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Article *in* Food Research International · January 2014

DOI: 10.1016/j.foodres.2013.11.039

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Flour of banana (*Musa* AAA) peel as a source of antioxidant phenolic compounds

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ARTICLE INFO

Article history:
Received 14 October 2013
Accepted 23 November 2013

Keywords:
Banana
Phenolic compounds
Flavonols
Proanthocyanidins
Antioxidant activity

ABSTRACT

Banana peel is an underused by-product that can be processed to obtain flour that is more easily stored for further uses. The extracts of banana peel flour exhibited a high total phenolic content (around 29 mg/g, as GAE) due to the occurrence of important amounts of flavonoid phenolics: highly polymerized prodelphinidins (around 3952 mg/kg), followed by decreasing lower contents of flavonol glycosides (mainly 3-rutinosides and predominantly quercetin-based structures, accounting for around 129 mg/kg), B-type procyanidin dimers and monomeric flavan-3-ols (jointly around 126 mg/kg). The high total phenolic content of extracts of banana peel flour is likely responsible for the very high antioxidant activity (μM/g, as Trolox equivalents) measured by three different methods: FRAP, around 14 μM/g; ABTS, around 242 μM/g; and ORAC, around 436 μM/g. All these results suggest the interest in going in depth of the good use of banana peel as a profitable source of bioactive phenolic compounds.

1. Introduction

The banana peel is a household and industrial food waste discarded in large quantities in nature. It represents about 35% of the total fresh mass of ripe fruit (Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008; Reyes, 1991) and there is not further involved in remarkable industrial applications (Aurore, Parfait, & Fahrasmane, 2009; Qiu et al., 2010). Brazil was the second world producer of banana over the last two decades, accounting for overall production of 6.3 million tons per year (FAOSTAT, 2012). Around 53% of Brazilian production of banana is industrially processed, thus representing a potential generation of 1.2 million tons of banana peel waste (Instituto Brasileiro de Geografia e Estatística (IBGE), 2009). Bananas are one of the fruits most produced and consumed worldwide and the potential use of the peel would be of great relevance. Some researchers have revealed that the banana peel has compounds and nutrients important for food and for food industry. The banana peel is rich in dietary fiber, protein, essential amino acids, polyunsaturated fatty acids and potassium (Emaga, Andrianaivo, Wathelet, & Paquot, 2007). It also contains antioxidant compounds including polyphenols, catecholamines and carotenoids (Kanazawa & Sakakibara, 2000; Nguyen, Ketsa, & Van Doorn, 2003; Someya, Yoshiki, & Okubo, 2002; Subagio, Morita, & Sawada, 1996). Antioxidant compounds present in banana peel, particularly their polyphenols, could contribute positively to human health. Dietary polyphenols, by means of their well-known antioxidant properties that go beyond the modulation of oxidative stress, play an important role in the prevention of degenerative diseases, particularly cardiovascular diseases and cancers (Scalbert, Johnson, & Salmarsh, 2005).

With the development of orcharding sector in Brazil, the increase in the number of food industries and the intensification of fruit processing arises the problem of waste production (Qiu et al., 2010). Consequently, it becomes necessary the use of these residues because their current destination is associated with inappropriate environmental problems as well as economic losses (Cordenunsi, Shiga, & Lajolo, 2008; Sena & Nunes, 2006). In the processing to obtain pulps and nectars, for example, about 40% are waste, such as bagasse, peel, seeds and parts improperly processed. These byproducts are discarded into the environment without any form of treatment or, in some cases, may be used as formulation for animal feeding (Sena & Nunes, 2006). Wastes of food industry should be considered as potential sources of bioactive compounds that are of importance both for the food industry and pharmaceuticals due to its antioxidant capacity, low cost and easy availability (Babbar, Oberoi, Uppal, & Patil, 2011).

In order to minimize the problem, it is indispensable to develop technology that enables the rational utilization of waste generated in

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http://dx.doi.org/10.1016/j.foodres.2013.11.039

food processing industries, contributing even to the sustainable development of the country. Therefore, it is necessary to study the composition of the waste through scientific and technological research in order to create possibilities for turning them into profitable processing industry and use by potential consumers. Thus, when considering the importance of the phenolic compounds and the high volume of waste generated during the processing of banana, this study aimed to examine the in vitro antioxidant activity of flour banana peel (*Musa* AAA) and identify and quantify the phenolic compounds responsible for such activity.

2. Material and methods

2.1. Chemicals

All solvents were of HPLC quality, and all chemicals were analytical grade (>99%). Water was of ultrapure quality (Milli-Q). The following commercial standards from PhytoLab (Vestenbergsgreuth, Germany) were used: (−)-epigallocatechin and (−)-gallocatechin. Commercial standards from Extrasynthese (Genay, France) were used: procyanidins B1 and B2; kaempferol, quercetin, isorhamnetin, myricetin, syringetin; the 3-glucosides of kaempferol, quercetin, isorhamnetin, and syringetin; and the 3-rutinoside of quercetin. The following commercial standards from Sigma (Tres Cantos, Madrid, Spain) were used: gallic acid, (+)-catechin, (−)-epicatechin, (−)-epicatechin 3-gallate, and (−)-gallocatechin 3-gallate. Finally, a sample of procyanidin B4 was kindly supplied by Prof. Fernando Zamora (Department of Biochemistry and Biotechnology, Universitat Rovira i Virgili, Spain).

