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Characterization of Polyphenoloxidase extracted from *Solanum tuberosum* Jasim

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Abstract Polyphenoloxidase (PPO) was extracted from *Solanum tuberosum* Jasim by various chromatographic methods and was subsequently purified and characterized. PPO was purified upto 78-fold from the crude extract. SDS-PAGE profile of the enzyme showed a major subunit of PPO with molecular weight of 40 kDa. The optimum pH and temperature for the maximum activity of PPO was 6.5 and 25°C, respectively. The enzyme was found to be quite stable between 10 and 40°C, whereas it was almost inactivated at 70°C when incubated for 30 min. Substrate specificity study indicated that catechol was the most suitable substrate for PPO isolated from purple-fleshed potato with a K_m value of 21.1 mM. The most effective inhibitor was ascorbic acid, followed by L-cysteine, citric acid, EDTA, and boric acid. Studies on the effect of metal ion on PPO activity showed that magnesium and copper were inhibitory, while iron and zinc ions increased the activity of PPO.

Keywords: *Solanum tuberosum* Jasim, polyphenoloxidase, characterization

Introduction

Polyphenoloxidase (PPO) is widely distributed in plants and is responsible for the browning of damaged fruits and vegetables by catalyzing oxidation of phenolic compounds (1, 2). PPO is a copper-containing enzyme that catalyzes 2 types of oxidative reactions: hydroxylation of monophenols to o-diphenols (monophenol monooxidase: EC 1. 14. 8. 1), and oxidation of o-diphenols to o-quinones (diphenol oxidase: EC 1. 10. 3. 2) in the presence of oxygen (3, 4). As enzymatic browning of vegetables and fruits by PPO leads to deterioration of nutritional quality and affects the appearance, the inactivation of PPO activity is pertinent for the preservation of food stuffs (5). Hence, PPO inhibition is required for quality control during processing and storage of food.

"Jasim" is one of the varieties of purple-fleshed potatoes, which is bred and produced in Korea, and has an accumulation of anthocyanin pigment in the flesh. Anthocyanins are responsible for red, purple, and blue colors of many fruits and vegetables, and they are known to possess antioxidative (6) as well as antimutagenic activity (7). Physiological functions of anthocyanins have been extensively studied (8-10). As purple-fleshed potatoes are considered as a functional food in Korea, their demand is constantly increasing.

The presence and extraction of PPOs from fruits like grape, banana, apple, pear, peach, papaya, and yam have been reported (11-17). The most likely function of PPO present in plants is its involvement in resistance mechanism against diseases and insect (18). Recently, PPO from purple-fleshed potato was isolated in our laboratory (19). However, its characterization has yet not been reported. Therefore, to elucidate the molecular properties of PPO isolated from purple fleshed potato, some of the characteristics such as optimum pH and temperature, pH stability, thermal stability, substrate specificity and K_m value, inhibitor and metal ion effects have been reported in the present study.

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Materials and Methods

Materials Purple-fleshed potatoes (*Solanum tuberosum* Jasim) were obtained from Korea Rural Development Administration. Resource Q column (6 mL, anion exchange column) was purchased from Amersham Biosciences (Uppsala, Sweden). Protein assay reagent was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). YM-10 membrane (Mw 10,000 cutoff) and YM-3 membrane (Mw 3,000 cutoff) were obtained from Millipore Co. (Bedford, Mass, U.S.A.). Sephacryl S-200 HR, catechol, standard marker proteins for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and other chemicals were purchased from Sigma Chemicals Co. (St. Louis, MO., U.S.A.). All the chemicals used were of analytical grade.

Enzyme purification PPO from 1.5 kg of purple-fleshed potato (*Solanum tuberosum* Jasim) was purified as described in our previous report (19). Briefly, PPO was purified using membrane concentration, ammonium sulfate fractionation, Resource Q ion exchange chromatography, and Sephacryl S-200 HR gel permeation chromatography.

