

High Content of Dopamine, a Strong Antioxidant, in Cavendish Banana

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A strong water-soluble antioxidant was identified in the popular commercial banana *Musa cavendishii*. It is dopamine, one of the catecholamines. For suppressing the oxygen uptake of linoleic acid in an emulsion and scavenging a diphenylpicrylhydrazyl radical, dopamine had greater antioxidative potency than glutathione, food additives such as butylated hydroxyanisole and hydroxytoluene, flavone luteolin, flavonol quercetin, and catechin, and similar potency to the strongest antioxidants gallic acid and ascorbic acid. Banana contained dopamine at high levels in both the peel and pulp. Dopamine levels ranged from 80–560 mg per 100 g in peel and 2.5–10 mg in pulp, even in ripened bananas ready to eat. Banana is thus one of the antioxidative foods.

Keywords: *Banana; antioxidant; dopamine; phytochemical; catecholamine*

INTRODUCTION

The products of lipid peroxidation are part of our daily diet. The digestive system of man possesses an ability to detoxify small amounts of these products (Kanazawa and Ashida, 1998a). However, those peroxides which cannot be detoxified are absorbed into the body (Kanazawa and Ashida, 1998b), where they can cause various degenerative diseases such as atherosclerosis (Nourooz-Zadeh et al., 1996), cancer (Chaudhary et al., 1994), diabetes mellitus (Novotny et al., 1994), Parkinson's disease (Yoritaka et al., 1996), and others (Kristal et al., 1996). It is important to our health to maintain the ability to detoxify in the digestive tract by eating antioxidative foods.

Plant foods, particularly vegetables, contain various antioxidants, tocopherols, ascorbic acid, carotenoids, and flavonoids. Strongly antioxidative phytochemicals also have been found; catechins in teas (Salah et al., 1995), sesaminols in sesame (Kang et al., 1998), chlorogenic and caffeic acids in coffee (Laranjinha et al., 1994), curcumin in the herb turmeric (Toda et al., 1988), rosmarinol, carnosol, and carnosic acid in rosemary (Inatani et al., 1983; Aruoma et al., 1992), and so forth. We are interested in fruit antioxidants. Wang et al. (1996) examined the antioxidative potency in 12 fruits and 5 fruit juices and reported that strawberry had the highest activity followed by plum, orange, etc., including banana. We had also examined the antioxidative potency of several fruits and found that tropical fruits had strong activity. For example, a banana water-extract suppressed the autoxidation of linoleic acid by 65–70% after a 5-day incubation in an emulsion system, as determined from the peroxide value and thiobarbituric acid reactivity. Tropical fruits grow under hot and intense sunlight that facilitates lipid peroxidation. We considered that bananas should contain antioxidants in pulp and peel to shield against peroxidizing factors. The

objective of the present study was an exploration of banana antioxidants.

We first examined the peel and identified dopamine as a strong water-soluble antioxidant, present at the level of 100 mg/100 g. Next, we analyzed the pulp and found it too contained dopamine at a few mg/100 g even in ripened bananas ready to eat.

MATERIALS AND METHODS

Materials. The most popular banana on the world market, *Musa cavendishii*, imported from two different plantations in the Philippines, was obtained from a local market outlet before ripening treatment with ethylene gas. Linoleic acid purchased from Nacalai Tesque, Inc. (Kyoto, Japan) was distilled under nitrogen in vacuo twice. Standard chemicals, dopamine, and other catecholamines, amino acids, and tocopherols were from Nacalai Tesque, Inc. (Kyoto, Japan), Wako Pure Chemical Industries Ltd. (Osaka, Japan), and Ezai Co., Ltd. (Tokyo, Japan), respectively. Flavonoids and catechins were from Funakoshi Co., Ltd. (Tokyo, Japan). All other chemicals were commercially available in high grade.

Separation of Banana Antioxidants. Banana peel (1050 g) was obtained from banana (3 kg) without fruit apex and stalk. The peel was allowed immediately to stand in boiling water for 20 s to inactivate polyphenol oxidases (Jiménez and García-Carmona, 1999). It was minced with a Waring blender and centrifuged at 8000g. The supernatant in a cellulose tube (dialytic molecular size, less than 12 000–14 000) was dialyzed to 10 L of water overnight. The diffusate after being condensed with an evaporator under nitrogen was weighed (29.84 g by dry weight) and was subjected to the following chromatographic separations. The part of pulp was cut into 1 cm pieces, and the 500 g was taken up randomly and then treated similarly.

