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Original Research Article

Effect of boiling on phenolic profiles determined using HPLC/ESI-LTQ-Orbitrap-MS, physico-chemical parameters of six plantain banana cultivars (Musa sp)

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ABSTRACT

The effect of boiling on pulp and peel phenolic profiles of five plantain cultivars and the plantain hybrid F568 was evaluated using high-performance liquid chromatography/electrospray ionisation-linear trap quadrupole-Orbitrap-mass spectrometry (HPLC/ESI-LTQ-Orbitrap-MS) and HPLC-diode array detector (DAD) analysis on sample extracts obtained by a solid/liquid extraction. Total soluble solids, pH, water, and total ash contents were also determined. After boiling the whole fruit, total phenolics decreased considerably in the peel (34.3% on average), whereas no significant change was observed in the pulp after boiling with or without peel. With regards to individual phenolic compounds, all the peel flavonols decreased except for myricetin-deoxyhexose-hexoside, with maximum losses of 61.3% and 59.8% of kaempferol-3-*O*-rutinoside and rutin, respectively, for the cultivar Red Yade. In both pulp and peel, ferulic acid was apparently released from its conjugated forms after boiling, with increases of 63.7% after boiling with peel and of 33.2% after boiling without peel in the pulp of the cultivar Moto Ebanga. Changes in water and total ash contents also suggested a protective effect of the peel. Finally, the principal component analysis revealed that peel total ash content and pH were strongly correlated to the decrease in phenolics.

1. Introduction

In vitro and *in vivo* studies have suggested that dietary phenolic compounds play an important role against a wide range of health disorders *e.g.* cancers, diabetes, neurodegenerative impairments, cardiovascular disorders, gastrointestinal lesions, and bone damages (Kishimoto et al., 2013; Rodríguez-Morató et al., 2015;

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Shen et al., 2012; Taamalli et al., 2012; Vadivel and Biesalski, 2011). These health benefits are believed to be the result of various biological activities such as antioxidant, anti-inflammatory, enzyme expression modulation, and chemoprevention (Manosroi et al., 2013). The search for food rich in phenolic compounds with potential health benefits has led to numerous studies investigating their abilities to exert these health-promoting activities in *in vitro* models. Surprisingly, very few studies considered that some food and plantain banana in particular, are cooked before their consumption.

Cooking induces modifications of the physical and chemical characteristics of the food, making it more palatable, hygienic, and digestible. Boiling seemed to negatively influence nutritional pattern of vegetables, leading to a general loss of carotenoids and phenolic compounds and a decrease of total antioxidant capacity (Mazzeo et al., 2011). Changes in phenolic compounds are of great complexity because they vary according to their structure, the original food material, and the cooking method. Mazzeo et al. (2011) reported variations of specific phenolic compounds depending on the raw material. They found that, after boiling carrots, chlorogenic acid, quercetin, and luteolin decreased, whereas kaempferol increased. Interestingly, they observed no change of the same compounds after the boiling of cauliflowers. Bellail et al. (2012) showed that increases of sweet potato phenolic acids after boiling depend on the cultivar and the type of compound. Wang et al. (2000) observed a decrease of epicatechin derivatives and an increase and a neoformation of catechin derivatives in green tea after a heat processing. They explained that high temperature induced isomerisation of epicatechin, which involved a change in the configuration of the anomeric carbon C2 leading to the formation of catechin. Among the four cooking methods (steaming, water boiling, microwaving and stir-frying) tested on white cauliflower by Ahmed and Ali (2013), water boiling was found to have the most negative impact on all phenolic compounds. Therefore, it is worth studying individual phenolic compound behaviour during cooking processes of fruits and vegetables.

