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An HPLC-MS Analysis of Phenolic Antioxidants in Banana Peel

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Banana peels are rich in many nutrients which allow for their use as food ingredients and biofertilizers in tropical agriculture. The phenomenon of rapid peel browning is a common occurrence, and is attributable to the peel's high polyphenol oxidase activity and high concentrations of polyphenols. An analysis of methanol extracts of banana peel shows a diversity of polyphenols, including proanthocyanidin oligomers (n = 1–4) and polymers, ferulic acid and *p*-coumaric acid conjugates, and flavonoids. Analysis of these classes of compounds is facilitated by separations accomplished by LH20 chromatography. The flavonoids occur mainly as flavonol glycosides, including conjugates of myricetin and quercetin. The numerous isomers of ferulic and *p*-coumaric acid conjugates (each n > 6) in banana peel occur as high molecular weight compounds, and their mass spectra exhibit similar patterns of neutral losses, suggesting that they occur with similar side groups. The tentative structures and degrees of polymerization of the proanthocyanidins were analyzed by electrospray ionization mass spectrometry. This is further facilitated by separations of these complexes by normal phase thin layer chromatography. This study illustrates the diversity of the polyphenols in banana peel, and is supportive of the use of this portion of the fruit as a high antioxidant food ingredient.

Banana (*Musa* sp.) is the major fresh fruit imported into the United States. Banana shipments to the United States in 2010 valued $1.64 billion, and represented a third of the total value of fresh fruit imported that year (Evans and Ballen, 2015). In contrast, U.S. banana production is small and occurs only on approximately 500 acres, having a crop value of $2 million. This production mainly targets specialty markets, including those for locally grown organic and processed bananas. Yet, banana is one of the most important food crops worldwide, with production in over 130 countries. The banana crop not only provides an important nutritious food to the worldwide human population, but is also critical to the economies of tropical agricultural regions, particularly those in Southeast Asia, Latin America, the Caribbean, and West Africa. In 2009, world production of bananas was an estimated 97.3 million metric tons, grown on 4.9 million hectares (FAO, 2014).

The banana (pulp and peel) is an excellent source of fiber, potassium (USDA, ARS, Database 2016), and natural dietary antioxidants (Fatemeh et al., 2012). Many of the antioxidants are polyphenols (Montelongo et al., 2010, Bennett et al., 2010; Fernando et al., 2014; Tsamo et al., 2015), and are evident by the rapid browning observed with cut peel and pulp. The polyphenols in banana have been studied for their potential health benefits (Sundara et al., 2011), as well as for other nonfood uses (Mohapatra et al., 2010). Often the polyphenols in banana show strong varietal differences, and the characterization of the profiles of these compounds in specific cultivars is important for determinations of optimal postharvest storage conditions, human nutrition and health-benefit studies, as well as co-product development. To study these profiles, an initial high pressure liquid chromatography-photodiode array-mass spectrometry (HPLC-PDA-MS) study of 'Cavendish' banana peel was conducted.

Materials and Methods

Extraction of banana peel polyphenolics. Bananas originating from Central America, variety 'Cavendish', were purchased at a local supermarket. The peels of four bananas at a ripeness stage of 3 were cut into 3 cm pieces and placed in 2 L of methanol. The peels were homogenized with a Brinkmann Polytron homogenizer (Kinematica, Lucerne, Switzerland) at medium speed for 3 min. The homogenate was vacuum filtered through a fiberglass filter and the residue was re-homogenized with a second 2-L portion of methanol. The homogenate was likewise filtered, and the combined filtrates were reduced in volume with a rotary evaporator (Buchi, Flawil, Switzerland) to 400 mL. The resulting aqueous methanol solution was extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate fractions were evaporated to dryness, and the remaining residue was dissolved in 30 mL of methanol.

