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# Phenolic profiling in the pulp and peel of nine plantain cultivars (*Musa* sp.)

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ABSTRACT

The present study investigated the phenolic profiles of the pulp and peel of nine plantain cultivars and compared them to those of two dessert bananas of commercial interest (Grand Nain and Gros Michel), alongside a newly created hybrid, resistant to black sigatoka disease (F568). Identification and quantification of phenolic compounds were performed by means of HPLC–ESI-HR-MS and HPLC-DAD. Hydroxycinnamic acids, particularly ferulic acid-hexoside with 4.4–85.1 μg/g of dry weight, dominated in the plantain pulp and showed a large diversity among cultivars. Flavonol glycosides were predominant in plantain peels, rutin (242.2–618.7 μg/g of dry weight) being the most abundant. A principal component analysis on the whole data revealed that the phenolic profiles of the hybrid, the dessert bananas and the pure plantains differed from each other. Plantain pulps and peels appeared as good sources of phenolics, which could be involved in the health benefits associated with their current applications.
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## 1. Introduction

Phenolic compounds are secondary metabolites produced in plants through the phenylpropanoid pathway and encompass a wide range of chemical classes, including phenolic acids, flavonoids, stilbenes and lignans (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). They are basically involved in plant defence mechanisms and are also known to exert numerous health promoting effects. They act as antioxidants and modulators of enzyme expression and thereby contribute to the alleviation of a wide range of chronic diseases, such as cancer, diabetes, skin damages, allergies, atherosclerosis and viral infections (Huang & Shen, 2012; Liu, 2004). Furthermore, phenolic compounds are exploited in food protection against alterations by microorganisms or by lipid oxidations (Maqsood, Benjakul, & Shahidi, 2013). They are therefore involved in the formulation of many dietary supplements, food additives and drugs and their natural sources are of great interest.

Plantain bananas as well as the other parts of the *Musa* sp. plant, which include roots, pseudostems, stems, leaves and flowers have long been used in folk medicine in Africa, India, Asia and America. Recent investigations highlighted their antioxidant, anti-bacterial, anti-ulcerogenic, anti-hypertension, anti-diabetic and anti-cancer activities (Imam & Akter, 2011). Phenolic compounds are believed to be partly responsible for these properties (Jawla, Kumar, & Khan, 2012). However, only scarce information on the phenolic composition of plantain bananas is currently available.

Three types of *Musa* sp. fruits can be distinguished: dessert, plantain cooking and non-plantain cooking bananas. Plantain banana cultivars are generally separated into two main groups, namely French and Horn, according to the presence or the absence of the male bud at harvest, respectively. In addition, new elite hybrids, with improved disease resistance, are currently being developed and increase plantain banana diversity.

Bananas have been reported as an important source of phenolic compounds (Schieber, Stintzing, & Carle, 2001). Flavonoids, including flavones (apigenin), flavanones (naringenin), and flavonols

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(myricetin, kaempferol and quercetin), were found in the sap of diverse dessert banana species (Pothavorn et al., 2010). Anthocyanin pigments (petunidin, malvidin, pelargonidin, delphinidin, cyanidin and peonidin) were identified in wild banana bracts (Kitdamrongsont et al., 2008). Epigallocatechin was identified as the major constituent of condensed tannins in the pulps of diverse dessert and plantain banana cultivars (Uclés Santos, Bakry, & Brillouet, 2010) and a leucocyanidin (flavan-3,4-diol) was identified in plantain banana pulp as the active anti-ulcerogenic molecule (Lewis, Fields, & Shaw, 1999). To the best of our knowledge, the whole phenolic profile of plantain pulp and peel has never been investigated.

In this study, we characterized for the first time the phenolic profile of the pulp and peel of diverse plantain cultivars. Their chemical patterns were further compared to the ones of two dessert banana cultivars and two samples of one newly created hybrid collected in two different pedo-climatic environments. The use of the plantain phenolic extracts in the food and pharmaceutical industry and the involvement of the identified phenolics in plantain diversity was finally discussed.

## 2. Materials and methods

### 2.1. Chemicals

Kaempferol-rutinoside, isorhamnetin-rutinoside, ferulic acid, and caffeic acid standards were purchased from Sigma–Aldrich (St. Louis, MO). Rutin was obtained from ExtraSynthese (Genay, France). Acetone of analytical grade was obtained from VWR-Prolabo (Briare, France). Methanol, acetonitrile, formic acid of HPLC grade were supplied by Biosolve (Valkenswaard, The Netherlands).

