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Inhibitory effect of onion extract on polyphenol oxidase and enzymatic browning of taro (*Colocasia antiquorum* var. *esculenta*)

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Abstract

The inhibitory effect of onion extract on polyphenol oxidase and enzymatic browning of taro was investigated. The polyphenol oxidase from taro was strongly inhibited by various reducing agents, such as L-ascorbic acid, L-cysteine, dithiothreitol, glutathione and sodium pyrosulfite. The enzyme was also inhibited by addition of onion extract. Regardless of substrates used, the addition of heated onion extract at 100 °C for 10 min, gave a stronger inhibitory effect on taro polyphenol oxidase activity than did fresh unheated extrtact. The inhibitory effect of onion extract was dependent on heating temperature and time. The addition of glucose, glycine, or both to the onion extract, during heating, stimulated the inhibitory effect of the onion extract, suggesting that non-enzymatic browning products, produced during heating, might be responsible for the stronger inhibitory action of the heated onion extract.

Keywords: Enzymatic browning; Polyphenol oxidase (PPO); Taro; Onion extract

1. Introduction

Browning in fruits and vegetables damaged by mechanical injury during harvesting, during post harvest storage or processing, is one of the main causes of quality loss (Flick, Ory, & St. Angelo, 1977; Mathew & Parpia, 1971; Perez-Gilabert & Carmona, 2000). This can involve different compounds and proceed through different chemical pathways. The major groups of reactions leading to browning are enzymatic browning and non-enzymatic browning (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001). Enzymatic browning is caused by activity of polyphenol oxidase (PPO, *o*-diphenol: O_2 oxidoreductase, EC 1.10.3.1) and plays an important role in fruit and vegetable processing and during storage of processed foods (Martinez & Whitaker, 1995). Polyphenol oxidase catalyzes two types of oxidative reactions: hydroxylation of monophenols to *o*-diphenols (cresolase activity) and oxidation to *o*-quinones (catecholase activity) (Kavrayan & Aydemir, 2001). Further condensation of quinones leads to brown melanin pigments (Martinez & Whitaker, 1995). The enzyme activity is high in plants and vegetables that are particularly sensitive to oxidative browning, such as potato, apple, mushroom, banana, and peach (Iyengar & McEvily, 1992). Consequently, the control of enzymatic browning has aroused strong interest in the food industry (Langdon, 1987).

Sulfite-containing additives have been extensively used as anti-browning agents to keep vegetables and fruits fresh looking (Langdon, 1987). However, consumer awareness of the risks associated with sulfites and increased regulatory scrutiny have created the need for substitutes (Iyengar & McEvily, 1992). There is increasing consumer demand to substitute synthetic compounds with natural substances as food ingredients (Jang, Sanada, Ushio, Tanaka, & Ohshima, 2002). The polyphenol oxidase inhibitors occurring in natural resources have been studied in several plants (Choi, Kim, Chang, & Sapers, 1997; Espin, Jolivet, Overeem, & Wichers, 1999; Jang et al., 2002), but the development of natural and efficient polyphenol oxidase inhibitors is needed.

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Taro (*Colocasia esculenta*) is a staple food in many tropical and subtropical areas of the world. Taro must be processed to be utilized commercially, and the control of enzymatic browning, due to polyphenol oxidase, seems to be necessary. In the present work, we have investigated the inhibitory effect of onion extract as a natural inhibitor of polyphenol oxidase and enzymatic browning of taro.

2. Materials and methods

2.1. Taro polyphenol oxidase preparation

Taro (*C. antiquorum* var. *esculenta*) was purchased from a local market in Busan, Korea. Peeled taro (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 (pH 6.8) and dialyzed overnight against the same buffer.

2.2. 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 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 onion extract at 100 °C for 10 min.

2.3. Polyphenol oxidase assay

The polyphenol oxidase activity was measured spectrophotometrically (Ultrospec 3000, Pharmacia Biotech). A certain amount of the enzyme solution was added to 1 ml of 0.2 M catechol solution to initiate the enzyme reaction. The reaction mixture, of 0.1 ml of taro polyphenol oxidase, 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. Then the mixture 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 (Zauberman et al., 1991). The total volume of assay for inhibition of polyphenol oxidase activity was 3.0 ml.

3. Results and discussion

3.1. Effect of anti-browning agents on taro polyphenol oxidase

Table 1 demonstrates the effect of various anti-browning agents on taro polyphenol oxidase with catechol as a substrate. The enzyme activity was most inhibited by addition of various reducing agents, such as L-ascorbic acid, 2-mercaptoethanol, dithiothreitol, glutathione and sodium pyrosulfite. The inhibition of polyphenol oxidase by various reducing agents has been reported in browning control in litchi fruit (Jiang & Fu, 1998), and fresh-cut pear wedges (Oms-Oliu, Aguilo-Aguayo, & Martin-Belloso, 2006). Although sulfites are effective for controlling enzymatic browning, they can be harmful to asthmatic patients (Taylor, Higley, & Bush, 1986). Ascorbic acid is an effective reducing agent and has been used as an antioxidant in the food industry. The choice of particular anti-browning agent depends on several factors, such as the efficacy, cost, method of treatment, and any effects on taste, flavour, texture or colour (Iyengar & McEvily, 1992).