All the above-mentioned standards were used for identification in HPLC analysis. Quantification (in mg/kg of banana peel flour) was done as equivalents of the most representative compounds for each family of phenolic compounds: quercetin 3-rutinoside for all flavonol 3-glycosides; (+)-catechin for polymeric flavan-3-ols (total proanthocyanidins); individual flavan-3-ol monomers and dimers by their corresponding standards, but their total sum as (+)-catechin equivalents.

The chemicals: 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridyltriazine (TPTZ), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and gallic acid were from Aldrich. Buffer salts and all other chemicals were of analytical grade.

2.2. Sample

Approximately 300 kg of banana (*Musa* AAA) Cavendish type was purchased in the market of Viçosa-MG (Brazil), with “degree of skin color, 5” (yellow with green tip), according to the Von Loesecke scale. In the Science Lab of Fruit and Vegetable Products, Federal University of Viçosa (MG, Brazil), bananas were washed and sanitized by spraying with a solution of chlorine at 50 mg L^{-1} for 15 min. The peels were manually removed from the pulp, cut into pieces of about 5 cm^2 and bleached at 95 °C for 5 min in jacketed pan (Groen MGF Co., USA, model ALL 20) to inactivate endogenous enzymes and subjected to drying at 55 °C in a tray drier with forced air up to reach constant weight. After drying, the peels were ground in a Wiley Mill, Brabender (Duisburg, Germany, model WI). The obtained flour, called ‘banana peel flour’, was stored at −18 °C in polyethylene bags and used as feedstock for all experiments in this study.

2.3. Sample preparation

A previously described method for grape phenolic extraction was adapted (Castillo-Muñoz et al., 2009). The samples (ca. 2.5 g) were immerged in 50 mL of a solvent mixture of methanol, water, and formic acid (50:48.5:1.5 v/v/v), the extraction was assisted by homogenization for 2 min and subjected to an ultrasonic bath for 5 min and subsequent centrifugation at 2500 *g* at 5 °C for 10 min. Two more extractions were performed with the resulting pellets using the same volume of the solvent mixture (50 mL), and the combined supernatants for each sample were maintained at −18 °C until analysis.

To analyze flavonols 10 mL of extract was dried in a rotary evaporator (35 °C) and re-dissolved in 1 mL of 20% methanol in water and directly injected onto the HPLC equipment.

The flavan-3-ols (monomers, B-type dimers, and polymeric proanthocyanidins) were isolated from banana peel flour extracts by SPE on C18 cartridges (Sep-Pak Plus C18, Waters Corp., Mildford, MA; cartridges filled with 820 mg of adsorbent). A mixture of 2 mL of extract with 12 mL of water was passed through the C18 cartridge, which had been previously conditioned with methanol (5 mL) and water (5 mL); after the cartridge was dried, methanol (15 mL) and ethyl acetate (5 mL) were added in order to recover adsorbed phenolics; after the solvent was evaporated in a rotary evaporator (35 °C), the residue was dissolved in methanol (2 mL) and stored at −18 °C for subsequent analyses.

2.4. HPLC-DAD–ESI-MS^n identification of occurring flavonols in banana peel flour

Flavonols were analyzed after adaptation of previously described method (Castillo-Muñoz et al., 2009) to the use of chromatography columns of narrow bore and smaller particle size (Rebello et al., 2013). The diluted extracts were injected (20 μL) for the analysis after filtration (0.20 μm, polyester membrane, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany) on a reversed-phase column ZORBAX Eclipse XDB-C18 (2.1 × 150 mm; 3.5 μm particle; Agilent, Germany), thermostated at 40 °C. Flow rate was 0.19 mL/min. The solvents were: solvent A (acetonitrile/water/formic acid, 3:88.5:8.5, v/v/v), solvent B (acetonitrile/water/formic acid, 50:41.5:8.5, v/v/v), and solvent C (methanol/water/formic acid, 90:1.5:8.5, v/v/v). The linear solvent gradient was: initially, 98% A and 2% B; 8 min, 96% A and 4% B; 37 min, 70% A, 17% B and 13% C; 51 min, 50% A, 30% B and 20% C; 51.5 min, 30% A, 40% B and 30% C; 56 min, 50% B and 50% C; 57 min, 50% B and 50% C; 64 min, 96% A and 4% B. For identification, ESI-MS/MS was used in negative ionization mode, setting the following parameters: dry gas, N_2, 8 L/min; drying temperature, 325 °C; nebulizer, N_2, 50 psi; ionization and fragmentation parameters were optimized by direct infusion of appropriate standard solutions (quercetin-3-rutinoside in negative ionization mode); and scan range is 50–1200 *m/z*. Identification was mainly based on spectroscopic data (UV–vis and MS/MS) obtained from authentic standards or previously reported (Castillo-Muñoz et al., 2009; Lago-Vanzela, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011a,b). For quantification, DAD-chromatograms were extracted at 360 nm. In the case of overlapping peaks in the DAD-chromatograms (peaks 6 and 7 in Fig. 1A), quantification was made with the help of extracted ion chromatograms (EIC) at the *m/z* values of the corresponding pseudomolecular ions of each overlapping compound: the EIC integral value was used for an estimation of the contribution of each individual overlapping compound to the joint DAD peak. All analyses were performed in triplicate.

