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Abstract

Banana ranks as the fifth most cultivated crop in the West Indies and more generally in the world. The banana's health-promoting benefits are correlated with its composition of bioactive compounds, such as phenolic molecules. Thus, the present study attempts to evaluate the potential health benefits of banana phenolic content. Banana peel and pulp were analyzed using reverse-phase HPLC coupled with diode array (HPLC-DAD) detection following thioacidolysis to quantify phenolic compounds. Proanthocyanidins were the main polyphenol class in both pulp and peel, with concentrations ranging from 0.042 to 0.096 g/100 g DW in the pulp and from 0.098 to 0.125 g/100 g DW in the peel. They were characterized by a very high average degree of polymerization (n), ranging around 17 to 37 in the peel and from 67 to 189 in the pulp, depending on the variety. They comprised mainly (-)-epigallocatechin and (-)-epicatechin, with a small proportion of (+)-catechin as the terminal unit. Dopamine was also abundant, with much higher concentrations in the peel than in the pulp (up to 0.780 g/100 g DW and 0.0060 g/100 g DW, respectively). Flavonols with varied aglycones (myricetin, quercetin, isorhamnetin, and kaempferol derivatives) also had higher concentrations in the peel. Hydroxycinnamic acids and anthocyanins could also be detected in the red banana peel variety.

Keywords: Musaceae; Polyphenol; Proanthocyanidin; Flavonol; Anthocyanin

Introduction

Banana (Musa spp.L.) is a tropical giant fruiting herb from the Musaceae family, cultivated in tropical and subtropical countries. The fruit is consumed either ripe or unripe as a vegetable after cooking. Banana pulp is a good source of various nutrients (1) with its high sugar content, vitamins, minerals, and many secondary metabolite contents, including phenolic compounds. The phenolic compounds family is numerically important and comprises many complex groups of compounds, varying from simple phenols to highly polymerized compounds such as tannins. These compounds are involved in fruit visual appearance (color, browning), taste (astringency), and defense against insects. They are also a source of many properties sought-after by consumers; it has been suggested that they are responsible for health benefits associated with antioxidant, anti-carcinogenic, antimicrobial, anti-allergic, anti-mutagenic, anti-inflammatory properties, and some kinds of prevention against cardiovascular diseases (2-6). The distribution of phenolic compounds in fruits is affected by maturity, cultivar, crop practices, geographic origin, growing season, post-harvest storage conditions, and processing procedures (7-10).

Studies have shown that there are significant levels of total phenolic contents in the pulp of ripe bananas, ranging from 11.8 to 90.4 mg of gallic acid equivalent per 100 g of fresh weight (11). Banana pulp also has significant levels of total cell wall-bound phenolics (4.4 mg of gallic acid equivalent per 100 g of cell wall) and cell wall-bound water-soluble phenolics (29.9 mg of gallic acid equivalent per 100 g of cell wall) (12). Many free phenolic compounds have been identified in ripe banana pulp: gallic acid, catechin, gallocatechin, and naringenin-7-0-hesperoside (13, 14). Biologically active compounds, such as dopamine, dopa, norepinephrine, and serotonin, have been identified in ripe banana pulp, which in turn may provide consumers with health-promoting benefits (15,16).

Several studies have suggested that consumption of unripe bananas confers beneficial effects on human health. Unripe bananas might be an important source of phenolic compounds that are regarded as natural antioxidants (17,18). Unripe dessert bananas (Musa cavendish L.) contain high levels of dopamine and norepinephrine in both the peel and pulp (respectively 235–930 and 38–43 mg per 100 g FW of peel, and 0.72-6.1 and 0.62-1.5 mg per 100 g FW of pulp) (16). A new banana hybrid (Florban 920) has higher levels of phenolics at all stages of fruit development than Grande Naine, a control banana (19).

