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Anthocyanins from banana bracts (*Musa X paradisiaca*) as potential food colorants

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Abstract

Banana (*Musa X paradisiaca*) bracts, abundant edible residues of banana production, were investigated as a potential source of natural colorant. Anthocyanins were extracted with acidified methanol, purified using C-18 resin, and characterized by UV-visible spectroscopy, physicochemical reactions, HPLC, and electrospray mass spectrometry. Monomeric anthocyanin content was 32.3 mg/100 g bracts on a cyanidin-3-rutinoside basis. Color characteristics (Hunter CIE L^*hc) of a solution (absorbance of 0.3, 520 nm, pH 3.5), were $L^* = 86.8$, $h = 44.2$ and $c = 12.7$. Cyanidin-3-rutinoside represented ~80% of the total pigment. Other anthocyanins were 3-rutinoside derivatives of delphinidin, pelargonidin, peonidin and malvidin. One acylated anthocyanin (~2% of the pigment) was found but not identified. Acid hydrolysis of anthocyanins revealed the concomitant presence of six more common anthocyanidins (delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin) suggesting that, besides being a good pigment source, it could also be a useful tool for anthocyanin identification.

Keywords: Banana bracts; *Musa X paradisiaca*; Anthocyanins; Natural colorants; Pigments

1. Introduction

Interest in anthocyanins has increased significantly due to their bright attractive colors, water-solubility, that facilitates incorporation into aqueous systems, and beneficial health effects (Timberlake & Henry, 1988; Lauro, 1991; Henry, 1996; Cao, Sofic & Prior, 1997; Wang, Cao & Prior, 1997). New sources of anthocyanins with high tinctorial power, stability and low cost are desired as natural food colorants (Francis, 1982; Henry, 1996).

Most bananas have red, purple or violet bracts although a few are acyanic — green or yellow. According to Simmonds (1962), the variation in bract color is correlated with the composition of the anthocyanins present, which is distinctive of species and subspecies. Therefore, anthocyanin chemistry was used as a taxonomic tool in species differentiation (Simmonds, 1962; Horry & Jay, 1988). All six of the common anthocyanidins have been detected in the bracts of different species. They occur as pelargonidin and cyanidin in *Musa coccinea*; as cyanidin and delphinidin in *Musa balbisiana*; *Musa velutina* and *Musa laterita*; as cyanidin, delphinidin, peonidin, petunidin and malvidin in *Musa acuminata;* and as peonidin and malvidin in *Musa flaviflora*, *Musa ornata* and *Musa violascens* (Simmonds, 1962; Horry & Jay, 1988). Horry and Jay (1988) separated nine anthocyanins from bracts of 59 banana varieties — hybrids and cultivars of *Musa acuminata* and *M. balbisiana*. Five of them were identified as 3-rutinosides, one was characterized as a cyanidin derivative and three were not identified. The samples analyzed were separated into five main chemotypes, based upon the ratio between delphinidin and cyanidin derivatives.

The potential of food plants as commercial sources of anthocyanins is generally limited by availability of raw

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material. From an economic perspective, the best potential commercial sources of anthocyanins are those from which the pigment is a by-product of the manufacture of other value-added products, e.g. grape skin extracts (Jackman & Smith, 1996). Banana, one of the most important fruit crops in Brazil, is produced throughout the year. Brazil is the second worldwide producer and the country with the highest *per capita* consumption — 50–60 kg/year (FAO, 1994). The majority of the bananas produced in Brazil belong to the species *Musa paradisiaca*, *Musa cavendishii* and *Musa sapientum*. In some regions of Brazil, banana bracts are used for culinary purposes (Haddad, Visentainer, Matsushita & Souza, 1992) and, in France, fresh bracts are available at supermarkets. In both countries they are cooked before consumption. They are also commercialized canned, especially in Oriental food stores. However, most of the bracts are considered residues, being disposed of during banana harvesting.

Since the bracts of banana are widely available and have been traditionally used as food without apparent toxic effect, they could be a potential source of anthocyanins. The objective of this study was to determine the anthocyanin pigment content and profile of the bracts of the banana species *Musa×paradisiaca*, for their potential application as a natural food colorant.