Plantain banana (*Musa sp*) (plantain), a source of carbohydrates, minerals, and vitamins of major importance in the diet of many African populations, has been found in a previous study to have high hydroxycinnamic acid and flavonol contents (Passo Tsamo et al., 2015). Boiling, roasting and frying are the main cooking methods applied on plantains with a preference for boiling in most African households (Ekesa et al., 2012; Odenigbo et al., 2013). Plantain can be boiled peeled or unpeeled according to the dishes (Yussif, 2013). A few studies followed physico-chemical changes during boiling of plantain pulp, revealing water absorption, decline of firmness, and pectin solubilisation associated with cell wall damages (Ngalani and Tchango Tchango, 1997; Qi et al., 2000). Ebun and Santosh (2011) noticed an increase of the total soluble phenolic compounds after boiling of plantain pulp. To our best knowledge, no report on the effect of boiling on specific phenolic compounds of plantain pulp and peel in their native forms has been published.

The current study investigated the fate of individual phenolic compounds in plantain banana pulp and peel after the boiling process. Relevant physico-chemical parameters known to change during this process were also evaluated. Principal component analysis was used to highlight major changes and the relationships between the studied parameters.

2. Materials and methods

2.1. Chemicals

Analytical grade acetone was acquired from VWR-Prolabo (Briare, France). Methanol, acetonitrile and formic acid were of HPLC grade, and purchased from Biosolve (Valkenswaard, The Netherlands). Sucrose (≥99%) was obtained from Aldrich (Milwaukee, WI, USA). Kaempferol-3-*O*-rutinoside (≥98%), isorhamnetin-3-*O*-rutinoside (≥99%), ferulic acid (≥99%), and caffeic acid (≥98%) standards were supplied by Sigma-Aldrich (St. Louis, MO, USA), and myricetin (≥99%) and rutin (≥99%) by Extrasynthèse (Genay, France).

2.2. Plant material

Six genotypes including five plantain cultivars (Mbeta 1, Red Yade, Essang, Moto Ebanga and Mbouroukou N°1), and one newly created plantain-like hybrid resistant to the black sigatoka disease (F568) were harvested in December 2011 in the living collection of the African Research Centre in Banana and Plantain (CARBAP) in Njombe (Cameroon). The pedo-climatic environment of Njombe town is characterised by an elevation of 80 m, a young volcanic soil, a mean temperature of 27 °C and an annual precipitation of 2600 mm. The plants were grown with a chemical fertilizer composed of minerals and with regular watering. Bunches were harvested by the breeding laboratory at physiological maturity according to their protocol. The fingers from the middle part of each bunch were removed and stored in a cold room at the recommended temperature for banana storage (14 °C). The fruit were further put into cardboards and allowed to ripen at room temperature. For each cultivar, three groups of ripe fruits were constituted. The first group (control) was not boiled; the second and the third groups were boiled with or without peel, respectively. For each group, the three fruits were treated separately.

2.3. Boiling treatments

The boiling treatment was conducted in conditions close to those commonly applied in African households. For each cultivar, fruit samples of an average weight of 500 g were formed and boiled in such a way that plantain fingers remained totally immersed by the water at the end of the boiling time. For that, 1.5 L of demineralized water was introduced into a stainless steel pan and brought to boiling on an electric hot plate. For each cultivar, the fruits were peeled, introduced into the boiling water and allowed to cook during 25 min. The same conditions were applied for the boiling with peel. The apical and the distal ends of the fruits were removed before the introduction of the unpeeled fruits into the boiling water. The boiled fruits were allowed to cool at room temperature. The boiling experiments were performed in triplicate and one fruit per replicate was prepared for further analyses.

The peel and the pulp were separately frozen in liquid nitrogen and further freeze-dried, ground, and kept at −20 °C under nitrogen.