However, the scientific literature on banana fruit detects a low concentration of phenolic compounds, while fairly high antioxidant capacities are reported. It can be assumed that there is a problem with sample treatment concealing the banana's real quantitative content of antioxidant compounds. Significant levels of tannins have been reported in unripe bananas. Plant polyphenols, or proanthocyanidins, are oligo- and polymeric phenolic compounds composed of flavan-3-ol units. Proanthocyanidins with a low degree of polymerization can be responsible for bitterness, while proanthocyanidins with a high degree of polymerization are

astringent (20). A chemotaxonomic analysis of plant polyphenols, without quantification, in the flesh of green bananas from different varieties was first reported by Ucles Santos et al. (21); their contents were between 0.3 and 2.1% of fresh matter, except for a Pacific plantain that was tannin-free. Leucocyanidin, a proanthocyanidin precursor, has been identified in the unripe pulp of plantain bananas (3.3 mg/g of fresh weight) (22). Tannins in banana fruit have been reported to be concentrated in latex vessels (lacticifers). The lacticifers of the unripe pulp appeared as brown stains with potassium dichromate but became pale pink during the early stages of ripening (16). Various stages of the synthesis of flavan-3-ols are known, but not all the steps have been established (21).

The aim of this work was to extract tannins, assess their average degree of polymerization (n), and characterize the phenolic composition of bananas harvested in the French West Indies. By using a method based on thiolysis in conjunction with reversed-phase HPLC analysis, this paper deals with the characterization and quantification of proanthocyanidins in pulp and peel (Musa spp.). Five unripe banana varieties were studied: two cooking bananas (Poto (PO) and Plantain (PL)) and three dessert bananas (Grande Naine (GN), the export Cavendish banana, Café (CAF), and Red Banana (RED).

Material and Methods

Plant Materials

The banana varieties Poto (PO), Plantain (Musa paradisiaca, PL), Cavendish Grande Naine (GN), Café (CAF), and Red Banana (RED) were obtained from plants grown in Guadeloupe (French West Indies). They were harvested between 90 and 100 days after flowering (agricultural custom of this region). The fruits were sampled and kept for 24 hours in a chamber ventilated with humidified air. The samples were a mixture of approximately 3 kg (15 fingers) of green fruits from each accession (PO, PL, GN, CAF, and RED). The bananas were peeled, and the pulp and the peel were separately sliced, immediately frozen in liquid nitrogen, and freeze-dried for 5 days. The dried pulp and peel were powdered using a commercial blender. The powder samples were stored at -80 °C until analysis.

Solvents and Reagents

Methanol and acetonitrile of HPLC-grade quality and glacial acetic acid were purchased from Fisher Scientific (Illkirch, France). Toluene-α-thiol was obtained from Merck (Darmstadt, Germany). High-purity water (18 MΩ) was prepared using a milli-Q water system (Millipore Corporation, New Bedford, MA).

Phenolic Standards

Ferulic acid, chlorogenic acid, (+)-catechin, (-)-epicatechin, and (-)-epigallocatechin were provided by Sigma-Aldrich Co. (Stenheim, Germany). Quercetin, rutin, isorhamnetin, kaempferol, myricetin, cyanidin, and peonidin were purchased from Extrasynthese SA (Lyon, France).

Thioacidolysis Conditions

Polyphenols were measured by High-Performance Liquid Chromatography (HPLC)/Diode Array Detection (DAD) after thioacidolysis using a method adapted from Guyot, Marnet, Laraba, Sanoner, & Drilleau [23], Guyot, Marnet, Drilleau [24] and Le Bourvellec, Bouzerzour, Ginies, Regis, Ple, & Renard (2011).

