

Antioxidant compounds from bananas (*Musa Cavendish*)

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Abstract

The antioxidant compounds from commercial bananas, *Musa Cavendish*, were studied. One of the antioxidants, gallo catechin, was identified in the banana. The gallo catechin was isolated (using HPLC) from the banana peel extract, which showed strong antioxidant activity. Gallo catechin was more abundant in peel (158 mg/100 g dry wt.) than in pulp (29.6 mg/100 g dry wt.). The antioxidant activity of the banana peel extract, against lipid autoxidation, was stronger than that of the banana pulp extract. This result was consistent with the gallo catechin analysis. The higher gallo catechin content may account for the better antioxidant effects. Thus, the antioxidant capacity of the bananas may be attributed to their gallo catechin content. Bananas should be considered as a good source of natural antioxidants for foods. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Bananas are one of the most popular fruits on the world market. It is well known that fruits contain various antioxidants, such as vitamin C, vitamin E, and β -carotene (Kanazawa & Sakakibara, 2000; Paul & Southgate, 1978). Antioxidant capacity of a fruit may be due to other antioxidants, such as flavonoids. Macheix, Fleuriet, and Billot, (1999) were interested in different types of antioxidants and studied total phenolic and tannin contents in banana pulp. However, the content of another flavonoid, catechin, in bananas has not yet been studied. A specific flavonoid, related to antioxidant activity of bananas, has neither been identified nor studied.

The objective of this study is to examine antioxidant activity in the commercial banana, *Musa Cavendish*. We also study and identify antioxidant compounds in the bananas.

2. Materials and methods

2.1. Materials

Musa Cavendish, commercial bananas, imported from Philippines, were purchased from local supermarkets. (–)-Gallo catechin, (+)-catechin, and (–)-epicatechin were purchased from Funakoshi Co., Ltd (Tokyo, Japan). Linoleic acid, gallic acid, ammonium thiocyanate, dimethyl sulfoxide-*d*₆ (DMSO-*d*₆), and iron (II) chloride were purchased from Nacalai Tesque Co. (Kyoto, Japan). All other reagents were of analytical grade.

2.2. Extraction

Fresh banana peel (300 g) was boiled in 900 ml of distilled water for 5 min. The peel was homogenized and extracted with water at 90 °C for 2 h. The extract was then concentrated to 300 ml of water extract. The extract was dissolved in water–chloroform (1:1, v/v) solution. The water phase was dissolved in water–ethyl acetate (1:1, v/v) solution. The ethyl acetate phase was concentrated and freeze-dried to give 3.07 g banana peel extracts. Banana pulp was treated similarly to give pulp extracts.

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2.3. Total phenolic analysis

The Folin–Denis method (Swain & Hillis, 1959) was used. Five millilitres of banana extracts and Folin–Denis reagent were mixed. After 3 min, 5 ml of 10% Na₂CO₃ were added and the mixture left at room temperature for 1 h. Absorbance was then measured at 760 nm and the result expressed in mg of (+)-catechin/100 g dry weight.

2.4. Antioxidant activities

Effects of the banana extracts on lipid autoxidation were evaluated using the ferric thiocyanate method. One milligramme per millilitre of peel extracts and pulp extracts, 0.5 mM of gallic acid, and distilled water, as control, were prepared as samples. Each sample (100 µl) was added to a mixture of 1.026 ml of ethanol containing 2.51% linoleic acid, 2.0 ml of 50 mM phosphate buffer (pH 7.0), and 0.974 ml of distilled water. The reaction mixture (100 µl) was used for an antioxidant assay with the method described by Inatani, Nakatani, and Fuwa (1983).

2.5. HPLC analysis

Antioxidant compounds were purified by HPLC (Shimazu LC-VP) (Shimazu Co. Ltd.) from the banana peel extracts: column, YMCA-pack ODS-AM323 (10×250 mm); mobile phase, 20% methanol; flow rate, 2.0 ml/min; column temperature was 35 °C; monitoring was with a UV detector set at 280 nm. The antioxidant compounds were concentrated and freeze-dried for further analysis.

HPLC analysis of the banana extracts was conducted on a YMCA-pack ODS-AM303 (4.6×250 mm) column with a flow rate of 0.9 ml/min.

2.6. MS spectroscopy

Molecular weight was determined using FAB-mass spectrometry (Jeol JMX HX-105) (Jeol Ltd.) with glycerol as a matrix.