Products in the diffusate were purified while monitoring the antioxidative potency. An alumina (aluminum oxide 90, Merck & Co., Inc., Darmstadt, Germany) column (i.d. 3.5 × 30 cm, 0.05 M borate buffer (pH 8.6) for the immobile phase) was first used (Drell, 1970). The elution with water (fraction A-I), 0.2 N acetic acid (A-II), and 2 N HCl (A-III) gave 16.95, 10.38, and 2.46 g dry weight, respectively. The A-I was subjected to an ion-exchange chromatography (Dowex 50W X 4, 200–400 mesh, Na form, i.d. 1.0 × 65 cm) (Dow Chemical Co., Midland,

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MI), and eluted with 0.2 M acetate buffer of pH 3.25 (I-a), the same buffer of pH 4.8 (I-n), and 0.02 N NaOH (I-b). The eluted matter weighed 9.00, 1.59, and 6.34 g dry weight, respectively. A-II was passed through a silica gel column (Wakogel C-100, i.d. 2.0×40 cm) (Wako Pure Chemical Industries Ltd., Osaka, Japan). The elution with a mixed solvent of 1-butanol:acetic acid:water = 4:2:1 as the immobile phase gave a colorless fraction (II-S1, dry weight 0.34 g) and yellow fraction (II-S2, 8.85 g), and then a wash with 2 N HCl gave fraction II-S3 (1.17 g). II-S3 was finally subjected to purification of antioxidant by cellulose (Avicel SF) thin-layer chromatography (TLC) ($0.25 \times 200 \times 200$ mm) twice, developing with 1-butanol:acetic acid:water = 4:2:1 and with ethanol:water = 63:37.

Evaluation of Antioxidative Potency. We employed an oxygen-absorption method using a Gilson differential respirometer (model GRP 14) (Gilson France), because one can directly evaluate the antioxidative potency from the suppression of oxygen uptake during the peroxidation of polyunsaturated fatty acid (Kanazawa et al., 1973). Twenty micromoles of linoleic acid was dispersed with 20% Tween 40 in 2 mL of 0.1 M phosphate buffer (pH 7.0) containing a certain concentration of banana products. While it was shaken in a water bath at 37 °C, the oxygen uptake was monitored and compared to that of the control without banana products.

Another evaluation method was used that employs a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The banana antioxidant was added to 250 nmol DPPH in 50% ethanol in 0.05 M acetate buffer (pH 5.5), and immediately fading at 517 nm was monitored, comparing to the fading abilities of 130 nmol each of standard chemicals.

Instrumental Analyses. The chemical structure of the purified antioxidant was elucidated using infrared spectrometry (Shimadzu IR-408) (Shimadzu Co. Ltd.), nuclear magnetic resonance spectrometry (Bruker AC-250) (Bruker Analytik GmbH), and gas chromatography–mass spectrometry (JEOL DX 500) (JEOL Ltd.) with a column of 3% OV-1 using an ionizing voltage of 20 eV.

High-Performance Liquid Chromatography (HPLC) for Determining Antioxidative Phytochemicals. The antioxidants in both banana peel and pulp were identified by HPLC (IRICA Σ871) (Irica Co. Ltd.) comparing with the respective standard chemicals. Levels of catecholamines were analyzed in the A-II fraction, modifying the method of Causon et al. (1981): column, Wakosil-II 5C18AR (i.d. 4.6×200 mm); mobile phase, 70 mM KH_2PO_4 containing 1 mM ethylenediamine tetraacetate disodium, 2 mM sodium 1-octanesulfonate, and 10% methanol; flow rate, 1.0 mL/min; column temperature, 35 °C; and monitoring with an electrochemical detector (IRICA Σ875) (Irica Co. Ltd.) set at +600 mV. Flavonoid content also was determined for A-II, using a column Shiseido Capcell UG120 (i.d. 4.6×250 mm) (Shiseido Co. Ltd.) maintained at 35 °C, eluting with 0.045 M potassium phosphate buffer (pH 3.3):methanol = 45:55, and monitoring with a photodiode array detector (Shimadzu SPD-M6A) (Shimadzu Co. Ltd.). Alternatively, banana peel or pulp was minced in ethanol containing 5% pyrogallol, saponified in 60% KOH for 3 min, and then extracted with diethyl ether or with methanol:dichloromethane = 1:2. The ether extract was passed through a Sep-Pak (Waters Florisil cartridges) (Nihon Waters K.K.) and submitted to tocopherol analysis on HPLC (Lumen and Fiad, 1982). Carotene contents were determined in methanol:dichloromethane extract (Miller et al., 1984). The determination of antioxidant levels was performed three times independently using 12 bananas each at the ripening stage.