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Column chromatography and thin layer chromatography. LH20 resin (GE Healthcare Life Sciences) slurry was prepared in methanol, and packed into a glass column (3 cm x 28 cm). The column was equilibrated with methanol. The 30-mL methanol banana peel extract was run on the column at a flow rate of 5 mL/min. Fractions (25 mL) were collected, and every fifth tube was analyzed by HPLC for polyphenol content. Fractions with similar compound profiles were pooled and evaporated to dryness. Combined column fractions were additionally analyzed by analytical and preparative thin layer chromatography (TLC). Silica gel plates (Analtech, Newark, Del.) with fluorescence indicator were developed with chloroform:methanol:water, 65:35:4 by volume. Compounds on the TLC plates were detected by long wavelength UV, and scraped and extracted from the silica gel with methanol. Detection of proanthocyanidin monomers, oligomers, and polymers was achieved by the TLC method of (Sun et al., 1998).

High pressure liquid chromatography-photodiode array-electrospray ionization-mass spectrometry (HPLC-PDA-MS). The banana peel polyphenolics were analyzed with a Waters 2695 Alliance high performance liquid chromatograph (HPLC) (Waters, Medford, MA) connected in parallel with a Waters 996 PDA detector and a Waters Micromass ZQ single-quadrupole mass spectrometer equipped with an electrospray ionization source. Compound separations were achieved with a Waters XBridge C8 analytical column (5 μm, 4.5 × 150 mm) with solvent conditions initially composed of aqueous 0.5% formic acid:acetonitrile at 10:90 (by volume), then changed in acetonitrile content to 20%, 25%, 40%, 70%, 70%, 10%, and 10% by volume acetonitrile content at 10, 15, 23, 40, 45, 53, and 60 min, respectively, at a flow rate of 0.75 mL·min^{-1} (Hijaz et al., 2016). A flow splitter (10:1) was used to simultaneously monitor UV and mass spectra of the eluting peaks. UV spectra were monitored between 400 to 240 nm. ZQ parameters were as follows: MS parameters were as follows: ionization mode, ES$^-$; capillary voltage 3.0 kV; extractor voltage 5 V; source temperature 100 °C; desolvation temperature 225 °C; desolvation N_2 flow 465 L·h^{-1}, cone N_2 flow 70 L·h^{-1}; scan range *m/z* 50–1000; scan rate 1 scan s^{-1}; cone voltages 20 and 40 V. Analysis of the chromatograms was performed by using ZQ calculated mass extracted total ion chromatograms (TIC) obtained in scanning mode, or in the single ion response mode. To normalize the mass spectrometer response during sequential runs, an internal standard, mangiferin, was used. Data handling was done with MassLynx software version 4.1.

Semi-preparative HPLC was run with Varian Star 210 pumps, a 335 UV/VIS photodiode array detector, and Varian Star software. Compound isolations were achieved with linear gradients of 0.5% aqueous formic acid and acetonitrile at 5 mL/min using a 2.5 cm x 25 cm Synergi Hydro-RP column (Phenomenex, Torrence, CA). Compound elution was monitored at 280 nm and 330 nm.

Results

The HPLC-PDA-ESI-MS analysis of the banana peel extracts showed four main classes of polyphenols, including flavonol glycosides, two separate sets of hydroxycinnamates, and proanthocyanidins. Excellent preparative-scale separations of these classes of compounds were achieved by LH20 chromatography run initially with methanol, and then with 70% aqueous acetone for the elution of anthocyanidins and proanthocyanidin polymers. Additional compound separations were achieved by preparative silica gel TLC and C18 reversed phase HPLC. The UV and MS spectroscopic analyses of these polyphenols are reported in the following section.