Freeze-dried peel and flesh banana powders directly underwent thiolysis without any purification step, as described by Guyot [24]. Precisely weighted amounts (300-400 mg) of banana powder were suspended in 400 µL of dried methanol acidified by concentrated HCl (3N), and 800 µl of toluene-α-thiol solution (5%, v/v in dried methanol) were added. The reaction was carried out at 40°C for 30 min, with agitation on a vortex every 10 min. Then, the vials were cooled in an ice bath for at least 5 minutes. After filtration (PTFE, 0.45 µm), the reaction medium was directly injected (20 µL) into the HPLC-DAD system. Analyses were conducted in duplicate.

Reverse-Phase HPLC-DAD Conditions

After thioacidolysis, no distinction can be made between native catechins and catechins coming from the terminal units of procyanidins [24]. For this reason, methanol extractions of polyphenols from freeze-dried samples without thioacidolysis were also performed to separately assay native catechin by reverse phase HPLC.

For this, the banana powders (300-400 mg) were suspended in 1200 µL of dried methanol acidified by acetic acid (1% v/v) and extracted in an ultrasound bath for 15 minutes. After filtration (PTFE, 0.45 µm), the reaction medium was directly injected (20 µL) into the HPLC system.

HPLC-DAD analyses were performed using an Ultra-Fast Liquid Chromatography Shimadzu Prominence system (Kyoto, Japan), including two pumps (LC-20AD), a UFLC Prominence Liquid Chromatograph, a DGU-20A5 Prominence degasser, a SIL-20ACHT Prominence autosampler, a CTO-20AC Prominence column oven, a SPD-M20A Prominence diode array detector, a CBM-20A Prominence communication bus module, and controlled by LC Solution software (Shimadzu, Kyoto, Japan).

Separation was performed using a (250 mm x 4 mm i.d.) Licrocart (Licrospher PR-18 5µm) column (Merck, Darmstadt, Germany) with a guard column (Licrospher PR-18 5µm column, Merck, Darmstadt, Germany) operated at 30°C.

The mobile phase consisted of water/acetic acid (97.5:2.5, v/v) (eluent A) and acetonitrile (eluent B). The flow rate was 1 mL/min. The education program was as follows: 3-9% B (0-5 min); 9-16% B (5-15 min); 16-50% B (15-45 min); 50-90% B (45-48 min); 90-90% B (48-52 min); 90-3% B (52-55 min); 3-3% B (55-60 min). Samples and standard solutions were maintained at 4°C before injection. Absorbance spectra were measured over the wavelength range of 280-800 nm. Phenolic compounds were identified by comparing their retention time and their UV-visible spectra against standards. Quantification was achieved by injecting standard solutions of known concentrations. The column effluent was monitored at 280 nm for flavan-3-ol monomers and thioether adducts, 320 nm for hydroxycinnamic acids, and 350 nm for flavonols.

Quantification was carried out by using external standard solutions of known concentrations. Thioether adducts, i.e., (-)-epigallocatechin benzyl thioether and (-)-epicatechin benzyl thioether, were quantified as (-)-epicatechin equivalent. An unknown polyphenol was quantified as quercetin equivalent due to its close retention times. Myricetin rutinoside was quantified as myricetin equivalent, rutin as quercetin equivalent, isorhamnetin dihexoside and isorhamnetin hexoside as isorhamnetin equivalent, kaempferol rutinoside 1 and 2 as kaempferol equivalent, cyanidin-3-rutinoside as cyanidin equivalent, and peonidin-coumaroyl-hexoside as peonidin equivalent.

UPLC-MS Analysis

UPLC-MS analyses were performed on the Acquity Ultra Performance LC™ (UPLC™) apparatus from Waters, equipped with an UV-visible diode array detector (DAD) and coupled with a Brucker Daltonics (Bremen, Germany) HCT ultra ion trap mass spectrometer with a negative electrospray ionization (ESI) mode. Separation was performed using the same column as described above, operated at 30 °C. The mobile phase consisted of water/formic acid (99.95:0.05, v/v) (eluent A) and acetonitrile (eluent B). The flow rate was 0.1 ml/min. The same elution program as for HPLC-DAD analysis was used. Samples were injected at a level of 2 µl. The column effluent was monitored at 280, 320 and 365 nm. The mass spectra were generated in the ultrascan mode in the m/z range of 100-900. Nitrogen was used as the nebulizing gas. Data were collected and processed using Bruker Compass Data Analysis software.