2. Material and methods

2.1. Materials

Bracts of banana *Musa×paradisiaca* were obtained from farms in Janaúba, state of Minas Gerais, Brazil. The colored layers of the bracts were taken and washed. Grape, strawberry and sweet cherry were used as references for the identification of anthocyanidins and anthocyanins. Succinic and malonic acids were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Extraction of anthocyanins

Anthocyanins were extracted with 0.15% HCl in methanol (Francis, 1982). The bracts were blended with ca. 2 l of solvent/kg and filtered on a Buchner funnel. The filter cake residue was re-extracted until a clear solution was obtained. The extracts were combined and concentrated on a Büchi rotavapor at 35°C. The aqueous extract was made up to a known volume with distilled water.

2.3. Monomeric anthocyanin content

The extract obtained was analyzed for the levels of monomeric anthocyanins using a pH differential method (Wrolstad, Culbertson, Cornwell & Mattick, 1982). A Shimadzu 160A UV spectrophotometer and 1 cm pathlength silica cells were used for spectral measurements at 420, 510 and 700 nm. Pigment content was calculated as cyanidin-3-rutinoside, using an extinction coefficient of 28,800 l cm^{-1} mg^{-1} and molecular weight of 445.2 g (Giusti, Rodriguez-Saona & Wrolstad, 1999).

2.4. Color characteristics

Hunter CIE L^* a^* b^* characteristics of anthocyanin containing solutions were determined using a ColorQuest Hunter colorimeter (Hunter Associates Laboratory, Reston, VA). Anthocyanin extracts (0.315 of absorbance at 520 nm, pH 3.5) were placed in a 1-cm pathlength optical glass cell (Hellma, Germany) and color parameters were measured in duplicate under total transmission mode, using illuminant C and 10° observer angle. The chroma (c) and hue angle (h) were calculated by the formulas $c=(a^{*2}+b^{*2})^{1/2}$ and $h=(\tan^{-1} a^*/b^*)$, respectively.

2.5. Anthocyanin purification

The aqueous extract was passed through a C-18 Sep-Pak cartridge (Water Associates, Milford, MA), previously activated with methanol followed by 0.01% aqueous HCl (Giusti & Wrolstad, 1996). Anthocyanins (and other phenolics) were adsorbed onto the Sep-Pak; sugars, acids and other water-soluble compounds were eluted with 2 volumes of 0.01% aqueous HCl. Anthocyanins were recovered with methanol containing 0.01% HCl (v/v). The methanol was evaporated using a Büchi rotavapor at 35°C and the pigments were dissolved in ultrapure water (Milli-Q, Millipore, Bedford, MA) containing 0.01% HCl.

2.6. Alkaline hydrolysis of anthocyanins

Purified pigment (ca. 10 mg) was hydrolyzed (saponified) in a screw-cap test tube with 10 ml of 10% aqueous KOH for 8 min at room temperature (ca. 23°C) in the dark, as described by Hong & Wrolstad (1990a). The solution was neutralized using 2 M HCl, and the saponified anthocyanins were purified using C-18 Sep-Pak cartridge (Waters Assoc., Milford, MA) and concentrated, as previously described.

2.7. Acid hydrolysis of anthocyanins

Fifteen ml of 2 M HCl were added to ca. 1 mg of purified saponified pigment in a screw-cap test tube, flushed with nitrogen and capped. The pigment was hydrolyzed for 45 min at 100°C, then cooled in an ice bath (Hong & Wrolstad, 1990b). The anthocyanidins were purified using C-18 Sep-Pak cartridge, as previously described.

2.8. HPLC separation of anthocyanins, saponified anthocyanins and anthocyanidins

A Perkin-Elmer Series 400 liquid chromatograph, equipped with a Hewlett-Packard 1040A photodiode array detector and a Hewlett-Packard 9000 computer system, was used. Detection was at 280, 320 and 520 nm, simultaneously. The spectra (detection wavelengths from 250 to 600 nm) were recorded for all peaks.

