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Inhibition of Polyphenol Oxidase and Peach Juice Browning by Onion Extract

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Abstract The inhibitory effect of onion extract on polyphenol oxidase (PPO) and browning of peach juice was investigated. Various reducing agents such as L-ascorbic acid, L-cysteine, dithiothreitol, glutathione, and sodium pyrosulfite strongly inhibited polyphenol oxidase extracted from peach. The enzyme was also inhibited by addition of water extract of onion. Regardless of substrates used, the addition of heated onion extract exhibited stronger inhibitory effect on peach polyphenol oxidase activity than that of the fresh one. The inhibitory effect of onion extract was dependent on heating temperature and time. The onion extract inhibited the peach polyphenol oxidase non-competitively. The heating of onion extract in the presence of glucose, glycine stimulated the inhibitory effect of the onion extract, which suggests non-enzymatic browning products produced during heating might be responsible for the stronger inhibitory action of the heated onion extract. The retardation of peach juice browning by onion extract seems to be caused by inhibition of peach PPO.

Keywords: browning, polyphenol oxidase, peach, onion

Introduction

Browning in fruits and vegetables damaged by mechanical injury during harvesting, post harvest storage, or processing is one of the main causes of quality loss (1), which may involve different compounds and proceed through different chemical pathways. The major groups of reactions leading to browning are enzymatic browning and non-enzymatic browning (2, 3). Enzymatic browning is caused by polyphenol oxidase (PPO, *o*-diphenol: O_2 oxidoreductase, EC 1.10.3.1) and the enzyme plays an important role in fruit and vegetable processing and during storage of the processed foods (4, 5). PPO is a copper containing enzyme and catalyzes either one or two reaction involving molecular oxygen. The first type of reaction is hydroxylation of monophenols, leading to formation of *o*-dihydroxy compounds. The second type of reaction is oxidation of *o*-dihydroxy compounds to quinone. Further condensation of quinones leads to brown melanin pigments (6). The PPO activity is high in plants and vegetables that are particularly sensitive to oxidative browning such as potato, apple, mushroom, and banana (7). Consequently, the control of enzymatic browning has created strong interest in food industry (8).

PPO activity has been successfully controlled by sulfites, but there have been concerns among consumers over the harmful effect (9). Consumer awareness of the risks associated with sulfites and increased regulatory scrutiny demand for substituting synthetic compounds with natural substances as food ingredients. The PPO inhibitors occurring in natural resources have been widely studied in several plants (10-13), but more development of natural and efficient polyphenol oxidase inhibitors is needed.

Onions (*Allium cepa*) are grown in every part of the world where plants are farmed and exhibited a great of diversity in form including color, shape, dry matter content, and pungency. Onion consumption is increasing significantly and this is partly because of heavy promotion that links flavor and health. Onions are rich in two chemical groups that have perceived benefits to human health. These are the flavonoids and the alk(en)nyl cysteine sulfoxides. Compounds from onion have been reported to have a range of health benefits which include anticarcinogenic properties, antiplatelet activity, antithrombotic activity, antiasthmatic, antioxidative, and antibiotic effects (14, 15). It was previously reported that onion extract effectively inhibited the PPO of potato (16). Onion extract provides not only beneficial effect to human health but it is effective inhibitor against PPO present in some plants. Therefore, we have investigated the inhibitory effect of onion extract as natural inhibitor on browning of peach juice.

Materials and Methods

Peach PPO preparation Peach was purchased from a local market in Busan, Korea. Peeled peach (140 g) was homogenized with 70 mL of a 50 mM potassium phosphate buffer at pH 6.8 for 5 min, and the homogenate was filtered through cheesecloth. The filtrate was centrifuged at 16,000×g for 20 min at 4°C. The supernatant after centrifugation was fractionated with ammonium sulfate (30-80% saturation), and the precipitate was collected by centrifugation at 16,000×g for 20 min and redissolved in 10 mL of 50 mM potassium phosphate buffer at pH 6.8 and dialyzed overnight against the same buffer.

Onion extract preparation Onion (100 g) was homogenized with 100 mL of a 50 mM potassium phosphate buffer at pH 6.8 for 5 min, and the homogenate was filtered through cheesecloth. The filtrate was centrifuged at

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16,000×g for 20 min at 4°C and the supernatant after centrifugation was used for this experiment. Heated onion extract was prepared by incubating the fresh extract at 100°C for 10 min.

