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# Prevention of enzymatic browning of pear by onion extract

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**Abstract**

Onion extract was prepared by extracting onion with water, and the effects of the extract on pear polyphenol oxidase and browning of pear were investigated. The polyphenol oxidase of pear was inhibited by onion extract, and the inhibitory effect of onion extract toward pear polyphenol oxidase was increased with the heated extract. The inhibitory effect of the extract was increased with increasing heating temperature and time. The browning of pear juice was retarded by addition of both fresh and heated onion extracts. The onion extract inhibited the pear polyphenol oxidase non-competitively. Therefore, the inhibitory effect of onion extract against pear browning seems to be due to the inhibitory effect of onion extract against pear polyphenol oxidase.


*Keywords:* Polyphenol oxidase; Pear; Onion extract; Inhibitor

## 1. Introduction

Browning usually occurs in certain fruits and vegetables during handling, processing and storage after harvest (Jang, Sanada, Ushio, Tanaka, & Ohshima, 2002). Enzymatic browning is a major factor contributing to quality loss in foods and beverages (McEvily, lyengar, & Otwell, 1992). The browning phenomenon usually impairs the sensory properties of products because of the associated changes in colour, flavour and softening (Martinez & Whitaker, 1995). Browning in fruits and vegetables is caused by the enzymatic oxidation of phenolic compounds by polyphenol oxidase (Martinez & Whitaker, 1995). Polyphenol oxidase(PPO, *o*-diphenol : $O_2$ oxidoreductase, EC 1.10.3.1) is a copper-containing enzyme, which is also known as catechol oxidase, catecholase, diphenol oxidase, *o*-diphenolase, phenolase and tyrosinase (Martinez & Whitaker, 1995, Negishi, Negishi, & Ozawa, 2002). PPO catalyzes either one or two reactions 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 quinines (Busch, 1999). It is impossible to disregard browning by PPO in food; prevention of browning by PPO is an important issue for the food industry. Browning by PPO can be prevented by the addition of sulfites, ascorbic acid and its analogues, as well as cysteine (Ding, Chachin, Ueda, & Wang, 2002, Jang et al., 2002, Martinez & Whitaker, 1995, Negishi & Ozawa, 2000). Although sulfites are very effective for preventing browning, they could be a problem for human health (Sapers, 1993). Accordingly, there is an increasing demand by consumers for substituting synthetic compounds with natural substances as food ingredients (Jang et al., 2002). Compounds of inherently natural origin would be widely accepted by consumers in the market (Jang et al., 2002). In this work, we attempt to evaluate onion extract as a natural inhibitor of pear browning by PPO.

## 2. Materials and methods

### 2.1. Onion extract preparation

Onion (500 g) was homogenized with water (500 ml) for 3 min, and the homogenate was centrifuged at 12,000*g* for 20 min at 4 °C. The supernatant after centrifugation was used for this experiment. Heated onion

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extract was obtained by boiling the extract for various time at 100 °C.

### 2.2. Pear PPO and juice preparation

Pear (200 g) was homogenized with 200 ml of a 50 mM phosphate buffer at pH 6.6 for 3 min, and the homogenate was centrifuged at 15,000*g* for 20 min, and the supernatant was collected. The supernatant was used as a pear enzyme throughout this experiment. All steps were carried out at 4 °C. For pear juice preparation, the pear was homogenized with water instead of buffer, and all other procedures were the same as for enzyme preparation.

### 2.3. PPO assay

The PPO activity was assayed with 0.2 M catechol as a substrate by a spectrophotometric procedure (Zauberman et al., 1991). In the procedure for inhibition reaction mixture was incubated for 5 min at 25 °C. The reaction mixture included 0.1ml of pear PPO, 0.9 ml of a 50 mM phosphate buffer at pH 6.8 and 1.0 ml of onion extract as inhibitor. Then 1.0 ml of 0.2 M catechol was added to the reaction mixture to initiate the enzyme reaction. The total volume of assay for inhibition on PPO activity was 3.0 ml. Absorbance, at 420 nm, was monitored continuously at 25 °C for 1 min.

## 3. Results and discussion

### 3.1. Effect of onion extract on pear PPO

Table 1 exhibits the inhibitory effect of various anti-browning agents on pear PPO activity, with catechol as substrate. The enzyme was strongly inhibited by ascorbic acid and cysteine. Under experimental conditions, the pear PPO activity was also partially inhibited by both fresh and heat-treated onion extract. The addition of the onion extract that had been heated at 100 °C for 10 min caused a stronger inhibitory effect on pear PPO than did the fresh one. Since heating of onion extract increased its inhibitory effect, we also investigated the effect of heating time on inhibitory effect of onion extract, as shown in Fig. 1. Similar results were also obtained when potato PPO was treated with heated onion extract (Lee et al., 2002). It was reported that Maillard reaction products were inhibitors of PPO, and the increase in inhibitory effect by heating the onion extract might be due to a synergy effect with Maillard reaction products produced during heating. These results suggested that the heated onion extract had a strong inhibitory effect on the browning of pear. Although mechanisms of enzymatic prevention on heated onion extract are not well understood, onion contained certain