For the separation of anthocyanins, saponified anthocyanins and anthocyanidins, a Supelcosil LC-18 column (5 μm), 250×5 mm i.d. (Supelco, Bellefonte, PA) was used, fitted with a ODS-10, 40×4.6 mm i.d., Micro-Guard column (Bio-Rad Laboratories). Solvent A was 100% HPLC grade acetonitrile, and B was 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water (Milli-Q, Millipore, Bedford, MA). The program followed a linear gradient from 0 to 30% A in 30 min. The flow rate was 1 ml/min and the injection volume 50 μl (Giusti & Wrolstad, 1996).

2.9. Electrospray mass spectroscopy (MS) of anthocyanins

Low-resolution MS was performed using electrospray MS. The instrument was a Perkin-Elmer SCIEX API III+ Mass Spectrometer, equipped with an Ion Spray source (ISV = 4700, orifice voltage of 80) and loop injection. A volume of 5 μl of partially purified anthocyanin, saponified anthocyanin and anthocyanidin extracts was injected directly into the system (Giusti & Wrolstad, 1996).

2.10. Organic acid analysis

Organic acids, obtained from the saponification of banana bracts anthocyanins, were separated by HPLC. A Perkin-Elmer Series 400 liquid chromatograph, equipped with a Hewlett-Packard 1040A photodiode array detector and a Hewlett-Packard 9000 computer system, were used. Detection was at 214 and 224 nm, simultaneously. Spherisorb ODS-2 and Spherisorb ODS (Supelco, Bellefonte, PA), both of 5 μm and 250×4.6 mm i.d., were connected in series. The mobile phase used was phosphate buffer (27.2 g KH_2PO_4/l with water), pH adjusted to 2.4 with concentrated phosphoric acid. The flow rate was 0.7 ml/min and the injection volume 50 μl (Giusti & Wrolstad, 1996). The spectra and retention times of the acids were compared to those of pure standards.

2.11. Physico-chemical reactions

The reaction with aluminium chloride was used to verify the existence of vicinal hydroxy-groups. The absorption spectra of a methanolic solution of the pigment were registered before and after addition of 5% methanolic $AlCl_3$ (Markakis, 1982; Gross, 1987).

The presence of fluorescence under ultraviolet radiation was checked for anthocyanins separated by paper chromatography, using 1% HCl and BAW as mobile phases to investigate the existence of substituent at C-5 (Francis, 1982).

3. Results and discussion

3.1. Monomeric anthocyanin content and color characteristics

The extraction of anthocyanins from banana bracts yielded 32 mg of monomeric anthocyanins/100 g, which corresponded to 250 mg/100 g on a dry weight basis. This anthocyanin content is higher than the one reported for red cabbage (25 mg/100 g), already commercially available as a food color extract (Timberlake, 1989). Considering that each bract contains ~600 g of colored bracts and that a hectare produces ~1500 plants (and therefore bracts), the potential pigment production, just from the waste generated by banana production, would be 290 g of anthocyanins per Ha. Therefore it could be commercially feasible to produce pigment from this source.

The Hunter CIE $L^*a^*b^*$ color characteristics of a solution of banana bracts' anthocyanins with absorbance of 0.315 at 520 nm and pH of 3.5 were $L^* = 86.8$, $a^* = +9.1$ and $b^* = +8.9$. The chroma and hue angle were 12.7 and 44.2, respectively. It had an attractive red hue.

3.2. HPLC analysis

Six anthocyanins were separated by HPLC as shown in Fig. 1a. Peak 3 represented about 80% of the total area at 520 nm, while the other peaks represented less than 3% each. The UV-visible spectral characteristics of the different pigments were determined. The $Abs_{440}/Abs_{\lambda max}$ ratios, calculated for each anthocyanin, were 29.9, 47.6, 32.2, 28.8, 34.5 and 31.1% for peaks 1 through 6, respectively. According to Harborne (1967) these values indicate glycosidic substitutions at positions 3 or 3,7 of the flavylium ring. The relatively low absorbance at the 310–320 nm range, indicated that anthocyanins were not acylated with hydroxylated aromatic organic acids (Harborne, 1967; Hong & Wrolstad, 1990a). Horry and Jay (1988) also observed absence of acylation on banana bracts' anthocyanins.

