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Inhibitory effect of banana polyphenol oxidase during ripening of banana by onion extract and Maillard reaction products

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Abstract

The inhibitory effect of onion extract on banana polyphenol oxidase activity during ripening of banana when stored at room temperature for 10 days was investigated. The addition of the onion extract that had been heated at 100 °C for 10 min exhibited a higher inhibitory effect on the banana polyphenol oxidase activity during ripening of banana than that of the fresh onion extract. When the onion extract that had been treated at a high temperature was added, the banana polyphenol oxidase activity was markedly inhibited. It was found that heat treated onion extract inhibited the banana polyphenol oxidase non-competitively. The MRP synthesized from arginine, cysteine, histidine and lysine significantly inhibited banana polyphenol oxidase. The enzyme activity was inhibited by addition of various anti-browning agents.

Keywords: Polyphenol oxidase; Banana; Ripening; Onion extract; MRP

1. Introduction

The banana belongs to the genus *Musa* of the family Musaceae (Cano et al., 1997). Banana, with its innumerable varieties, is a tropical fruit of commercial significance and undergoes textural, color transformations as they pass through the ripening process (Prabha & Bhagyalakshmi, 1998). Biochemical, physiological and compositional changes associated with ripening and resulting softening of bananas have been reviewed extensively (Marriott, 1980). Lodh, Ravel, Selvaraj, and Kohli (1971) have reported on the chemical changes in the banana fruit during various stages of development.

Plant polyphenol oxidases (PPO) are responsible for the enzymatic browning reactions occurring during the handling, storage and processing of fruits and vegetables (Dincer, Colak, Aydin, Kadioglu, & Guner, 2002). In plant tissues, the browning pigments lead to organoleptic and nutritional modifications, thus depreciating the food product (Friedman, 1996). The degree of browning in banana, after cutting, was correlated with polyphenol oxidase activity and the concentration of free phenolic substrates (Weaver & Charley, 1974). Several methods such as the addition of antioxidants and the exclusion of oxygen as well as thermal processing have been used to inhibit enzymatic browning(Sun, Lee, & Song, 2002). Many papers have reported the inhibition activity of polyphenol oxidase by Maillard reaction products or anti-browning agents (Tan & Harris, 1995). However, for inactivation of polyphenol oxidase, thermal processing has limits like loss of sensory and nutritional quality of food products(Sun et al., 2002). Also consumer awareness of the risks associated with sulfite-containing anti-browning agents and increased regulatory scrutiny have created the need for substitutes (Iyengar & Mcevily, 1992). Therefore, the development of alternative safe and efficient anti-browning agents has become crucial in order to preserve or minimize the loss of fresh fruits and vegetables (Tan & Harris, 1995). However, little research has been conducted on the inhibitory action of the polyphenol oxidase produced by a natural food source. The objective of this work is to study the inhibitory action of onion extract on banana polyphenol oxidase during ripening of banana.

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2. Materials and methods

2.1. Materials

Bananas (*Musa acuminata*, Cultivar Jejudo) and onion were purchased from a local market in Pusan, Korea. All amino acids, sugars, catechol, citric acid, potassium sorbate, sodium chloride, ascorbic acid, sodium pyrosulfite were obtained from Sigma Chemical Co.

2.2. Preparation of samples

Onion (200 g) was homogenized with 200 ml of a 50 mM phosphate buffer at pH 6.6 for 3 min. The homogenate was centrifuged at 15,000*g* for 20 min, and the supernatant was collected. The supernatant after centrifugation was used as a fresh onion extract, the yield of the extract being 9.4 g from 200 g of onion. The heat-treated onion extract was prepared by heating the extract at 100 °C for 10 min.

A batch of bananas were allowed to ripen at room temperature. Two fruits were sampled every 2 days or 3 days during ripening. They were homogenized with 50 mM phosphate buffer at pH 6.8 for 3 min. The homogenate was centrifuged at 15,000*g* for 20 min, and the supernatant was collected. All steps were carried out at 4 °C.