RESULTS

Isolation of Antioxidative Ingredients in Banana Peel. Figure 1 shows the isolating procedures for antioxidants in banana peel. The antioxidative potency of isolated products was monitored from the suppressing effect on oxygen uptake of linoleic acid. Linoleic acid absorbs 1 mol of oxygen to form its hydroperoxides and another mole or a little more for decomposition of the hydroperoxides (Kanazawa et al., 1973). Finally, 20

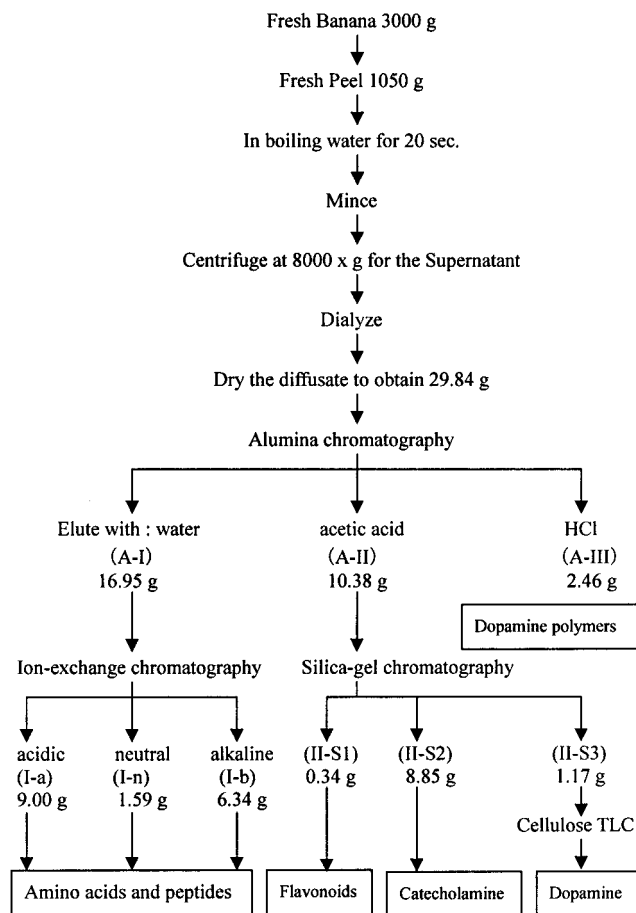


Figure 1. Isolation of banana antioxidants. Details are given in the Materials and Methods section. Numbers next to the fraction names are the yields in grams as dry weight. The products are identified in the boxes.

μmol of linoleic acid used here was estimated to absorb around 50 μmol (1120 μL) of oxygen. Figure 2 compares the oxygen uptake of linoleic acid with and without banana products. In a control without product, the uptake initiated after around 60 h incubation, which is the induction period of autoxidation. Then, linoleic acid absorbed oxygen actively, and the autoxidation almost finished at around of 230 h. In the linoleic acid emulsion, 50 μg of dry matter of the isolated fractions was added. The diffusate prolonged the induction period to around of 200 h and suppressed the oxygen uptake to 570 μL after 300 h (Figure 2A).

It had been reported by Udenfriend et al. (1959) that banana contained catecholamines. The strong antioxidative potency of banana diffusate might be attributed to the catecholamines. For the isolation of catecholamines, alumina column chromatography has been reported to be suitable (Drell, 1970). We then employed the alumina chromatography and separated the diffusate into three fractions. Fraction A-I had weak antioxidative potency, whereas A-II and A-III were strong. A-I was further separated through an ion-exchange column chromatography, but still gave no active fractions (Figure 2B). In contrast, A-II passed through a silica gel column gave a strong fraction, II-S3. Products in II-S3 were purified on cellulose TLC twice to II-S3C. The A-III fraction formed a dark-brown precipitate during a few hours' standing. These isolated fractions, purified products, and precipitates were elucidated instrumentally as to chemical structure.