Saponification of banana anthocyanins produced a chromatographic profile (Fig. 1b) similar to the one of the anthocyanins (Fig. 1a), with the exception of peak 1, which disappeared after saponification with the formation of a new peak, labeled as peak 1s (Fig. 1b). The shift in retention time, combined with the low absorbance at the 310–320 nm range, suggested that the anthocyanin corresponding to peak 1 was acylated with

an aliphatic organic acid. Typically, acylation with common aliphatic or cinnamic acids will render the molecule more non-polar, with longer retention times under reversed phase chromatography. The case of peak 1 was unusual because of its high polarity, revealed by its short retention time.

Acid hydrolysis of saponified anthocyanins yielded six anthocyanidins. The retention times and UV-visible spectra of the peaks matched those of delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin obtained by the acid hydrolysis of samples commonly used as reference — grape and strawberry (Ribereau-Gayon, 1982; Hong & Wrolstad, 1990a).

3.3. Organic acid analysis

The saponified anthocyanin extract was submitted to HPLC analysis of aliphatic organic acids in an attempt to determine the acylating group esterified to the sugars of anthocyanin peak 1. HPLC analysis revealed the presence of succinic acid in the saponified anthocyanin fraction. However, it was not possible to determine whether succinic acid was produced by the saponification of anthocyanins or by saponification of other compounds present in the banana bracts extracts. Further studies would be needed to elucidate the source of this organic acid, and to determine the acylating group esterified to anthocyanin peak 1.

3.4. Electrospray mass spectroscopy of anthocyanidins and anthocyanins

Electrospray mass spectrometry of anthocyanins and hydrolyzed anthocyanins produced, primarily, intact molecular ions. The low resolution mass spectrum of banana bracts anthocyanidins showed m/z values of 270.8, 286.8, 300.8, 302.8, 316.8 and 330.8, which matched the molecular mass of the anthocyanidins pelargonidin (271), cyanidin (287), peonidin (301), delphinidin (303), petunidin (317) and malvidin (331). The presence of the six most frequently found anthocyanidins in one single product is of great interest. Such a product could be used as a reference in the identification of anthocyanidins.

The electrospray mass spectrum of banana bract anthocyanins produced ions with m/z ratios of 578.8, 594.8, 608.8, 610.8, 624.8 and 638.8, matching the molecular mass of rutinoside derivatives of pelargonidin (579.2), cyanidin (595.2), peonidin (609.2), delphinidin (611.2), petunidin (625.2) and of malvidin (639.2). Horry and Jay (1988) detected five of these compounds (the exception being pelargonidin) in *M. acuminata* and *M. balbisiana*.

