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Investigations of the inhibition kinetics of some drugs and chemicals on enzyme of polyphenol oxidase purified from Apricot's (*Salak*)

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ABSTRACT

Polyphenol oxidase (PPO) was purified from Igdir apricot, with a 367 fold purification of PPO by affinity chromatography being achieved. Amount of the protein was determined according to Bradford method. Vmax and Km values were found by means of Lineweaver- Burk graphs. Asetly salisilic acid, paracetamol, ascorbic acid (vitamin C), sodium sulphate, copper sulphate, glucose, sodium nitrite, sodium chlorure, glisine, sodium azide, 2-merkaptoethanol, tyrosine, citric acid, etilendiamin tetra acetic acid (EDTA) ve p-amino benzoic acid were used as inhibitor. Inhibition constants Ki of each inhibitor were found from Lineweaver-Burk graphs. It was found that the p-aminobenzoik acid function showed the highest inhibitory effect.

Key words : Polyphenol oxidase, characterization, purification, inhibitor, kinetics.

INTRODUCTION

Polyphenol oxidase (PPO) (monophenol, dihydroxy-Lphenylalanine: oxygen oxidoreductase, EC 1.14.18.1) is a widely distributed copper-containing enzyme, which is associated with undesirable browning reactions in fruits and vegetables. Polyphenol oxidase has been widely studied in many fruits and vegetables to determine how to prevent the browning which results in the loss of their marketability. Enzymatic browning occurs in many vegetables and fruits after brushing or cutting or during storage. This results from oxidation of phenolic compounds to quiononesby polyphenol oxidase in the presence of oxygen[1-3] . Therefore, enzymatic browning is an economic problem for processors and consumers. Browning has been attributed to oxidation of phenolics by polyphenoloxidase, brown colored by products[4-7]. For these reasons there are considerable losses in the market value of this fruit. The first reason for skin browning is the oxidation of phenolic compounds by molecular oxygen as a result of enzymatic catalysis of polyphenol oxidase. The phenolic compounds and PPO are components of the skin tissue of fruit[8]. inhibition studies have gained more importance for these types of reactions in food and vegetable processing technology. PPO has been given more attention in food technology in this regard[9,10].

The objective of this study was to characterize the PPO from Igdir apricot and properties enzyme of some kinetic were investigated. This work will be useful in devising effective methods for inhibiting browning during storage.

MATERIAL AND METHODS

Materials

Igdir apricot was obtained from the vegetable garden from Igdir-Turkey.

Chemicals

Catechol, benzoic acid, sodium azide, HCl, ascorbic acid, sodium phosphate were obtained from Sigma Chemicals. All other chemicals and solvents used in this study were of analytical grade.

Extraction and purification of apricot PPO by affinity chromatography

To purify polyphenol oxidase enzyme obtained from Igdýr apricot, phosphate buffer at 7.3 pH was used, necessary centrifuging and other processes were carried out and the homogenate to be applied to the column was prepared. The homogenate was applied to activated Sepharose 4B-Tyrosine-p-aminobenzoic asid affinity column. Activity showing fractions obtained from column, for quantitative protein analyse was performed at 595 nm with Coomassie-Blue method.

Determination of protein

Protein concentration was determined according to the dye binding method of Bradford[11] with bovine serum albumin as standard.

PPO activity assay

The assay mixture consisted of 2.8 mL of 0.001 M sodium phosphate buffer (NaPi), pH,7.3, 0.2 ml of 0.1 M catechol and 0.2 mL of enzyme extract. The increase in absorbance at 420 nm was measured. One unit of enzyme activity is defined as the amount of the enzyme that causes an increase in absorbance of 0.001 min at 25 °C (12).

Enzyme kinetics

V_{max} and K_m values of polyphenol oxidase were determined by Lineweaver-Burk graphs.

RESULTS AND DISCUSSION

Polyphenol oxidase (PPO) (EC 1.14.18.1), which is widely distributed in the plant and animal kingdoms, is a copper-containing enzyme and is responsible for the enzymatic browning reaction occurring in many plants and vegetables damaged by improper handling, resulting in bruising, compression or indentations[21].Although browning reactions, in some food products, result in good

Table 1: Ki values and inhibition modes for 5 inhibitors for PPO

Inhibitors	K_i (mM)	Type of inhibition
Amoksiline	0.819	Non-competitive
Asetly salicilic acid	0.167	Non-competitive
Paracetamol	0.308	Non-competitive
sodium Sulphate	0.278	Non-competitive
Copper Sulphate	0.552	Non-competitive
Glucose	Active	-
sodium Nitrite	0.0822	Non-competitive
sodium Chlorure	0.166	Non-competitive
Glisine	0.221	Non-competitive
sodium Azide	0.404	Non-competitive
Ascorbic Acid	0.304	Non-competitive
2-mercaptoethanol	0.271	Non-competitive
Tyrosine	Active	-
Citric Acid	0.0594	Non-competitive
EDTA	0.255	Non-competitive
p-aminobenzoic Acid	1.018	Non-competitive

appearance in terms of colour, these kinds of reactions, in general, lead to undesirable results with respect to texture, sweetness, and overall flavour. Therefore, inhibition studies have gained more importance for these types of reactions in food and vegetable processing technology[8,10].