Flavonol glycosides. Flavonols are a widely occurring class of flavonoids, particularly in fruits and vegetables, consisting of flavones with hydroxyl substituents at the 3-position of the middle C ring. Flavonols often exhibit powerful antioxidant and metal chelating properties (Hopia and Heinonen 1999, Heim et al., 2002), and some have been shown to possibly inhibit important cell activation/signaling events in human chronic diseases (Manthey et al., 2000, Montano et al., 2011). Previously, Tsamo et al., (2015) reported partial structures of seven flavonols in banana fruit, including glycosides of isorhamnetin, rhamnetin, kaempferol, quercetin, myricetin, and a methylmyricetin. Chemical structures of these flavonol aglycones are shown in Fig. 1. The protonated molecular weights $[M+H]^+$ of these compounds are 317, 317, 287, 303, 319, and 333 *m/z*, respectively. We used these mass ions to detect and analyze the partial structural characterizations of 'Cavendish' banana peel flavonol glycosides (Table 1). MS data in Table 1 indicate the presence of glycosides of quercetin, myricetin, methylmyricetin, and kaempferol in these peel extracts. Most of these compounds showed neutral losses of 162 and 146 atomic mass units (amu), suggestive of the occurrence of glucose and rhamnose substituents, respectively. Final determinations of the structures of these compounds await NMR analyses and comparisons with known standards.

Hydroxycinnamates. Two distinct classes of hydroxycinnamates were detected in the HPLC-PDA-MS analyses of the peel extracts. The first class of hydroxycinnamates eluted off the LH20 column at similar times, and exhibited UV/vis spectra nearly identical to that of *p*-coumaric acid (Fig. 2). Additionally, the MS of these compounds showed prominent 147 *m/z* fragment ions, which is also indicative of *p*-coumaric acid [165-H_2O]. These compounds showed high molecular weights, ranging from 611 to 737. When this hydroxycinnamate fraction is run on silica gel TLC, four major bands occur, where each band contained compounds with protonated molecular weights of either 737, 695, 653, or 611, each cluster differing by 42 amu. FTIR spectra of each band of compounds obtained from the preparative TLC

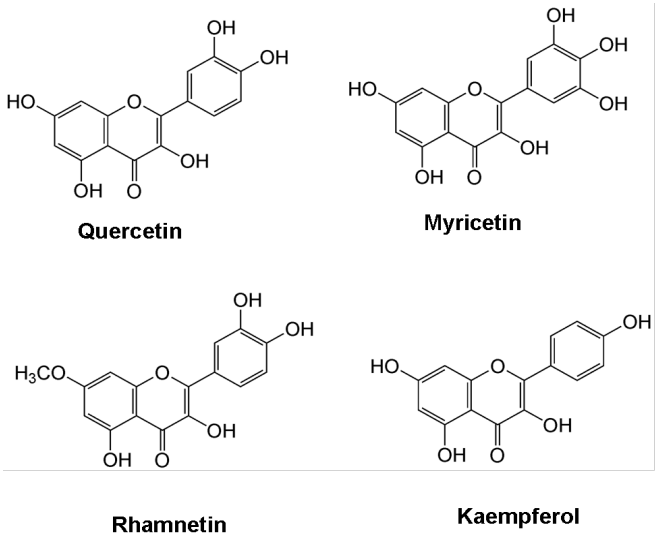

Fig. 1. Chemical structures of common plant flavonols.

Table 1. HPLC-PDA-MS analyses of UV/vis spectra and mass ion fragments of banana (*Musa* sp. 'Cavendish') peel flavonol glycosides. The UV/vis are the wavelength maxima (nm) of the optical spectra.

Elution time	UV/vis	*m/z*	Proposed composition[z]
9.1 min	260,356	757/627/481/319	M+G+R
10.8 min	255,353	611/465/303	Q+G+R
11.2 min	255,353	611/465/303 and 641/495/333	Q+G+R and MM+G+R
12.3 min	265,337	737/595/449/327,287	K+G+R
13.1 min	264,345	595/449/317,287	K+G+R and RH-glyc
14.1 min	265,340	347/303/287	K-glyc, Q-glyc
15.5 min	269,349	493/331	UK+G

Abbreviations: M (myricetin); Q quercetin; MM methylmyricetin; K (kaempferol); RH (rhamnetin); UK (unknown flavonol); glyc (unknown glycoside); G (glucose); and R (rhamnose).