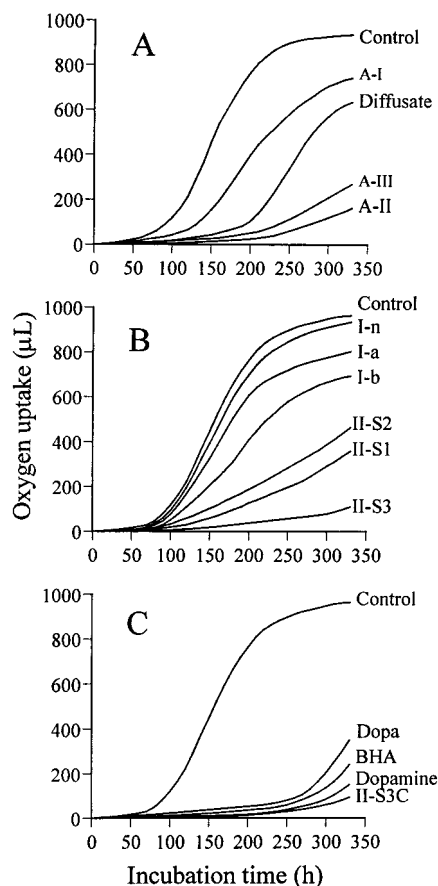


Figure 2. Antioxidative potency of the isolated banana ingredients: (A) the diffusate and isolated fractions obtained by the alumina column chromatography; (B) the fractions on ion exchange and silica gel column chromatographies; (C) a comparison of the antioxidative potency of 10 μg of purified fraction II-S3C and 50 nmol each of standard chemicals dopa, dopamine, and BHA (butylated hydroxyanisole).

Identification of Banana Antioxidants. Fraction A-I was positive for ninhydrin reagent and was then analyzed by amino acid analyzer (Hitachi model 835) (Hitachi Ltd.) directly and after hydrolysis. It was composed mainly of peptides and free amino acids (data not shown). The most active I-b among the three A-I fractions included basic peptides and amino acids, particularly 1.77 mg of free arginine in 6.34 g of I-b fraction (Figure 1). The basic amino acids have been known to be more antioxidative than acidic and neutral amino acids (Matsushita and Ibuki, 1965). The activity of I-b was probably due to arginine. In I-n fraction (1.59 g), 0.30 mg of free tyrosine and 0.44 mg of phenylalanine were detected, but Figure 2B showed that I-n fraction was not antioxidative. This indicated that antioxidative potency of the neutral amino acids were weaker than arginine.

In the most antioxidative A-II fraction, the II-S3C purified from II-S3 showed a phenolic OH (ν_{max} 3300 cm^{-1}), amine (ν_{max} 1600 cm^{-1}), and aromatic ring (ν_{max} 1495 cm^{-1}) in an infrared spectrum in KBr. The mass spectrum after acetylation with trifluoroacetic acid detected a molecular ion peak at m/z 441 (M^+) and fragment ion peaks; at m/z 372 ($-\text{CF}_3$), 329 ($-\text{NHCOCF}_3$) as a base ion peak, 315 ($-\text{CH}_2\text{NHCOCF}_3$), 301 ($-\text{CH}_2\text{CH}_2\text{NHCOCF}_3$), and 126 ($\text{CH}_2\text{NHCOCF}_3^+$) as the secondary peak. The spectrum of ^1H NMR in dimethyl sulfoxide- d_6 was assigned as follows; at δ 2.66 (t, $J = 7.8$) for H-1', δ 2.91 (t, $J = 7.8$) for H-2', δ 6.48 (m) for

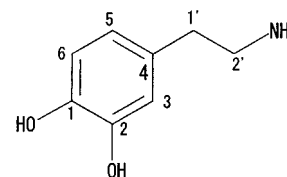


Figure 3. Chemical structure of dopamine.

H-4, δ 6.63 (d, $J = 7.9$) for H-5, and δ 6.66 (d, $J = 8.0$) for H-6. The ^{13}C NMR signals were as follows: at δ 32.7 for C-1', δ 41.0 for C-2', δ 116.3 for C-3, δ 116.5 for C-6, δ 120.2 for C-5, δ 128.6 for C-4, δ 144.3 for C-1, and δ 145.5 for C-2. Every analytical datum completely coincided with that of standard dopamine (Figure 3). In addition, this II-S3C exhibited strong potency for anti-oxidant similar to the standard dopamine (Figure 2C).