Protein characterization

To check the PPO-preparation purity, and SDS-PAGE electrophoresis experiment was performed according to method of Gauillard and Richard-Forget[22,23].

Enzyme kinetics

Lineweaver-Burk Graph analysis of this enzyme preparation showed K_m value of 14088 mM for catechol. This value for catechol was different from that of tea leaf 12.52 mM(13), amasya apple 34 mM(14), Stanley plum 20 mM (15), peyrus communis 5.55 mM (8), and herb 25 mM (16). It has been reported that K_m values for pyrogallol are follows; apple, 27mM (17); peach ,0.2mM (18); spinach, 15.7mM (19); and tea leaf, 17.8 mM (20). Vmax value were 8.17 EU/ml for catechol. In an earlier work, its reported a 344.58.17 EU/ml Vmax value for pear PPO with catechol substrates[8].

Effect of inhibitors

Inhibitor effects on PPO activity were studied by using the following inhibitors: amoksiline, Asetly salicilic acid, paracetamol, ascorbic acid (vitamin C), sodium sulphate, copper Sulphate, glucose, sodium nitrite, sodium chlorure, glisine, sodium azide, 2-mercaptoethanol, tyrosine, citric acid, etilendiamin tetra acetic asid (EDTA) ve p-aminobenzoic acid. Percent activity graphs were drawn from these results to find both I_{50} values. Later, using five different concentrations of the substrates, PPO activities were measured at three constant inhibitor concentrations with the inhibitors indicated above. 1/V and 1/[S] values obtained from these activity measurements were used for drawing Lineweaver–Burk graphs. Finally, inhibition constants Ki of each inhibitor were found from Lineweaver-Burk graphs.

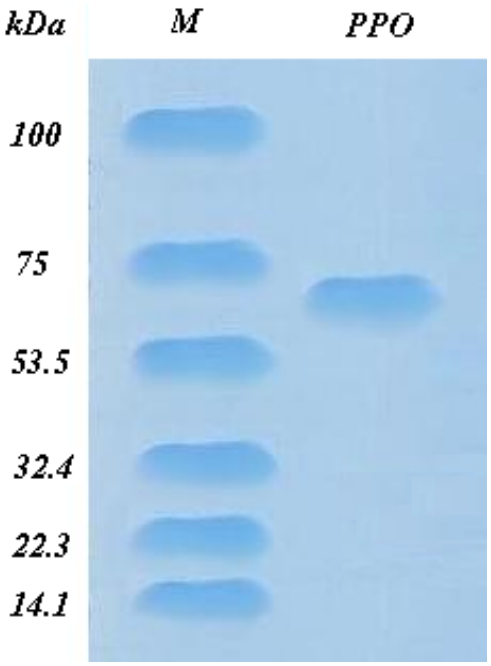

Fig. 1: Band SDS-PAGE of PPO purified by affinity chromatographthy

Table 2: I_{50} values for 5 inhibitors for PPO

Drugs and chemicals	I_{50} (mM)
Amoksiline	0.846
Asetly salicilic acid	4.131
Paracetamol	2.243
sodium Sulphate	2.487
Copper Sulphate	1.327
Glucose	Activator
sodium Nitrite	8.432
sodium Clorure	4.159
Glisine	3.138
sodium Azide	1.717
Ascorbic Acid	2.276
2-mercaptoethanol	2.552
Tyrosine	Activator
Citric Acid	11.666
EDTA	2.719
p-aminobenzoic Acid	0.681

I_{50} values are shown in Table 1 for each inhibitor. Ki values and inhibition modes for 5 inhibitors are given in Table 2 From the Ki constants, it was concluded that the inhibition mode all of inhibitors is non-competitive for Igdir apricot. Inhibition by members of the benzoic and cinnamic acid series has previously been investigated[24-28]. The inhibition of palmito (Acanthophoenix rubra) polyphenol oxidase (PPO) is reported[29]. Enzymatic browning can be prevented by bisulphite, ascorbic acid and its analogies, and cysteine[30-36]. Ascorbic acid has also been reported to cause irreversible inhibition[30]. Similar inhibitory effects of acetic acid, ascorbic acid and citric acid were found in the browning of head lettuce[36,38]. These results suggest that L-ascorbic acid, citric acid and acetic acid are good inhibitors of enzymatic browning of artichoke [38, 39]. In addition, ascorbic acid, citric acid, $FeSO_4$ and acetic acid have been used as inhibitor[5]. Similar inhibitory effects of some chemicals on PPOs were also reported in banana peel, head lettuce and banana pulb[4,37,40]