2.5. Identification and quantification of occurring flavan-3-ols in banana peel flour by HPLC–ESI-MS/MS Using Multiple Reaction Monitoring (MRM)

For the identification and quantification of diverse flavan-3-ols we used standards of: the monomers (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-gallocatechin, and (−)-epicatechin 3-gallate; and the dimers procyanidins B1, B2 and B4. The total polymeric proanthocyanidin content was also quantified as (+)-catechin equivalents and their structural features were characterized (molar percentage of extension and terminal subunits; mean degree of polymerization; molar percentage of galloylation; and molar percentage of

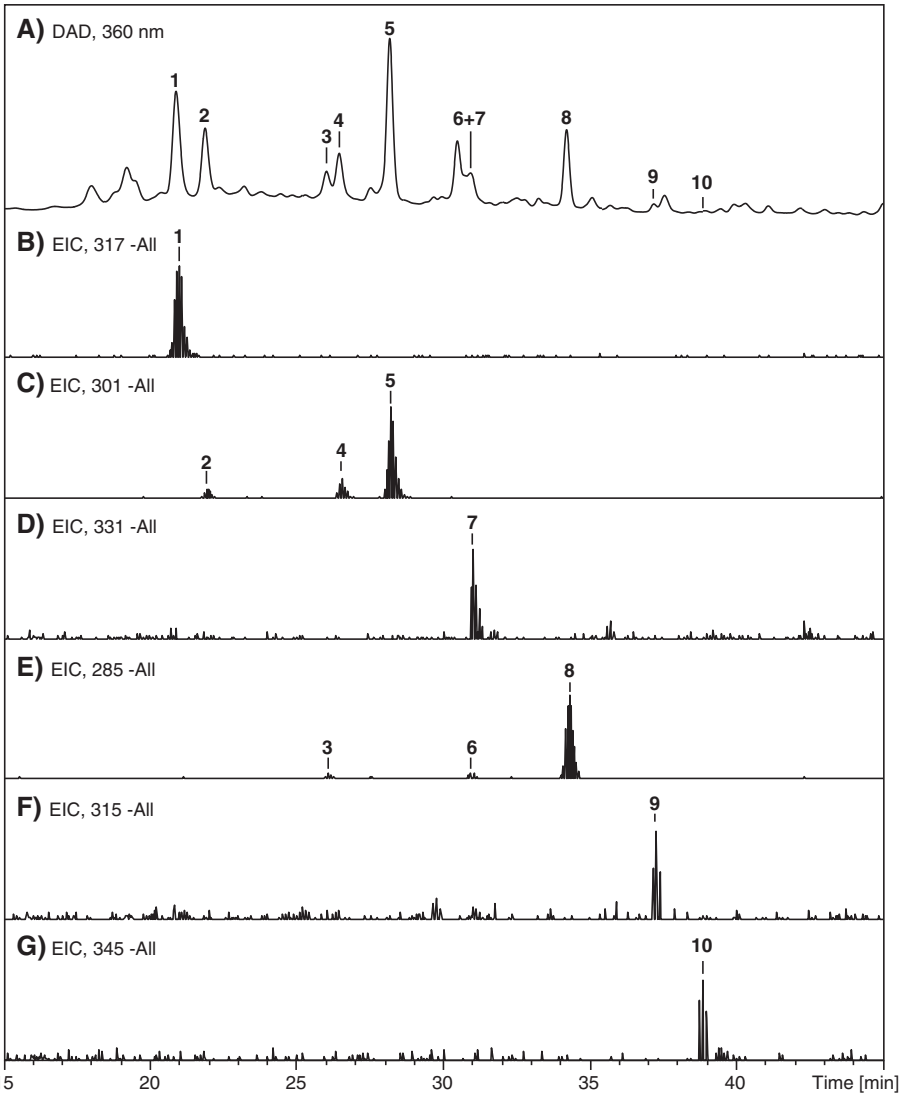

Fig. 1. HPLC-chromatographic profile of flavonols present in banana peel flour extracts: A) DAD-chromatogram at 360 nm; B) Extracted ion chromatogram (EIC) at $m/z = 317$, corresponding to myricetin-based flavonols; C) EIC at $m/z = 301$, corresponding to quercetin-based flavonols; D) EIC at $m/z = 331$, corresponding to laricitrin-based flavonols; E) EIC at $m/z = 285$, corresponding to kaempferol-based flavonols; F) EIC at $m/z = 315$, corresponding to isorhamnetin-based flavonols; and G) EIC at $m/z = 345$, corresponding to syringetin-based flavonols.

prodelphinidins). We followed a previously developed method (Rebello et al., 2013) based on the use of the −EMS (enhanced mass spectrum; MS conditions) scan mode for identification, MRM (multiple reaction monitoring; MS/MS conditions) scan mode for quantification, (+)-catechin as external standard, and acid-catalyzed depolymerization induced by pyrogallol for structural characterization of proanthocyanidins.

