IJABBR- 2014- eISSN: 2322-4827

International Journal of Advanced Biological and Biomedical Research

Journal homepage: www.ijabbr.com

Original Article

Effect of Anti Browning Agents on Partial Purified Polyphenol Oxidase of Hawthorn *(Crataegus Spp*

Shahriar Saeidian*

Assistant professor of Biochemistry, Department Of Biology, Payame Noor University ,Iran

ARTICLE INFO

Article history:
Received: 05 June, 2014
Revised: 25 June, 2014
Accepted: 16 July, 2014
ePublished: 30 August, 2014

Key words:
Inhibition
Polyphenol oxidase
Hawthorn
Crataegus spp
L-cysteine
Citric acid,
Ascorbic acid

ABSTRACT

Objective: Polyphenol oxidase or PPO (EC 1.14.18.1) is considered the enzyme responsible for quality deterioration and browning in different fruits during postharvest period. The objective of this study was to evaluate the antibrowning (inhibition of polyphenol oxidase activity) effect of Cysteine, Ascorbic acid, citric acid, kojic acid and glycine at two different pH (6 and 7) in hawthorn. **Methods:** Partial purified polyphenol oxidase was mixed with Cys, ascorbic acid, citric acid, glycine and kojic acid, to assess their effect on PPO. **Results:** The most effective PPO inhibitors was kojic acid. IC50 for cysteine was earned 0.48 and 0.58, for ascorbic acid, 0.8 and 1, for citric acid, 0.75 and 0.85 and for kojic acid was 0.3 and 0.4 at pH 6 and 7, respectively Partial purified polyphenol oxidase was mixed with Cys, ascorbic acid, citric acid, glycine and kojic acid, to assess their effect on PPO. The most effective PPO inhibitors was kojic acid. IC50 for cysteine was earned 0.48 and 0.58, for ascorbic acid, 0.8 and 1, for citric acid, 0.75 and 0.85 and for kojic acid was 0.3 and 0.4 at pH 6 and 7, respectively.

1.INTRODUCTION

Enzymatic browning reactions in fruits are catalyzed by polyphenol oxidases (PPO) followed by nonenzymatic formation of melanins (Martinez and Whitaker, 1995). Extensive research has been focused on browning control of fresh-cut apples, and many approaches have been explored for this purpose (Soliva-Fortuny and Martin-Belloso, 2003). However, few browning inhibitors have shown potential for use in the food industry because of concerns over off-flavors and off-odors, food safety, economic feasibility, and effectiveness of inhibition (McEvily and et.al, 1992; Park, 1999). When cell membrane integrity is disrupted, phenolic substrates encounter the enzyme and are converted to o-quinones in a two-step process of hydroxylation of monophenols to diphenols (monophenolase activity), followed by oxidation of diphenols to o-quinones (diphenolase activity) (Espin et al., 1998). PPO has been implicated in the formation of pigments, oxygen scavenging (Trebst and Depka, 1995) and defense mechanism against plant pathogens, (Mohammadi and Kazemi, 2002) and herbivory insects (Constabel et al, 2000). Phenolic compounds serve as precursors in the formation of physical polyphenolic barriers, limiting pathogen translocation. The quinones formed by PPOs can bind plant proteins, reducing protein digestibility and their nutritive value to herbivores (Ryan, 2000). On the other

***Corresponding Author:** Shahriar Saeidian, Assistant professor of Biochemistry, Department Of Biology, Payame Noor University , Iran. (saeedyan@pnu.ac.ir)