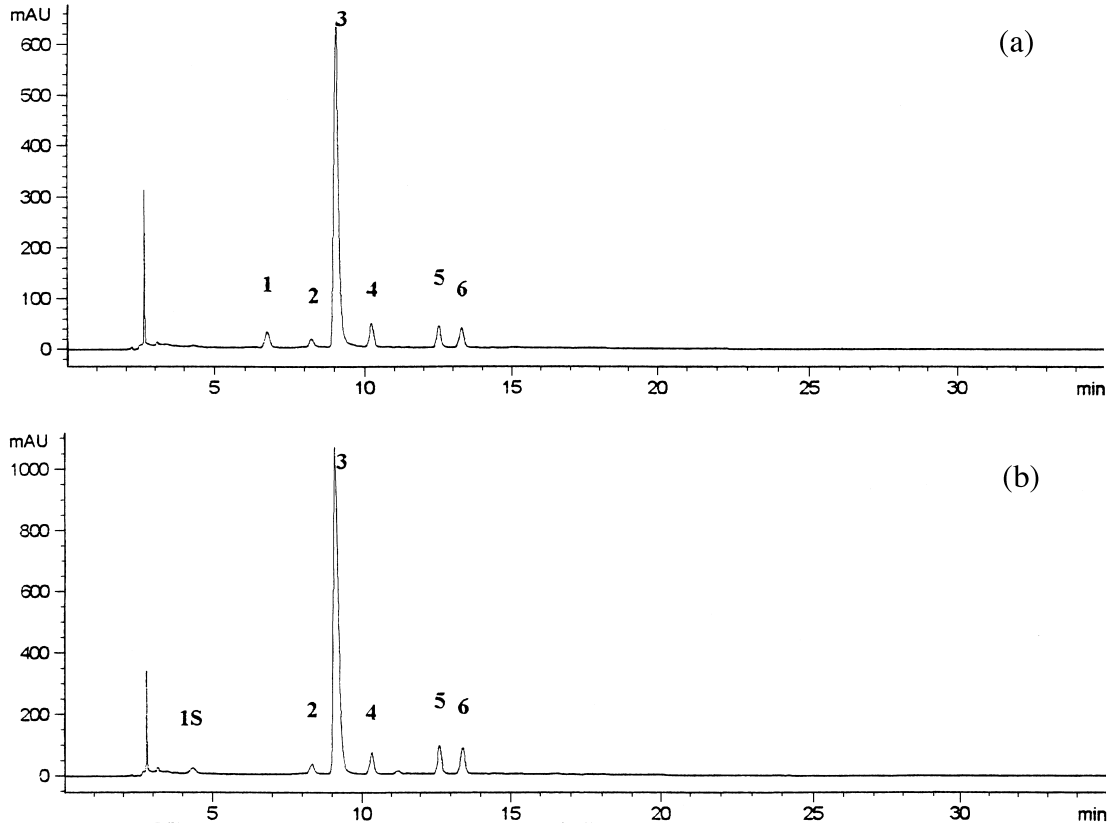

Fig. 1. HPLC separation of (a) anthocyanins and (b) saponified anthocyanins from banana bracts. Supelcosil LC-18, 5 μm, 250×5 mm. Solvent A: 100% acetonitrile; B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. Linear gradient of 0–30% A in 30 min. Flow rate: 1 ml/min. Injection volume: 50 μl. Detection at 520 nm.

An additional peak was detected with a m/z 694.8, and that peak disappeared after saponification, suggesting that it corresponded to the molecular mass of the acylated anthocyanin. As seen before, succinic acid was detected in the saponified anthocyanin extract. The rutinoside of cyanidin acylated with succinic acid shows such molecular mass; however, this compound should present a longer retention time under reverse phased HPLC as compared to the non acylated derivative. Horry and Jay (1988) also reported the presence of a derivative of cyanidin other than cyanidin-3-rutinoside in some varieties of *M. acuminata;* however, they did not identify it.

3.5. Peak identification

The tentative identities of banana bracts anthocyanins are summarized on Table 1. The data obtained suggested the presence of rutinoside derivatives of six anthocyanidins. According to the $Abs_{440}/Abs_{\lambda max}$ ratio, the glycosidic bond is located at C-3 of the aglycone, the preferential location of sugar substitutions on anthocyanidins (Harborne, 1967). These results are similar to those described by Horry and Jay (1988), who reported the presence of 3-rutinosides of delphinidin, cyanidin, petunidin, peonidin and malvidin in the bracts of different varieties of *M. acuminata* and *M. balbisiana*.

Sweet cherry contains cyanidin-3-rutinoside and peonidin-3-rutinoside anthocyanins (Wrolstad, Hong, Boyles & Durst, 1993), and was used as a reference. Peak 3, the major anthocyanin in banana bracts, matched the retention time and spectral characteristics of cyanidin-3-rutinoside from sweet cherry. The predominance of this anthocyanin was confirmed by the bathochromic shift observed after addition of $AlCl_3$ to the extract (Markakis, 1982; Gross, 1987; Jackman & Smith, 1996). Also, using cherry as reference, peak 5 matched the retention time and spectral characteristics of peonidin-3-rutinoside. Its typical maximum absorbance wavelength — 520 nm — was also observed.

