バナナ追熟過程のポリフェノール成分の変化

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Change of Polyphenol Compounds in Banana Pulp during Ripening

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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 2×10^5 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 2×10^5 and an astringency disappeared during ripening. However, no change was seen in those polyphenol compounds with a MW below 2×10^5. These results suggest that the disappearance of astringency by ripening treatment is resulted from the polymerizing insolubilization of polyphenol compounds with a MW above 2×10^5, the astringent components.

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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℃. 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℃ 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 ripening[1]. 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℃ 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

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was filtrated, and then concentrated under reduced pressure below 40℃ 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 : 2×10^5) from Advantec Toyo Co., Ltd. (Tokyo), the polyphenol extract was filtrated under a nitrogen gas pressure of 4 kg/㎠ and fractionated into two fractions of MW above 2×10^5 and MW below 2×10^5.

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

Polyphenol fractions were applied to a column made by connecting two columns of 2.5×90㎝ filled with CM-Sepharose CL-6B and elution was performed using 0.08 M borate buffer (pH 7.8)[2]. The polyphenol compounds in the eluate were colored by the method of FOLIN-DENIS[3] and then determined by the absorbance at wavelength of 700 ㎚.

7. Determination of polyphenol compounds

Polyphenol compounds were determined by the method of FOLIN-DENIS[3] 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 (10×10㎝) 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 potassium

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[4].

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)[5], *n*-butanol-acetic acid-water (4 : 1 : 5 in volume ratio, upper layer)[5] and n-butanol-2 N HCl (1 : 1 in volume ratio, upper layer)[6] 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 ㎖ eluate of 0.01% HCl-ethanol was mixed with 3 drops of 5% $AlCl_3$-ethanol and its absorption spectrum was measured again to calculate the shift of spectral maximum caused by the addition of $AlCl_3$[7].

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

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[8].

Table 1 Polyphenol content of banana pulp before ripening

Fraction	Polyphenol	
	Content (㎎/100 g)*	Fractionation (%)
Extract	103.0	100
MW above 2×10^5	61.0	59.2
MW below 2×10^5	41.5	40.3

* concentration of dry weight.

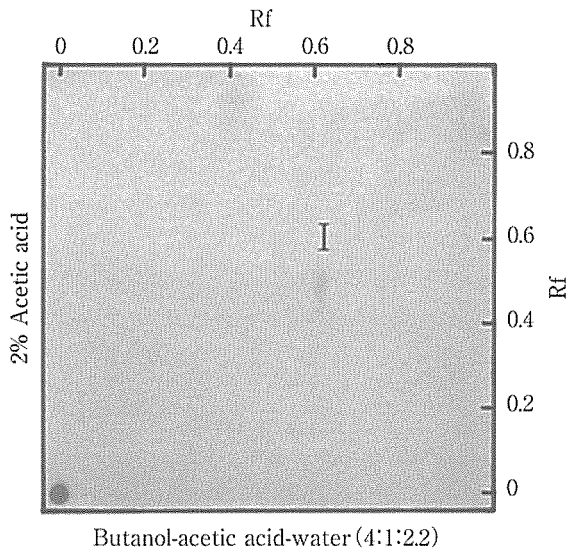

Photo 1 Two-dimensional cellulose thin-layer chromatogram of polyphenol compounds in the pulp of mature green bananas before ripening

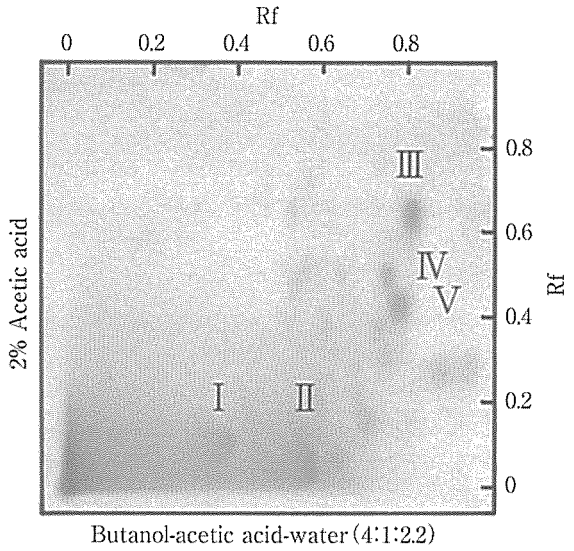

Photo 3 Two-dimensional cellulose thin-layer chromatogram of polyphenol compounds released by the hydrolysis of the fraction of MW above 2×10^5

Hydrolysis of the fractions of MW above 2×10^5 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.