2.4. Total soluble solids, pH, water content and total ash content determination

The pulp and the peel of each fruit were separately analysed. A mixture of the sample in distilled water (1:3, w:w) was blended for 2 min. The mixture was centrifuged at 20 °C, for 5 min at 5000 × *g* in a Jouan BR4i centrifuge device (St Herblain, France). The supernatant was used for the measurement of the pH using a bench top pH metre (Inolab, Weilheim, Germany). The refraction index was read using a visual handheld refractometer (Atago, Tokyo, Japan). A five-point calibration curve with sucrose as standard was used. The total soluble solid was expressed in mg sucrose equivalent/100 g of dry weight (DW).

The water and ash contents were determined according to the methods described in our previous work (Passo Tsamo et al., 2014).

Water content was determined by oven drying for 24 h at 105 °C. The total ash content was determined by calcinating the dried samples at 550 °C during 16 h. The results were expressed in mg/100 g DW.

2.5. Phenolic compound determination by HPLC-ESI-HR-MS and HPLC-DAD

2.5.1. Sample extraction

Pulp and peel extracts were prepared as described previously (Passo Tsamo et al., 2015) by applying three successive extractions with 10 mL of acetone: water: acetic acid (50:49:1, v:v:v) containing 0.2 mM of ascorbic acid on approximately 0.5 g of the freeze-dried sample. For each extraction, the mixture was vortexed for 1 min, put in a water-bath under agitation for 1 h and centrifuged at 5000 × *g* during 20 min. The supernatants were combined and evaporated to dryness using a ThermoFisher SpeedVac (Asheville, NC, USA).

2.5.2. Identification of phenolic compounds

The pulp and peel extracts of the sample boiled with peel of the cultivar Red Yade were used for the identification of the phenolic markers by high performance liquid chromatography-electrospray ionisation-high resolution-mass spectrometry (HPLC-ESI-HR-MS). For this, 10 fractions of each extract were produced by stepwise elution onto a pre-conditioned C18 SPE column with increasing ethanol concentrations (0, 5, 7, 10, 15, 20, 25, 50, 75 and 100% ethanol), as described by Lai et al. (2013). The fractions were dried, resuspended in 1 mL of methanol:water (50:50) and filtered through a 0.2 μm hydrophilic polypropylene membrane filter (Acrodisc; Osaka, Japan) supplied by VWR before being analysed using HPLC-ESI-HR-MS. The pulp and peel crude extracts were also resuspended in 1 mL of methanol:water (50:50), filtered and further analysed using HPLC-ESI-HR-MS.

The HPLC-ESI-HR-MS system used was described by (Lai et al., 2013) and consisted of an Accela UHPLC system (Thermo Fisher scientific, Bremen, Germany), composed of a quaternary pump with an integrated degasser, an autosampler, a column oven and a photodiode array detector (PDA). The analytical column consisted in a Waters XSelect CSH C18 column (100 mm × 3 mm; 2.5 μm particle size) equipped with a guard column (3 mm × 20 mm; 3.5 μm particle size) of the same type (Milford, MA, USA). The UHPLC system was connected to a LTQ-Orbitrap-XL mass spectrometer providing multi-stage mass spectra (MS^n), accurate masse of the molecular ion and related fragments, and their corresponding molecular formula. For each fraction and each crude extract, an aliquot of 20 μL of the extract was injected onto the column in the UHPLC system. Phenolic compounds were separated using the mobile phases A (water) and B (acetonitrile), all with 0.1% formic acid under the following elution gradient: 0–10 min, 0–15% B; 10–20 min, 15% B; 20–30 min, 15–35% B; 30–35 min, 35–100% B; 35–40 min, 100% B; 40–41 min, 100–0% B; 41–46 min, 0% B at a flow rate of 0.75 mL/min. The mass spectra were obtained in negative mode using rutin for the tuning of the ESI source under the following 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.