These results suggest that amoksiline, asetly salisilic acid, paracetamol, ascorbic acid (vitamin C), sodium sulphate, copper sulphate, sodium nitrite, sodium chlorure, glisine, sodium azide, 2-mercaptoethanol, citric acid, etilendiamin tetra asetic acid (EDTA) ve p-aminobenzoic acid are able to be used as good inhibitors in enzymatic browning for Igdir apricot. Of all inhibitors used in the study, p-aminobenzoik acid was the most effective inhibitor on the observed Igdir apricot PPO activity, followed by amoksiline and copper sulphate.

REFERENCES

1. Ridgway, T.J., Tucher, G.A., Procedure for the partial purification of apple leaf polyphenol oxidase suitable for commerical application, *Enzyme and Mic. Tech.* **24**: 225-231 (1998).
2. Mayer, A.M., Nagai,T., Suzuki, N., Partial characterization of polyphenol oxidase from Chinese cabbage Brassica rapa, *J. Agric. Food Chem.* **49**: 3922-3926 (2001).
3. Harel, E., Procedure for the Partial characterization of apple leaf polyphenol Polyphenol oxidases in plants, Phytochemistry, **24**: 193-215 (1979).
4. Yang, C.P., Fujita, S., Ashrafuzzaman, M.D., Nakamura, N., Hayashi, N., Purification and characterization of polyphenol oxidase from banana peel, *J. Agric. Food Chem.* **49**: 1446-1449 (2001).
5. Demir, H., Purification and characterization of polyphenol oxidase from the Ercis apple, *Bulletin of Pure and Applied Sciences Chemistry.,* **23**: 1-4 (2004) .
6. Das, R.J., Bhat, G.S., Gowde, R.L., Purification and characterization of polyphenol oxidase from the Kew cultivar of Indian pineapple fruit, *J. Agric. Food Chem.,* **45**: 2031-2035 (1997).
7. Jang, Y.M., Purification and some properties of polyphenol oxidase of Longan Fruit, *Food Chemistry.,* **66**: 75-79 (1999).
8. Ziyan, E., Pekyardimci, S., Purification and characterization of Pear polyphenol oxidase *Turk. J. Chem.* **28**: 547-557 (2004).
9. Vamos-Vigyazo, L., Polyphenol oxidase and peroxidase in fruits and vegetables. *CRC Critical Reviews in Food Science and Nutrition.*, **15**: 49 (1981) .
10. Erat, M., Sakiroglu, H., Kufrevioglu, O.I., Purification and characterization of polyphenol oxidase from Ferula sp. *Food Chemistry.,* **95**: 503-508 (2006).
11. Bradford, M. M., A rapid and sensitive method for the quantitation of protein utilizing the principle of protein dye binding. *Anal. Biochem.*, **72**: 248 (1976).
12. Das, R.J., Bhat, G.S., Gowde, R.L., Purification and characterization of polyphenol oxidase from the Kew cultivar of indian pineapple fruit, *J. Agric. Food Chem.* **45**: 2031-2035 (1997).
13. Halder, J., Tamuli, P., Bhaduri, A.N., Isolasion and characterization of polyphenol oxidase from Indian tea leaf (*Camellia sinensis*),

