Change of Polyphenol Compounds in Banana Pulp during Ripening

MURA Kiyoshi*1 and TANIMURA Wahachiro*1

*1 Faculty of Applied Bioscience, Tokyo University of Agriculture
1-1-1, Sakuragaoka, Setagaya-ku, Tokyo 156–8502

In mature green bananas before ripening, 103mg/100g of polyphenol compounds were contained in the pulp and about 60% of those polyphenol compounds have a MW above $2 \times 10^5$ and an astringency. These high molecular weight polyphenol compounds produced cyanidin, delphinidin and catechin on hydrolysis with HCl and were proanthocyanidins consisting of leucoanthocyanidin and catechin. The changes in polyphenol compounds during ripening have been studied with separating by ultrafiltration and ion-exclusion chromatography and the result showed that the polyphenol compounds with a MW above $2 \times 10^5$ and an astringency disappeared during ripening. However, no change was seen in those polyphenol compounds with a MW below $2 \times 10^5$. These results suggest that the disappearance of astringency by ripening treatment is resulted from the polymerizing insolubilization of polyphenol compounds with a MW above $2 \times 10^5$, the astringent components.

In Japan, bananas are fruits greatly consumed next to citrus and apples and most of them are imported from the tropical regions like the Philippines, Taiwan and Ecuador. Bananas are harvested as mature green fruits in the producing area, packed into cases and shipped to Japan under a low temperature about 13°C. After arriving in Japan, the bananas are treated with hydrogen cyanide gas to prevent epidemics, then unloaded and transported to various places by cooling cars. Ripening treatment is usually carried out in a place near the consumers. A ripening room is kept in a temperature about 20°C and a relative humidity of 90~95%. Ripening is performed for several days by treating the bananas with ethylene for a half or one day and then leaving till the peels become yellow and the bananas edible.

There is a strong astringency in the pulp of mature green bananas before ripening and this astringency is resulted from the polyphenol compounds contained plentifully in the idioblasts called tannin cells. It is considered that these polyphenol compounds in tannin cells become insoluble and the astringency in the pulp disappears with the progress of ripening2. In this study, we investigated in detail the change of polyphenol compounds in the pulp of mature green bananas during ripening and the relationship between astringency disappearance and change in polyphenol compounds.

Materials and Methods

1. Bananas and ripening treatment
Mature green bananas imported from the Philippines were used as materials and ripening treatment was carried out at 20°C in a room of constant temperature.

2. Measurement of chromaticity of peels
The chromaticity of banana peels was measured in eight levels from level 1 of dark green immature fruits to level 8 of dark yellow fully mature ones according to the Banana Chromaticity Table (KyoKuto Fruit Co., Ltd.).

3. Measurement of astringency in pulp
The astringency in banana pulp was measured by tasting test in five levels from level 1 of mature fruits almost without astringency to level 5 of immature ones with a strong astringency.

4. Extraction of polyphenol compounds
To the banana pulp which has been cut into small pieces, 10 volumes of 80% ethanol was added immediately and the mixture was extracted under reflux for 30 min in boiling water bath. Extraction was repeated for three times. The combined extract...
was filtrated, and then concentrated under reduced pressure below 40°C to remove ethanol. The concentration was regulated as the need arises and the solution was used as polyphenol extract.

5. Fractionation of polyphenol compounds by ultrafiltration

Using ultrafilter of Q 2000 (MW cutoff: $2 \times 10^5$) from Advantec Toyo Co., Ltd. (Tokyo), the polyphenol extract was filtrated under a nitrogen gas pressure of 4 kg/cm² and fractionated into two fractions of MW above $2 \times 10^5$ and MW below $2 \times 10^5$.

6. Separation of polyphenol compounds by CM-Sepharose CL-6 B

Polyphenol fractions were applied to a column made by connecting two columns of 2.5×90cm filled with CM-Sepharose CL-6 B and elution was performed using 0.08 M borate buffer (pH 7.8). The polyphenol compounds in the eluate were colored by the method of Folin-Denis and then determined by the absorbance at wavelength of 700 nm.