In the analysis of flavan-3-ol monomers and B-type dimer procyanidins, 0.25 mL of the SPE-C18 extract was diluted with 4.75 mL of water:formic acid (98.5:1.5 v/v) in a chromatographic vial that was sealed, and the extract was then injected. The structural information of proanthocyanidins was obtained after acid-catalyzed depolymerization was induced by pyrogallol. In this study, 0.50 mL of pyrogallol reagent solution (100 g/L of pyrogallol and 20 g/L ascorbic acid in methanolic 0.3 N HCl) was added to 0.25 mL of SPE-C18 extract, and the mixture was then maintained at 30 °C for 40 min. After the reaction was finalized with the addition of 2.25 mL of 67 mM sodium acetate and 2 mL water, the reaction mixture was then injected.

The analysis was performed using an Agilent 1200 series system equipped with DAD (Agilent, Germany), and coupled to an AB Sciex 3200 Q TRAP (Applied Biosystems) electrospray ionization mass spectrometry system (ESI-MS/MS). The chromatographic system was managed by the Agilent ChemStation (version B.01.03) data-processing station. The mass spectra data was processed with the Analyst MSD software (Applied Biosystems, version 1.5).

Analysis of samples before depolymerization allowed the determination of the concentration of monomeric flavan-3-ols and B-type procyanidin dimers. Data of initial concentrations of flavan-3-ol monomers, obtained before depolymerization reaction, was used for correction of the concentrations of released flavan-3-ol monomers (terminal subunits of polymeric proanthocyanidins) during the depolymerization reaction of proanthocyanidins.

2.6. Determination of total phenolics (TPH)

The total phenolic content in the samples was determined using the Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999). The standard curve ranges of 0.01–0.07 mg of gallic acid per mL. The absorbance was measured at 740 nm after 120 min at environment temperature. The absorbance values were then compared with those of standards of known gallic acid concentrations. All

values were expressed as the mean (milligrams of gallic acid equivalents per 100 g of dry sample) ± SD for three replications.

2.7. Antioxidant activity

2.7.1. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure described in the literature (Benzie & Strain, 1996). Briefly, the FRAP reagent was prepared from sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM iron(III) chloride solution in a volume ratio of 10:1:1, respectively. The FRAP reagent was prepared freshly daily and warmed to 37 °C in a water bath before use. 240 μL water and 80 μL of the diluted sample were added to 2400 μL of the FRAP reagent. The absorbance of the mixture was measured at 593 nm in Pró-Análise UV 1600 spectrophotometer, after 15 min. The standard curve was constructed using Trolox (160–720 μM) solution.

2.7.2. 2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay

The ABTS assay was carried out according to the method established in the literature (Re et al., 1999). Briefly, the $ABTS^+$ stock solution was prepared from 7 mM ABTS in potassium persulfate (2.45 mM), and then incubated in the dark for 16 h at room temperature. The $ABTS^+$ working solution was prepared by diluting the stock solution with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm (Pró-Análise UV 1600 spectrophotometer). All samples were diluted to provide approximately 20–80% inhibition of the blank absorbance. The 30 μL of the diluted sample was mixed with 3.0 mL $ABTS^+$ working solution. The absorbance of the mixture was measured at 734 nm after 25 min of incubation at 30 °C temperature, and the percent of inhibition of absorbance at 734 nm was calculated. The standard curve was constructed using Trolox (2000–20 μM) solution.

2.7.3. Oxygen radical absorbance capacity (ORAC) assay

The ORAC procedure used an automated plate reader with 96-well plates (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). Analyses were conducted in system composed of one probe, fluorescein; a peroxyl radical generator, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH); and Trolox as a control standard. All reagents were prepared in 75 mM potassium phosphate buffer (pH 7.4) just before analysis. After an addition of 20 μL of diluted samples, it was added to 120 μL of fluorescein (0.378 μg/mL) and 60 μL of AAPH (0.108 g/L) in each well, 80 min for reaction completion at 37 °C in automated microplate reader BMG Labtech. Fluorescence conditions were as follows: excitation at 485 nm and emission at 520 nm. The standard curve was constructed using Trolox (80–1500 μM) solution. The area under de curve (AUC), relative fluorescence versus incubation time, was calculated as shown in Eq. (1). The AUC differences between the extract and blank were taken and used for calculations.