PPO assay The PPO activity was measured spectrophotometrically (Ultrospec 3000; Pharmacia Biotech, Uppsala, Sweden). A certain amount of the enzyme solution was added to 1.0 mL of 0.2 M catechol solution to initiate the enzyme reaction. The reaction mixture of 0.1 mL of peach PPO, 0.9 mL of a 50 mM phosphate buffer at pH 6.8, and 1.0 mL of onion extract as inhibitor was incubated for 5 min at 25°C. And then the mixture was transferred to a cuvette, and 1.0 mL of 0.2 M catechol was added. The absorbance at 420 nm was recorded continuously at 25°C for 1 min (17, 18). The total volume of assay for inhibition of PPO activity was 3.0 mL.

Measurement of peach browning Fresh peach juice was squeezed from peeled peach pulp, and was mixed with equal volume of distilled water, fresh onion extract, or heated onion extract. The degree of browning was evaluated by measuring the absorbance at 400 nm.

Results and Discussion

Effect of anti-browning agents on peach PPO Table 1 demonstrates the effect of various anti-browning agents on peach PPO with catechol as a substrate. The enzyme activity was most inhibited by addition of various reducing agents such as L-ascorbic acid, L-cysteine, dithiothreitol, and sodium pyrosulfite. The inhibition of PPO by various reducing agents was reported in browning control in litchi fruit (19). Although sulfites are effective to control enzymatic browning, they can be harmful to asthmatic patients (9), and alternative natural products without toxic effect are needed to replace the chemicals.

Table 1. Inhibitory effect of various anti-browning agents on peach PPO[1)]

Anti-browning agents	Relative activity (%)[2)]
Control	100.0
L-Ascorbic acid	0
L-Cysteine	0
Dithiothreitol	0
Glutathione	56.6±1.1
Sodium pyrosulfite	0
Citric acid	64.0±1.9
Sodium chloride	97.6±5.5
Potassium sorbate	93.0±6.8
EDTA	80.3±4.2
Sodium azide	92.2±1.0
Urea	94.0±2.2

[1)]Anti-browning agents were used at a final concentration of 0.1 mM. The enzyme activity was measured at 25°C for 1 min using the spectrophotometric procedure.
[2)]Triplicate determination±SE.

Table 2. Effect of substrates on inhibition of peach PPO by onion extract[1)]

Substrate (10 mM)	Relative activity (%)[2)]		
	Control	Fresh onion	Heated onion
Catechol	100.0	77.2±2.2	53.2±1.5
4-Methylcatechol	186.4±3.5	141.7±2.1	108.6±4.7
Pyrogallol	199.3±4.9	163.9±3.5	139.4±7.1
Resorcinol	-	-	-
Hydroquinone	-	-	-
(+)-Catechin	42.4±1.9	32.2±4.9	24.8±1.8

[1)]The final concentration of each substrate was 10 mM. The amount of onion extract was 60.0 mg/mL. Heated onion extract was incubated for 10 min at 100°C. The enzyme activity was measured at 25°C for 1 min using the spectrophotometric procedure at 420 (catechol), 410 (4-methylcatechol), 334 (pyrogallol), 400 (resorcinol), and 475 nm (hydroquinone and (+)-catechin).
[2)]Triplicate determination±SE.

Effect of onion extract on peach PPO Table 2 exhibits the inhibitory effect of onion extract with various substrates on peach PPO. The peach PPO was most active with pyrogallol followed by 4-methylcatechol, catechol, and (+)-catechin as a substrate, whereas the enzyme showed poor substrate specificity toward resorcinol and hydroquinone. This result suggests that peach PPO may use *o*-diphenol (catechol, 4-methylcatechol) or trihydroxyphenol (pyrogallol) as a substrate, but it may not use *m*-diphenol (resorcinol) or *p*-diphenol (hydroquinone). It was also reported that *Fuji* apple PPO was most active with *o*-diphenols such as chlorogenic acid, (+)-catechin, and catechol as a substrate (20). However, it was reported that the longan PPO was most active with pyrogallol, followed by 4-methylcatechol and catechol (21), and peppermint PPO exhibited maximum activity toward catechol (22).

Regardless of substrate used, the heated onion extract at 100°C for 10 min exhibited more inhibitory effect on peach PPO than that of the fresh onion extract. Figure 1 and 2 demonstrate the inhibitory effect of onion extract

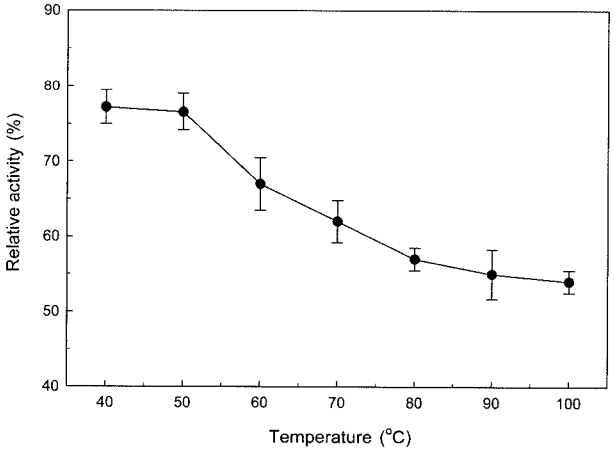

Fig. 1. Effect of heat treatment temperature of onion extract on inhibitory effect of peach PPO. The amount of the onion extract was 60 mg/mL.