2.3. Synthesis of Maillard reaction product (MRP)

MRP of different amino acids were obtained by heating equal volumes of 1.5 M various amino acid solution and 1.5 M glucose solution at 90 °C for 7 h. MRPs formed from various sugars were obtained by heating equal volumes of 1.5 M glycine and 1.5 M various sugars at 90 °C for 7 h. The MRP formation was evaluated by measuring absorbance at 420 nm.

2.4. Measurement of polyphenol oxidase activity

Banana polyphenol oxidase activity (Zauberman et al., 1991)was assayed with 0.2 M catechol as a substrate by a spectrophotometric procedure (Ultrospec 3000, Pharmacia Biotech). The assay mixture contained 0.1 ml of banana PPO, 0.9 ml of a 50 mM phosphate buffer at pH 6.6, 1 ml of onion extract(fresh or heat-treated) was incubated for 5 min at 25 °C. After this incubation, 0.2 M catechol was added to the assay mixture, and the increase in absorbance at 420 nm and 25 °C was recorded automatically for 1 min. The total assay volume was 3 ml.

3. Results and discussion

3.1. Inhibitory effect of onion extract on banana polyphenol oxidase

Fig. 1 shows the inhibitory effect of onion extract(fresh or heat-treated) on banana polyphenol oxidase during ripening of banana when stored at room temperature for 10 days. Polyphenol oxidase activity of banana decreased during storage at room temperature and was inhibited by addition of onion extract. The addition of the onion extract that had been heated at 100 °C for 10 min exhibited a higher inhibitory effect on the banana polyphenol oxidase activity during ripening of banana than that of the fresh onion extract. The amount of the onion extract was 3.1 mg/ml. Variations in polyphenol oxidase activity differ according to fruit species. As previously mentioned, there is generally a change from bound to more soluble enzyme forms during maturation, but activity of the latter form is always lower than observed in young fruits (Lee & Whitaker, 1994).

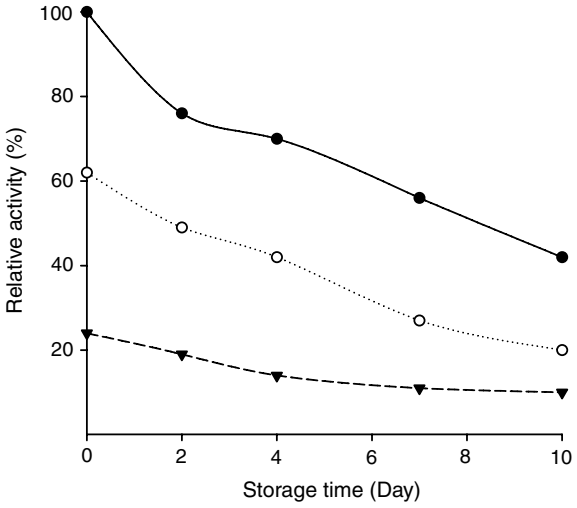

Fig. 1. The inhibitory effect of onion extract on banana polyphenol oxidase during ripening of banana. The banana was stored at room temperature for 10 days. The heated onion extract was incubated for 10 min at 100 °C. The amount of the onion extract was 3.1 mg/ml. The enzyme activity was measured at 25 °C for 1 min by the spectrophotometric procedure. PPO activity (●–●); addition of fresh onion extract (○–○); addition of heated onion extract (▼–▼).

3.2. Effect of heat treatment of onion extract on the inhibitory effect of banana polyphenol oxidase

The inhibitory effect of onion extract after heating at various temperatures on banana polyphenol oxidase during ripening of banana when stored at room temperature for 0 day and 10 days are exhibited in Fig. 2. When the onion extract that had been treated at a high temperature was added, the banana polyphenol oxidase activity was markedly inhibited. As shown in Fig. 3, Lineweaver–Burk plots of banana polyphenol oxidase in the presence of the heat treated onion extract when stored at room temperature for 10 days was investigated. It was found that heat treated onion extract inhibited the banana polyphenol oxidase non-competitively.