Enzyme assay Activities of PPO were determined by spectrophotometer (Milton Roy Co. Rochester, N.Y., U.S.A.) according to the method of Flurkey and Jen (20) with slight modification (21). One mL of 50 mM potassium phosphate buffer (pH 6.5) and 0.3 mL of 0.2 M catechol solution in 50 mM potassium phosphate buffer (pH 6.5) as a substrate were added to 0.05 mL of the enzyme solution. One unit of PPO activity was defined as an increase in the

absorbance of 0.001/min at 420 nm and 25°C. Enzyme activities were measured thrice for reproducibility.

Protein assay The protein concentration was determined according to the method of Bradford (22) by using bovine serum albumin as standard.

SDS-PAGE SDS-PAGE was performed according to the method of Laemmli (23). Equal amount of the protein samples (10 μg) were loaded onto each lane of gel. Proteins were resolved on a 12.5% acrylamide gel, and stained with Coomassie Brilliant Blue. The following molecular weight markers were used: bovine serum albumin (66 kDa), egg albumin (45 kDa), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36 kDa), bovine erythrocytes carbonic anhydrase (29 kDa), bovine pancreas trypsinogen (24.1 kDa), soybean trypsin inhibitor (20.1 kDa), and bovine milk á-lactalbumin (14.2 kDa).

Optimum pH and pH stability Optimum pH of PPO was determined in 50 mM sodium citrate buffer (pH 3.0 and 4.0), 50 mM sodium acetate buffer (pH 5.0), 50 mM potassium phosphate buffer (pH 6.0, 6.5, 7.0, and 7.5), 50 mM Tris-HCl buffer (pH 8.0), and 50 mM sodium borate buffer (pH 9.0). One mL of each of the buffer solution at various pH values (pH 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0) and 0.3 mL of 0.2 M catechol solution as a substrate were added to 0.05 mL of the enzyme solution. To determine pH stability of PPO, 50 μL of the enzyme solution was incubated with one mL of buffer solution of various pH values (pH 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0) at 25°C for 30 min. After incubation, 0.2 M catechol solution was then added. The residual activities of PPO were measured at 25°C (24).

Optimum temperature and thermal stability Activities of PPO were assayed at various reaction temperatures (20, 25, 30, 35, 40, 50, and 60°C) in a circulating water bath. One mL of 50 mM potassium phosphate buffer (pH 6.5) containing 0.05 mL of the enzyme solution was incubated at a given temperature. After incubation for 5 min, 0.2 M catechol solution was added to the enzyme solution.

To determine the thermal stability of PPO, 1 mL of 50 mM potassium phosphate buffer (pH 6.5) containing 0.05 mL of the enzyme solution was incubated at various temperatures (20, 30, 40, 50, 60, and 70°C) in a circulating water bath. After incubation for 30 min, the buffer solution was cooled rapidly in ice, and was then added to 0.2 M catechol solution. The residual activities of PPO were measured at 25°C.

Substrate specificity and K_m value Ten phenolic compounds were used as substrates for PPO: L-tyrosine, p-coumaric acid, p-cresol, DL-DOPA, catechol, resorcinol, phloroglucinol, hydroquinone, pyrogallol, and gallic acid. One mL of 50 mM potassium phosphate buffer (pH 6.5) and 0.3 mL of a 10 mM substrate solution except L-tyrosine, p-coumaric acid, and p-cresol (2.5 mM in phosphate buffer) were added to 0.05 mL of the enzyme solution.

Michaelis-Menten constant (K_m) was determined using various concentration (0.001, 0.005, 0.01, 0.015, 0.02, and 0.05 M) of catechol solution as a substrate. The results were plotted according to the method of Lineweaver and Burk (25).

Inhibitor and metal ion effects To determine inhibitor effects, ascorbic acid, boric acid, citric acid, and ethylenediaminetetraacetic acid (EDTA) were used at various concentrations. Each of the inhibitor was dissolved in 1 mL of 50 mM potassium phosphate buffer (pH 6.5) containing 0.2 M catechol solution. Fifty μL of the enzyme solution was then added to the substrate containing the respective inhibitors.