$$AUC = 1 + \frac{f2}{f1} + \frac{f3}{f1} + \frac{f4}{f1} + \frac{fn}{f1}, \quad \text{where } f \text{ is the fluorescence reading.} \tag{1}$$

3. Results and discussion

The antioxidant activity of banana peel flour determined by three different methods (FRAP, ABTS and ORAC) and total phenolic content are presented in Table 1. Comparing the values obtained by the different methods used for the determination of antioxidant activity, one can observe the highest value for the ORAC method, followed by ABTS and FRAP. This fact may indicate that the compounds present in the flour act more efficiently by the mechanism of hydrogen atom transfer (HAT) than electron transfer for cation radical ABTS and the ferric ion. Because of the lack of data dealing with ripe banana peel flour, we were only able to compare our results with those obtained for green (unripe) banana flour. Thus, results of antioxidant activity by FRAP and ORAC methods obtained for unripe banana flour, *nanicão* variety, were lower (Menezes et al., 2011): 3.59 μM Trolox eq/g DW for FRAP; and 2.61 μM Trolox eq/g DW for ORAC. Moreover, the ABTS antioxidant activity data obtained for green banana flour was also lower (Sarawong, Schoenlechner, Sekiguchi, Berghofer, & Ng, 2014): 34.10 μM Trolox eq/g DW. These differences could be attributed to two reasons: on the one hand, antioxidant activity values are expected to be lower for green (unripe) banana peel, since the content of bioactive compounds and therefore the antioxidant activity are usually increased over fruit maturation (Barcia, Jacques, Pertuzatti, & Zambiazi, 2010); on the other hand, the reference found data corresponded to flour obtained from banana pulp, that is, free of solid parts (e.g., peel and bark) that usually are richer in antioxidant compounds (Someya et al., 2002; Sulaiman et al., 2011).

Table 1
Total phenolic (TPH) content and antioxidant activity (mean value ± standard deviation; n = 3) by FRAP, ABTS and ORAC methods found in banana peel flour. Units: GAE, gallic acid equivalents; Trolox-eq, Trolox equivalents.

TPH (mg GAE/g)	FRAP (μM Trolox-eq/g)	ABTS (μM Trolox-eq/g)	ORAC (μM Trolox-eq/g)
29.2 ± 0.8	14.0 ± 1.7	242.2 ± 34.8	435.5 ± 60.2

The antioxidant activity and the presence of bioactive compounds in extracts of banana (*Musa acuminata* Colla AAA) revealed that the high content of catecholamines, dopamine and L-dopa provides antioxidant activity to the extract (González-Montelongo, Lobo, & González, 2010). Other bioactive compounds were analyzed as potentially responsible for the antioxidant activity (ascorbic acid, tocopherols and phytosterols) but were not detected in the extraction conditions used. These researchers also showed that factors such as time and extraction temperature affected the levels of bioactive compounds, thus suggesting more research is needed on the influence of bioactive compounds on the antioxidant activity showed by banana flour.

Kanazawa and Sakakibara (2000) studied the presence of dopamine in *Musa cavendish* revealing that dopamine occurs in large amounts in both the pulp and peel (80 to 560 mg/100 g and 2.5–10 mg/100 g banana, respectively) and banana can be considered a rich source of antioxidants. They also observed that 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 gallocatechin gallate and ascorbic acid.

In addition, research has demonstrated that extracts of banana bark showed significant antioxidant activity, and this residue may be a source for extraction of antioxidant compounds, especially flavanols (González-Montelongo et al., 2010; Someya et al., 2002; Kanazawa & Sakakibara, 2000). Studies conducted in order to identify the compounds present in banana peel extracts showed that these byproducts are a potential source of provitamin A (carotenoids like *trans*-α-carotene, *trans*-β-carotene and β-cryptoxanthin) and antioxidant compounds including carotenoids, catecholamines and phenolic compounds (González-Montelongo et al., 2010; Kanazawa & Sakakibara, 2000; Nguyen et al., 2003; Someya et al., 2002; Subagio et al., 1996).

Among the antioxidant compounds present in banana peel are phenolic compounds which have been intensively studied in the last two decades with regard to the effects of dietary polyphenols on human health. These studies strongly support their role in the prevention of degenerative diseases, particularly cardiovascular diseases and cancers; the well-known antioxidant properties of polyphenols have been widely studied, but it has become clear that the mechanisms of action of polyphenols go beyond the modulation of oxidative stress (Scalbert

et al., 2005). Flour banana peel evaluated in this study showed a higher content of total phenolic compounds of 29.17 mg GAE/g DW, which is considered quite high since it is higher than the total phenolic content of fruits known for their high content phenolics, such as blackberry (Jacques, Pertuzatti, Barcia, Zambiazi, & Chim, 2010), blueberry (Moraes, Pertuzatti, Corrêa, & Salas-Mellado, 2007) and grapes (Yang, Martinson, & Liu, 2009) and even higher than 3.8 mg GAE/g DW found in banana residue (Babbar et al., 2011).