2.5.3. Quantification of phenolic compounds

The high performance liquid chromatography with a diode array detector (HPLC-DAD) system used for the quantification of the phenolic compounds and described by Lai et al. (2013) was composed of a Thermo Separation product system (San Jose, CA, USA), equipped with a P200 pump, an AS 100 autosampler, the same type of column as the one used for the HPLC-ESI-HR-MS analysis, an SN 4000 interface and a UV 6000 LP DAD. For all the cultivars, the crude dried extracts were resuspended in 1 mL of methanol:water (50:50) containing 0.1% formic acid, filtered. A 20 μL aliquot of the filtered extract was injected into the column and the elution of the phenolic compounds was conducted with the same solvent and flow rate as described for the HPLC-ESI-HR-MS analysis and with 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% B; 35–40 min, 25–100% B; 40–45 min, 100% B; 45–50 min, 100–0% B; 50–55 min, 0% B. Simultaneous record of the data was set at 280 nm for benzoic acids, 320 nm for hydroxycinnamic acids, and 350 nm for flavonols.

2.5.4. Identification and quantification methodology

Putative identifications using HPLC-ESI-HR-MS data were proposed by comparing the absorption spectra, the fragmentation pattern of the precursor ions with the literature and reference data from PubChem compound data base (http://pubchem.ncbi.nlm.nih.gov). The proposed identification was considered as valid when the mass error was below 4 ppm. The identification of some compounds were further confirmed using pure standards and by adding the retention time to the other criteria.

After the HPLC analysis, 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, 3.13–50 μg/mL for kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside and 6.25–100 μg/mL for rutin and myricetin. The calibration curves were prepared at each analysis and $R^2 > 0.9$ were obtained. The detection and quantification limits were 0.02 μg/mL and 0.08 μg/mL for caffeic acid, 0.01 μg/mL and 0.02 μg/mL for ferulic acid, 0.07 and 0.24 μg/mL for rutin, 0.11 and 0.38 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 flavonoid 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 ± 3.6% in the pulp and 93.7% ± 4.5 in the peel. The results are expressed in μg standard equivalent/g dry weight (DW). For each compound the standard used for the quantification are indicated as footnote below the tables presenting the results.

2.6. Statistical analysis

Statistical analyses of the data were performed with the JMP 10 Statistical Discovery software from SAS supplied by SAS Institute Inc. (Carry, NC, USA). Analysis of variance (ANOVA) was used to evaluate the significance of the boiling process on physico-chemical characteristics and phenolic compounds. The Student's *t*-test and the Tukey's test were used for mean value comparisons. The differences observed were considered as significant at *p*-values under or equal to 0.05. Principal component analysis was performed to compare the different treatments according to the phenolic profiles of the different cultivars.

3. Results and discussion

3.1. Changes in plantain pulp and peel water content, total soluble solids, total ash, pH and total phenolics induced by the boiling treatments

Table 1 presents the effects of the boiling process on water content, total soluble solids, total ash content, pH, and total

Table 1
Water content, total soluble solids, total ash content, pH and total phenolic compound content of plantain cultivar pulps and peels resulting from the analyses of three experimental replicates ($n = 3$) each analysed in duplicate.