7. Determination of polyphenol compounds

Polyphenol compounds were determined by the method of Folin-Denis and calculated as D- (+) catechin.

8. TLC for polyphenol compounds

Polyphenol compounds were separated by two-dimensional thin-layer chromatography (TLC) using a HPTLC cellulose plate (10×10cm) from MERK (Darmstadt, Germany). As development solvents, n-butanol-acetic acid-water (4:1:2.2 in volume ratio) and 2% acetic acid were used. After development, polyphenol compounds were detected by the blue color on the plate after spraying with ferricyanide-ferric chloride reagent. Besides, the developed plate was also sprayed with vanillin-HCl reagent and flavanol compounds were detected by the red color on the plate.

9. Identification of anthocyanidins

Anthocyanidins were separated by TLC using a HPTLC cellulose plate. Three kinds of solvents, acetic acid-concentrated HCl-water (30:3:10 in volume ratio), n-butanol-acetic acid-water (4:1:5 in volume ratio, upper layer) and n-butanol-2 N HCl (1:1 in volume ratio, upper layer) were used in development.

The anthocyanidins separated by TLC developed in acetic acid-concentrated HCl-water were scratched up from the plate, eluted in 0.01% HCl-methanol and 0.01% HCl-ethanol respectively, and the absorption spectra of the eluates were measured. In addition, 4 ml eluate of 0.01% HCl-ethanol was mixed with 3 drops of 5% AlCl₃-ethanol and its absorption spectrum was measured again to calculate the shift of spectral maximum caused by the addition of AlCl₃.

Results and Discussion

1. Polyphenol compounds in banana pulp before ripening

The polyphenol compounds extracted from the pulp of mature green bananas before ripening were fractionated by ultrafiltration into two fractions of MW above $2 \times 10^5$ and MW below $2 \times 10^5$, and the components of polyphenol in the two fractions were determined (Table 1). In the pulp of mature green bananas before ripening, 103mg/100g of polyphenol compounds were detected and about 60% were those with a rather high molecular weight above $2 \times 10^5$ and the other 40% were those with a MW below $2 \times 10^5$. The astringency in the two fractions was tested and a strong astringency was found in the fraction of MW above $2 \times 10^5$ but nearly no astringency was sensed in the fraction of MW below $2 \times 10^5$, indicating that the astringency components in the mature green bananas were polyphenol with a rather high molecular weight above $2 \times 10^5$.

Photo 1 shows the TLC results of polyphenol compounds contained in the pulp of mature green bananas before ripening. Most of the polyphenol compounds did not move from the starting point but spot I was consistent with the position of dopamine. Dopamine is the main component of low molecular weight polyphenol in banana pulp and is considered to be a major substrate of enzymatic browning.

### Table 1 Polyphenol content of banana pulp before ripening

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Content (mg/100g)</th>
<th>Fractionation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>103.0</td>
<td>100</td>
</tr>
<tr>
<td>MW above $2 \times 10^5$</td>
<td>61.0</td>
<td>59.2</td>
</tr>
<tr>
<td>MW below $2 \times 10^5$</td>
<td>41.5</td>
<td>40.3</td>
</tr>
</tbody>
</table>

* concentration of dry weight.
Photo 1  Two-dimensional cellulose thin-layer chromatogram of polyphenol compounds in the pulp of mature green bananas before ripening.

Photo 2  Hydrolyzate of the fraction of MW above $2 \times 10^5$.

Hydrolysis of the fractions of MW above $2 \times 10^5$ was performed with 2 N HCl in boiling water for 30 min. Polyphenol compounds were detected by the blue color on the plate after spraying with potassium ferricyanide-ferric chloride reagent.

Photo 3  Two-dimensional cellulose thin-layer chromatogram of polyphenol compounds released by the hydrolysis of the fraction of MW above $2 \times 10^5$.