2.7. NMR spectrometry

¹H, ¹³C, ¹H–¹H COSY, and ¹³C–¹H COSY NMR spectra of the sample dissolved in DMSO-*d*₆ were recorded on a NMR spectrometer (Varian Unity INOVA 500) (Varian, Inc.).

3. Results and discussion

3.1. Antioxidant activities

Total phenolics were more abundant in peel (907 mg/100 g dry wt.) than in pulp (232 mg/100 g dry wt.). This

result was consistent with the antioxidant activity. The peel extract showed 2.2 times stronger antioxidant activity than the pulp extract when the incubation times were compared (Fig. 1). The difference in the antioxidant activities between the peel extract and the pulp extract may be attributed to their phenolic contents.

3.2. Identification of antioxidants

Twenty microlitres of the sample (5 mg of banana peel extracts/1 ml of distilled water) were injected into the HPLC, and compounds A, B, and C were detected at 6.9, 17.2, and 41.5 min retention times (Fig. 2). The compounds A, B, and C in the banana peel extract corresponded with retention times of gallic acid, catechin, and epicatechin, respectively. The pulp extract was also analyzed similarly for the identification.

Gallic acid content in both peel and pulp were quantified by comparison to the respective standard chemicals. Gallic acid was more abundant in peel (158 mg/100 g dry wt.) than in pulp (29.6 mg/100 g dry wt.). This result was consistent with the total phenolic analysis and the antioxidant activity. The higher gallic acid content of the banana peel may account for the better antioxidant effects.

The mass spectrum of compound A showed molecular ion peaks at *m/z* = 307 [M+H]⁺ and at *m/z* = 305 [M–H][–]. The mass spectrum of compounds B and C showed molecular ion peaks at *m/z* = 291 [M+H]⁺ and at *m/z* = 289 [M–H][–] for compound B, and at *m/z* = 291 [M+H]⁺ and at *m/z* = 289 [M–H][–] for compound C. Thus, the molecular weight of compounds A, B, and C were determined to be 306, 290, and 290, respectively. These results coincided with the molecular weights of the standard chemicals.

NMR analysis was conducted only for compound A, since the antioxidant effect of gallic acid was considered to be much stronger than those of catechin and epicatechin (Nanjo, Goto, Seto, Suzuki, Sakai, & Hara,

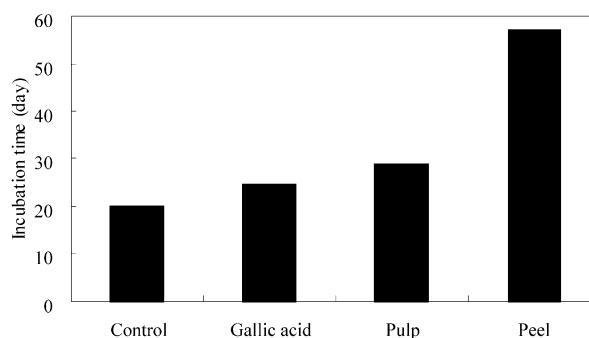


Fig. 1. Effects of banana extracts on lipid autoxidation. Banana pulp extracts (1 mg/ml; pulp), 1 mg/ml of banana peel extracts (peel), 0.5 mM of gallic acid (gallic acid), and distilled water (control) were used for the antioxidant assay. Incubation times are compared at 0.3 absorbance (500 nm).

	Compound A	Compound B	Compound C
Positive-ion-mode			
[M + H] ⁺	307	291	291
Negative-ion-mode			
[M - H] ⁻	305	289	289