### 2.2. Plant materials

Banana fruits harvested in 2011 were provided by the African Research Centre on Banana and Plantain (CARBAP, Njombe in Cameroon), which is hosting the world largest field *Musa* collection (Tomekpe, Kwa, Dzomeku, & Ganry, 2011). The collected fruits were composed of 8 plantain cultivars, including 2 French plantains (Red Yade and Mbeta 1) and 6 Horn plantains (Big Ebanga, Moto Ebanga, Batard, Essang, Mbouroukou N° 1, and Mbouroukou N° 3), 1 dessert banana (Gros Michel) and 2 samples of the same hybrid (F568 resistant to the black sigatoka disease) harvested at two different pedo-climatic environments: Njombe (an elevation of 80 m, a young volcanic soil, a mean temperature of 27 °C and an annual precipitation of 2600 mm) and Bansoa (an elevation of 1400 m, an old volcanic soil, a mean temperature of 19 °C and an annual precipitation of 1500 mm). The sampling was completed by a commercialized plantain from Colombia and the commercialized dessert banana Grand Nain, known as "Banane Chiquita", was purchased in local markets in Belgium. For each cultivar, 3 ripe fruits were treated separately. The peel and the pulp were separately frozen in liquid nitrogen and further freeze-dried, ground (particle size < 1 mm), and kept at −20 °C under nitrogen.

### 2.3. Identification of phenolic compounds of plantain pulp and peel

#### 2.3.1. Extraction

Approximately 0.5 g of freeze-dried pulp or peel, were extracted with 10 ml of acetone:water:acetic acid (50:49:1; v:v:v) containing 0.2 mM of ascorbic acid. The mixture was vortexed for 1 min and the extraction was carried out in a water bath under agitation at 40 °C for 1 h. The extract was centrifuged at 4 °C, for 20 min at 5000 g and the supernatant was collected. The residue was extracted two more times. The supernatants were combined and evaporated to dryness with a rotary evaporator at 40 °C.

#### 2.3.2. HPLC–ESI-HR-MS analysis

The dried extracts of the cultivar Red Yade (used as plantain reference) were solubilized in water and poured onto a pre-conditioned C18 SPE column. Then fractions were produced by stepwise elution with increasing ethanol concentrations (0%, 5%, 7%, 10%, 15%, 20%, 25%, 50%, 75%, and 100%) as described by Lai et al. (2013). Fractions were evaporated to dryness in a SpeedVac, resuspended in 1 ml (for peel) and 0.5 ml (for pulp) of methanol 50% in water, and filtered through a 0.45 μm syringe filter (Macherey–Nagel, Düren, Germany), before being analysed by high performance liquid chromatography (HPLC) negative electrospray ionisation high-resolution mass spectrometry (ESI-HR-MS).

HPLC–ESI-HR-MS analysis was performed as described by Lai et al. (2013), using a HPLC system connected to a LTQ-Orbitrap-XL mass spectrometer. Briefly, an aliquot of 20 μl of each fraction was injected onto a Waters XSelect CSH C18 column (100 × 3 mm; 2.5 μm particle size) equipped with a guard column of the same type (Milford, MA). The mobile phases A (water) and B (acetonitrile, all with 0.1% formic acid) were used under the following elution gradient: 0–10 min, 0–15% B; 10–25 min, 15% B; 25–30 min, 15–25% B; 30–35 min, 25–100% B; 35–40 min, 100% B; 40–45 min, 100–0% B; 45–50 min, 0% B, at a flow rate of 0.75 ml/min with a 50:50 flow-split just before the mass detector. All the operating parameters were the same as described by Lai et al. (2013) except that the tuning of the ESI source was done with rutin with the following ESI conditions: 5 V of spray voltage; sheath gas ($N_2$) flow rate of 18 and auxiliary gas ($N_2$) flow rate of 30 arbitrary units; temperature of the capillary set at 275 °C; capillary voltage of −43 V; tube lens of −143 V.

### 2.4. Quantification by HPLC-DAD analysis

For the quantification, the dried extracts were resuspended in 1 ml of 50% methanol in water and filtered. The HPLC-Diode Array Detector (DAD) system described by Lai et al. (2013) was used. Simultaneous monitoring was set at 280 nm for benzoic acids, 320 nm for hydroxycinnamic acids, and 350 nm for flavonols. Phenolic compounds were identified by their retention times and spectral data and were quantified using five-point calibration curves. Caffeic acid, ferulic acid, rutin, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside and myricetin were used for the calibration curves with the working ranges of 0.8–12.5 μg/ml for caffeic acid and ferulic acid, 3.1–50 μg/ml for kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside and 6.25–100 μg/ml for rutin and myricetin. The detection and quantification limits were 0.024 μg/ml and 0.08 μg/ml for caffeic acid, 0.007 μg/ml and 0.022 μg/ml for ferulic acid, 0.07 and 0.24 μg/ml for rutin, 0.11 and 038 μg/ml for kaempferol-3-*O*-rutinoside, 0.10 and 0.34 μg/ml for isorhamnetin-3-*O*-rutinoside and 0.16 and 0.54 μg/ml for myricetin. The recovery rates (mean ± SD, $n = 4$) were determined for the phenolic acid ferulic acid and for the flavonol rutin. The values obtained for ferulic acid were 82.6 ± 1.7% in the pulp and 92.6 ± 4.3% in the peel. Those obtained for rutin were 100.5 ± 3.6% in the pulp and 93.73 ± 4.5% in the peel.

### 2.5. Statistical analysis

Statistical analyses of the data were performed by JMP 9.0 Statistical Discovery software from SAS and SAS Entreprise Guide 4.3. Tukey's test was used for mean value comparisons. Principal component analysis was performed to compare the phenolic profiles of the different banana cultivars.

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