriculture and Food Chemistry*, **53**, 776–785.

Doğan, S., Turan, P., Doğan, M., Arslan, O. & Alkan, M. (2005a). Purification and characterization of *Ocimum basilicum* L. polyphenol oxidase. *Journal of Agriculture and Food Chemistry*, **53**, 10224–10230.

Doğan, S., Turan, P. & Doğan, M. (2006). Some kinetic properties of polyphenol oxidase from *Thymbra spicata* L. var. *spicata*. *Process Biochemistry*, **41**, 2379–2385.

© 2008 The Authors. Journal compilation © 2008 Institute of Food Science and Technology Trust Fund

Doğan, S., Turan, P., Doğan, M., Arslan, O. & Alkan, M. (2007). Variations of peroxidase activity among Salvia species. *Journal of Food Engineering*, **79**, 375–382.

Doğan, S., Turan, P., Doğan, M., Alkan, M. & Arslan, O. (2007). Inhibition kinetics of polyphenol oxidase by glutamic acid. *European Food Research and Technology*, **225**, 67–73.

Doğan, S., Turan, P., Doğan, M., Alkan, M. & Arslan, O. (in press) Partial characterization of peroxidase from the leaves of *Thymbra* plant (*Thymbra spicata* L. var. *spicata*). *European Food Research and Technology*.

Dorman, H.J.D., Bachmayer, O., Kosar, M. & Hiltunen, R. (2004). Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. *Journal of Agriculture and Food Chemistry*, **52**, 762–770.

Dudley, E.D. & Hotchkiss, J.H. (1989). Cysteine as an inhibitor of polyphenol oxidase. *Journal of Food Biochemistry*, **13**, 65–75.

Espin, J.C., Jolivet, S. & Wichers, H.J. (1998). Inhibition of mushroom PPO by agartine. *Journal of Agriculture and Food Chemistry*, **46**, 2976–2980.

Fayad, N., Marchal, L., Billaud, C. & Nicolas, J. (1997). Comparison of cyclodextrin effect on polyphenol oxidation catalyzed bypurified polyphenol oxidase from different sources. *Journal of Agriculture and Food Chemistry*, **45**, 2442–2446.

Ferrar, P.H. & Walker, J.R.L. (1996). Inhibition of diphenol oxidases: a comparative study. *Journal of Food Biochemistry*, **20**, 15–30.

Fried, R. (1976). Enzymatic oxidation of diethyldithiocarbamate by xanthine oxidase and its colorimetric assay. *Annals of New York Academy of Science*, **273**, 212–218.

Garcia-Carmona, F., Valero, E. & Cabanes, J. (1988). Effect of L-proline on mushroom tyrosinase. *Phytochemistry*, **27**, 1961–1964.

Gündoğmaz, G., Doğan, S. & Arslan, O. (2003). Some kinetic properties of polyphenol oxidase obtained from various *Salvia* species (*Salvia viridis* L., *Salvia virgata* Jacq. and *Salvia tomentosa* Miller). *Food Science and Technology International*, **9**, 309–315.

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

Kurkcuoglu, M., Tumen, G. & Baser, K.H.C. (2001). Essential oil constituents of *Satureja boissieri* from Turkey. *Chemical Nature and Composition*, **37**, 329–331.

Lambrecht, H.S. (1995). Sulfite substitutes for the prevention of enzymatic browning in foods. In: *Enzymatic Browning and its Prevention* (edited by C.Y. Lee & J.R. Whitaker). Pp. 313–323. Washington, DC: American Chemical Society.

Langdon, T.T. (1987). Preventing of browning in fresh prepared potatoes without the use of sulfiting agents. *Food Technology*, **41**, 64–67.

Lund, D.B. (1977). Design of thermal processes for maximizing nutrient retention. *Food Technology*, **31**, 71–78.

Mason, H.S. & Peterson, E.W. (1965). Melanoproteins I. Reactions between enzyme-generated quinones and amino acids. *Biochimica Biophysica Acta*, **111**, 134–146.

Matheis, G. & Whitaker, J.R. (1984). Modification of proteins by polyphenol oxidase and peroxidase and their products. *Journal of Food Biochemistry*, **8**, 137–162.

McEvily, A.J., Iyengar, R. & Otwell, W.S. (1992). Sulfite alternative prevents shrimp melanosis. 4-hexylresorcinol controls enzymatic browning in shrimp and has potential for application in a variety of other foods and beverages. *Critical Review of Food Science and Nutrition*, **32**, 253–273.

Pizzale, L., Bortolomeazzi, R., Vichi, S., Uberegger, E. & Conte, L.S. (2002). Antioxidant activity of sage (*Salvia officinalis* and *S. fruticosa*) and oregano (*Origanum onites* and *O. indercedens*) extracts related to their phenolic compound content. *Journal of Science of Food Agriculture*, **82**, 1645–1651.

Richardson, T. & Hyslop, D.B. (1985). *Food Chemistry*, 2nd edn. (edited by O.R. Fennema). Pp. 445–447. New York: Dekker.

Robert, C., Rouch, C. & Cadet, F. (1997). Inhibition of palmito (*Acanthophoenix rubra*) PPO by carboxylic acids. *Food Chemistry*, **59**, 355–360.

Sapers, G.M. (1993). Browning of foods: control by sulfites, antioxidants and other means. *Food Technology*, **47**, 75–84.

Taylor, S.L. (1986). Sulfites as food ingredients. *Food Technology*, **40**, 47–52.

Voet, D. & Voet, J.G. (2003). *Biochemistry*. USA: John Wiley & Sons, Inc.

Weaver, R.F., Rajagopalan, K.V. & Handler, P. (1972). Mechanism of action of a respiratory inhibitor from the gill tissue of sporulating common mushroom, *Agaricus bisporus*. *Archives of Biochemistry and Biophysics*, **149**, 541–548.

Weaver, R.F., Rajagopalan, K.V., Handler, P., Jeffs, P., Byrne, W.L. & Rosenthal, D. (1970). Isolation of g-L-glutaminyl 4-hydroxybenzene and g-L-glutaminyl 3,4-benzoquinone: a natural sulfhydryl reagent, from sporulating gill tissue of the mushroom *Agaricus bisporus*. *Proceedings of the National Academy of Sciences (USA)*, **67**, 1050–1056.

Wuyts, N., De Waele, D. & Swennen, R. (2006). Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata Grande naine*) roots. *Plant Physiology and Biochemistry*, **44**, 308–314.

 © 2008 The Authors. Journal compilation © 2008 Institute of Food Science and Technology Trust Fund