Phenolic compounds, including catecholamines, phenolic acids and flavonoids (anthocyanins; flavan-3-ols monomers and polymers, the latter also known as tannins) have been found in banana, considering different fruit parts (pulp and peel) and other plant parts like the pseudostem (Aurore et al., 2009; Bennett et al., 2010; Emaga et al., 2007; González-Montelongo et al., 2010; Saravanan & Aradhya, 2011). The content of phenolic compounds in banana peel is generally greater than that found in banana pulp: gallocatechin was more abundant in peel (158 mg/100 g DW) than in pulp (29.6 mg/100 g DW) (Someya et al., 2002); total phenolic content was higher in aqueous extracts of dried peel (3.98–13.00 mg GAE/g DW) than in dried pulp (0.24–7.77 mg GAE/g DW) in seven Malaysian banana cultivars, with the only exception of Raja cultivar (76.37 vs. 12.27 mg GAE/g DW for pulp and peel, respectively) (Sulaiman et al., 2011). Some studies have reported on the phenolic content of banana peel, but we did not find literature dealing with the phenolic content of banana peel flour. As banana peel flour is obtained by drying with heated air flow, it is expected that phenolic content was diminished by well-known thermal degradation of phenolic compounds.

In our study, we only found two types of flavonoids within the phenolic compounds of banana peel flour: flavan-3-ols and flavonols. With regard to flavan-3-ols the following single compounds were identified (Table 2): three monomers, namely, (+)-catechin, (−)-epicatechin and (−)-gallocatechin; and three B-type procyanidin dimers, namely, procyanidins B1, B2 and B4. The latter compounds accounted for a total amount of around 126 mg/kg as (+)-catechin equivalents. B-type procyanidins accounted for higher molar proportions than monomeric flavan-3-ols, the most abundant single compounds being procyanidin B2 and (−)-gallocatechin, respectively. Gallocatechin has been reported to account in banana peel extracts (158 mg/100 g DW, vs. 29.6 mg/100 g DW in pulp) and positively correlated to the antioxidant potential of the extracts (Someya et al., 2002).

Flavan-3-ols of polymeric nature, known as tannins or proanthocyanidins, were also present in banana peel flour, accounting for a total concentration of around 3952 mg/kg, as (+)-catechin equivalents, the content being much higher than that of respective flavan-3-ol monomers and dimers (Table 3). These proanthocyanidins were structurally characterized, showing a high degree of polymerization (mDP, average of around 19) and a high contribution of prodelphinidin-type units: in the case of extension units, (−)-epigallocatechin was the dominant structural unit (molar percentage of around 70%); in contrast, (−)-epicatechin dominated within terminal units (molar percentage of around 65%) although (−)-gallocatechin and (−)-epigallocatechin contributions were remarkable (molar percentage of around 24 and 8%, respectively). It is well established that the higher the polymerization degree of proanthocyanidins the higher the perceived feel-in-mouth known as astringency (Chira, Pacella, Jourdes, & Teissedre, 2011; Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009; De Freitas & Mateus, 2001). In contrast, monomeric flavan-3-ols and their smaller oligomers (e.g., B-type procyanidin dimers) are mainly responsible of bitterness (Peleg, Gacon, Schlich, & Noble, 1999). Moreover, other families of phenolic compounds, such as flavonols and hydroxycinnamic acids, could also be related with bitterness (Preys et al., 2006). To the best of our knowledge, this is the first time that the occurrence of B-type procyanidins and the structural characterization of proanthocyanidins have been reported for banana peel.

Flavonols are a type of flavonoid phenolic compounds easily found in many fruits, but there is a lack of literature data dealing with their identification in banana. These compounds were detected in the studied banana peel flour (Table 4) in amounts around 129 mg/kg (as quercetin-3-rutinoside equivalents). Their identification was mainly based on

Table 2
Total content (as (+)-catechin equivalents) and molar percentage (mean ± standard deviation; n = 3) of monomeric flavan-3-ols and B-type procyanidin dimers found in banana peel flour.

Flavan-3-ols	Molar %
(+)-Catechin	1.34 ± 0.27
(−)-Epicatechin	2.55 ± 0.09
(−)-Gallocatechin	4.20 ± 0.39
Procyanidin B1	1.27 ± 0.49
Procyanidin B2	81.95 ± 0.01
Procyanidin B4	7.90 ± 0.62
Total (mg/kg)	125.8 ± 7.7

Table 3
Structural characterization of the proanthocyanidins (molar percentages: mean ± standard deviation) found in banana peel flour.