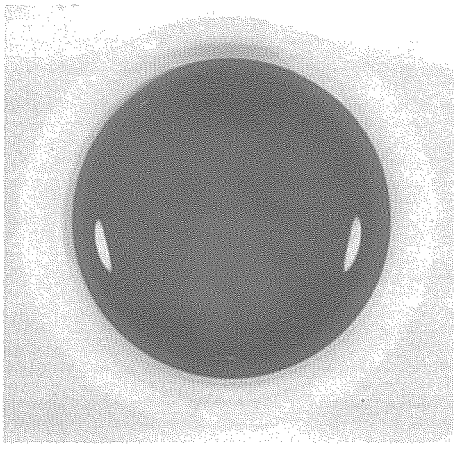

Photo 2 Hydrolyzate of the fraction of MW above 2×10^5

Hydrolysis of the fractions of MW above 2×10^5 was performed with 2 N HCl in boiling water for 30 min.

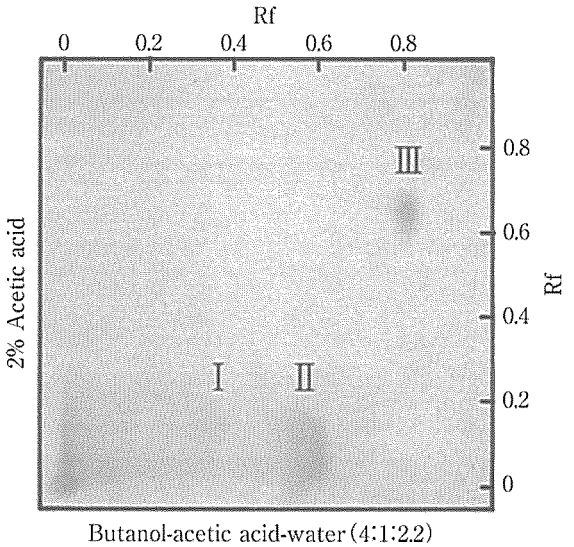

Photo 4 Two-dimensional cellulose thin-layer chromatogram of flavanol compounds released by the hydrolysis of the fraction of MW above 2×10^5

Hydrolysis of the fractions of MW above 2×10^5 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.

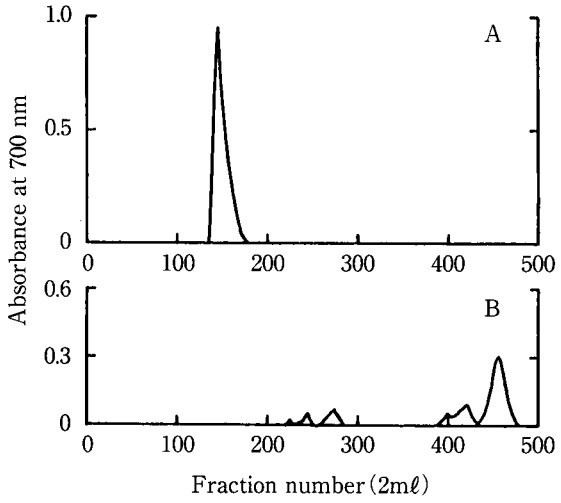

Fig. 1 Ion-exclusion chromatograms of fractions of MW above 2×10^5 and MW below 2×10^5 on CM-Sepharose CL-6B

The fractions were put on a CM-Sepharose CL-6B column (2.5×90cm and 2.5×90cm) and eluted with 0.08 M borate buffer, pH 7.8. Polyphenols were detected using FOLIN-DENIS method.
A : fraction of MW above 2×10^5,
B : fraction of MW below 2×10^5.

The polyphenol compounds extracted from the pulp of mature green bananas before ripening were fractionated by ultrafiltration into two fractions of MW above 2×10^5 and MW below 2×10^5 and the two fractions were further separated by ion-exclusion chromatography (Fig. 1). In the fraction of MW above 2×10^5, 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 2×10^5

The astringency components in unripe bananas are considered to be high molecular weight proanthocyanidins consisting of leucoanthocyanidin and catechin[9]. We hydrolyzed the polyphenol compounds that have a molecular weight above 2×10^5 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 2×10^5 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[5),7)] and BATE-SMITH[6)] (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 $AlCl_3$ 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[10]. As anthocyanidins have been produced in the hydrolysis with HCl, the polyphenol compounds with

Table 2 *R*f values and spectral maxima of the anthocyanidins released by hydrolysis of the fraction of MW above 2×10^5 in banana pulp before ripening