The typical order of elution of different anthocyanins with similar glycosylation patterns, under the experimental HPLC conditions, would be determined by the polarity of the aglycone. Therefore, they would be expected to follow the same elution order as shown by anthocyanidins: first would be delphinidin, followed by cyanidin, petunidin, pelargonidin, peonidin, and last malvidin. The typical order of elution would suggest that peaks 2 and 6 could correspond to rutinosides of delphinidin and malvidin, respectively. Spectral data from peak 6 indicated maximum absorbance at 530 and 535 nm, respectively, which is characteristic of malvidin-3-rutinoside, previously reported by Horry and Jay (1988) in bracts of different banana varieties. Supporting this identification is the longer retention time, due to the lower polarity of the malvidin derivative compared to the other anthocyanins, due to the presence of two methoxyl groups in ring B of the anthocyanin (Hong & Wrolstad, 1990a). Similarly, the wavelength of maximum absorbance for peak 2 was 530 nm, in agreement with the tentative identification of the pigment as delphinidin-3-rutinoside.

The elution order of peak 4 could correspond to either petunidin or pelargonidin. However, the spectral characteristics of the pigment, with a wavelength of maximum absorbance of 530 nm, support the identification of the pigment as petunidin-3-rutinoside. Typical wavelength of maximum absorbance of non-acylated pelargonidin derivatives is between 500 and 505 nm (Hong & Wrolstad, 1990b; Giusti et al., 1999).

Even though the anthocyanidin pelargonidin was detected, it was not possible to identify the corresponding anthocyanin. This could be due to its low concentration compared to the other anthocyanins, evidenced by the low relative intensity detected during MS analysis and HPLC separation of anthocyanidins. However, pelargonidin-3-rutinoside could correspond to the small peak at retention time 11.2 min (Fig. 1b), eluted between petunidin-3-rutinoside and peonidin-3-rutinoside. Such elution, order observed for the anthocyanins, would correspond to the polarity criteria and also to the findings by Horry and Jay (1988).

Peak number 1 was a highly polar anthocyanin acylated with organic acids; however, its identity was not

Table 1
Peak assignment for anthocyanin pigments from bracts of *Musa X paradisiaca*

Peak number[a]	Peak area (%)	Retention time (min)	λ max (nm)	$A_{440}/A_{\lambda\ max}$	Molecular ion (m/z)	Peak assignment[b]
1[c]	2.9	5.8	528	29.9	694.8	cyd-3-rha-7-glu
2	1.5	8.3	530	47.6	611.2	dpd-3-rut
3	82	9.1	528	32.2	595.2	cyd-3-rut
4	4.9	10.2	530	28.8	625.2	ptd-3-rut
4a	4.4	11.2	–	–	579.2	pgd-3-rut
5	4.0	12.8	520	34.5	609.2	pnd-3-rut
6	0.3	13.5	535	31.1	639.2	mvd-3-rut

[a] As indicated on Fig. 1
[b] cyd, cyanidin; dpd, delphinidin; ptd, petunidin; pgd, pelagornidin; pnd, peonidin; mvd, malvidin.
[c] Tentative identification.

elucidated. The possibility of being a pelargonidin derivative did not seem likely since the wavelength of maximum absorbance was much higher than that of pelargonidin, and acylation with aliphatic acids does not cause the bathochromic shift typical of cinnamic acid acylation (Hong & Wrolstad, 1990b; Giusti et al., 1999). Horry and Jay (1988) also detected one anthocyanin, which they considered to show unusual chromatographic behavior, with reverse phase HPLC elution order similar to the one obtained in this study for peak number 1. They suggested it to be a cyanidin derivative. MS results suggested that a possible identity for the peak would be a derivative of cyanidin rutinoside acylated with succinic acid. However confirmation of this suggestion is needed.

4. Conclusions

Banana, *Musa X Paradisiaca*, bracts' anthocyanin content and profile were investigated. Banana bracts contained 32 mg anthocyanin/100 g bracts. Non-acylated rutinoside derivatives of five different anthocyanidins accounted for ~97% of the total pigment, cyanidin-3-rutinoside being the major anthocyanin (~80%); present, the other four were 3-rutinoside derivatives of delphinidin, petunidin, peonidin and malvidin. One additional anthocyanin (~2% of total pigment) was acylated with aliphatic acids, but its chemical structure was not elucidated. Banana bracts showed great potential as an economical source of natural pigments because of their edible character and abundance as a residue of banana production, coupled with the attractive hue of the pigments. Furthermore, the concomitant presence of the six most common anthocyanidins makes it a useful tool in anthocyanin identification.

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