hand, the oxidation of phenolic substrates by PPO is thought to be the major cause of the brown coloration of many fruits and vegetables during ripening, handling, storage and processing. Now a days, several methods have been used to inhibit the PPO activity, such as the use of Anti-browning agents, removal of its one of the necessary component: O2, Cu2 etc or by thermal processing to inactivate PPO activity is limited due to loss of sensory and nutritional quality of fruits and vegetables4 . Removal of oxygen from PPO can also check enzymatic browning but browning may restart when oxygen is available (Langdon, 1987). So, the best way to prevent enzymatic browning is the use of anti-browning agents. These agents act on the enzyme or react with the substrate and/or products of enzymatic catalysis and thereby formation of browning pigment is inhibited (Arslan and Dogan, 2005). Several Anti-browning agents such as sulphites (Sulphur dioxide, sodium or potassium metabisulfite, sodium or potassium bisulfite); acidulants such as citric, malic or phosphoric acid; chelators (EDTA); reducing agents (ascorbic acid alone or in combination, Cysteine); 4-hydroxyrecercinol etc have been extensively used for preventing browning of Foods (Fayad et al, 1997; Sapers, 1993). Ascorbic acid reduces o-quinones to diphenols and prevents the formation of browning pigments (McEvily et al, 1992). Sometimes ascorbic acid is oxidized completely and browning may occur due to formation of melanins (Walker et al, 1995). Acidifiers or acidulants can inhibit PPO activity by reducing pH and or chelating copper in fruits and vegetables (Richardson, and Hyslop, 1985). Cysteine reacts with O- quinones and forms a colourless compound and thereby reduces PPO activity by delaying discolorations (Dudley et al, 1989). Another anti-browning agent, 4- hydroxyresorcinol binds with PPO to form an inactive complex and thereby reduces the browning reaction (Lambrecht, 1995). The objective of this study was to evaluate the anti-browning (inhibition of PPO activity) effect of Cysteine, ascorbic acid, citric acid, glycine and kojic acid, at tho different pH (6 and 7) in hawthorn cultivated in kurdistan (Iran).

2. MATERIALS AND METHODS

The hawthornes used in this study were obtained from Kurdistan of Iran and frozen at -25 °C until used. pyrogallol was purchased from Merck (Darmstadt, Germany). Acetone, ammonium sulphate, L-cysteine, kojic acid, L-glycine, polyethylene glycol, phenylmethylsulfonyl fluoride (PMSF), cellulose membrane (76x49mm) and DEAE-cellulose were purchased from Sigma-Aldrich (St. Louis, USA). All chemicals were of analytical grade.

2.1. Enzyme Extraction

200 grams of hawthornes were homogenized in 150 mL of 0.1M phosphate buffer (pH 6.8) containing 10 mM ascorbic acid and 0.5% polyvinylpyrrolidone with the aid of a magnetic stirrer for 1h. The crude extract samples were centrifuged at 20000 g for 20 min at 4ºC. Solid ammonium sulphate (NH4)2SO4 was added to the supernatant to obtain 30 and 80% (NH4)2SO4 saturation, respectively. After 1 h, the precipitated proteins for each stage were separated by centrifugation at 20000 g for 30 min. The precipitate was redissolved in a volume of distilled water and dialyzed at 4ºC against distilled water for 24 h with 4 changes of the water during dialysis.

2.2. Ion Exchange Chromatography

The dialysate was applied to a column (2.5 cm x 30 cm) filled with DEAE-cellulose, balanced with 10 mM phosphate buffer, pH 6.8. In order to remove non adsorbed fractions the column was washed with 200 mL of the same buffer at the flow rate of 0.5 mL/min. Then, a linear gradient of phosphate buffer concentration from 20 to 180 mM was applied. 5 mL fractions were collected in which the protein level and PPO activity towards pyrogallol as substrate were monitored. The fractions which showed PPO activity were combined and were used as enzyme source in the following experiments.

2.3. PPO Assay

Enzymatic activity was determined by measuring the increase in absorbance at 420 nm for pyrogallol with a spectrophotometer (6305 JENWAY). The sample cuvette contained 3 ml of substrate pyrogallol in constant concentrations and in presence of different concentration of anti-browning agents, prepared in the phosphate buffer. Assays were carried out by addition of 150 µl of extracts to the sample cuvette, and changes in absorbance 420 nm were recorded. The reference cuvette contained just 3 ml of substrate solution. PPO activity was assayed in presence of concentrations of 0.5, 1 and 1.5 M of anti browning agents. Polyphenol oxidase activity was determined by measuring the amount of quinone produced, using an extinction coefficient of 12 $M^{-1}cm^{-1}$ for pyrogallol. Enzyme activity was calculated from the linear portion of the curve. Assays were carried out at room temperature and results are the averages of at least three assays.

2.4. Inhibition of PPO Activity by anti browning agents at pH 6 and 7

Inhibition of PPO activity was conducted in a disposable cuvette containing 3 mL of the standard reaction mixture.