J. Nutr. Biochem., **9**: 75-80 (1998).

14. Oktay, M., Kufrevioolu, I., Kocaçaliskan, I., Sakiroglu, H., Polyphenoloxidase from Amasya apple, *J. Food Sci.* **60**: 494-496 (1995).
15. Siddiq, M., Sinha, N.K., Cash, J.N., Characterization of polyphenoloxidase from Stanley plums, *J. Food Sci.,* **57**: 1177-1179 (1992).
16. Arslan, O., Temur, A., Tozlu, I., Polyphenol oxidase from Allium sp, *J. Agric. Food Chem.*, **45**: 2861-2863 (1997).
17. Oktay, M., Küfrevioðlu, I., Kocaçaliskan, I., Sakiroglu, H., Polyphenoloxidase from Amasya apple, *J. Food Sci.* **60**: 494-496 (1995).
18. Flurkey, W.H., Jen, J.J., Purification of peach polyphenoloxidase in the presence of added protease inhibitors, *J. Food Biochem.,* **4**: 29-41 (1980).
19. Golbeck, J.H., Cammarata, K.V., Spinach thylakoid polyphenoloxidase. Isolasion and activation and properties of the native chloroplast enzyme, *Plant Physiol*, **67**: 877-884 (1981).
20. Halder, J., Tamuli, P., Bhaduri, A.N., Isolasion and characterization of polyphenol oxidase from Indian tea leaf (*Camellia sinensis*), *J. Nutr. Biochem.* **9**: 75-80 (1998).
21. Zawitowski, J., Bilideris, C. G., & Eskin, N. A. M. Polyphenol Oxidase. In D. S. Robinson & N. A. M. Eskin (Eds.), Oxidative enzymes in foods. New York: Elsevier, 217–273 (1991).
22. Gauillard, F. Richard-Forget, Polyphenol oxidase from Williams pear (Pryus communis L.cv Williams):activation, purification and some properties. *J. Agric. Food Chem.,* **74** : 49-54 (1997).
23. Laemmli, D.K., Cleavage of Structural Proteins During in Asembly of the Head of Bacteriophage T_4- *Nature*. London. 227-244 (1970).
24. Walker, J.R.L., The control of enzymic browning in fruit juices by cinnamic acids. *J. Food Technol.,* **11**: 341-345 (1976).
25. Gunata, Y. Z., Sapis, J. C. & Moutonet, M. Substrates and aromatic carboxylic acid inhibitors of grape polyphenoloxidases. *Phytochemistry,* **26**(6): 1573-1575 (1987).
26. Janovitz-Klapp, A. H., Richard, F. C., Goupy, P. M. &Nicolas, J. Inhibition studies on apple polyphenol oxidase. *J. Agric. Food Chem.,* **38**: 926-931 (1990).
27. McEvily, A.J., Iyengar, R. & Gross, A. T. Inhibition of polyphenol oxidase by phenolic compounds. *In Phenolic Compounds in Food and their Effects on Health,* Vol. I, *Analysis, Occurrence and Chemistry*, eds Chi-Tang Ho, Chang Y. Lee & Mou-Tang Huang. American Chemical Society, Washington, DC, 318-325 (1992).
28. Kermasha, S., Goetghebeur, M. & Monfette, A. Studies on inhibition of mushroom polyphenol oxidase using chlorogenic acid as substrate. *J. Agric. Food Chem.,* **41**: 526-531 (1993).
29. Robert, C., Rouch, C. and Cadet, F.C., Inhibition of palmito (Acanthophoenix rubra) polyphenol oxidase by carboxylic acids, *Food Chemistry.,* **59**(3): 355-360 (1991).
30. Golan-Goldhirsh, A. and Whitaker, J. R. Effect of ascorbic acid, sodium bisulfite, and thiol compounds on mushroom polyphenol oxidase. *Journal of Agriculture and Food Chemistry.,* **32**: 1003-1009 (1984).
31. Sayavedra-Soto, T. A. and Montgomery, M. W. Inhibition of polyphenoloxidase by sulfite. *Journal of Food Science.,* **51**: 1531-1536 (1986).
32. Sapers, G.M. and Ziolkowski, M.A. Comparison of erythorbic and ascorbic acids as inhibitors of enzymatic browning in apple. *Journal of Food Science.,* **52**: 1732-1733 (1987).
33. Hus, A. F., Shieh, J. J., Bills, D. D., White and K., Inhibition of mushroom polyphenol oxidase by ascorbic acid derivatives. *Journal of Food Science* **53**: 765-767 (1988).
34. Walker, J. R. L. and Reddish, C. E. S. Note on the use of cysteine to prevent browning in apple products. *Journal of Science and Agriculture.,* **15**: 902-904 (1964).
35. Dudley, E. D. and Hotchkiss, J. H. Cysteine as an inhibitor of polyphenol oxidase. *Journal of Food Biochemistry.,* **13**: 65-75 (1989).
36. Richard-Forget, F. C., Goupy, P. M. and Nicolas, J. J. Cysteine as an inhibitor of enzymatic browning. 2. kinetic studies. *Journal Agriculture Food Chemistry.,* **40**: 2108-2113 (1992).

37. Castner, M., Gil, M. I., Artes, F. and Tomas-Barberan, F. A. Inhibition of browning of harvested head tettuce. *Journal of Food Science.,* **61**: 314–316 (1996).
38. Aydemir, T., Partial purification and characterization of polyphenol oxidase from artichoke (Cynara scolymus L.). *Food Chemistry,* **87**: 59–67 (2004).
39. Lattanzio, V., Linsalata, V., Sandro, P., & VanSumere, C. F. The deneficial effect of citric and ascorbic acid on the phenolicbrowning reaction in stored artichoke (*Cynara scolymus* L) heads. *Food Chemistry,* **33**: 93-106 (1989).
40. C.P. Yang, S. Fujita, M.D. Ashrafuzzaman, N. Nakamura, N. Hayashi, Purification and characterization of polyphenol oxidase from banana (*Musa sapientum,* L.) pulp, *J. Agric. Food Chem*. **48**: 2732-2735 (2000).