Table 1
The inhibitory effect of various anti-browning agents on pear PPO

| Anti-browning agent | Relative activity (%) |
|---|---|
| None | 100 |
| Fresh onion | 73.3 |
| Heated onion | 45.9 |
| Ascorbic acid | 0.68 |
| Citric acid | 96.7 |
| Cysteine | 0.16 |
| Potassium sorbate | 83.0 |

The amount of the onion extract was 60.0 mg/ml. The heated onion extract was incubated for 10 min at 100 °C. All anti-browning agents were present at a final concentration of 0.005%. The enzyme activity was measured at 25 °C for 1 min by a spectrophotometric procedure.

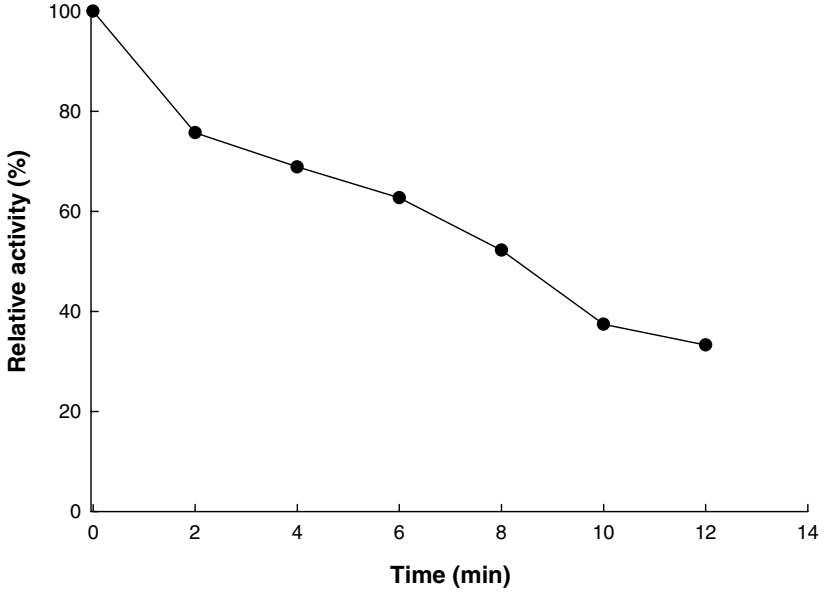


Fig. 1. Effect of heating time on the inhibitory effect of onion extract. The onion extract was heated for various times at 100 °C, and the pear PPO activity was then assayed in the presence of the heated onion extract. The amount of the onion extract added was 60.0 mg/ml.

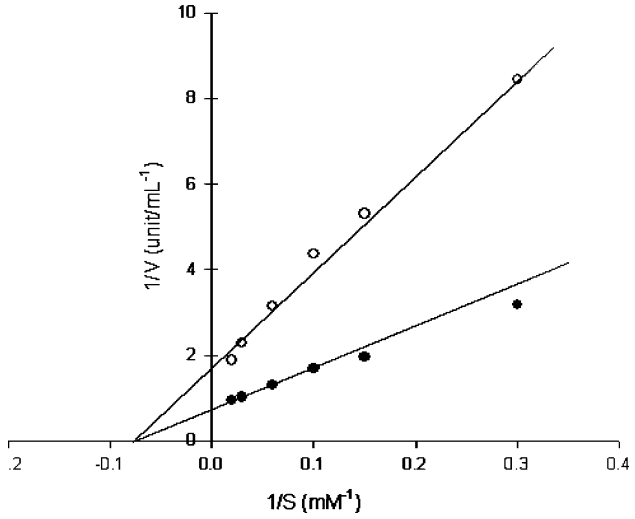


Fig. 2. Lineweaver–Burk plot of pear PPO in the presence of heated onion extract. Catechol was used as a substrate. The concentration of onion extract was 60.0 mg/ml. (○) – control, (●) – with heated onion extract.

compounds which effectively inhibited PPO activity of pear. It was reported that various volatile sulfur compounds, including thiols, were present in *Allium* species such as onion (Negishi et al., 2002). Since thiol compounds are reported to inhibit PPO (Ding et al., 2002, Negishi & Ozawa, 2000), we can assume that the thiol compounds in onion might be the active components responsible for the inhibitory effect of onion extract. When the onion extract was dialyzed, the inhibitory effect against pear PPO was completely eliminated, suggesting low molecular compounds. Fig. 2 shows that the heated onion extract inhibited the pear PPO non-competitively.

