バナナ追熟中における果皮のフェノール化合物とポリフェノールオキシダーゼ活性の変化
Changes in concentrations of phenolic compounds and polyphenol oxidase activity in banana peel during storage

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Phenolic compounds were analyzed in the outer, middle and inner layers of the peel and in the pulp of bananas during ripening from the ripening-initiation. The major phenolic compound found was dopamine followed by 3,4-dihydroxyphenylalanine (L-DOPA), tyramine and tyrosine in all portions of the bananas. These compounds located mainly in the outer layer of peel followed by the middle and inner layers of the peel and the pulp. The concentration of dopamine decreased during ripening in all portions of the fruit, particularly in the outer layer of the peel in which senescent spotting appeared at a late stage of storage. On the other hand, those of the other compounds, namely L-DOPA, tyrosine and tyramine increased during ripening. Polyphenol oxidase (PPO) activity was detected in all portions of the fruit; however, it increased only in the outer layer of the peel. Short-term (24h) nitrogen-gas treatment of the ripening-initiated banana delayed the induction of senescent spotting and the decrease in dopamine content, while it increased the PPO activity of the nitrogen-gas-treated bananas to the same extent as that of air-treated bananas. These results indicate that dopamine is used as substrate of PPO for the formation of brown pigments of senescent spotting.

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Material and Methods

1. Plant material and preparations
Bananas (Musa cv. Sucrifer, locally known as kluai khai; diploid AA), a high export potential in Thailand, were harvested at commercial ripeness (80% maturity based on their shape and development) from Petchaburi in Western Thailand and transported to the postharvest laboratory (Kasetsart University, Kamphaeng Saen Campus) within 1 day after harvest. Banana hands were dipped for 2 min in 500 ppm ethephon, air dried, placed in plastic baskets in sealed polyethylene bags and stored in a cool room at 25°C for 24h to initiate ripening. They were then removed from the bags and stored at 25°C until they reached stage 3 (peel, more green than yellow).
2. Phenolic compounds in various portions of bananas during ripening at 20°C

After the ripening treatment, the banana hands (stage 3) were placed in corrugated cardboard boxes and stored in a cool room at 20°C. For phenolic compound (e.g., tyrosine, tyramine, L-DOPA and dopamine) determination, the outer (surface tissues consist of epidermis and photosynthetic parenchymatous cells), middle and inner layers of the peel and outer portion of the pulp of the bananas were periodically sampled at 3 ~ 4 -days intervals for up to day 7. PPO activities were analyzed from frozen stocks of these samples.

Phenolic compounds were extracted from a sample tissue (1 g) with 0.1M perchloric acid containing 4 mM sodium bisulfite. The compounds were analyzed by HPLC (Shimadzu LC-10 ADvp) with a column ODS C18 YMC (size, 250.0 x 4.6 mm ID). The mobile phase was prepared with a mixture of 0.02 M potassium phosphate monobasic (pH 7.0) and 60% methanol in a ratio of 66:34, and pumped at 1.0 mL/min. The wavelength at 280 nm was monitored by a SPD-10 Avp detector (Shimadzu).

3. PPO assay

Procedure for PPO assay was modified from that of YANG et al. A sample tissue (2 g) was ground in 8 mL of 0.1 M phosphate buffer (pH 7.0) and 0.1 g of polyvinylpyrrolidone with a chilled mortar and pestle. The homogenate was filtered through four layers of gauze and centrifuged at 13,000 rpm for 20 min at 4°C. During the preparation of the enzyme extract, the temperature was maintained at 4°C. The supernatant was used to determine PPO activity by a colorimetric method. The assay medium contained 1.9 mL of 0.1M phosphate buffer (pH 7.0), 0.5 mL of 0.02M dopamine, and 0.1 mL of crude enzyme extract. The reference cuvette contained 2.4 mL of 0.1M phosphate buffer (pH 7.0) and 0.1 mL of enzyme extract. PPO activity was determined from measuring the increase in the absorbance of the mixture at 420 nm (1 cm light path) 30 sec after adding the enzyme extract. Since preliminary experiments showed that the absorbance increased linearly until 30 sec, a reaction time of 30 sec was selected for measurement.

To determine the substrate specificity of PPO by the method of MONTGOMERY and SGARBERT, L-DOPA and tyrosine were used as substrates. The reaction mixture consisted of 2.9 mL of 5 mM L-DOPA or 5 mM tyrosine, 0.1 mL of crude enzyme extract and 0.1 mL of 0.1M phosphate buffer (pH 7.0). The reference cuvette contained 2.9 mL of the same substrate solution and 0.1 mL of 0.1M phosphate buffer. The absorbance of the mixture at 420 nm was measured 30 sec after adding the enzyme.

One unit of the enzyme activity was defined as an increase in the absorbance of the mixture at 420 nm per minute per milliliter of the enzyme solution.

4. Nitrogen (N2)-gas treatment

After the ripening treatment, the banana hands (stage 3, approx. 1,100 g) were separated into two parts. One half were kept in a 23-liter vacuum desiccator with 100% N2 gas for 24h. The other half were treated with air as control. During these treatments, the O2 concentrations in the desiccators for N2 and air treatments were 0.1~0.3 and 17~21%, respectively. The bananas were placed in a room at 20°C after N2 or air treatment to assess the PPO activity in the banana peel (outer and inner layers) and pulp at 1 ~ 2-day intervals for up to day 8.

5. Protein determination

Protein concentration was determined by the method of BRADFORD, using bovine serum albumin as standard (BSA 0 ~ 100 μg/mL). The enzyme solution (1.0 mL) was mixed with 4.0 mL of 0.01% Coomassie blue. After incubating the mixture at room temperature (27°C) for 20 min, its absorbance at 595 nm was measured. Specific PPO activity was expressed as units per milligram protein.

