Study of Senescent Spotting of Banana Peel

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Senescent spots which developed on the yellow banana peel at later shelf storage occur as physiological denature. The initiation and development of the spotting were investigated by microscopic observations and change of phenolic content. Several layers of cells from the surface of banana peels showed browning from the vertical view, and brown particles in the cells were observed from the horizontal view by light microscopy. Scanning electron microscopy (SEM) revealed that the browning part of spots are sunken like craters from normal cell area and stomata were located in their center. Dopamine content of outmost peel was more abundant than the other parts of peel and pulp. However the content of dopamine in the area decreased during ripening, particularly in the area of senescent spots.

Banana peel browning during senescence, which is called senescent spotting or sugar spotting, is observed just after bananas become fully ripened and their taste reaches the maximum acceptability. However, the phenomenon prevents consumer's choice when such bananas decorate the shelf of the retail market. Senescent spotting has been studied by many researchers. These indicate that senescent spotting is a physiological disorder, not a pathogenic disease. Spots produced can be visualized by phenol polymerization catalyzed by polyphenoloxidase. KANAZAWA and SAKAKIBARA reported that dopamine is a powerful antioxidant in bananas and that dopamine level in bananas depends on their ripening stage. ROMPHOPHAK et al. also suggested that dopamine level in bananas negatively correlates with spotting. Other phytochemicals, such as dopa and tyrosine, had also been determined in bananas at various ripening stages. So far, the relationship between initiation of senescence spotting and phenolic compound remains to be elucidated.

In this study, we investigated development of the senescent spotting by morphological observation with two kinds of microscopes for the first time and by analyzing the phenolic compound at spotting and normal area in the banana peel.

Materials and Methods

1. Material and storage conditions
Mature green bananas (Musa sapientum L.) imported from Ecuador, were purchased from a wholesaler before ethylene treatment. The bananas were treated with 1,000 ppm ethylene for 24 hours and stored in perforated (6 mm) polyethylene bags at 20 ºC. The bananas were observed at 2-day intervals up to 11 days after ethylene treatment.

2. Observation of brown spots by optical microscopy and scanning electron microscopy (SEM) during senescence
The outer tissue from the surface of a banana peel of 0.5 mm thickness with or without senescent spots was observed by optical microscopy (IX 70 (Olympus) equipped with a CCD camera (DP 11 - N, Olympus)). Small senescent spots (about 2 mm in diameter) were chosen for this observation.

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cross sections of the banana peel that showed senescent spotting were also observed.

For SEM (S-800, Hitachi) a banana surface peel was cast in Impregum and the mold was treated with epoxy resin and Debucon before coating with platinum (Pt) and lead (Pd) vapor. The banana peel was observed at 2-day intervals up to 9 days after the ripening treatment. When senescent spots appeared, we tried to sample from small senescent spots as small as possible to observe the initial stage of spotting.

3. Controlling brown spots by sanitizing peel with ethanol

Bananas were dipped in 50% ethanol for 30 sec everyday and dried in air during storage at 20°C.

4. Dopamine (3, 4-dihydroxyphenylethylamine), L-DOPA (3, 4-dihydroxyphenylalanine) and tyrosine analyses of banana surface peel during ripening

10 mg of the surface portion of a banana peel (0.5 mm depth) was macerated with 10 ml of 0.1 M perchloric acid containing 4 mM sodium bisulfite. The resulting mixture was centrifuged at 15,000 g for 15 min and then passed through a Millipore filter (hydrophilic type, pore size 0.45 μm). Twenty μl of the filtrate was injected through a sample loop into HPLC (Shimadzu LC-9 A) equipped with an Inertsil ODS-2 column (size, 250.0 × 4.0 mm ID).

The mobile phase used was prepared with a mixture of 0.02 M potassium phosphate (monobasic) containing 1 g/l of heptanesulfonic acid sodium salt, adjusted to pH 3.3 with phosphoric acid, and 60% methanol in a 66 : 34 ratio; it was pumped at 0.8 ml/min. The excitation beam was used at 290 nm and fluorescence at 330 nm was monitored by a photofluorescent detector (FP - 210)7.

5. Volatile-compound treatment of peel after separation from pulp

The peels of bananas at the full-yellow stage but showing no senescence spots were separated from the banana pulp, placed in a petri dish at 20°C for observation of spot formation in the browning region. Thirteen chemical compounds (5 ~ 100 μl) that have been detected in bananas fruit were placed in the bottle and covered with the separated banana peel fixed with false-tooth gum, and browning symptoms were observed on the peel.