The concentration of L-glycine was 0, 0.4, 0.7, 0.9, 1.1, 1.4, 1.8 and 2 M in an phosphate buffered reaction mixture with pH of 6 and 7, and PPO activity for the oxidation of pyrogallol at a final concentration of 25 mM was determined at pH 6 and 7 respectively. This concentration for L-cysteine, citric acid, ascorbic acid and kojic acid were similar of glycine in an phosphate buffered reaction mixture with pH of 6 and 7.

2.5. Kinetic Study

Various concentrations of pyrogallol (0-25 mM) and each of anti browning agents (0-2 M) were prepared in 0.1 M phosphate buffer (pH 6.7). These various concentrations for pyrogallol were 4, 8, 10, 12, 14, 16, 20 and 25 mM. These various concentrations for pyrogallol and anti browning agents at pH 6 and 7 were similar for all of anti browning agents. The reaction mixture and PPO activity assay were the same as those for the standard reaction. The inhibition kinetics of L-glycine, L-cysteine, citric acid, ascorbic acid and kojic acid on PPO activity were determined by Lineweaver-Burk plots (Marangoni, 2002).

3. RESULTS AND DISCUSSION

3.1. Effect of anti browning agents on PPO activity in hawthorn extract

L-glycine, L-cysteine, ascorbic acid, citric acid and kojic acid inhibited the PPO activity detectable with pyrogallol as substrate. The concentration of L-glycine inhibiting PPO activity by 50% (IC50) was 0.45 and 0.65 mM at pH 6 and 7, respectively. IC50 for L-cysteine inhibiting PPO activity was 0.48 at pH 6 and 0.58 at pH 7. IC50 for ascorbic acid, citric acid and kojic acid inhibiting PPO activity were 0.8, 0.75 and 0.3 at pH 6 and 1, 0.85 and 0.4 at pH 7, respectively. (Table 1). Fig 1 represents kojic acid-mediated inhibition of hawthorn PPO activity.

3.2. Inhibition Kinetic of anti browning agents on PPO Activity at pH 6 and 7

Inhibition of partial purified polyphenol oxidase investigated in presence of concentrations of 0.5, 1 and 1.5 mM of anti browning agents (glycine, cysteine, citric acid, ascorbic acid and kojic acid) (table 2). Activity of enzyme in control condition (absence of anti browning agents) were 2.2 and 2.5 mM/min.mg protein at pH 6 and 7 respectively. These activity decreased and reached to 1.5 at pH 6 and 1.7 at pH 7 in presence of glycine (0.5 mM), so a decrease of 32% observed at both of pH. Decresing of activity in presence of 1 mM of glycine were 50% at pH 6 and 56 % at pH 7, in presence of 1.5 mM glycine, 73% and 68 %; in presence of 0.5 mM cysteine, 46% and 48%; 1 mM of cysteine, 57% and 50%; 1.5 mM cysteine, 80% and 74%; 0.5 mM kojic acid, 55% and 56%; 1 mM kojic acid, 69% and 62%; 1.5 mM kojic acid, 85% and 82%; 0.5 mM citric acid, 16% and 12%; 1 mM citric acid, 25% and 30%; 1.5 mM citric acid, 39% and 41%; 0.5 mM ascorbic acid, 50% and 44%; 1 mM ascorbic acid, 62% and 56%; 1.5 mM ascorbic acid, 80% and 69%, at pH 6 and 7 respectively.