3.3. Inhibitory effect of Maillard reaction products (MRP) during ripening of banana

The inhibitory effect of Maillard reaction products (MRP) synthesized from different amino acids with

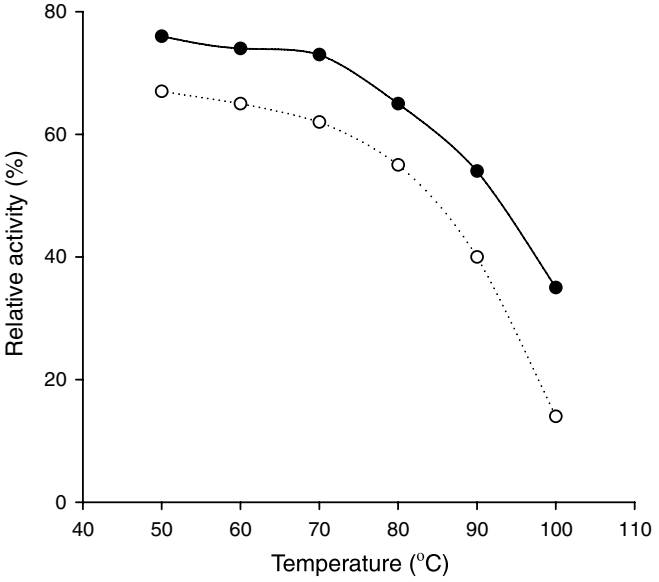

Fig. 2. The inhibitory effect of onion extract after heating at various temperature on banana polyphenol oxidase. The onion extract was heated at various temperature for 10 min. The amount of the onion extract was 3.1 mg/ml. PPO activity when stored at room temperature for 0 day (●–●) ; PPO activity when stored at room temperature for 10 days (○–○).

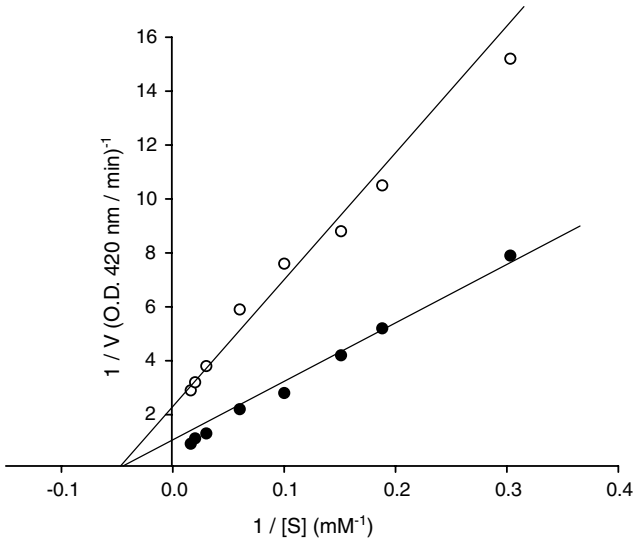

Fig. 3. Lineweaver–Burk plots of banana polyphenol oxidase in the presence of heated onion extract. Catechol was used as a substrate. The enzyme activity was assayed when stored for 10 days at room temperature.The concentration of the onion extract used was 1.55 mg/ml. Control (●–●); addition of heated onion extract (○–○).

constant amount of glucose (1.5 M) on banana polyphenol oxidase during ripening of banana are exhibited in Table 1. The MRP synthesized from arginine was most inhibitory of banana polyphenol oxidase, followed by cysteine, histidine, lysine and glycine. MRP from basic amino acids such as arginine, histidine, or lysine with reducing sugars have been shown to have very strong activities in vitro (Lingnert & Ericksson, 1980). However, the arginine or lysine-glucose solutions will be limited in their use as polyphenol oxidase inhibitors due to the high color intensity associated with them (Tan & Harris, 1995).

Table 1
The inhibitory effect of MRP synthesized from various amino acids with glucose during ripening of banana

Compounds	Relative activity (%)				
	0 (day)	2 (days)	4 (days)	7 (days)	10 (days)
Control	100.0	100.0	100.0	100.0	100.0
Valine	20.2	21.4	20.8	21.9	19.2
Glycine	14.6	12.9	13.5	13.8	12.7
Serine	27.6	26.5	28.6	27.3	29.4
Cysteine	0.2	0.1	0.1	0.0	0.1
Asparagine	40.0	38.9	37.8	39.2	38.5
Lysine	5.7	5.2	5.1	5.0	5.4
Arginine	0.0	0.0	0.0	0.0	0.0
Histidine	0.5	0.6	0.8	0.7	0.6

The MRP was obtained by heating equal volume of each 1.5 M amino acid and 1.5 M glucose at 90 °C for 7 h. The banana was stored at room temperature for 10 days.