Five metal ions ($CuSO_4$, $MgCl_2$, $FeSO_4$, $ZnSO_4$, and $CaCl_2$) were used to examine the effect of metal ion on the activity of PPO. Each metal ion was dissolved in 1 mL of 50 mM potassium phosphate buffer (pH 6.5) containing 0.2 M catechol solution. Subsequently, 50 μL of the enzyme solution was added, and the residual activity of PPO was measured.

Results and Discussion

Purification of PPO from purple-fleshed potato A crude extract of purple-fleshed potatoes was membrane-concentrated and subjected to Resource Q anion exchange chromatography to purify PPO. An elution profile of the column showed that there were 6 major fractions (Table 1). Each fraction was assayed for PPO activity, and the results revealed that F4 had the highest specific activity (Table 1). F4 was pooled and then lyophilized for further purification.

The concentrated F4 fraction was subjected to Sephacryl S-200 HR gel permeation chromatography (Table 1). Two major fractions were collected from the column. Amongst the two, P1 had the highest specific activity (Table 1). The results of the purification experiment of PPO using the crude extract of purple-fleshed potatoes are summarized in Table 1. PPO was purified 78-fold from crude extract of purple-fleshed potatoes by Sephacryl S-200 gel permeation chromatography as a final purification step.

To confirm the homogeneity of the purified PPO, SDS-PAGE was performed on a 12.5% separating gel. A typical molecular weight profile of fractions obtained at each purification step is shown in Fig. 1. SDS-PAGE results showed that the major subunit molecular weight of the purified PPO was 40 kDa with minor impurities, which could not be removed even by further purification using a Mono Q ion exchange column chromatography (data not shown). Native-PAGE also had a similar pattern, where it had a single major band of PPO (data not shown). Mazzafera and Robinson (26) have previously reported that proteolysis can occur during the extraction process, resulting in 40-45 kDa PPO patterns which retain full enzyme activity. This could explain numerous reports of PPO of 40-45 kDa purified from various plants such as apple and banana (27), where there had been proteolysis involved during extraction.

There have been many studies on the purification of PPO isolated from various plant sources (17). Molecular weights of PPOs isolated form different source have been reported like for: spinach (40 kDa), sunflower seeds (42 kDa), cabbage (39 kDa), banana (41 kDa) (28-31). Our results were in good agreement with the reported data,

Table 1. Purification profile of PPO extracted from purple-fleshed potatoes

	Volume (mL)	Total protien (mg)	Total activity (units)	Specific activity (unit/mg protein)	Recovery (%)	Purification (fold)
Crude	2495	12220.96	1,149,014	94.02	100.0	1
Membrane-Concentration	851	2060.83	1,045,128	507.14	91.0	5
$(NH_4)_2SO_4$ precipitation	113	502.20	495,887	987.43	43.2	11
Resoure		**Q column**				**fractions**
F1	19	16.44	0	0.00	0.0	0
F2	19	21.17	6,514	307.75	0.6	3
F3	19	36.48	48,949	1341.91	4.3	14
F4	19	30.49	62,480	2049.50	5.4	22
F5	19	54.81	39,819	726.50	3.5	8
F6	19	75.22	27,375	363.92	2.4	4
Sephacryl S-200		**HR column**				**fractions**
P1	19	6.05	44,361	7334.84	3.9	78
P2	19	23.63	6640	281.06	0.6	3

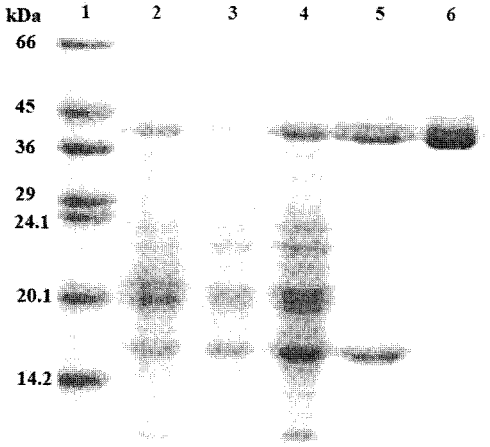

Fig. 1. SDS-PAGE profile of PPO fractions. Molecular weight marker (1); crude extract (2); membrane-concentrated solution (3); 80% $(NH_4)_2SO_4$ precipitate (4); Resource Q peak F4 (5); Sephacryl S-200 HR peak P1 (6).