Statistical Analysis

Samples (each variety) were analysed in duplicate before and after thioacidolysis. The results are presented as mean values, and the reproducibility of the results was expressed as a pooled standard deviation. Pooled standard deviations were calculated for each series of replicates using the sum of individual variances weighted by the individual degrees of freedom.

Results and Discussion

Identification and Quantification of Phenolic Compounds in Banana

The polyphenols were identified from UPLC-MS and UV-Vis spectral data, and retention times against authentic standards. Concerning the main compounds detected, flavan-3-ol families were found at higher concentrations than other phenolic compounds.

Three phenolic groups (flavan-3-ols, flavonols and anthocyanins) with a total of eight identified individual compounds were quantified (Table 1). The sum of phenolics determined by HPLC ranged from 1,057 g/ 100g Dry Weight (DW) (cv. Red banana) to 2,415 g/100 g DW (cv. Café banana) in the peel. For all varieties, in the pulp, the total polyphenols were significantly lower than in the peel, ranging from 0,4901 g/ 100 g DW (cv. Cavendish) to 1,0528 g/ 100 g DW (cv. Red banana). These higher phenolic compound concentrations in the peel than in the pulp are found in many other fruits as observed in the apple (23), pear (24), grape (25), quince (26) and persimmon (27). The total polyphenols detected in the pulp were slightly higher than total polyphenols determined using a modified Folin Ciocalteu colorimetric method in previous works for the banana: 0,2320 g/ 100 g DW (28); 0,0391 g/ 100 g DW (29). The total polyphenols in the peel were slightly higher, but within the range of previous works for banana pericarps (0,346-1,260 g/ 100 g DW) (28).

Monomeric flavan-3-ols were assayed by HPLC-DAD without prior thioacidolysis to differentiate them from the flavan-3-ols terminal units of procyanidins. The procedure is also able to assay the other main polyphenols classes such as hydroxycinnamic acids, flavonols and dihydrochalcones (23). For each variety, Table 1 presents quantitative data of dopamine and phenolic compounds found in the peel and in the pulp.

Table 1: *Concentrations of Minor Phenolic Compounds in Bananas. Data Represent Mean ± Standard Deviation Expressed in Mg/G Dry Weight (n=2; degree of freedom = 5).*

	Dop	HCA	Flavonols						Anthocyanins	
			Rut.MYR	RUT	diHex.ISO	Rut.KMP1	Rut.KMP2	Hex.ISO	Cya-3-GLU	Peo-COU
Cav. GN (Pulp)	0.05±0	2.37±0.64	0.11±0.01	0.35±0.02	ND	ND	ND	ND	ND	ND
Poto (Pulp)	0.06±0	1.87±0.84	0.08±0.01	0.24±0.02	0.01±0	0.01±0	0.04±0	ND	ND	ND
Plantain (Pulp)	0.01±0.07	1.92±0.05	0.04±0	0.53±0.01	0.37±0.01	0.02±0.01	0.14±0	ND	ND	ND
Café (Pulp)	0.04±0	0.58±0.01	0.03±0	ND	ND	ND	ND	ND	ND	ND
Red (Pulp)	0.05±0	0.12±0.01	0.05±0	ND	ND	ND	ND	ND	ND	ND
Cav. GN (Peel)	2.72±0.06	5.93±1.30	5.06±0.27	4.57±0.13	ND	ND	1.76±0.09	ND	ND	ND
Poto (Peel)	1.9±0.08	8.05±5.24	5.69±2.24	20.68±6.25	ND	0.57±0.17	3.98±1.15	0.02±0	ND	ND
Plantain (Peel)	7.8±0.03	1.75±1.46	0.37±0.02	28.38±4.32	ND	ND	ND	0.05±0	ND	ND
Café (Peel)	4.24±0.02	0.74±0.01	0.08±0	ND	ND	0.11±0	ND	ND	ND	ND
Red (Peel)	3.47±0.05	0.12±0	0.07±0	0.1±0	ND	ND	0.02±0	ND	0.34±0	0.38±0