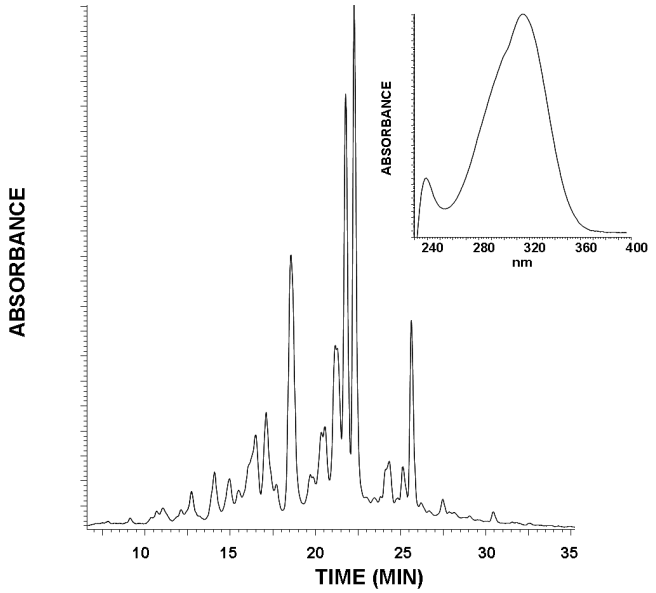

Fig. 2. HPLC chromatogram (320 nm) of *p*-coumaric acid-containing LH20 column fraction. Each peak exhibited a UV spectrum similar to *p*-coumaric acid (insert).

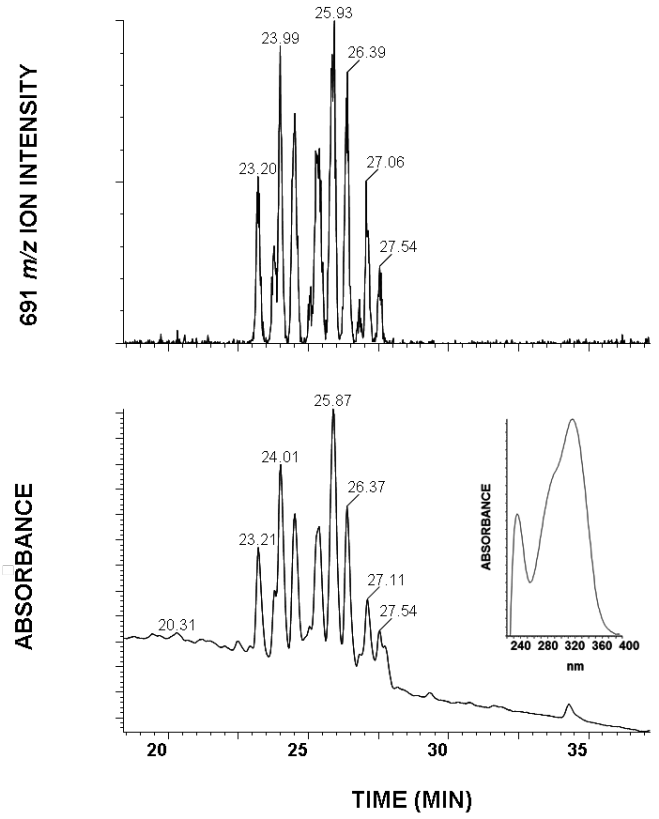

Fig. 3. HPLC chromatogram (320 nm) of ferulic acid-containing LH20 column fraction. Each peak (lower chromatogram) exhibited a UV spectrum similar to *p*-coumaric acid (insert). Each of these peaks also showed mass extracted fragment ion peaks at 691 *m/z* (upper chromatogram).

showed evidence of long chain alkanes, with partial unsaturation (data not shown) as constituents of these compounds. Further compound isolations and spectroscopic analyses are needed to gain a better understanding of the structures of these compounds.

A second group of hydroxycinnamates, consisting of 10 compounds, exhibits UV/vis spectra suggestive of ferulic acid as the primary phenolic constituent (Fig. 3). The MS of these compounds show identical protonated molecular mass ions at 691 *m/z* (Fig. 3), as well as a prominent fragment ion at 177 (data not shown). The latter is further evidence of ferulic acid as a constituent of these compounds, and these similarities in the spectroscopic properties of these compounds provide an indication of their close similarities in chemical structures.