Table 1
The inhibitory effects of various anti-browning agents on taro polyphenol oxidase

Anti-browning agents	Relative activity (%)
Control	100
L-Ascorbic acid	0
L-Cysteine	0
Dithiothreitol	0
Glutathione	0
Sodium pyrosulfite	0
Citric acid	92.1
Sodium chloride	97.9
Potassium sorbate	84.1
EDTA	84.4

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.

3.2. Effect of onion extract on taro polyphenol oxidase

Table 2 shows the inhibitory effect of onion extract (with various substrates) on taro polyphenol oxidase. The taro polyphenol oxidase was most active with 4-methylcatechol

Table 2
Effects of substrates on inhibition of taro polyphenol oxidase by fresh and heated onion extract

Substrate (10 mM)	Relative activity(%)		
	Control	Fresh onion	Heated onion
Catechol	100	88.2	46.2
4-Methylcatechol	320	241	126
Pyrogallol	113	107	58.9
Resorcinol	0.0	0.0	0.0
Hydroquinone	1.1	0.0	0.0
(+)-Catechin	74.6	65.9	33.6
L-DOPA	135	117	58.5

Heated onion extract was prepared by incubating fresh onion extract at 100 °C for 10 min. The final concentration of all substrates was 10 mM. The polyphenol oxidase activity was assayed by a spectrophotometric procedure at 420 nm (catechol), 410 nm (4-methylcatechol), 334 nm (pyrogallol), 400 nm (resorcinol), and 475 nm (hydroquinone, (+)-catechin, L-DOPA(L-β-3,4-dihydroxyphenylalanine)). The relative activity of taro polyphenol oxidase with catechol as a substrate was 100%. The amount of onion extract used was 3.1 mg/ml.

as a substrate, followed by pyrogallol, and L-DOPA, whereas, the enzyme showed poor substrate specifies toward resorcinol and hydroquinone. It was reported that 4-methyl catechol was the preferred substrate for plant polyphenol oxidases (Walker, 1995). However, the polyphenol oxidase from longan was most active with pyrogallol, followed by 4-methylcatechol and catechol (Jiang, 1999), and peppermint PPO exhibited maximum activity toward catechol (Kavrayan & Aydemir, 2001).

Regardless of the substrate used, the heated onion extract (at 100 °C for 10 min) exhibited stronger inhibitory effect on taro polyphenol oxidase than did the fresh onion extract. As shown in Fig. 1, the inhibitory effect of onion extract, after heating at various temperatures, on taro polyphenol oxidase, was investigated. Onion extract treated at higher temperature exhibited stronger inhibition toward taro polyphenol oxidase. Fig. 2 also shows the effect of heating time on inhibition of taro polyphenol oxidase. The inhibitory effect of onion extract was increased with higher temperature and longer reaction time. When onion extract, treated at high temperature, was added to potato polyphenol oxidase, similar results were obtained, (Lee et al., 2002).

It was reported that various volatile sulfur compounds, including thiols, were present in *Allium* species, such as onion (Negishi, Negishi, & Ozawa, 2002). Since inhibition of enzymatic browning with thiol compounds, such as cysteine and dithiothreitol, was reported by Negishi and Ozawa (2000), the thiol compounds contained in onion might be responsible for the inhibition of browning in taro. The inhibition of onion extract on taro polyphenol oxidase was dramatically reduced by dialysis (molecular weight cutoff; 12,000). The relative activity of taro polyphenol oxidase by addition of heated onion extract was 46.2%, with

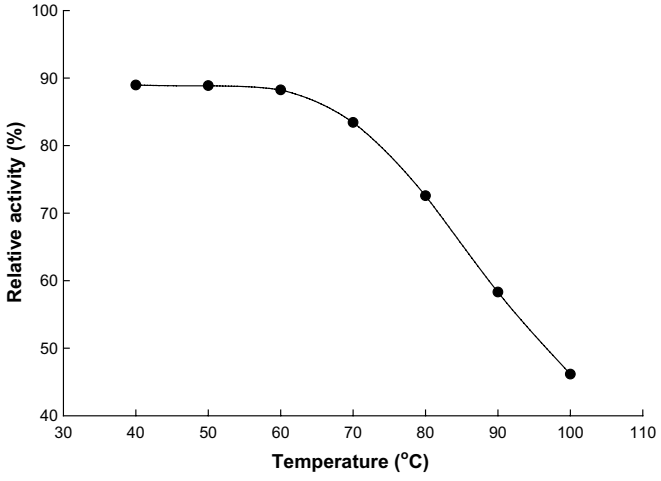

Fig. 1. Influence of heat treatment temperature of onion extract on inhibitory effect of taro polyphenol oxidase. The onion extract was heated at various temperature for 10 min, and the taro polyphenol oxidase activity was then assayed in the presence of the heated onion extract. The amount of the onion extract was 3.1 mg/ml.