Pigment	Visible color	*R*f values			Spectral maximum in MeOH-HCl (nm)	$AlCl_3$ sift (nm)
		AHW*	BAW**	BHW***		
I	purple	0.33	0.42	0.16	552	27
II	magenta	0.51	0.68	0.25	539	20
Delphinidin	purple	0.32	0.42	0.13	546	23
Cyanidin	magenta	0.49	0.68	0.22	535	18

* 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 2×10^5 were thought to contain leucoanthocyanidins and to be proanthocyanidins consisting of leucoanthocyanidin and catechin[11]. In addition, the astringency component in unripe bananas was estimated to be proanthocyanidins with a rather high molecular weight above 2×10^5.

3. Change of polyphenol compounds in banana pulp during ripening

We performed ripening of mature green bananas at 20℃ 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 2×10^5 and MW below 2×10^5 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 2×10^5 and without astringency. The polyphenol compounds with a MW above 2×10^5 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 2×10^5 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 2×10^5. With the progress of ripening, the polyphenol compounds of MW above 2×10^5 disappeared whereas no increase was seen in the polyphenol compounds of MW below 2×10^5. This result indicates that the

Table 3 Change of the chromaticity of peels, the astringency, moisture and polyphenol content of banana pulp during ripening

Ripening period (days)	Peel	Pulp		
	Chromaticity*	Astringency**	Moisture (%)	Polyphenol (mg/100 g) ***
0	2	5	76.0	103.0
3	3	2	75.2	53.2
8	8	1	76.1	44.2

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

Table 4 Change of the polyphenol content of banana pulp during ripening

Ripening Period (days)	Fraction	Polyphenol	
		Content (mg/100 g)*	Fractionation (%)
0	extract	103.0	100
	MW above 2×10^5	61.0	59.2
	MW below 2×10^5	41.5	40.3
8	extract	44.2	100
	MW above 2×10^5	0.9	2.0
	MW below 2×10^5	40.0	90.5

* concentration of dry weight.

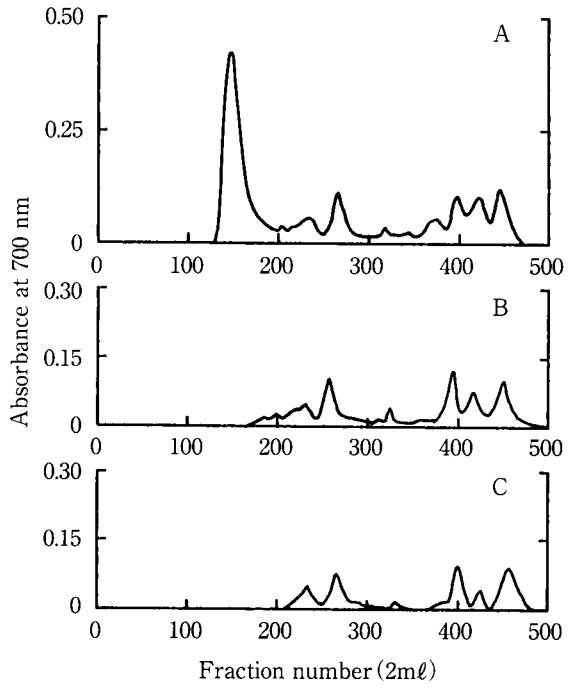

Fig. 2 Ion-exclusion chromatograms of the extracts of banana pulp during ripening on CM-Sepharose CL-6B

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.

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.

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バナナ追熟過程のポリフェノール成分の変化

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追熟前の緑熟果のバナナ果肉には103mg/100gのポリフェノール成分が含まれ，その約60%は分子量2×10^5以上のポリフェノール成分であり，その分子量2×10^5以上のポリフェノール成分が渋味成分であることが確認された。この渋味を呈するきわめて高分子のポリフェノール成分は，塩酸での加水分解によりシアニジン，デルフィニジン，カテキンなどを生成し，ロイコアントシアニジンやカテキンからなるプロアントシニジンであることが知られた。また，追熟過程におけるポリフェノール成分の変化を限外濾過法およびイオン排除クロマトグラフィーで分画して調べた結果，渋味を呈する分子量2×10^5以上のポリフェノール成分は追熟過程で消失したが，分子量2×10^5以下のポリフェノール成分は追熟過程での変化がみられず，追熟による渋味の消失は，渋味成分である分子量2×10^5以上のポリフェノール成分の重合による不溶化に起因することが示唆された。

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