Proanthocyanidins. Catechin, epicatechin and gallocatechin have been shown to be major polyphenols in 'Cavendish' peel, with gallocatechin occurring at 158 mg · 100 g dry weight (Someya et al., 2002). The concentrations of these compounds occur at much higher concentrations in the peel than in the pulp. In our current study a number of proanthocyanidins, including catechin monomer, oligomers, and polymer, were also detected in 'Cavendish' banana peel extracts. The occurrence of these compounds was suggested by the UV spectra of well-resolved HPLC peaks, and further confirmed by the mass spectra showing protonated mass ions at 291 *m/z* (monomer), 579 *m/z* (dimers), 867 *m/z* (trimers), and 1155 *m/z* (tetramers) (Fig. 4). Identifications of catechin and the dimer B1 are supported by HPLC peak overlaps with authentic standards. Use of the TLC separations of the monomers through polymers, developed by Sun et al., (1998) further demonstrated the presence of these compounds.

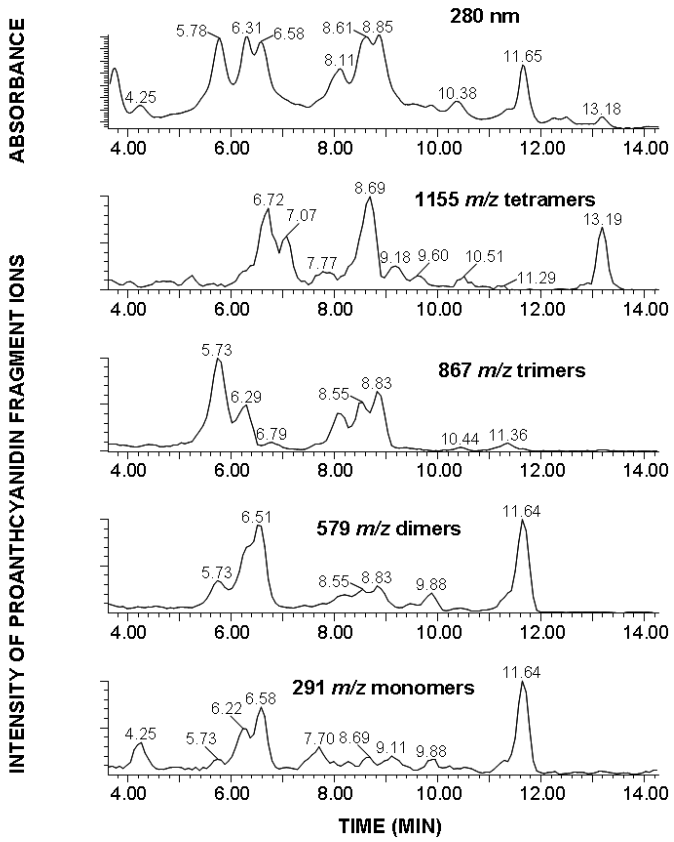

Fig. 4. Specific mass ion extracted detection of proanthocyanidin monomers, dimers, trimers, and tetramers at 291, 579, 867, and 1155 *m/z*, respectively. The UV chromatogram (top) at 280 nm, shows peaks exhibiting UV spectra similar to catechin.

Discussion

This analysis of the methanol-extracted polyphenols in banana peel shows a diversity of chemical structures, yet also strong similarities in chemical structures among the different classes of compounds. The conjugates of *p*-coumaric and ferulic acids are unknown, but evidence suggests that long-chain hydrocarbon substituents may be present. The identification of these soluble polyphenols will provided important clues into the chemical structures of the cell wall bound polyphenols, which constitute the major portion of these compounds in the whole fruit (Bennett et al., 2010). It is our goal to use this information on the chemical structures on banana polyphenols to better understand how these dietary compounds assist in improving human health, and how these compounds can be used as potential value-added materials generated from the whole crop production.