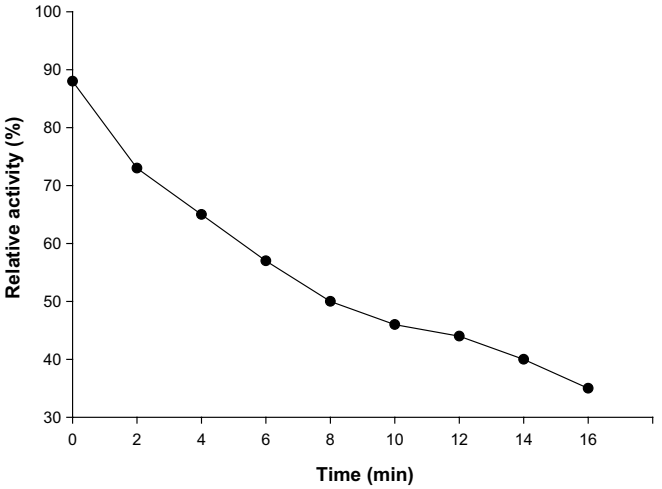

Fig. 2. Influence of heat treatment time of onion extract on inhibitory effect of taro polyphenol oxidase. The onion extract was heated at 100 °C for various times, and the taro polyphenol oxidase activity was then assayed in the presence of the heated onion extract. The amount of the onion extract was 3.1 mg/ml.

catechol as a substrate, as shown in Table 2, whereas, that of the enzyme with addition of heated onion extract after dialysis was found to be 87.5%. Therefore, the anti-browning activities in onion extract may be compounds of low molecular weight.

A possible explanation for the strong inhibitory effect of heated onion extract on taro polyphenol oxidase was sought by addition of glucose, glycine or both to the onion extract (Table 3). Neither glucose nor glycine alone had any significant effect on taro polyphenol oxidase in the fresh or heated state. 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 has been reported that Maillard reaction products had an inhibitory effect on polyphe-

Table 3
The inhibitory effect of the heated onion extract with added glucose and glycine on taro polyphenol oxidase

Compound	Relative activity (%)	
	Fresh	Heat-treated
Onion extract	88.2	46.2
Onion + glucose + glycine	76.6	31.5
Onion + glucose	83.5	41.8
Onion + glycine	81.4	37.0
Glucose + glycine	81.8	60.3
Glucose	95.9	91.5
Glycine	89.5	74.1

The amount of the onion extract was 3.1 mg/ml. The heat treatment was performed at 100 °C for 10 min. Glucose and glycine were each added at a final concentration of 25 mM. The enzyme activity was measured at 25 °C for 1 min by a spectrophotometric procedure.

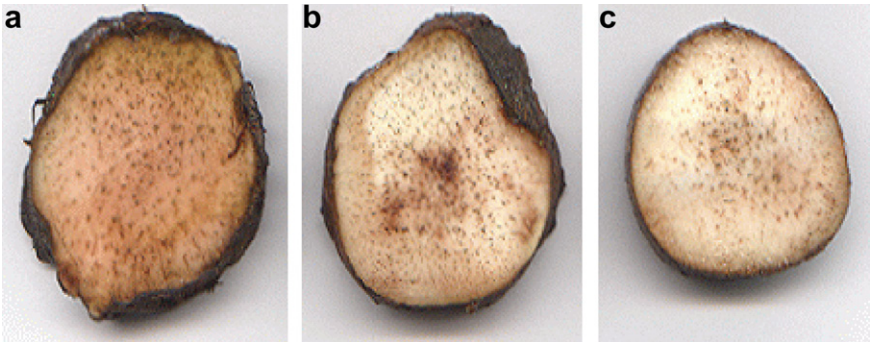

Fig. 3. Effect of onion extract on browning of taro. All slices were observed after incubating at room temperature for 25 min. The amount of onion extract was 3.1 mg/ml. (a) Addition of substrate solution (1 ml of 0.2 M catechol). (b) Addition of substrate solution (1 ml of 0.2 M catechol) after dipping in fresh onion extract. (c) Addition of substrate solution (1 ml of 0.2 M catechol) after dipping in heated onion extract.

nol oxidase from potato (Lee & Park, 2005) and apple (Tan & Harris, 1995).

3.3. Effect of onion extract on enzymatic browning of taro

The inhibitory effect of onion extract on browning of taro is shown in Fig. 3. Taro was cut into 5 mm slices, and each slice were immersed for 20 min in distilled water, fresh onion extract and heated onion extract prepared at 100 °C for 10 min. Then, 1.0 ml of 0.2 M catechol was spread over the whole surface of each slice. The addition of heated onion gave a higher inhibitory effect on the taro polyphenol oxidase activity than did the fresh unheated extract. The taro slice immersed in distilled water (Fig. 3a) showed a rapid change to a brown colour, whereas, that immersed in fresh (Fig. 3b) or heated onion extract (Fig. 3c) showed reduced intensity of the brown colour formation. The results reported here are in good agreement with the result of a previous study of potato polyphenol oxidase (Lee et al., 2002).

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

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