The II-S1 showed two large peaks on HPLC, having 3.9 and 4.1 min as retention times. The mass spectra analyzing with a liquid chromatography/APCI-mass spectrometry (Hitachi M-1200H) (Hitachi Ltd.) at -50 V for ionizing voltage, showed ion peaks at m/z 577 ($M - 1 - 2\text{H}$) and m/z 271 ($M - 1 - \text{rhamnoglucoside}$) for the former, and at m/z 609 ($M - 1$) and m/z 301 ($M - 1 - \text{rutinoside}$) for the latter. The analytic data coincided with those of standard flavonoid glycosides, naringin, and rutin, respectively. Flavonoids have been recognized to be potent antioxidants (Jovanovic et al., 1994; Cao et al., 1997). The activity of II-S1 was due to these flavonoids (Figure 2B). II-S2 gave two major and several minor peaks on HPLC. The major peaks had 6.8 and 19.5 min of retention times and gave signals at m/z 168 ($M - 1$) and m/z 152 ($M - 1$), respectively, with a liquid chromatography/APCI-mass spectrometry. The information coincided with that of standard chemicals, and then they were identified as norepinephrine and dopamine, respectively.

The precipitate from A-III had almost the same spectrum as dopamine in the infrared analysis. This fraction was suggested to include a large amount of dopamine and to form oxidized products such as dopamine polymers during the preparation.

Then, because of the instability of dopamine, the antioxidant contents of banana were determined using fresh peel and pulp.

Antioxidant Contents of Banana at Various Ripening Stages. The peel contained antioxidative arginine, flavonoids, and catecholamines, and probably others. The amounts of these compounds and other known antioxidative phytochemicals were determined in both peel and pulp at the various ripening stages (Table 1). The ripening stage was evaluated according to the common classification in the market. The classification defines eight stages by color score: all green, light green, half-green, half-yellow, green chip, full yellow, star, and duffel, and by sugar content, around 1, 3, 5, 9, 14, 17, 19, and 19%, respectively. Stages 1–3 were just imported and had not received ripening treatment. Stages 4–8 were after the treatment with ethylene gas. Banana is usually served at a ripening stage of 6 or 7. At stages 1–3, it is not usually eaten because it is green and very hard. At stage 8, it is overripe and muddy. Banana contained a large amount of dopamine in both peel and pulp. The amounts decreased a little with ripening and remained at levels between 80 and 560 and between 2.5 and 10 mg, per 100 g of peel and pulp, respectively, even at the edible stages. The variance was due to the difference of the

Table 1. Levels of Antioxidative Phytochemicals in Banana at Various Ripening Stages

	ripening stage ^a			
	1-3	4-6	6-7	7-8
	In Peel (mg/100 g) ^b			
dopamine	865-1940 (1290 ± 420)	185-705 (430 ± 210)	80-560 (380 ± 160)	235-930 (500 ± 270)
dopa	14-30	3.5-10	1.1-8.0	2.5-15
norepinephrine	55-118	8.2-14	ND ^c -24	38-43
naringin	120-260	17-120	28-95	42-72
rutin	16-23	11-14	11-16	15-17
ascorbic acid ^d	6.8-8.2	6.6-8.7	5.8-8.0	3.2-7.9
carotenes ^d	0.43-1.2	0.55-0.87	0.28-0.78	0.11-0.66
tocopherols ^d	2.4-2.6	3.2-5.3	1.5-5.8	5.5-7.8
	In Pulp (mg/100 g) ^b			
dopamine	4.7-10 (7.0 ± 2.0)	6.1-15 (9.1 ± 3.1)	2.5-10 (7.3 ± 2.4)	0.72-6.1 (3.4 ± 2.2)
dopa	1.3-1.9	1.3-1.9	1.0-1.5	0.95-1.4
norepinephrine	0.80-1.7	0.84-2.1	0.82-1.6	0.62-1.5
naringin	ND ^c -65	ND ^c	ND ^c -3.3	ND ^c
rutin	ND ^c -4.8	ND ^c	ND ^c	ND ^c
ascorbic acid ^d	12-13	11-13	6.9-11	5.4-10
carotenes ^d	0.09-0.12	0.06-0.12	0.03-0.12	0.02-0.05
tocopherols ^d	ND ^c	ND ^c -0.29	ND ^c	ND ^c -0.45

^a The ripening stage was determined as mentioned in the text.