Literature Cited

Bennet, R.N., T.M. Shiga, N.M.A. Hassimoto, E.A.S. Rosa, F.M. Lajolo, and B.R. Cordenunsi. 2010. Phenolics and antioxidant properties of fruit pulp and cell wall fractions of postharvest banana (*Musa acuminata* Juss.) cultivars. J. Agr. Food Chem. 58:7991–8003.

Evans, E. and F. Ballen. 2015. Banana market. Extension Publ. FE 901. Florida Cooperative Extension Service, University of Florida, Gainesville.

Fatemeh, S. R., R. Saifullah, F.M.A. Abbas, and M.E. Azhar. 2012. Total phenolics, flavonoids and antioxidant activity of banana pulp and peel flours: influence of variety and stage of ripeness. Intern. Food Res. J. 19:1041–1046.

FAO [Food and Agriculture Organization of the United Nations and Horticulture and Tropical (RAMHOT) Products Team]. 2014. Banana Market Review and Banana Statistics 2012–2013. Market and Policy Analyses of Raw Materials Accessed June 14, 2016. <http://www.fao.org/economic/est/est-commodities/bananas/en/>.

Fernando, H. R. P., V. Srilaong, N. Pongprasert, P. Boonyaritthongchai, and P. Jitareerat. 2014. Changes in antioxidant properties and chemical composition during ripening in banana variety 'Hom Thong' (AAA group) and 'Khai' (AA group). Intern. Food Res. J. 21:749–754.

Heim, K.E., A.R. Tagliaferro, and D.J. Bobilya. 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13:572–584.

Hijaz, F., J.A. Manthey, D. Van der Merwec, and N. Killiny. 2016. Nucleotides, micro- and macro-nutrients, limonoids, flavonoids, and hydroxycinnamates composition in the phloem sap of sweet orange. Plant Signaling & Behavior. 11(6), e1183084. 11 p. doi: 10.1080/15592324.2016.1183084.

Hopia, A., and M. Heinonen. 1999. Antioxidant activity of flavonol aglycones and their glycosides in methyl linoleate. J. Amer. Oil Chem. Soc. 76:139-144.

Manthey, J.A., N. Guthrie, and K. Grohmann. 2001. Biological properties of citrus flavonoids pertaining to cancer and inflammation. Curr. Med. Chem. 8:135–153.

Mohapatra, D., S. Mishra, and N. Sutar. 2010. Banana and its utilization: An overview. J. Sci. Industrial Res. 69:323–329.

Montano, J.M.C., E.B. Moron, C.P. Guerrero, and M.L. Lazaro. 2011. A review on the dietary flavonoid kaempferol. Mini-Rev. Med. Chem. 11:298-344.

Montelongo, R.G., M.G. Lobo, and M. González. 2010. Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds. Food Chemistry 119:1030–1039.

Someya, S., Y. Yoshikib, and K. Okubo. 2002. Antioxidant compounds from bananas (Musa 'Cavendish'). Food Chemistry 79:351–354

Sun, B., C. Leandro, J.M. Ricardo da Silva, and I. Spranger. 1998. Separation of grape and wine proanthocyanidins according to their degree of polymerization. J. Agr. Food Chem. 46:1390–1396.

Sundara, S., S. Shadma Anjum, P. Dwivedi, and G. Kumar Rai. 2011. Antioxidant activity and protective effect of banana peel against oxidative hemolysis of human erythrocyte at different stages of ripening. Appl. Biochem. Biotechnol. 164:1192–1206.

Tsamo, C.V.P., M.-F. Herent, K. Tomekpe, T. H. Emaga, J. Quetin-Leclercq, H. Rogez, Y. Larondelle, and C. Andre. 2015. Phenolic profiling in the pulp and peel of nine plantain cultivars (*Musa* sp.). Food Chem. 167:197–204.

United States Department of Agriculture, Agricultural Research Service. 2016. National Nutrient Database for Standard Reference Release 28. Basic Report: 09040, Bananas, raw.