### 3.2. Effect of onion extract on browning of pear

Inhibitory effect of onion extract on browning of pear juice squeezed from whole pear was also investigated. The extracted pear juice was immediately mixed with onion extract, and the colour change due to enzymatic browning was measured at 400 nm by a spectrophotometric procedure. Fig. 3 shows the time course of browning in the presence of fresh or heated onion extract. Both fresh and heated onion extracts decreased the browning of pear, and the heated onion extract was more effective in prevention of pear browning. The inhibition of browning in pear juice by onion extract seems to be due to the PPO inhibitor present in onion extract. Since heated onion extract was more powerful inhibitor against pear PPO, it could be more effective in preventing browning in pear. Fig. 4 shows the degree of browning in pear juice after mixing with various concentrations of heated onion extract. As the onion extract concentration increased, the lag period lengthened and the extent of browning decreased. These results support the conclusion that addition of heated onion extract is efficient for preventing browning of pear juice. This result demonstrates that the heated onion extract effectively inhibits the browning of pear, and the onion extract contains certain potential inhibitors of pear PPO. Although the active compound for inhibitory effect has not been identified, utilization of onion extract will be possible as a natural food additive for the prevention of browning caused by PPO.

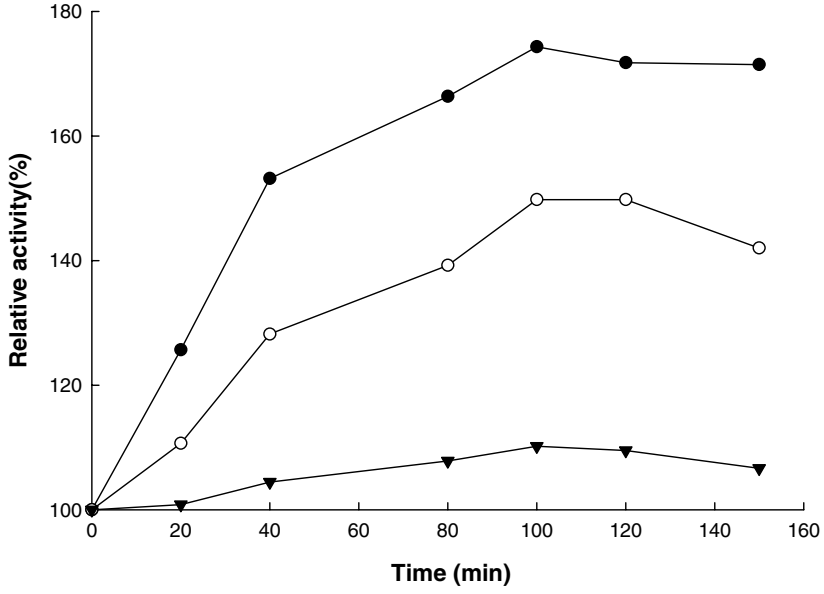


Fig. 3. Time course of pear browning in the presence of fresh and heated onion extracts. The concentration of onion extract was 100 mg/ml. (●) – control, (○) – with fresh onion, (▼) – with heated onion.

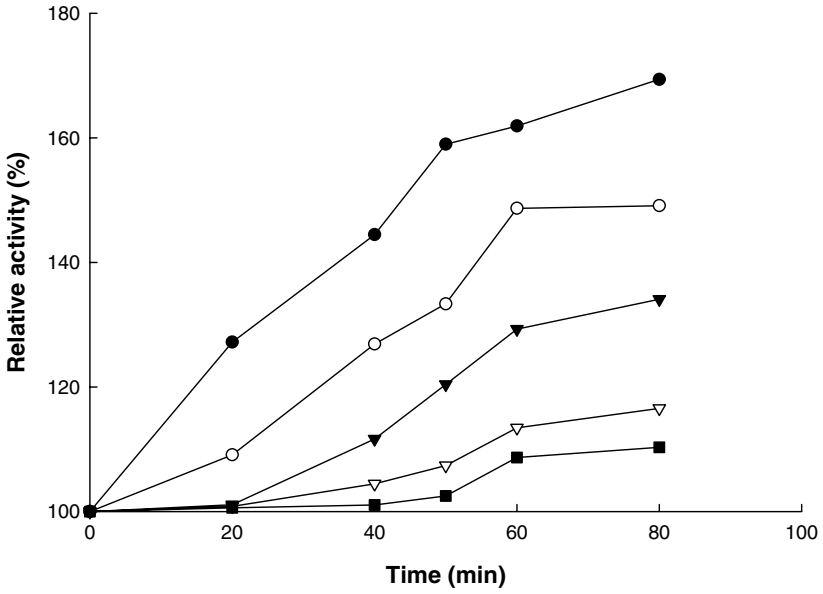


Fig. 4. Time course of pear browning in the presence of various amounts of heated onion extract. The onion extract was heated for 10 min at 100 °C. (●) – control, (○) – 40.0 mg/ml, (▼) – 60.0 mg/ml, (▽) – 80.0 mg/ml, (□) – 100 mg/ml.

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