6. Statistical analysis

The treatments were carried out in triplicate. Each replicate consisted of 2 ~ 3 hands (4 ~ 5 fruits/cluster or hand). For the analysis of phenolic compounds and PPO, 8 ~ 15 individual fruits were used from various hands or clusters for each treatment. The experiments were carried out 2 ~ 3 times. Data were subjected to analysis of variance using the Statistical Analysis System (SAS).

Results and Discussion

1. Phenolic compounds in different layers of peel and in pulp

The concentrations of phenolic compounds were determined in different layers of the peel and in the pulp. Evaluation was conducted before the ripening treatment and 4 or 7 days after the ripening treatment. Samples on day 4 and 7 showed a
yellow peel with mild and severe senescent spotting, respectively.

The bananas contained higher concentrations of phenolic compounds (e.g., dopamine, L-DOPA, tyrosine and tyramine) in the outer layer of the peel, followed by the middle and inner layers and the pulp (Fig. 1). The concentration of dopamine, the major component, decreased during ripening, while these of the other phenolic compounds generally increased in each layer of the peel and in the pulp during ripening except in the middle layer of the peel on day 7. At this late stage, the middle layer of the peel could not be distinguished from the other layers.

Only in the outer layer of the peel where higher concentrations of phenolic compounds than in the other portions were observed, senescent spotting symptom was observed. The concentrations of tyrosine and L-DOPA, which are precursors of dopamine (Fig. 2), increased during ripening whereas that dopamine decreased. The decrease in dopamine concentration correlated with the induction of senescent spotting, and the lost dopamine might have been used as substrate for brown spotting in the outer layer of the peel.

2. PPO activities in peel and pulp with different substrates

Diphenols (e.g., dopamine and L-DOPA) were oxidized by PPO, but monophenols such as tyrosine was not oxidized from any portions of the bananas. No reaction with tyrosine at 2.5~5.0 mM was observed up to 3 min after mixing the enzymes of the peel and the pulp (data not shown). However, Montgomery and Scabrier reported that tyrosine is oxidized at a very low rate by pulp extracts and but not by peel extracts. They also reported that PPO from banana peel reacted with catechol, and that the PPO activity in the peel is less than that in the pulp. The PPO activities in the outer layer of the peel of Sucrèr bananas with both dopamine and L-DOPA as substrates gradually increased during ripening, and became higher than those of the inner layer of the peel and of the pulp (Fig. 3). The enzyme activity was lower with L-DOPA than with dopamine substrate by about 30~60%, 20~40% and 10~20% in the outer and inner layers of the peel and in the pulp, respectively, during ripening. However, Yang et al. found that the relative PPO activities with L-DOPA in the peel and pulp extract are 8 and 12%, respectively, of dopamine.

Fig. 1 Phenolic compounds (mg/100 gFW) in peel and pulp of banana during ripening

X means on day 7, when middle layer of peel could not be distinguished from other layers. “Before” means that the sample was extracted before ripening treatment.

Fig. 2 Dopamine synthesis pathway in banana

(Yang et al., 1996)
as substrate. The difference between our result from YANG et al.'s may be because of the difference in the variety of bananas used.

The above results showed a strong association between increasing PPO activity and senescent spotting in the outer layer of the peel at a late stage of ripening, which is the same as that of CHOEHOM et al. As shown in Fig. 3, the PPO activity with L-DOPA in the outer layer of the peel was high (about 30 ~ 60% of the activity with dopamine in the outer layer of peel) and L-DOPA has the second highest concentration among phenolic compounds in bananas. Therefore L-DOPA might have a role in senescent browning, although dopamine is a major substrate of PPO.

The protein concentration in the outer layer of peel was higher than that in the inner layer; however, both increased from the day before treatment up to day 7. Therefore, the specific PPO activities in all layers of the peel and the pulp are almost identical (data not shown).

3. PPO activity from different layers of peel and pulp treated with N\textsubscript{2} gas

During ripening, PPO activity increased both in the peel and pulp, although the PPO activities in the inner layer of the peel and the pulp were lower than that in the outer layer of the peel. Both air and N\textsubscript{2}-gas-treated bananas increased their PPO activities during ripening to the same extent (Fig. 4). N\textsubscript{2}-gas-treated bananas have a delayed appearance of senescent spotting and extended shelf life up to 5 ~ 6 days compared with 3 ~ 4 days for air-treated bananas (ROMPHOPHAK et al). Therefore, an increase in PPO activity in the banana peel is not directly related to the severity of senescent spotting, although presence of PPO is important for the browning of the spots during such spotting.

**Conclusions**

Dopamine concentration in the outer layer of the banana peel decreased as senescent spots developed during ripening. Detailed analysis of the phenolic compounds in the peel and pulp revealed that dopamine is the major compound against L-DOPA, tyrosine and tyramine, and it is located mainly in the outer layer of the peel in which senescent spotting occurs. Interestingly, only dopamine decreased in concentration in all layers of the peel; the other phenolic compounds increased their concentrations in the peel. PPO activity was also found to be located mainly in the outer layer of the peel, and it increased during ripening. When bananas were treated with oxygen at a low concentration, development of the senescent spotting was generally delayed. However, PPO equally developed its activity in air and under low-O\textsubscript{2} conditions.

The above result implies that dopamine is the main source of brown pigments in senescent spotting. However, from the results of low-O\textsubscript{2} treatments, PPO development in the outer layer of the banana peel during ripening did not explain the initiation of senescent spotting. Some other factors are necessary in the reaction of PPO with dopamine for the initiation of senescent spotting.

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