Proanthocyanidins' characteristics[a]	
Total PA (mg/kg)	3952 ± 405
mDP	19.2 ± 2.8
% terminal-prodelphinidin	31.74 ± 2.06
% terminal-C	3.22 ± 0.44
% terminal-EC	65.05 ± 1.75
% terminal-GC	24.23 ± 1.41
% terminal-EGC	7.51 ± 0.67
% extension-prodelphinidin	72.12 ± 5.66
% extension-C	0.66 ± 0.18
% extension-EC	27.21 ± 5.51
% extension-GC	1.76 ± 0.13
% extension-EGC	70.36 ± 5.53

[a] Total PA, total concentration of proanthocyanidins, as (+)-catechin equivalents, calculated by total sum of the concentrations of all the extension and terminal subunits; mDP, mean degree of polymerization; % prodelphinidin, % of epigallocatechin subunits; and % of each of the flavan-3-ol monomers found as terminal and extension subunits; C, (+)-catechin; EC, (−)-epicatechin; GC, (−)-gallocatechin; EGC, (−)-epigallocatechin.

Table 4
Chromatographic and spectroscopic (UV–vis and MS/MS spectra) characteristics of the flavonols identified in flour banana peel by HPLC–DAD–ESI-MS/MS (negative ionization mode), molar proportions (mean value ± standard deviation, n = 3), and total concentration (as quercetin 3-rutinoside equivalents). Peak numbers as shown in Fig. 1.

Peak	Assignation[a]	Rt (min)	UV–vis (nm)	Pseudomolecular ion; product ions (m/z)	Molar %
1	M-3-rutinoside	20.84	260, 305(sh), 357	625; 317, 316	22.50 ± 0.50
2	Q-3/7-rutinoside-3/7-rhamnoside[b]	21.81	~355	755; 609, 301, 300	12.91 ± 0.14
3	K-3/7-rutinoside-3/7-rhamnoside[b]	25.95	265, 290, 351	739; 593, 285, 284	5.32 ± 0.10
4	Q-7-rutinoside	26.40	253, 265 (sh), 300 (sh), 343	609; 301, 300	8.78 ± 0.15
5	Q-3-rutinoside	28.11	256, 264 (sh), 295 (sh), 352	609; 301, 300	29.87 ± 0.07
6[c]	K-7-rutinoside	30.88	~350	593; 447, 285, 284	4.12 ± 0.01
7[c]	L-3-rutinoside	30.88	265, 356	639; 331, 330	2.22 ± 0.05
8	K-3-rutinoside	34.15	265, 295 (sh), 349	593; 285	12.35 ± 0.20
9	I-3-rutinoside	37.11	~353	623; 315	1.31 ± 0.09
10	S-3-rutinoside	38.70	~357	653; 345	0.63 ± 0.01
Total (mg/kg)					128.7 ± 12.8

[a] Abbreviations: M, myricetin; Q, quercetin; L, laricitrin; K, kaempferol; I, isorhammetin; S, syringetin.
[b] Substituents at positions 3 and 7 could be interchanged.
[c] Overlapping peaks.

UV–vis and MS^n data and the suggested assignations corresponded to only glycosides (Fig. 1). The complete series of 3-rutinosides of the six common flavonoid structures within flavonols was found: the B-ring monosubstituted, kaempferol; the B-ring disubstituted, quercetin and isorhamnetin; and the B-ring trisubstituted, myricetin, laricitrin and syringetin. The assignation of flavonol structures was suggested on the basis of the matching of chromatographic and spectral properties of an authentic standard of quercetin-3-rutinoside and the rest of rutinoside series derivatives were assumed to be also 3-rutinosides. In addition, two minor rutinoside isomers of kaempferol and quercetin were found and they were assigned as 7-rutinosides (Fig. 2B, C, E and F), since these compounds have been found in other plant materials (Williams, 2006). Finally, other two glycosides of kaempferol and quercetin were assigned as 3/7-rutinoside-3/7-rhamnosides (Fig. 2A and D; the linking positions of rutinoside and rhamnoside moieties were suggested to be also C-3 or C-7 and may be interchanged). Quercetin-based glycosides were the most important flavonols in banana peel flour, accounting for around of the half of total flavonol pool.

4. Conclusion

Banana peel is an underused by-product of industrial banana processing that is rich in total phenolic compounds. Banana peel can be processed to obtain flour that is more easily stored for further uses. The high phenolic content of extracts of banana peel flour is likely responsible for the very high antioxidant activity shown by it, as measured by three different methods (FRAP, around 14 μM/g; ABTS, around 242 μM/g; ORAC, around 436 μM/g; all of them as Trolox equivalents). The extracts of banana peel flour exhibited a high phenolic content due to the occurrence of important amounts of flavonoid phenolics: mainly, highly polymerized proanthocyanidins (around 3952 mg/kg, as (+)-catechin) and followed by decreasing lower contents of flavonol glycosides (around 129 mg/kg, as quercetin 3-rutinoside), B-type procyanidin dimers and monomeric flavan-3-ols (jointly around 126 mg/kg, as (+)-catechin). As far as we knew, the phenolic composition of banana peel and related products (e.g., banana peel flour) has not been previously characterized in detailed knowledge as we do. The