^b The values are expressed as a range between the lowest and highest contents. In the lines for dopamine, values in parentheses are the mean ±SD. ^c Not detectable. ^d The ascorbic acid is the sum of reduced and oxidized forms. Carotenes are the sum of α - and β -carotenes, mainly α -carotene. Tocopherols are the sum of α -, γ -, and δ -tocopherols, mainly α -tocopherol.

plantations in the Philippines. Interestingly, the metabolizing precursor dopa was less abundant than dopamine in peel and pulp. Naringin and rutin contents were a few 10 mg in the peel and negligible in pulp. The ascorbic acid content was constant at around 10 mg/100 g in both peel and pulp, regardless of the ripening stage. Carotenes and tocopherols were appreciable in peel and less in pulp. Thus, the strong antioxidative potency of banana is attributable to dopamine, which is much more abundant than other antioxidants in this fruit.

Strong Antioxidative Potency of Dopamine. The antioxidative potency of dopamine was compared with that of standard chemicals (Figure 2C). Dopamine was stronger than dopa and the food additive BHA. In Figure 4, another evaluation method was used with a DPPH radical. Dopamine scavenged the DPPH radical actively, more so than glutathione and another food additive, BHT. The activity was also stronger than that of flavone luteolin and flavonol quercetin. Catechins have been found to be the strongest antioxidants in phytochemicals (Salah et al., 1995). Dopamine had a faster radical-scavenging rate than catechin and was similar to gallic acid gallate. Ascorbic acid is the strongest water-soluble antioxidant. Dopamine exhibited similar activity to ascorbic acid.

DISCUSSION

The present study revealed that banana contained a strong antioxidant, dopamine, in large amounts. The antioxidative potency of dopamine was greater than that of BHA, BHT, flavonoids, glutathione, and catechin, and similar to that of the strong antioxidants gallic acid gallate and ascorbic acid (Figures 2C and 4). Bors et al. (1990) describes that the strong activity requires an *o*-dihydroxy (catechol) structure. Dopamine, one of the catecholamines, has this structure, and its amino resi-

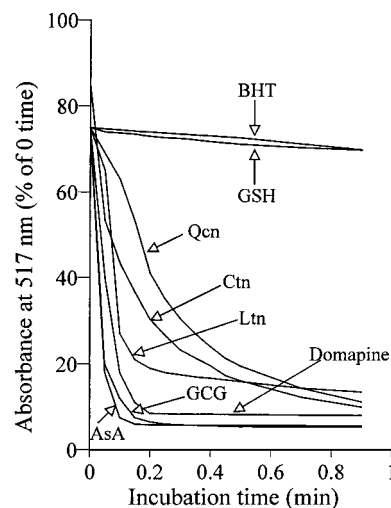


Figure 4. Scavenging activity of dopamine toward a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Abbreviations: AsA, ascorbic acid; GSH, glutathione reduced form; BHT, butylated hydroxytoluene; Ltn, flavone luteolin; Qcn, flavonol quercetin; Ctn, catechin; and GCG, gallic acid gallate.

due facilitates the hydrophilic character. Thus, dopamine is a powerful water-soluble antioxidant like ascorbic acid. Edible banana pulp contained dopamine and ascorbic acid at high levels (Table 1). Banana is therefore one of the best sources of antioxidants.

The peel contained dopamine at the 10 mg level, and the antioxidative phytochemicals flavanone glycoside naringin and flavonol glycoside rutin at the 10 mg level per 100 g at all ripening stages. Banana, a tropical plant, may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidants. A few milligrams of dopamine existed in the pulp at the stage that it is usually eaten. Dopamine has been found to protect against intestinal mucosal injury by modulating eicosanoid synthesis (MacNaughton and Wallace, 1989; Alanko et al., 1992). Banana may contribute to the antiinflammation.

Dopamine is considered to be easily absorbed through a dopamine transporter and used in the body (Abi-Dargham et al., 1998). Although dopamine plays important roles as a neurotransmitter and precursor for norepinephrine and epinephrine, an accumulation of the oxidized products of dopamine such as its quinone in brain with age can cause neurocells to undergo apoptosis, a process which is associated with Parkinson's disease (Luo et al., 1998; Cadet and Brannock, 1998). It is a remaining question whether a few milligrams of dopamine in pulp has a favorable or unfavorable effect on human health.

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