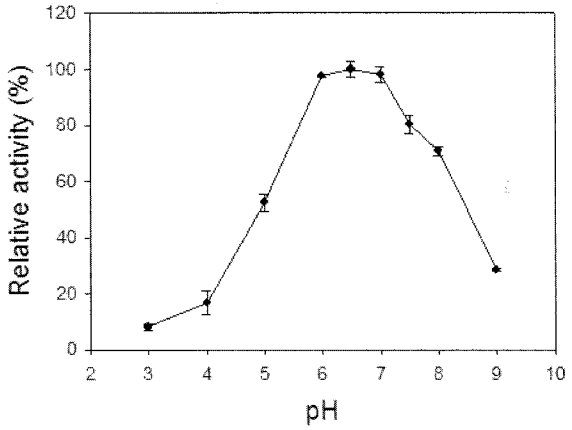

Fig. 2. Optimum pH profile of PPO extracted from purple-fleshed potatoes.

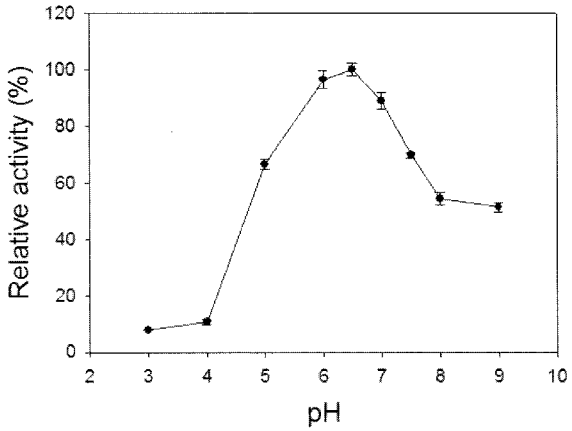

Fig. 3. Stability of PPO at various pH values.

though the source of enzyme was different.

Optimum pH and pH stability PPO activities were measured at various pH values (pH 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0) at 25°C (Fig. 2). The pH profile showed that PPO from purple-fleshed potato has the highest activity at pH 6.5, and was most stable between pH 6.0 and 7.0. Similar results were reported in previous studies (26, 32), but there were some differences when compared to Chinese cabbage (pH 5.0), raspberry fruit (pH 8.0 and pH 5.5), Niagara grapes (pH 5.5), and bean sprouts (pH 9.0) (4, 33-35). At pH 5.0, the activity of PPO was decreased by 50%. The activity of PPO was also remarkably decreased below pH 4.0 or above pH 8.0.

To study the pH stability of PPO, enzyme solutions were incubated at various pH values (pH 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0) at 25°C for 30 min, and the residual activities of PPO were measured using 50 mM potassium phosphate buffer (pH 6.5) as described previously (Fig. 3). The results showed that the most stable pH for PPO activity was 6.5 and it was quite stable between pH 6.0 and 7.0. At pH 3.0 and 4.0, activities of PPO decreased to 7 and 11%, respectively when compared with the control. Rivas and Whitaker (12) reported that PPO from pear was not stable below pH 3.5 and Siddiq and others (36) have

also reported that PPO from plum was not stable below pH 4.5. Our results were in good agreement with their reported data. At pH 8.0 and 9.0, the activity of PPO decreased to 54 and 51% of its original activity, respectively. These results were in good agreement with other studies (3, 16).

Optimum temperature and thermal stability Activities of PPO were measured at various temperatures (10, 20, 25, 30, 35, 40 50, and 60°C). The optimum temperature of PPO was observed to be 25°C (Fig. 4). This result was in good agreement with other studies (26, 32, 34, 37, 38). However, it was different from studies on Chinese cabbage (50°C), Stanley plum (18°C), *Jerusalem Artichoke* Tuber (40°C), and oriental pear fruits (40°C) (4, 38-40).