3.3. Type of Inhibition of anti browning agents on PPO Activity at pH 6 and 7

Inhibition of PPO by anti browning agents were determined separately, in the presence of different concentrations of these agents, for three fixed concentrations of pyrogallol at pH 6.0 and 7. Lineweaver burk plots used to analyze inhibition kinetics of ascorbic acid and citric acid at pH 6 and pH 7 and cysteine at pH 7 show that the extrapolated lines for $1/V$ versus 1/[pyrogallol] intersect each other on the y-axis, indicating that these anti browning agents are competetive type inhibitors of PPO (table 1). These agents as a competetive inhibitor can bind at the active site of the enzyme to form an PPO-inhibitor complex. They block the active site, and pyrogallol as substrate cannot bind until the inhibitor dissociates. Since, these inhibitors and pyrogallol compete for the same site, raising the pyrogallol concentration can eventually overcome them, and Vmax can be achieved, but they raises Km, indicating that the affinity of PPO for pyrogallol is lower in the presence of them. To further investigate whether the inhibition of PPO activity by ascorbic acid is attributable to the inhibitor's effect on PPO, the substrate, or both, preincubation of ascorbic acid with PPO or pyrogallol was carried out before the inhibition reaction started. Figure 3 shows the effect of different durations of preincubation of the enzyme with ascorbic acid at pH 6.0 and at three concentrations of these inhibitors, So with increasing time of incubation , activity of enzyme decreased. 10 minute Incubation of ascorbic acid (1.5mM) with PPO caused 80% inhibition in activity. An 5-min preincubation of PPO with 1mM ascorbic acid resulted in a 42% loss in PPO activity compared to control (without inhibitor) (Fig. 4). Incubation of pyrogallol with ascorbic acid showed the same effect on activity in comparision to presence of PPO and ascorbic acid and pyrogallol without incubation. This finding suggests that ascorbic acid inhibits PPO activity by acting directly on the PPO rather than on the substrate and these finding confirmed that ascorbic acid is competitive inhibitor of hawthorn PPO at pH 6. Lineweaver burk plots used to analyze inhibition kinetics show that the extrapolated lines for $1/V$ versus 1/[pyrogallol] intersect each other on the x-axis, indicating that L-cysteine, glycine and kojic acid at pH 6 and kojic acid at pH 7 are noncompetetive type inhibitor. These anti browning agents as noncompetetive inhibitors can bind at an allosteric site on the PPO and leave the active site unblocked. Pyrogallol as substrate has an identical affinity for both the inhibitors-PPO complex and PPO. In presence of these inhibitors as noncompetitive inhibitor of PPO, the Km value is unchanged , while V_{max} is decreased. An 5-min

preincubation of PPO with 1mM kojic acid resulted in a 70% loss in PPO activity compared to control. Interestingly, preincubation of kojic acid with pyrogallol for 5 min resulted in no additional loss of PPO activity compared to that without incubation (Fig. 5). This finding suggests that these anti browning agent inhibit PPO activity by acting directly on the PPO rather than on the substrate and confirmed that kojic acid is non competitive in hibitor of PPO in hawthorn at pH 7. Lineweaver burk plots used to analyze inhibition kinetics show that the extrapolated lines for $1/V$ versus 1/[pyrogallol] are parallel and don't intersect each other near or on the *y and x*-axis, indicating that L-glycine at pH 7 is a uncompetetive type inhibitor. L-glycine as a uncompetetive inhibitor are thought to bind the the PPO-pyrogallol complex and not the PPO. The effect of L-glycine is to decrease both Vmax and Km. A lower Km corresponds to a higher affinity. The presence of L-glycine as uncompetitive inhibitor increases the affinity of the enzyme for the pyrogallol.

3.4. Kinetic parameters of PPO activity in presence of inhibitors

The Michaelis-Menten constant (Km) and maximum rate (V_{max}) values for PPO activity in hawthorn extract were determined by performing activity assays at pH 6 and pH 7, in the presence of extract aliquots and various concentrations of either pyrogallol as substrate and various concentrations of L-glycine, L-cysteine, ascorbic acid, citric acid and kojic acid as inhibitors. The rate of pyrogallol oxidation to its corresponding o-quinone was measured by monitoring the absorbance increase at 420 nm in a 3-ml reaction mixture containing 0.9 mg extract protein. The maximum rate (V_{max}) for pyrogallol oxidation at pH 6 and in absence of anti browning agents was 0.48 ΔA min^{-1}, with a Km of 21 mM. The catalytic efficiency calculated per milligram protein in the extract was 2.5 units mg^{-1} prot mM^{-1} (Table 3). The maximum rate (V_{max}) and Km for pyrogallol oxidation at pH 6 and in presence of L-glycine (1 mM) was 0.31 ΔA min^{-1} and 19 mM, but catalytic efficiency decreased to 1.78 units mg^{-1} prot mM^{-1}. The maximum rate (V_{max}) for pyrogallol oxidation at pH 7 and in absence of L-glycine was 0.62 ΔA min^{-1}, with a Km of 18 mM. V_{max} in presence of L-glycine (1 mM) decreased and reached to 0.47ΔA min^{-1} and Km decreased to 17 mM. Catalytic efficiency at pH 7 in presence of L-glycine decreased from 3.8 to 3.05 units mg^{-1} prot mM^{-1}. Data in table 2 shows that catalytic efficiency decreased for pyrogallol oxidation in presence of anti browning agents at pH 6 and pH 7. Catalytic efficiency at pH 6 in absence of anti browning agents and in presence of L-glycine, cysteine, ascorbic acid, citric acid and kojic acid decreased and were earned 2.5, 1.78, 2.2, 2.25, 2.5 and 1.15 units mg^{-1} prot mM^{-1} .