GN: Cavendish Grande Naine; PL: Plantain; PO: Poto, CAF: Café; RED: Red; Dop: Dopamine ; Myr-der: Myricetin rutinoside quantified as myricetin equivalent; Rut: Rutine; Isorham-di-hex: isorhamnetin dihexoside quantified as isorhamnetin equivalent; Kaem-der-1: Kaempferol hexoside-deoxyhexose 1 quantified as kaempferol equivalent; Kaem-der-2: Kaempferol hexoside-

deoxyhexose 2 quantified as kaempferol equivalent; Cya-3-RUT Cy: Cyanidin-3-O-rutinoside quantified as cyanidin equivalent; Peo-COU: Peonidin coumaroyl hexoside quantified as peonidin equivalent; ND: not detected.

Flavan-3-Ols Monomers

(-)-Epicatechin (EC), an isomer of catechin (CAT), was identified by HPLC-DAD. It was found as a free monomer in the peel of two studied varieties, Café (CAF) and Red, with respectively 0,0010 and 0,0014 g/100 g DW. Another monomer of flavan-3-ols, -gallocatechin (GC), is present in Café banana (0,0034 g/100 g DW).

Flavan-3-Ols Polymers

The thioacidolysis reaction in the presence of toluene-α-thiol was carried out on the pulp and peel of the five banana varieties. Reversed-phase HPLC following the thiolysis reaction allows the determination of the nature and proportions of proanthocyanidin constitutive units, making the distinction between terminal and extension units. In this way, the average degree of polymerization (n) can be calculated (23, 30). Solvolysis coupled with HPLC-DAD also produces quantitative information on the proanthocyanidin content, provided the reaction is performed with a good yield, which is the case for thioacidolysis with toluene-α-thiol [23]. Monomeric flavan-3-ols were assayed by HPLC-DAD without prior thioacidolysis to differentiate them from the flavan-3-ols terminal units of procyanidins. For each variety, Table 2 presents quantitative data on flavan-3-ol polymers, the proanthocyanidins, found in the peel and in the pulp assayed by the thioacidolysis-HPLC method.

Table 2: *Concentrations of Flavanols in Bananas. Data Represent Mean ± Standard Deviation Expressed in Mg/G Dry Weight (n=2; degree of freedom = 5).*

	Flavan-3-ol Polymer		Flavan-3-ol Monomers	
	Procyanidins	(DPn)	EPI	GC
Cav. GN (Pulp)	4.79±0.05	109±0.6	ND	ND
Poto (Pulp)	9.6±0.03	67±0.04	ND	ND
Plantain (Pulp)	4.23±0.02	88±0.51	ND	ND
Café (Pulp)	8.46±0	187±0.95	ND	ND
Red (Pulp)	0.46±0.02	114±2.21	ND	ND
Cav. GN (Peel)	13.14±0.16	29±0.28	ND	ND
Poto (Peel)	12.52±0.73	35±1.5	ND	ND
Plantain (Peel)	9.89±0.01	19±0.34	ND	ND
Café (Peel)	19.71±0.10	31±0.01	0.14±0	0.34±0
Red (Peel)	10.36±0.02	17±0.04	0.46±0.01	ND

GN: Cavendish Grande Naine; PL: Plantain; PO: Poto, CAF: Café; RED: Red; EPC: (-)-epicatechin; $\overline{DP}$ n: average degree of polymerization of procyanidins.