To elucidate temperature stability of PPO, enzyme solutions were incubated at various temperatures (20, 30, 40, 50, 60, and 70°C) for 30 min. After cooling, the residual activities of PPO were measured at 25°C (Fig. 5). The results showed that the most stable temperature of PPO was 20°C. The activity of PPO was retained to be more than 75% between 20°C and 40°C. Similar results have been reported in other studies also (4, 32). However, PPO activity decreased by more than 50% when incubated above 50°C. After incubation at 70°C for 30 min, PPO was mostly inactivated.

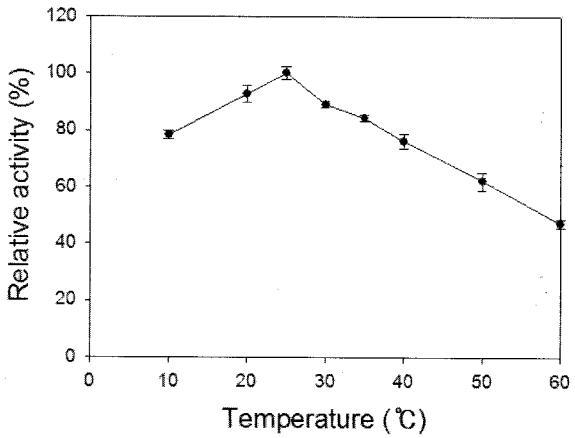

Fig. 4. Optimum temperature profile of PPO extracted from purple-fleshed potatoes.

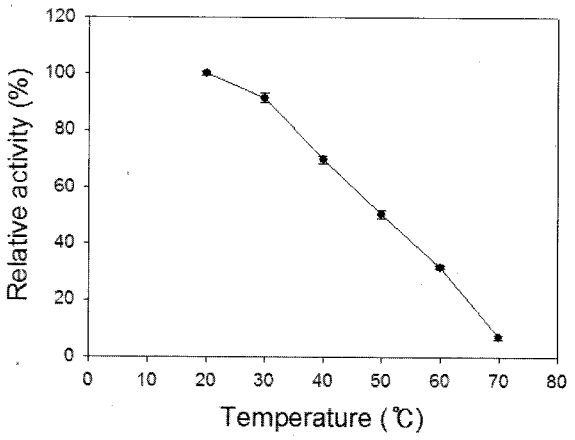

Fig. 5. Thermal stability of PPO at various temperatures.

Substrate specificity and K_m value Substrate specificity of PPO was determined using 10 different substrates (L-tyrosine, p-coumaric acid, p-cresol, DL-DOPA, catechol, resorcinol, phloroglucinol, hydroquinone, pyrogallol, and gallic acid) (Table 2). The results showed that catechol was the most suitable substrate for PPO. Kavarayan and Aydemir (3) and Nagai and Suzuki (4) have also reported the same results, although the enzymatic source was different. PPO exhibited lower activity towards pyrogallol (38.5%) and DL-DOPA (22.6%) than catechol. However, PPO had no specificity towards other substrates.

Michaelis-Menten constant (K_m) was determined using various concentrations (0.001, 0.005, 0.01, 0.015, 0.02, and 0.05 M) of catechol solution under optimized condition (25°C, pH 6.5) (Fig. 6). Lineweaver-Burk plot analysis showed that K_m value of PPO was 21.1 mM for catechol. It was quite similar with that of Stanley plum (20 mM) (36),

Table 2. Substrate specificity of PPO extracted from purple-fleshed potatoes

Substrate	Concentration(mM)	Relative activity (%)
Monophenols		
L-tyrosine	3	0
ρ-courmaric acid	3	0
ρ-cresol	3	0
Diphenols		
σ-diphenols		
DL-DOPA	10	23
Catechol	10	100
m-diphenols		
resorcinol	10	0
pkloroglucinol	10	0
ρ-diphenols		
hydroquinone	10	0
Triphenol		
Pyrogallol	10	39
Gallic acid	10	0