CONCLUSION

Our results showed that anti browning agents have different effects on PPO activity in hawthorn, So kojic acid is a potent inhibitor and ascorbic acid showed the least effect of inhibitory on activity of enzyme. Kinetic studies via lineweaver-Burk plots indicate that Glycine is uncompetitive inhibitor of PPO at pH 6 and 7, ascorbic acid and citric acid are competitive inhibitor of hawthorn PPO at pH 6 and 7 and kojic acid is a noncompetitive inhibitor of enzyme, but cysteine showed different inhibitory at pH 6 and 7, sothat cysteine is noncompetitive inhibitor at pH 6 and competitive inhibitor at pH7 for hawthorn PP. In according of our results, inhibition of PPO activity is pH and inhibitor dependent. Investigation of kinetics parameters showed that best catalytic efficiency for PPO activity earned in presence of citric acid, although kojic acid caused a decrease of 48% and 37% in catalytic efficiency of enzyme in control condition at pH 6 and pH 7, respectively. As reported for other plants (Ho K-K , 1999), (Escribano et al, 2002), multiple isoforms of PPO were detected in saffron (Saeidian, et al, 2007), so we can conclude that PPO in hawthorn (Crataegus spp) maybe have atleast two isoforms, because of different kinetic properties at pH 6 and 7.

AKNOWLEGMENTS

This work was supported in part by the University of Payame Noor, and was done in exploratory laboratory of biochemistry in payame noor university.

REFERENCES

Arslan, O and Dogan, S. (2005). Inhibition of Polyphenol Oxidase obtained from Various Sources by 2,3-Diaminopropionic Acid, J. Sci. Food Agri., 85, 1499-1504.

Constabel, C.P., Lynn, Y., Patton, J.J., Christopher, M.E. (2000). Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. Plant Physiol. *124*, 285–295.

Dudley, E.D and Hotchkiss, J.H. (1989). Cysteine as an Inhibitor of Polyphenol Oxidase, J. Food Biochem., 13, 65-75 (1989).

Escribano J, Gandı´a-Herrero F, Caballero N, Pedren˜o MA. (2002). Subcellular localization and isoenzyme pattern of peroxidase and polyphenol oxidase in beet root (Beta vulgaris L.). J Agric Food Chem 50:6123–6129.

Espin, J.C., Garcia-Ruiz, P.A., Tudela, J, Varon, R and Garcia-Canovas, F. (1998). Monophenolase and Diphenolase Reaction Mechanisms of Apple and Pear Polyphenol Oxidases. *J. Agric. Food Chem*. **46 (8)**: 2968-2975.

Fayad, L., Marchal, C., Billaud and J. Nicolas. (1997). Comparison of Beta-Cyclodextrin Effect on Polyphenol Oxidation Catalyzed by Purified Polyphenol Oxidase from Different Sources, J. Agri. Food Chem., 45, 2442-2446.

Ho K-K. (1999). Characterization of polyphenol oxidase from aerial roots of an orchid, Aranda ''Christine 130''. Plant Physiol Biochem 37:841–848.

Lambrecht, H. S. (1995). Sulfite Substitutes for the Prevention of Enzymatic Browning in Foods, in C. Y. Lee and J. R. Whitaker (Eds.), Enzymatic Browning and its Prevention, American Chemical Society USA (1995) pp. 313-323.