Proanthocyanidins represented the major class of compounds both in the peel and in the pulp of all five banana varieties. Pulp proanthocyanidin concentrations were lower than tannin content in banana pulp, as reported by Uclés Santos et al. (21). They reported very high concentrations of proanthocyanidins in the banana pulp, ranging from 0.3 g/100 g to 2.1 g/100 g FW, being that the dry matter represents about 1/4 of the fresh banana pulp (1), concentrations ranging from 1.2 to 8.4 g/100 g DW. Depending upon the variety in question and the tissue zone, proanthocyanidins accounted for 99.0-99.4% of the total polyphenols in the pulp and for 94.7-98.5% of the polyphenols in the peel. However, their concentrations varied greatly. Thus, proanthocyanidin concentrations in the peel were 2–3 times higher than in the pulp, and the concentration in the pulp of Poto was twice that in the plantain variety. Proanthocyanidin characteristics of the five studied varieties are presented in Table 3, with proportions of the constitutive flavan-3-ol units. (+)-Catechin was only found as terminal units and accounted for 0.00–0.3% of the total units in the pulp and for 0.2-0.6% of the total units in the peel. Other units corresponding to (-)-epicatechin and (-)-epigallocatechin were found as terminal and extension units. Uclés Santos et al. also found (-)-epicatechin and (-)-epigallocatechin as terminal and extension units of proanthocyanidin from banana. However, they do not report (+)-catechin as a terminal unit. The detection in a very small proportion of (+)-catechin as a terminal unit could be due to the epimerization reaction of (-)-epicatechin in our experimental conditions of thioacidolysis. Prieur and Guyot [23,25]reported respectively a rate of conversion of 15% for (-)-epicatechin, 4% for catechin, 17.5% for (-)-epicatechin, and 4.5% for (+)-catechin, so that the proportion of (+)-catechin in terminal units may be overestimated. However, in an HPLC study using a postcolumn derivation procedure, de Pascual-Teresa and collaborators identified, in a methanolic extraction of banana pulp, a gallocatechin-catechin dimer (31). Tanaka et al. also found amounts of (-)-epigallocatechin associated with (-)-epicatechin and dimer A-2 and traces of (-)-epiafzelechin in thiolacidolysis degradation products from acetone-insoluble pulp tannins from ripe bananas (32). However, neither we nor Uclés Santos et al. (21) detected these two last compounds in the pulp samples. The major extension unit of the proanthocyanidins was (-)-epigallocatechin,

which accounted in the peel for 52.3–74.5% and in the pulp for 61.6–88.2% of the total terminal and extension units. Epicatechin accounted for 0.5–1% and 22–37% of the total terminal and extension units, respectively, in the pulp and for 0.5–4.8% and 24.4–41.1% of the total terminal and extension units, respectively, in the peel.

(-)-Epigallocatechin accounted for 0.3-0.7% and for 62–88% of the total terminal and extension units, respectively, in the pulp and for 0.4–1.1% and for 52–75% of the total terminal and extension units, respectively, in the peel. However, Tanaka et al. (32) and Uclès Santos et al. (21) found major amounts of (-)-epigallocatechin (88–98%) associated with minor quantities of (-)-epigallocatechin (2–12%) in the pulp of green bananas from a wide range of genotypes. Uclès Santos et al. (22) report an (-)-epigallocatechin/(-)-epicatechin mass ratio of 6.41 to 64.36 in banana pulp, depending on the variety. This ratio varied here from 1.7 to 8 in the pulp and from 1.1 to 2.7 in the peel (21). The average degree of polymerization of the proanthocyanidins (n) increases according to the extraction method; indeed, large-sized tannins will be more difficult to extract than small-sized tannins. The studied samples were freeze-dried. It is likely that this treatment affected the extraction of the large tannins. The DPn corresponds to the average of all the extractible tannins in the samples. A more thorough study could be conducted on the residue after extraction to determine whether a large part of the tannins remain unextracted, probably those of very high molecular weight or those bound to fibers and sugar in the cell walls.