Hydrolysis of the fractions of MW above $2 \times 10^5$ was performed with 2 N HCl in boiling water for 30 min. Polyphenol compounds were detected by the blue color on the plate after spraying with potassium ferricyanide-ferric chloride reagent.

Photo 4  Two-dimensional cellulose thin-layer chromatogram of flavanol compounds released by the hydrolysis of the fraction of MW above $2 \times 10^5$.

Hydrolysis of the fractions of MW above $2 \times 10^5$ was performed with 2 N HCl in boiling water for 30 min. Flavanol compounds were detected by the red color on the plate after spraying with vanillin-HCl reagent.
The polyphenol compounds extracted from the pulp of mature green bananas before ripening were fractionated by ultrafiltration into two fractions of MW above $2 \times 10^5$ and MW below $2 \times 10^5$ and the two fractions were further separated by ion-exclusion chromatography (Fig. 1). In the fraction of MW above $2 \times 10^5$, the polyphenol compounds were eluted in a peak and a strong astringency was found in this peak.

2. Hydrolysates of polyphenol compounds with a MW above $2 \times 10^5$

The astringency components in unripe bananas are considered to be high molecular weight proanthocyanidins consisting of leucoanthocyanidin and catechin. We hydrolyzed the polyphenol compounds that have a molecular weight above $2 \times 10^5$ and an astringency using HCl and investigated the hydrolysis products. Hydrolysis was carried out by adding 2 N HCl to the freeze-dried fraction of MW above $2 \times 10^5$ and heating in boiling water bath for 30 min. As shown in Photo 2, the hydrolyzate was red in color and a red-brown precipitate was produced. Low molecular weight polyphenol compounds produced by hydrolysis were detected by TLC (Photo 3). Spot I was a purple pigment and spot II a red one and both became blue after spraying with potassium ferricyanide-ferric chloride reagent, suggesting that both of them were anthocyanidins. Besides, in the polyphenol compounds detected by potassium ferricyanide-ferric chloride reagent, other three major spots have been found in addition to the above two ones of anthocyanidins and the spot III was similar to D-(+) -catechin. Then detection of flavanol compounds was performed by spraying the plate with vanillin-HCl reagent. The spot III became red, confirming that it was a flavanol compound and possibly a catechin (Photo 4).

As the spot I and spot II on TLC have been confirmed to be anthocyanidins, we further identified by the methods of Harborne and Bate-Smith (Table 2). According to the Rf value in TLC in various solvents, the spectral maximum in HCl-methanol and the shift of spectral maximum by AlCl₃ addition, the purple spot I was identified to be delphinidin and the red spot II to be cyanidin. By heating in HCl, leucoanthocyanidin changes to anthocyanidin, leucodelphinidin to delphinidin and leucocyanidin to cyanidin respectively. As anthocyanidins have been produced in the hydrolysis with HCl, the polyphenol compounds with

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Visible color</th>
<th>Rf values</th>
<th>Spectral maximum in MeOH-HCl (nm)</th>
<th>AlCl₃ shift (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>purple</td>
<td>0.33</td>
<td>0.42 0.16</td>
<td>552</td>
</tr>
<tr>
<td>II</td>
<td>magenta</td>
<td>0.51</td>
<td>0.68 0.25</td>
<td>539</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>purple</td>
<td>0.32</td>
<td>0.42 0.13</td>
<td>546</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>magenta</td>
<td>0.49</td>
<td>0.68 0.22</td>
<td>535</td>
</tr>
</tbody>
</table>

* acetic acid-conc.HCl-water (30 : 3 : 10).
** n-butanol-acetic acid-water (4 : 1 : 5, upper layer).
*** n-butanol-2 N HCl (1 : 1, upper layer).
MW above $2 \times 10^5$ were thought to contain leucoanthocyanidins and to be proanthocyanidins consisting of leucoanthocyanidin and catechin. In addition, the astringency component in unripe bananas was estimated to be proanthocyanidins with a rather high molecular weight above $2 \times 10^5$.