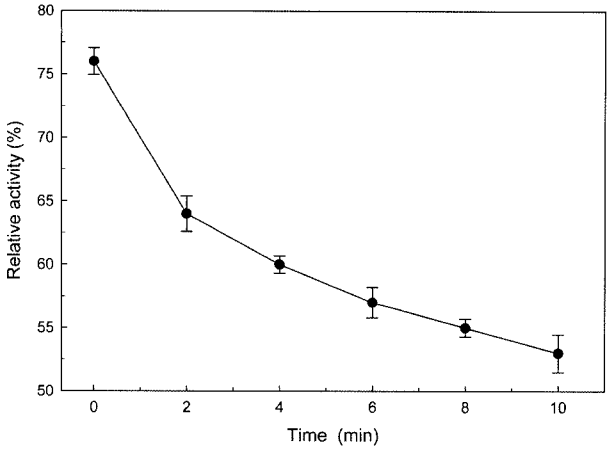

Fig. 2. Effect of heat treatment time of onion extract on inhibitory effect of peach PPO.

was dependent upon heating temperature and reaction time.

The inhibitory effect of onion extract was increased with higher temperature and longer reaction time. Similar results were also observed with the potato PPO activity (16).

The Michaelis-Menten constant (K_m) of peach PPO was measured at pH 6.8 and 25°C. From double reciprocal plot, the K_m values of peach PPO in the absence and presence of onion extract were measured. Figure 3 shows a plot of 1/V against 1/[catechol] in the absence and presence of onion extract. Both reciprocal plots that intersect on the horizontal axis at a value of $-1/K_m$ are obtained. From the plots, K_m value of peach PPO with catechol as a substrate was determined as 35.7 mM. Since the K_m value of peach PPO in the presence of heated onion extract was not changed as shown in Fig. 3, the heated onion extract seems to be non-competitive inhibitor against peach PPO.

It was reported that various volatile sulfur compounds

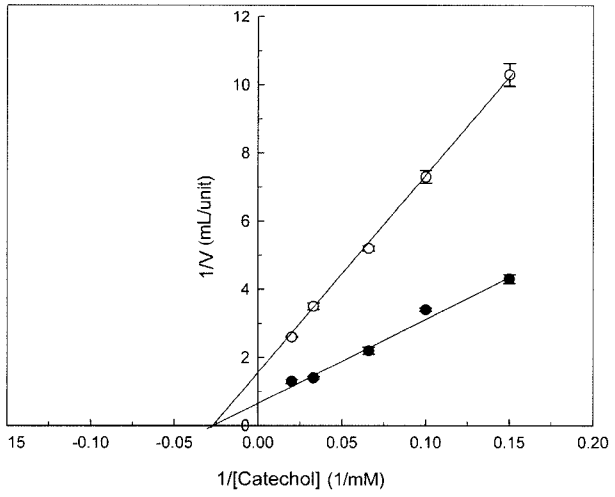

Fig. 3. Lineweaver-Burk plot of peach PPO in the presence of heated onion extract. The concentration of onion extract was 60 mg/mL. ○ , Control; ● , with heated onion extract.

Table 3. Inhibitory effect of onion extract in the presence of glucose and glycine on peach PPO[1)]

Compound	Relative activity (%)[2)]	
	Fresh	Heat-treated
Onion extract	77.2±2.2	53.2±1.5
Onion + glucose + glycine	64.3±5.6	31.7±1.4
Onion + glucose	74.3±0.9	46.5±2.8
Onion + glycine	70.9±4.2	45.5±1.4

[1)]The amount of the onion extract was 60.0 mg/mL. The heat treatment was performed at 100°C for 10 min. Glucose and glycine were added at a final concentration of 12.5 mM. The enzyme activity was measured at 25°C for 1 min by the spectrophotometric procedure.
[2)]Triplicate determination±SE.

including thiols were present in *Allium* species such as onion (23), and the inhibition of enzymatic browning with thiol compounds such as cysteine and dithiothreitol was also reported (24). The thiol compounds in onion might be responsible for the inhibition of browning in peach, and they may be compounds of low molecular weight since the inhibitory effect of onion extract was eliminated after dialysis (molecular weight cutoff; 12,000) (data not shown).