Langdon, T.T. (1987). Preventing of Browning in Fresh Prepared Potatoes without the use of Sulfiting Agents, Food Technol., 41(64), 66-67.

Lowry, O.H. Rosebrough, N.J. Farr, A.L and Randall, R.J.(1951). Protein measurement with the Folin-Phenol reagents*. J. Biol. Chem*. **193**: 265-27.

Marangoni, A. G. (2002). Reversible enzyme inhibition. In *Enzyme Kinetics: A Modern Approach*; Wiley: Hoboken, NJ ; pp 61-65.

Martinez, M. V., Whitaker, J. R. (1995). The biochemistry and control of enzymatic browning Trends Food Sci. Technol. 6, 195-200.

Mohammadi, M., Kazemi, H. (2002). Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Sci. *162*, 491–498.

McEvily, A.j., Iyengar, R and Otwell, W.S. (1992). Inhibition of Enzymatic Browning in Foods and Beverages, Critical Rev. Food Sci. Nut., 32, 253-273.

McEvily A. J., Iyengar, R., Otwell, W. S. (1992). Inhibition of enzymatic browning in foods and beverages. Crit. ReV. Food Sci. Nutr. 32, 253-273.

Park, H. J. (1999). Development of advanced edible coatings for fruits. Trends Food Sci. Technol. 10, 254-260.

Richardson, T and Hyslop, D.B. (1985). Enzymes, in, O. R. Fennema (Ed.), Food Chemistry, 2nd Ed., USA, Marcel Dekker pp. 371-476.

Ryan, C.A. (2000). The systemin signaling pathway: differential activation of plant defensive genes. Biochim. Biophys. Acta **.** *1477*, 112–121.

Saeidian, S. Keyhani, E and Keihani, J. (2007). Polyphenol oxidase activity in dormant saffron (Crocus Sativus L.) corm. Acta *Physiol Plant*. **29**: 463-471.

Saeidian, S. Keyhani, E and Keihani, J. (2007). Effect of Ionic Detergents, Nonionic Detergents, and Chaotropic Agents on Polyphenol Oxidase Activity from Dormant Saffron (*Crocus sativus* L.) Corms. *J. Agric. Food Chem.* 55(9): 3713-3719.

Sapers, G.M. (1993). Browning of Foods : Control by Sulfites, Antioxidants and other Means. Food Technol., 47, 75-84.

Soliva-Fortuny, R. C., Martin-Belloso, O. (2003). New advances in extending the shelf life of fresh-cut fruits: a review. Trends Food Sci. Technol. 14, 341-353.

Trebst, A., Depka, B. (1995). Polyphenol oxidase and photosynthesis research. Photosynth. Res. *46*, 41–44.

Walker, J. R. L., Lee, C. Y and Whitaker J. R. (1995). Enzymatic Browning in Fruits, in Enzymatic Browning and Its Prevention, USA : American Chem. Soc. pp. 8-22.

Table 1:

Effect of anti browning agents on the PPO activity in hawthorn

Substrate (pyrogallol)	pH 6	Inhibitor	IC50 (M)	Type of inhibition
		Glycine	0.45	Uncompetetive
		Ascorbic acid	0.8	competetive
		Citric acid	0.75	competetive
		Kojic acid	0.3	Noncompetetive
		Cysteine	0.48	Noncompettive
	pH 7	Inhibitor	IC50 (M)	Type of inhibition
		Ascorbic acid	1	competetive
		Citric acid	0.85	competetive
		Kojic acid	0.4	noncompetetive
		Glycine	0.65	Uncompetetive
		Cysteine	0.58	Competetive

Table 2 .