3. Change of polyphenol compounds in banana pulp during ripening

We performed ripening of mature green bananas at 20°C and have investigated the relation between astringency disappearance and the change in polyphenol compounds with the progress of ripening. Table 3 shows changes in the chromaticity of peels, the astringency, moisture and polyphenol content of the pulp during ripening. On the third day of ripening, the chromaticity of peels was level 3, not so much progress, but the polyphenol content in the pulp decreased to about 50% of that before ripening and the astringency decreased to level 2. On the eighth day of ripening, the chromaticity of peels increased to level 8 of fully mature and nearly no astringency was felt in the pulp. However, more than 40% of the polyphenol compounds had remained and no change was seen in the moisture in the pulp during ripening.

The polyphenol compounds in the pulp before and after ripening were fractionated by ultrafiltration into two fractions of MW above $2 \times 10^5$ and MW below $2 \times 10^5$ and determined respectively. Table 4 shows the results. In the pulp after ripening, more than 40% of polyphenol compounds had remained but most of them were polyphenol compounds with a MW below $2 \times 10^5$ and without astringency. The polyphenol compounds with a MW above $2 \times 10^5$ and an astringency have almost disappeared. The polyphenol compounds from the pulp before ripening, on the third day, and on the eighth day of ripening were separated by ion-exclusion chromatography and their changes were shown in Fig.2. The peak of fraction No.150 seen in the pulp before ripening was the polyphenol components with a MW above $2 \times 10^5$ and an astringency and this peak has almost disappeared on the third day of ripening. Nevertheless, no great change was seen during ripening in the peaks of polyphenol compounds with a MW below $2 \times 10^5$. With the progress of ripening, the polyphenol compounds of MW above $2 \times 10^5$ disappeared whereas no increase was seen in the polyphenol compounds of MW below $2 \times 10^5$. This result indicates that the

<table>
<thead>
<tr>
<th>Ripening period (days)</th>
<th>Peel</th>
<th>Pulp</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chromaticity*</td>
<td>Astringency**</td>
<td>Moisture (%)</td>
<td>Polyphenol (mg/100 g) ***</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>5</td>
<td>76.0</td>
<td>103.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>2</td>
<td>75.2</td>
<td>53.2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>1</td>
<td>76.1</td>
<td>44.2</td>
<td></td>
</tr>
</tbody>
</table>

* chromaticity was graded in 8 degrees (1 ~ 8).
** astringency was graded in 5 degrees (1 ~ 5).
*** concentration of dry weight.

<table>
<thead>
<tr>
<th>Ripening Period (days)</th>
<th>Fraction</th>
<th>Polyphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Content (mg/100 g)*</td>
<td>Fractionation (%)</td>
</tr>
<tr>
<td>extract</td>
<td>103.0</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>MW above $2 \times 10^5$</td>
<td>61.0</td>
</tr>
<tr>
<td></td>
<td>MW below $2 \times 10^5$</td>
<td>41.5</td>
</tr>
<tr>
<td>extract</td>
<td>44.2</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>MW above $2 \times 10^5$</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>MW below $2 \times 10^5$</td>
<td>40.0</td>
</tr>
</tbody>
</table>

* concentration of dry weight.
disappearance of astringency with ripening is due to the polymerizing insolubilization but not to the decomposition of polyphenol compounds with a MW above 2×10^5 and an astringency.

References
3) FOLIN, O. and DENIS, W.: A colorimetric method for the determination of phenols (and phenol derivatives) in urine, J. Biol. Chem., 22, 305 (1915)
11) NISHIKA, I.: Chemistry of tannin — recent studies, KASEAA, 42, 48 (1986)

Fig. 2 Ion-exclusion chromatograms of the extracts of banana pulp during ripening on CM-Sepharose CL-6B

The extracts were put on a CM-Sepharose CL-6B column (2.5 × 90 cm and 2.5 × 90 cm) and eluted with 0.08 M borate buffer, pH 7.8. Polyphenols were detected using FOLIN-DENIS method. A: before ripening, B: after ripening for 3 days, C: after ripening for 8 days.