The average degree of polymerization of the procyanidins (n) was calculated following the thiolysis reaction from the ratio of terminal to extension units. Proanthocyanidin n in the peel varied from 17 to 35 and in the pulp from 67 to 114 (Table 3). Some studies on banana polyphenols have identified proanthocyanidin dimers, trimers, and oligomers of procyanidins (28, 31), but never polymers. Other fruits such as apples, persimmons, grapes, and pears also have highly polymerized proanthocyanidins (27, 33-35). Guillevic and Avrolles apples differ markedly from the other cider apple varieties, with an exceptional pulp proanthocyanidin n of 40 and 50, respectively (33). Moreover, Guyot and collaborators reported proanthocyanidin n values for Avrolles aqueous acetone fractions extracted from pulp and eluted on normal-phase HPLC ranging from 47 to 190 (34). Li et al. showed that the proanthocyanidins n range from 19 to 47 for persimmon pulp. Mané [26] found proanthocyanidins n for grape pulp ranging from 18 to 21, and for grape skin from 23 to 39. Ferreira [27] reported a proanthocyanidin n of pear pulp of 26.

Table 3: *Comparison and Characteristics of the Procyanidins of the Different Banana Varieties Determined By HPLC-DAD Following Thioacidolysis Degradation.*

			Flavan-3-ol terminal units			Flavan-3-ol extension units		EGC/EC
Variety	Compartment	DPn	%CAT	%EGC	%EPC	%EGC	%EPC	
GN	pulp	109	0.04	0.3	0.5	76.8	22.3	3.4
PL	pulp	67	0.3	0.7	0.8	61.6	36.6	1.7
PO	pulp	88	0.1	0.4	1	66.8	31.7	2.1
CAF	pulp	187	0	0.2	0.3	88.2	11.3	8
RED	pulp	114	0.0	0.4	0.5	74.5	24.6	3
SD			0.05	0.07	0.11	4.07	3.87	
GN	peel	29	0.3	1.1	4.5	56.7	37.5	1.4
PL	peel	35	0.4	1.4	4.8	52.3	41.1	1.2
PO	peel	19	0.4	0.6	2.5	64.7	31.8	1.9
CAF	peel	31	0.2	0.6	1.8	73.1	24.4	2.7
RED	peel	17	0.6	0.4	0.5	74.5	24.6	1.4
SD			0.02	0.11	0.35	3.18	1.33	

GN: Cavendish Grande Naine; PL: Plantain; PO: Poto; CAF: Café; RED: Red; CAT: (+)-catechin; EPC: (-)-epicatechin, EGC: epigallocatechin; $\overline{DP}$ n: average degree of polymerization of procyanidins; SD: standard deviation, degree of freedom =5.

Usually, degrees of polymerization are higher in the peel than in the pulp (23, 25), which was not verified here.

No flavan-3-ol monomers were detected, in disagreement with previous results. In an HPLC study using a postcolumn derivation procedure, de Pascual-Teresa et al. identified (-)-epicatechin and (-)-epigallocatechin in banana pulp (31), while Someya et al. and Bennett et al. detected (+)-catechin, (+)-gallocatechin, and (-)-epicatechin in an extract from banana pulp or banana peel (11, 28). The absence of monomers of flavan-3-ols in parallel with the presence of highly polymerized proanthocyanidins also occurs in some apple cider varieties like Avrolles and Guillevic, which contain highly polymerized procyanidins with only trace amounts of flavan-3-ols monomers and few dimers (33). In the case of bananas and the negative correlation between highly polymerized proanthocyanidins and flavan-3-ols, monomers might be related to fruit physiology and especially to the biosynthesis of proanthocyanidins.