Result

1. Observation by optical microscopy

The peel changed from green to light yellow after 2 days of ethylene treatment. After 7 ~ 9 days, the peel began to develop senescent spots
after 11 days, the peel became thin and developed senescent spots severely.

On day 9, a 2-mm-diameter portion of a senescent spot on the banana peel was cut vertically and observed by optical (light) microscopy. The browning part was about 0.25 mm in depth, located within 7 ~ 8 layers of cells from the surface. Therefore, the senescent spot was only observed at the upper layer of the peel (Fig. 1 a, b). When cells from a horizontally sliced were observed at browning spots by optical microscopy, 2 ~ 3 large brown particles in the cells were observed (Fig. 1 d), no such particles were observed in cells from a normal part of a banana peel (Fig. 1 c). The above observation shows that cells in senescent spots are not uniformly brown but contains brown particles.

2. Observation SEM

The observation of the peel surface by SEM revealed that the surface was covered with rectangular cells like tiles of similar sizes, and with stomata arranged 300 ~ 1,000 μm apart in the early stage of storage (Fig. 2 a, c). However, 9 days after the ethylene treatment, some wilting and wrinkled structures were observed in the cells (Fig. 2 b, d). The browning part sunk compared with the normal-cell area forming craters and more wilted cells were observed in the crater on day 7 (Fig. 3 a, b). Fig. 3 c, d showed a more expanded senescent spot on day 9. At the center of each small senescent spot, stomata were clearly observed, most of kept typical stomatal features. When senescence of the peel proceeded, all senescent spots coalesced to form a large brown area at the overripe stages. No hyphae were observed on the area of senescent

Fig. 2  Surface view of healthy cells in yellow-banana peel by SEM after 1 day (a, c) and 9 days (b, d) of storage
spotting by SEM.

3. Sanitizing of peel by ethanol
   Bananas were dipped in 50% ethanol for 30 sec and dried everyday to avoid infection by microorganisms during storage. There is no difference in the initiation of browning between ethanol- and non-ethanol-treated bananas. Senescent spotting started at the same time on day 7 and developed to the same extent thereafter (photo not shown).

4. Phenolic compound of banana peel
   Tyrosine and L-DOPA were not detected in both browning and normal areas in the surface part of the peel. A fourfold lower dopamine content was detected in the browning tissue than in the normal tissue (Fig. 4).

5. Browning with volatile chemicals
   The full-yellow-banana peels that were separated from the banana pulp 6 days after ethylene treatment when browning has not yet occurred...
were stored for 10 days at a high humidity. Senescent spots did not develop on the peels even after 7 days from peeling, as observed in the two replication samples shown in Fig. 5.

The volatile chemicals (5~100 µl) reported earlier were used to treat separated surface peels described in Materials and Methods. Many chemicals affected on peel browning (Table 1). Browning on the peels appeared in a much wider area than spotting. Typical senescent spots on the peels did not appear in all cases.

**Discussion**

Fungal hyphae were not detected by microscopic observation, and daily ethanol treatment had no effect browning. These results reinforced that this browning was physiological senescence. Ketsa (2000) reported the preharvest and postharvest applications of benomyl, carbendazim and prochloraz do not control senescent spotting in bananas.

Spotting parts showed lower dopamine content than surrounding normal part in the peel. This result indicated the relationship of the decrease of dopamine content and the increase in the number of senescent spots during ripening.

When spotting peel surfaces were observed by SEM (Fig. 3), they appeared sunken and contained craters. Stomatal structures were observed at the center of these craters. It seems that senescence browning is initiated in stomata. Interestingly, the full-yellow-banana peels separated from the banana pulp did not show senescent spots 7 days after

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**Table 1** Effect of chemicals on peel browning

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(%) means percentage of browning area on peel surface.
peeling. One hypothesis formed from the above results is as follows: Volatile compounds from the banana pulp leak out through stomata the banana peel at the senescence stage and converts polyphenols to a brown pigment brown spots. The model test on pure volatile compounds in bottles covered with separated peels showed that many chemicals that were seen in banana volatile caused browning in the cover peels (Fig. 5). However, browning did not show typical spotting. Further study is needed in elucidating the relation between senescent spotting and volatile compounds in bananas.

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References