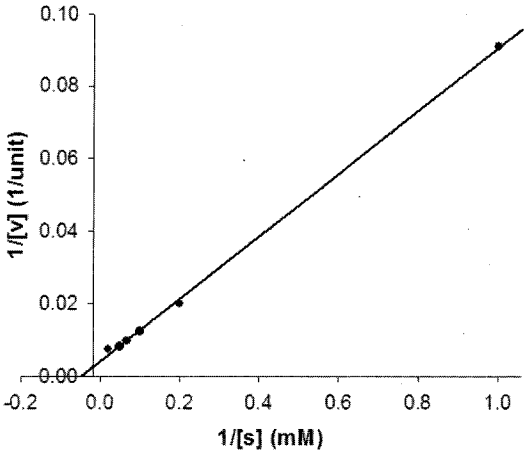

Fig. 6. Lineweaver-Burk plot to derive Michaelis-Menten constant value for PPO.

but lower than those of *Allium sp.* (25 mM) and amasya apple (34 mM) (39, 42) and higher than those of peach (2 mM), spinach (3 mM), and tea leaf (12.52 mM) (43-45). These results indicated that PPO isolated from purple-fleshed potato had lower affinity for catechol than that isolated form peach, spinach, and tea leaf, whereas it had higher affinity for catechol than than that from *Allium sp.* and amasya apple.

Table 3. Effect of inhibitors on the activity of PPO towards catechol

Inhibitor	Concentration (mM)	Relative activity (%)
Ascorbic acid		
	10	0
	1	0
	0.1	2.5
	0.01	84.7
	0.001	94.6
Boric acid		
	40	55.0
	30	64.4
	20	78.9
	10	87.7
Citric acid		
	10	27.0
	1	55.9
EDTA		
	10	69.4
	1	86.9
L-cysteine		
	1	23.4
	0.1	79.3
Non-treatment		100.0

Table 4. Effect of metal ions on the activity of PPO towards catechol

Salt	Concentration (mM)	Relative activity (%)
$CuSO_4$		
	10	66.4
	1	78.8
	0.1	102.7
$MgCl_2$		
	10	64.9
	1	87.7
$FeSO_4$		
	10	865.8
	1	841.9
	0.1	240.5
$ZuSO_4$		
	10	248.6
	1	101.8
$CaCl_2$		
	10	106.3
	1	96.4
Non-treatment		100.0

Inhibitor and metal ion effects Effect of inhibitors such as ascorbic acid, boric acid, citric acid, EDTA, and L-cysteine on the PPO activity was also determined (Table 3). The most effective inhibitor was ascorbic acid, followed by L-cysteine, citric acid, EDTA, and boric acid. As shown in Table 3, at low concentration (1 mM) of ascorbic acid, PPO activity was completely inhibited. Because ascorbic acid is a reducing agent, it reduces quinones generated by PPO. One mM L-cysteine and 10 mM citric acid inhibited PPO activity by 67 and 63%, respectively. In contrast, 40 mM boric acid and 10 mM EDTA inhibited PPO activity by less than 50%.

Effects of metal ions, such as Cu^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, and Ca^{2+} on the activity of PPO were examined (Table 4). Copper ion at a concentration of 1 and 10 mM decreased PPO activity, whereas 0.1 M concentration did not affect the activity. Similarly, magnesium ion decreased the activity of PPO, whereas iron and zinc ions increased PPO activity significantly. However, calcium ion did not had any influence on the enzyme activity. These results were in good agreement with other reports (38).