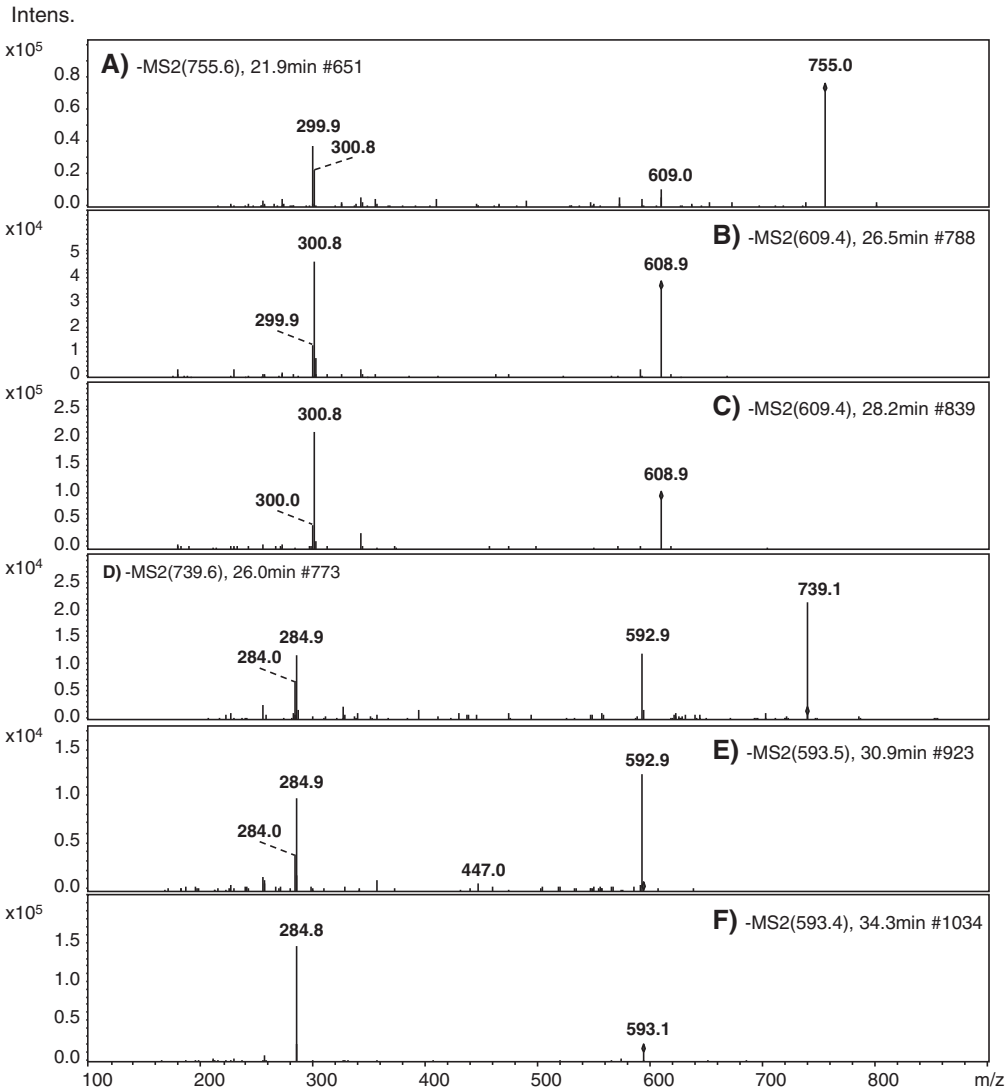

Fig. 2. MS/MS spectra in negative ionization mode (−MS2) obtained from the pseudo-molecular ($[M-H]^-$) parent ions corresponding to the quercetin- and kaempferol based flavonols found in banana peel flour extracts (the m/z value is indicated): A) quercetin 3/7-rutinoside-3/7-rhamnoside (pseudo-molecular parent ion, $m/z = 755$); B) quercetin 7-rutinoside ($m/z = 609$); C) quercetin 3-rutinoside ($m/z = 609$); D) kaempferol 3/7-rutinoside-3/7-rhamnoside ($m/z = 739$); E) kaempferol 7-rutinoside ($m/z = 593$); and F) kaempferol 3-rutinoside ($m/z = 593$).

results have demonstrated that flavonoids are important phenolic compounds of banana peel flour, in agreement with other antioxidant-rich foodstuffs (e.g., berries and wine). All these results suggest the interest in going in depth of the good use of banana peel as a profitable source of bioactive compounds because it is a cheap by-product with a high content of antioxidant flavonoids.

Acknowledgments

Authors Rebello, L. P. G. and Barcia, M.T. thank the following Brazilian institutions: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their scholarship in the Program of Doctoral Sandwich Abroad (PDSE); the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Cnpq); and the Fundação de Amparo à Pesquisa do estado de Minas Gerais. Author Castillo-Muñoz, N. thanks the Fondo Social Europeo and the Junta de Comunidades de Castilla-La Mancha for co-funding his contract (project POII10-0061-4432). Author Hermosín-Gutiérrez, I. thanks Spanish Ministerio de Economía y Competitividad for financial support (project AGL2011-29708-C02-02).

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