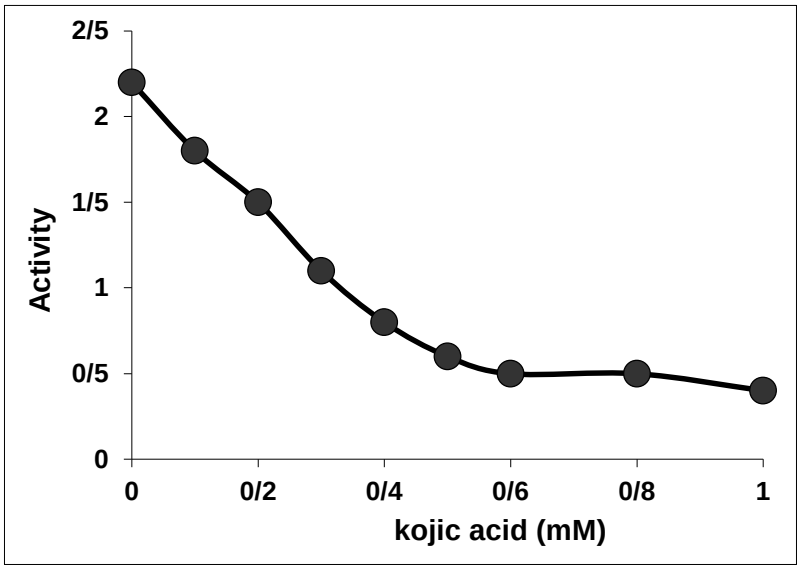
Effect of concentrations of 0.5, 1 and 1.5 mM of anti browning agents on PPO activity.

Antibrowning agents		**Enzyme activity**	
		pH	
agent	**Concentration (mM)**	**6**	**7**
control	0	2.2 mM/min.mg protein	2.5 mM/min.mg protein
Glycine	0.5	1.5	1.7
	1	1.1	1.4
	1.5	0.6	0.8
Cysteine	0.5	1.2	1.3
	1	0.95	1.25
	1.5	0.45	0.66
Kojic acid	0.5	1	1.1
	1	0.7	0.85
	1.5	0.32	0.45
Citric acid	0.5	1.85	2.2
	1	1.65	1.75
	1.5	1.35	1.48
Ascorbic acid	0.5	1.1	1.4
	1	0.85	0.98
	1.5	0.45	0.78

Table 3:

Kinetics parameters for the PPO activity detected in hawthorn.

pH	Substrate	Inhibitors	V_{max}	Activity	Km (mM)	Catalytic efficiency
6	Pyrogallol	----------	0.48	53	21	2.5
		Glycine	0.31	34	19	1.78
		cyestein	0.36	40	18	2.2
		Ascorbic acid	0.41	45	20	2.25
		Citric acid	0.39	43	17	2.5
		Kojic acid	0.21	23	20	1.15
7	pyrogallol	----------	0.62	69	18	3.8
		Glycine	0.47	52	17	3.05
		Cysteine	0.5	55	16	3.4
		Ascorbic acid	0.55	61	17	3.58
		Citric acid	0.5	56	15	3.7
		Kojic acid	0.38	42	17	2.4



1

Figure 1. kojic acid -mediated inhibition of hawthorn PPO activity by the oxidation of pyrogallol.

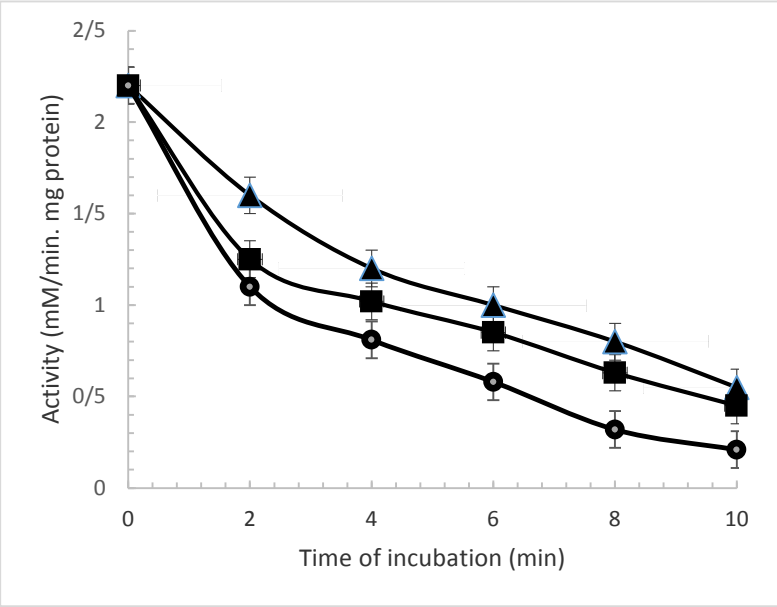


Figure 2. Preincubation duration for three concentrations of ascorbic acid with PPO at pH 6. The bars represent the standard errors of three replicates. 0.5 mM ascorbic acid, (dark filled triangle), 1mM ascorbic acid, (dark filled square), 1.5mM ascorbic acid, (dark filled circle).