References

1. Sun N, Song KB. Effect of γ-irradiation on the Molecular Properties of Mushroom Polyphenoloxidase. Food Sci. Biotechnol. 11: 694-696 (2002)
2. Vamos VL. Polyphenol oxidase and peroxidase in fruits and vegetables. CRC Crit. Rev. Food Nutr. 15: 49-127 (1981)
3. Kavrayan D, Aydemir TA. Partial purification and characterization of polyphenoloxidase from peppermint (*Mentha piperita*). Food Chem. 74: 147-154 (2001)
4. Nagai T, Suzuki N. Partial purification of polyphenol oxidase from Chinese cabbage *Brassica rapa* L. J. Agric. Food Chem. 49: 3922-3926 (2001)
5. Hendrickx M, Ludikhuyze L, Van Den Broeck, I., and Weemaes, C. Effect of high pressure on enzymes related to food quality. Trends Food Sci. Technol. 9: 197-203 (1998)
6. Suda I, Furuta S, Nishiba Y, Yamakawa O, Mastsugano K, Sugita K. Hepato-protective activity of purple-colored sweet potato juice. Sweet Potato Research Front (KNAES, Japan) 4: 3 (1997)
7. Yoshimoto M, Okuno S, Yoshinaga M, Yamakawa O, Yamaguchi M, Yamada H. Antimutagenicity of sweet potato (*Ipomoea batatas*) roots. Biosci. Biotech. Biochem. 63: 537-541 (1999)
8. Bettini V, Fiori A, Martino R, Mayellaro R, Ton P. Study of the mechanism whereby anthocyanosides potentiate the effect of catecholamines on coronary vessels. Fitoterapia 54: 67-72 (1985)
9. Vincieri FF, Romain A, Baldi M, Mulinacci N, Alberti, MB. Analysis HPLC of anthocyanins present in fluid extracts from Malva Sylvestris L. flowers and leaves. Bull Liaison-Groupe Polyphenols 16: 339-342 (1992)
10. Saija A. Pharmacological effects of anthocyanins from blood orange juice. Essenze-Deriv Agrum 64: 229-233 (1994)
11. Wong TC, Luh BS, and Whitaker JR. Isolation and characterization of polyphenol oxidase isozymes of Clingstone peach. Plant Physiol. 48: 19-23 (1971)
12. Rivas NDJ, Whitaker JR. Purification and some properties of two polyphenol oxidase from Bartlett pears. Plant Physiol. 52: 501-507 (1973)
13. Anosike EO, Ayaebene AO. Purification and some properties of polyphenol oxidase from yam tubers, *Dioscorea bulbifera*. Phytochemistry. 20: 2625-2628 (1981)
14. Galeazzi MAM, Sgarbieri VC, Constantinides SM. Isolation, purification and phsicochemical characterization of PPO from a dwarf variety of banana (*Musa cavensdishii*, L.). J. Food. Sci. 46: 150-155 (1981)
15. Yokotsuka K, Makino S, Singleton VL. Polyphenol oxidase from grapes: Precipitation, re-solubilization and characterization. Am. J.