To investigate the possible mechanism of increased inhibitory effect of onion extract after heat treatment, glucose, glycine, or both were added to the onion extract (Table 3). Both glucose and glycine had little effect on peach PPO. However, the heated onion extract in the presence of glucose and glycine stimulated the inhibitory effect of onion extract. This suggests that non-enzymatic browning products produced during heating might have been responsible for the stronger inhibitory action of the heated onion extract. It was also reported that Maillard reaction products inhibited apple and potato PPO (17, 25). Reductone moiety present in the Maillard reaction

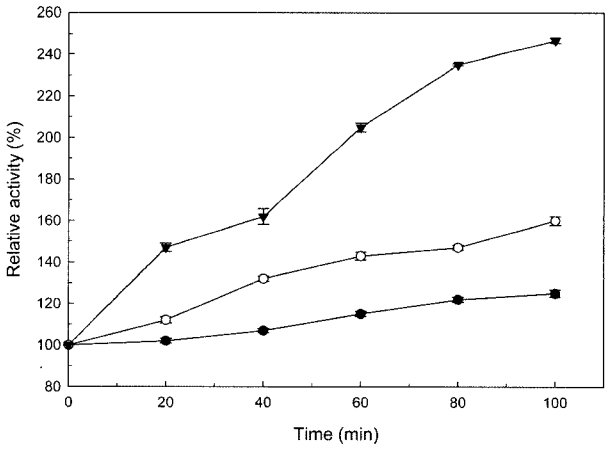

Fig. 4. Time dependent browning of peach juice in the presence of onion extract. The degree of browning of pear juice was measured spectrophotometrically by measuring the absorbance of the juice at 400 nm. The concentration of onion extract was 60 mg/mL. ● , Control; ○ , with fresh onion; ▼ , with heated onion extract.

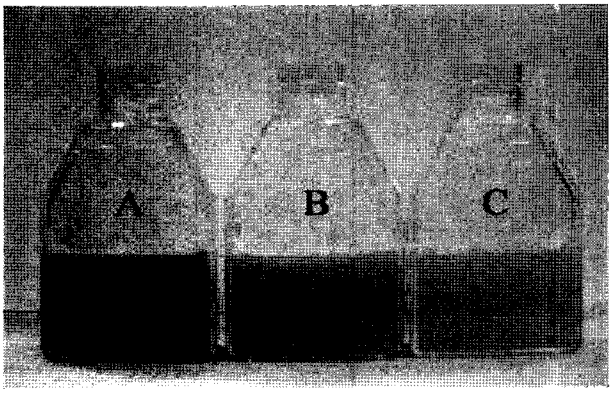

Fig. 5. Inhibitory effect of onion extract on browning of peach juice. A 100 g portion of peach pulp was homogenized with 100 mL of distilled water (A), fresh onion extract (B), and heated onion extract (C) at 100°C for 10 min and kept at 25°C for 30 min.

products has been reported to exhibit both reducing and chelating properties (26) in addition to oxygen scavenging properties (27). Therefore, the reductone moiety in the melanoidine's structure may prevent browning by reducing the copper of PPO. Another possibility is that the reductone in the Maillard reaction product may be capable of converting the quinone back to diphenol, thus preventing the polymerization of quinone (27). It was also reported that ethanol extract of seaweeds such as *Ecklonia cova* and *Sargassum siliquastrum* exhibited anti-browning activity by inhibiting tyrosinase (28).

Effect of onion extract on browning of peach juice Inhibitory effect of onion extract on browning of peach juice squeezed from whole peach was examined. The extracted peach juice was immediately mixed with onion extract, and the time-dependent color change was measured at 400 nm. Figure 4 shows the time course of browning of peach juice in the presence of fresh and heated onion extract. Both fresh and heated onion extract exhibited inhibitory effect on peach browning, and the heated onion extract was more effective in peach browning due to enzymatic browning. Figure 5 shows the browning of peach juice without onion extract (A), but the browning was inhibited by addition of fresh onion extract (B), and heated onion extracts (C). The peach juice without addition of onion extract (Fig. 5A) showed a rapid change to a brown color, whereas those with fresh (Fig. 5B) or heated onion extract (Fig. 5C) reduced the intensity of the brown color formation. Since the addition of heated onion extract exhibited higher inhibitory effect on peach PPO activity than that of the fresh one, the browning of peach was also slower in peach juice containing heated onion extract as shown in Fig. 5. This result is in good agreement with those of a previous study of potato PPO (16).

Since the browning of peach was inhibited effectively by the heated onion extract, onion extract has potential to be used commercially as a natural inhibitor of browning in various plants and vegetables.

Acknowledgments

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