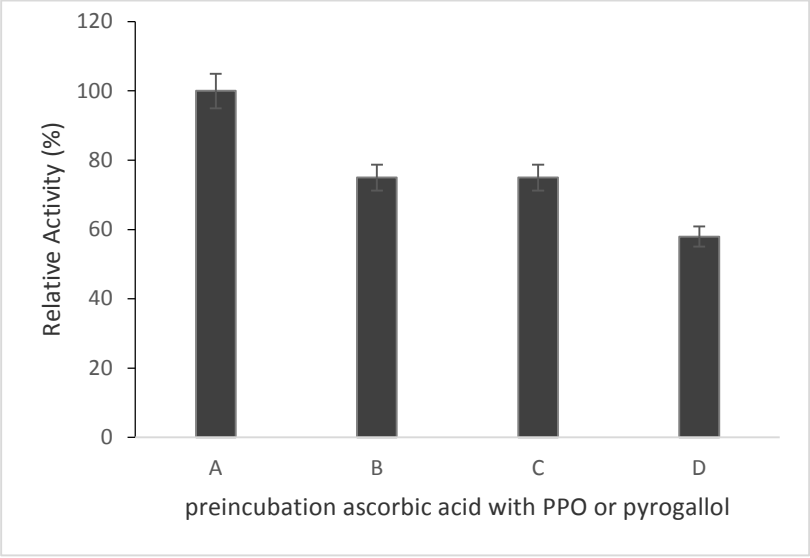


Figure 3. Preincubation of ascorbic acid with PPO or pyrogallol on the inhibition of hawthorn PPO activity at pH 6. Activities were expressed as percent relative activity to that determined without preincubation: no ascorbic acid or preincubation (A); 1mM ascorbic acid, no preincubation (B); preincubation of ascorbic acid with pyrogallol (C); preincubation of ascorbic acid with PPO (D). The vertical bars represent the standard errors of three replicates.

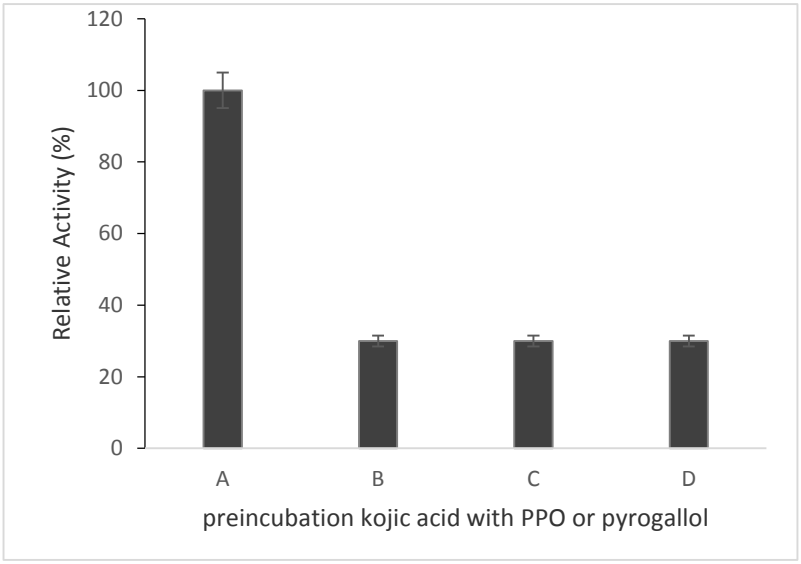


Figure 4. Preincubation of kojic acid with PPO or pyrogallol on the inhibition of hawthorn PPO activity at pH 6. Activities were expressed as percent relative activity to that determined without kojic acid or preincubation: no kojic acid or preincubation (A); 1mM kojic acid, no preincubation (B); preincubation of kojic acid with pyrogallol (C); preincubation of kojic acid with PPO (D). The vertical bars represent the standard errors of three replicates.