Enol. Vitic. 39: 293-302 (1988)
16. Zhou P, Smith NL, Lee CY. Potential purification and some properties of Monroe apple peel polyphenol oxidase. J. Agric. Food Chem. 41: 532-536 (1993)
17. Cano MP, Lobo MG, Ancos B, Galeazzi M. Polyphenol oxidase from Spanish hermaphrodite and female papaya fruits (*Carica papaya*). J. Agric. Food Chem. 44: 3075-3079 (1996)
18. Ray H, Hammerschmidt R. Responses of potato tuber to infection by *Fusarium sambucinum*. Physiol. Mol. Plant Pathol. 53: 81-92 (1998)
19. Jang J, Song KB. Purification of polyphenoloxidase from the purple-fleshed potato (*Solanum tuberosum* Jasim) and its secondary structure. J. Food Sci. 69: 648-651(2004)
20. Flurkey WH, Jen JJ. Peroxidase and polyphenol oxidase activities in developing peaches. J. Food Sci. 43:1826-1828 (1978)
21. Sun NK, Song KB. Effect of nonthermal treatment on the molecular properties of mushroom polyphenoloxidase. J. Food Sci. 68: 1639-1643 (2003)
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantitaties of protein utilizing the principle protein-dye binding. Anal. Biochem. 72: 248-254 (1976)
23. Laemlli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685 (1970)
24. Pontoh J, Low NH. Purification and characterization of β-glucosidase form honey bees (*Apis melifera*). Insect Biochem. Mol. Biol. 32:672-690 (2002)
25. Lineweaver H, Burk D. The determination of enzyme dissociation constants. J. Am. Chem. Sci. 56: 658-666 (1934)
26. Mazzafera P, Robinson SP. Characterization of polyphenol oxidase in coffee. Phytochem. 55: 285-296 (2000)
27. Marques L, Fleurict A, Cleyet-Marel JC, Macheix JJ. Purification of an apple polyphenoloxidase isoform resistant to SDS-proteinase K digestion. Phytochem. 36: 1117-1121 (1994)
28. Vaugham PFT, Eason R, Paton J. Molecular weight and amino acid composition of purified spinach beet phenolase. Phytochem. 14: 2383-2386 (1975)
29. Raymond J, Rakariyathan N, Azanza JL. Purification and some properties of polyphenol oxidases from sunflower seeds. Phytochem. 34: 927-932 (1993)
30. Fujita S, Saari NB, Maegawa M, Tetsuka T, Hayashi N, Tono T. Purification and properties of polyphenol oxidase from cabbage (*Brassica oleracea* L.). J. Agric. Food Chem. 43: 1138-1142 (1995)
31. Yang CP, Fujita S, Ashrafuzzaman MD, Nakamura N, Hayashi N. Purification and characterization of polyphenol oxidase from banana (*Musa sapientum* L.). J. Agric. Food Chem. 48: 2732-2735 (2000)
32. Duangmal K, Apenten RKO. A comparative study of polyphenol-oxidase from taro and potato. Food. Chem. 64: 351-359 (1999)
33. González EM, De Ancos B, Cano MP. Partial characterization of polyphenol oxidase activity in raspberry fruits. J. Agric. Food Chem. 47: 4068-4072 (1999)
34. Wissemann KW, Lee CY. Characterization of PPO from Ravat 51 and Niagara grapes. J. Food Sci. 46: 506-508 (1981)
35. Nagai T, Suzuki N. Polyphenol oxidase from bean sprouts (*Gylcine max* L.). J. Food Sci. 68: 16-20 (2003)
36. Siddiq M, Sinha NK, Cash JN. Characterization of polyphenol-oxidase from Stanley plums. J. Food Sci. 57: 1177-1179 (1992)
37. Matheis G. 1987. Polyphenol oxidase and enzymatic browning of potatoes (*Solanum tuberosum*) I. Properties of potato polyphenol oxidase. Chem. Mikrobiol. Technol. Lebensm. 11: 5-12 (1987)
38. Pyo H, Son D, Lee C. Purification and Characterization of polyphenol oxidase from *Flammulina velutipes*. Korean J. Food Sci. Technol. 34: 552-558 (2002)
39. Arslan O, Temur A, Tozlu I. Polyphenol oxidase from *Allium sp.* J. Agric. Food Chem. 45: 2861-2863 (1997)
40. Park KB, Lee JS, Choi EH. Isolation and characteristic of polyphenol oxidase from *Jerusalem Artichoke* Tuber. Korean J. Food Sci. Technol. 23: 414-419 (1991)
41. Seo JH, Hwang YS, Chun JP, Lee JC. Changes of phenolic compounds and occurrence of skin browning, and characterization of partially purified polyphenol oxidase in oriental pear fruits. J. Korean Soc. Hort. Sci. 42: 184-188 (2001)
42. Oktay M, Küfrevioglu I, Kocaçaliskan I, Sakiroglu H. Polyphenol-oxidase from amasya apple. J. Food Sci. 60: 494-496 (1995)
43. Flurkey WH, Jen JJ. Purification of peach polyphenoloxidase in the presence of added protease inhibitors. J. Food Biochem. 4: 29-41 (1980)
44. Golbeck JH, Cammarata KV. Spinach thylakoid polyphenol oxidase. Isolation, activation and properties of the native chloroplast enzyme. Plant Physiol. 67: 877-884 (1981)
45. Halder J, Tamuli P, Bhaduri AN. Isolation and characterization of polyphenol oxidase from Indian tea leaf (*Camellia sinensis*). J. Nutr. Biochem. 9: 75-80 (1998)