The inhibitory effect of MRP synthesized from different sugars with constant amount of glycine (1.5 M) on banana polyphenol oxidase activity are shown in Table 2. The enzyme was most inhibited by addition of fructose. Bell reported that in addition to considering the most frequently discussed variables (i.e. amino acid and sugar type, amino acid and sugar concentration, amine-sugar ratio, pH, temperature), the buffer type and concentration also have a significant impact on the Maillard reaction(Bell, 1997). Nicoli, Elizalde, Pitotti, and Lerici (1991) reported that Maillard reaction products showed two different effects on polyphenol oxidase: an inhibition of the enzyme activity and the appearance of an initial lag phase, due to the chelating properties of polyphenol oxidase on Cu^{2+} and the reducing properties of the Maillard reaction products.

3.4. The inhibitory effect of various anti-browning agents during ripening of banana

Table 3 shows the inhibitory effect of various anti-browning agents on banana polyphenol oxidase during ripening of banana with catechol as a substrate. The enzyme was most inhibited by the addition of sodium pyrosulfite. Inhibition assays indicate that thiol compounds, such as cysteine and

Table 2
The inhibitory effect of MRP synthesized from various sugars with glycine during ripening of banana

Compounds	Relative activity (%)				
	0 (day)	2 (days)	4 (days)	7 (days)	10 (days)
Control	100.0	100.0	100.0	100.0	100.0
Glucose	14.4	12.7	12.2	13.0	11.4
Lactose	23.2	21.5	19.9	22.4	26.9
Sucrose	27.4	26.4	23.2	26.8	27.8
Fructose	8.1	7.6	6.9	6.1	7.2
Maltose	24.3	20.9	20.4	21.1	22.2

The MRP of various sugars were obtained by heating equal volume of each 1.5 M sugar and 1.5 M glycine at 90 °C for 7 h. The banana was stored at room temperature for 10 days.

Table 3
The inhibitory effect of various anti-browning agents during ripening of banana

Anti-browning agents (final conc.)	Relative activity (%)				
	0 (day)	2 (days)	4 (days)	7 (days)	10 (days)
Control	100.0	100.0	100.0	100.0	100.0
Citric acid (100 mM)	80.1	78.3	79.7	75.2	77.2
Potassium sorbate (100 mM)	67.7	63.3	62.5	65.5	64.7
NaCl (100 mM)	88.0	86.5	87.2	85.1	86.5
Ascorbic acid (10 mM)	0.5	0.8	0.7	0.6	0.8
Sodium pyrosulfite (10 mM)	0.3	0.1	0.1	0.1	0.2

The banana was stored at room temperature for 10 days. The enzyme activity was measured at 25 °C for 1 min by the spectrophotometric procedure.

metabisulfite with very low K_i values, are potent inhibitors of the medlar enzyme (Dincer et al., 2002). Pizzocaro, Torreggiani, and Gilardi (1993) reported that concentrations between 0.5% and 1% of sodium chloride had an inhibiting effect on the enzymatic browning of whole apples or apple pieces but only concentrations of about 20% inactivated polyphenol oxidase isolated from the apple. The inhibitory effect of sodium chloride is attributed to the anion chloride: the action is of the non-competitive type, as shown for purified polyphenol oxidase from apples (Janovitz-Klapp, Richard, Goupy, & Nicolas, 1990). Son reported that among the compounds tested, oxalic acid, oxalacetic acid, ascorbic acid-2-phosphate, cysteine, glutathione, *N*-acetylcysteine, kojic acid and 4-hexyl resorcinol belonged to a group that showed the highest inhibitory activity on apple browning (Son, Moon, & Lee, 2001).

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