Flavonols

Kaempferol hexosides, myricetin and isorhamnetin glycosides were identified by UPLC/MS. The protonated molecular ions of MS fragmentation were m/z 593, 625, 640, respectively. The aglycones were detected with protonated molecular ions at m/z 285, 271 and 316; the loss of m/z 162 and 229 suggested hexosides residue for kaempferol hexosides and isorhamnetin glycosides; the loss of m/z 316 from myricetin glycoside suggested a rutinosyl group, a disaccharide of glucose and rhamnose. The daughter molecular ion of the MS2 was m/z 316, and that of MS3 was 271, 179 and 151.

Flavonols quantified in banana peel and pulp, depending on the variety, were myricetin hexose-deoxyhexose; rutin, isorhamnetin-hexoside, isorhamnetin dihexoside, and two kaempferol hexoside-deoxyhexose. Kevers [28] also identified flavonols in banana as glycosides of myricetin, quercetin and kaempferol. Total flavonol levels (Table 2) were in the range 0,0031 g/ 100 g DW (cv. plantain) to 0,0460 g/ 100 g DW (cv. Cavendish) in banana pulp, and 0,0190- 0,3094 g/ 100 g DW in peel (36). The results were consistent with previously published quantitative data dealing with flavonols in banana varieties (16). In all of the varieties, the flavonols were concentrated in the peel. The dominant form in all varieties was myricetin hexose-deoxyhexose in pulp and rutin in peel, except for the Cavendish cultivar. The individual flavonol conjugates were not necessarily distributed in the same proportions between the fruit pulp and peel, and depended on the variety, as in fruits such as apple (37).

Anthocyanins

Anthocyanins were also naturally present in the fruits as glycosides. In the studied banana varieties, only the red banana (RED) contains anthocyanins, essentially in the peel.

Two anthocyanins were identified in the unripe red banana peel: a glycoside of cyanidin, cyanidin-3-rutinoside (0,03380 ± 0,00015 g/100 g DW), and one of peonidin, peonidin-coumaroyl-hexoside (0,03769 ± 0,00011.9 g/100 g DW) (Table 3).

Other Phenolic Compounds

One hydroxycinnamic acid was detected in the five banana varieties; its structure contained the cinnamoyl system, which is responsible for the band I absorption (310-325 nm) seen in the UV-spectra. This hydroxycinnamic acid had m/z 353 and a fragment corresponding to the cinnamoyl molecule (cinnamoyl+ ion) due to the loss of esterified acid at m/z 191 and 179, respectively.

Banana peel contained large amounts of dopamine, ranging from 0,1900 (cv. plantain) to 0,7800 g/100 g DW (cv. poto). Dopamine content in banana pulp ranged from 0,0012 (cv. plantain) to 0,0060 g/100 g DW (cv. poto) and was lower than in the peel. The results were consistent with previously published quantitative data dealing with dopamine in banana varieties (14, 16).

Conclusion

The combination of HPLC-DAD and UPLC-ESI MS allowed the characterization of the phenolic compounds in banana pulp and peel. Proanthocyanidins represented the major class of compounds both in the peel and in the pulp of all five banana varieties. Pulp proanthocyanidin concentrations were lower than the tannin content of banana pulp. (+)-Catechin was only found as terminal units and accounted for 0.00–0.3% of the total units in the pulp and for 0.2-0.6% of the total units in the peel. Other units that corresponded to (-)-epicatechin and (-)-epigallocatechin were found as terminal and extension units. The major extension unit of the proanthocyanidins was (-)-epigallocatechin. To our knowledge, this paper is the first to report the number of proanthocyanidins in banana pulp and peel.

This study confirmed the occurrence of dopamine, a hydroxycinnamic acid, two kaempferol glucosides, an isorahmnetin glucoside, rutine, and myricetin rutinoside. Two anthocyanins were identified in the immature red banana, but essentially in the peel. However, further investigations into the fate of phenolic compounds during storage or processing are necessary before the use of banana extracts in food or in food supplements.

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