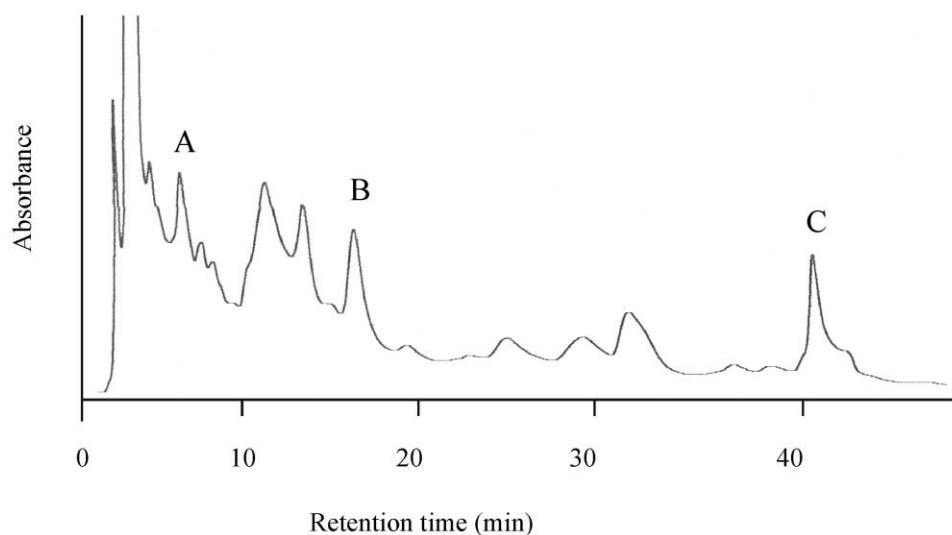


Fig. 2. Main peaks in FAB-MS and HPLC chromatography of antioxidants from banana peel extracts.

1996; Yoshiki, Kahara, Okubo, Igarashi, & Yotsuhashi, 1996). The ¹H NMR spectral assignments of compound A in DMSO-*d*₆ were as follows; at δ4.42 for H-2, δ3.78 for H-3, δ2.60 for H-4, δ5.69 for H-6, δ5.88 for H-8, δ6.24 for H-2', and δ6.24 for H-6'. The ¹³C NMR signals were as follows; at δ81.08 for C-2, δ66.35 for C-3, δ27.47 for C-4, δ155.34 for C-5, δ93.83 for C-6, δ156.46 for C-7, δ95.04 for C-8, δ156.22 for C-9, δ98.98 for C-10, δ129.83 for C-1', δ105.98 for C-2', δ145.67 for C-3', δ132.50 for C-4', δ145.67 for C-5', and δ105.98 for C-6'. All analytical data coincided with that of the standard gallocatechin (Fig. 3). From HPLC, MS, and NMR analyses, compound A in the banana extract was determined to be gallocatechin. Catechins are known to epimerize easily by heating above 80 °C (Seto, Nakamura, Nanjo, & Hara, 1997; Wang & Helliwell, 2000). Since compound A was isolated by heating at 90 °C for 2 h, which could lead to epimerization, the amounts of the epimers ((+)-gallocatechin or (-)-gallocatechin) of natural gallocatechin in the bananas could not be determined.

One of the antioxidant compounds in the bananas was determined to be gallocatechin, which was related to the antioxidant activity of the banana extract. Thus, the antioxidant capacity of the bananas may be attributed to their gallocatechin content.

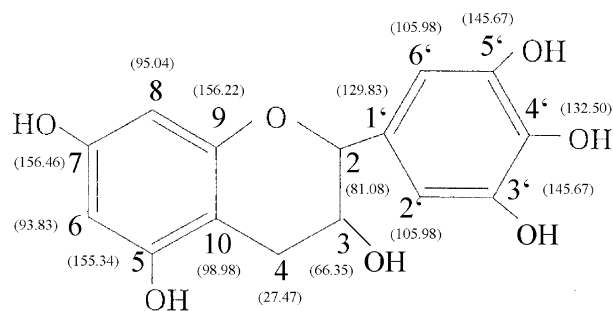


Fig. 3. Chemical structure of gallocatechin and ¹³C NMR spectral assignments.

4. Conclusion

Catechins show strong antioxidant effects against lipid peroxidation (Kondo, Kurihara, Miyata, Suzuki, & Toyoda, 1999), and protective effects against diseases such as cancer and cardiovascular disease (Hertlog, Feskens, Hollman, Katan & Kromhout, 1993; Middleton & Kondasmami, 1992; Renaud & de Lorgeril, 1992). The protection which fruits provide against diseases may be attributed to the antioxidants contained in them (Ames, 1983; Brown, 1980).

In this study, one of the antioxidant compounds, gallocatechin, was identified in the popular fruit, bananas, *Musa Cavendish*. The banana peel extract, which contained more gallocatechin than the pulp, showed stronger antioxidant activity than the pulp extract. Thus, the antioxidant capacity of the bananas may be attributed to the gallocatechin content. Bananas should be considered to be a good source of natural antioxidants for foods. Banana peel, which is usually discarded, should also be considered to be a functional food source against cancer and heart disease, since the banana peel is rich in gallocatechin.

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