Cultivar and treatment	Pulp water content (g/100 g DW)	Peel water content (g/100 g DW)	Pulp total soluble solids* (g/100 g DW)	Peel total soluble solids* (g/100 g DW)	Pulp total ash content (g/100 g DW)	Peel total ash content (g/100 g DW)	Pulp pH	Peel pH	Pulp total phenolics (μg/g DW)	Peel total phenolics (μg/g DW)
Mbeta 1										
Raw	191 ± 2.9^{b}	353 ± 96.4^{a}	32.8 ± 1.3^{a}	24.5 ± 3.5^{a}	2.3 ± 0.0^{a}	9.3 ± 1.2^{a}	4.9 ± 0.0^{a}	6.5 ± 0.3^{a}	163 ± 14.3^{a}	1056 ± 346^{a}
Boiled without peel	241 ± 19.7^{a}	/	23.2 ± 5.6^{a}	/	1.7 ± 0.2^{b}	/	4.9 ± 0.1^{a}	/	177 ± 31.7^{a}	
Boiled with peel	197 ± 11^{b}	306 ± 10.9^{a}	23.3 ± 9.1^{a}	31 ± 3.3^{a}	2.5 ± 0.0^{a}	8.7 ± 0.8^{a}	5.1 ± 0.2^{a}	5.4 ± 0.0^{b}	150 ± 14.8^{a}	724 ± 159^{a}
Red Yade										
Raw	193 ± 10.9^{b}	518 ± 26.3^{a}	59.3 ± 5.6^{a}	60.7 ± 10.8^{a}	2.5 ± 0.1^{a}	12.6 ± 0.8^{a}	4.7 ± 0.0^{a}	5.8 ± 0.1^{a}	53.7 ± 6.9^{a}	1195 ± 399^{a}
Boiled without peel	246 ± 16.4^{a}	/	49.2 ± 5.2^{a}	/	1.4 ± 0.2^{b}	/	4.6 ± 0.0^{c}	/	53.3 ± 5.5^{a}	
Boiled with peel	225 ± 13.5^{ab}	380 ± 69^{b}	57 ± 1.1^{a}	47.1 ± 2.9^{a}	1.9 ± 0.3^{b}	7.3 ± 0.8^{b}	4.7 ± 0.0^{b}	5.2 ± 0.0^{b}	63.8 ± 3.3^{a}	556 ± 24.4^{b}
Essang										
Raw	164 ± 12^{b}	364 ± 4.8^{a}	42.8 ± 6.4^{a}	49.9 ± 6.3^{a}	2.3 ± 0.1^{a}	10.1 ± 0.6^{a}	5 ± 0.2^{a}	6.3 ± 0.2^{a}	109 ± 5.1^{a}	680 ± 186^{a}
Boiled without peel	214 ± 14.2^{a}	/	32.9 ± 5.8^{a}	/	1.73 ± 0.1^{b}	/	4.8 ± 0.2^{a}	/	116 ± 5.9^{a}	
Boiled with peel	171 ± 8^{b}	319 ± 13.5^{b}	37 ± 7.7^{a}	42.9 ± 5.4^{a}	2.5 ± 0.3^{a}	7.4 ± 0.6^{b}	4.9 ± 0.1^{a}	5.3 ± 0.0^{b}	119 ± 25.8^{a}	577 ± 48.1^{a}
Moto Ebanga										
Raw	143 ± 5.7^{a}	473 ± 27.3^{a}	39.1 ± 6^{a}	49 ± 6.6^{a}	2.3 ± 0.1^{b}	15.8 ± 1^{a}	5 ± 0.1^{a}	6.2 ± 0.2^{a}	57 ± 0.8^{b}	1191 ± 76.3^{a}
Boiled without peel	174 ± 16.3^{a}	/	32.4 ± 4.1^{a}	/	2 ± 0.1^{c}	/	4.9 ± 0.1^{a}	/	60.9 ± 3.7^{ab}	
Boiled with peel	178 ± 28.2^{a}	389 ± 55.3^{a}	40.9 ± 6.7^{a}	46.7 ± 6.3^{a}	2.7 ± 0.2^{a}	10.2 ± 0.9^{b}	4.9 ± 0.1^{a}	5.4 ± 0.1^{b}	64.5 ± 2.4^{a}	810 ± 227^{b}
Mbouroukou N°1										
Raw	202 ± 3.4^{b}	655 ± 36.4^{a}	52.2 ± 3.2^{a}	46.3 ± 0.8^{b}	2.6 ± 0.1^{a}	11.4 ± 1.4^{a}	4.7 ± 0.0^{a}	5.9 ± 0.1^{a}	66.1 ± 1.7^{a}	904 ± 51.6^{a}
Boiled without peel	257 ± 19.8^{a}	/	50.1 ± 11.1^{a}	/	2.6 ± 0.3^{a}	/	4.6 ± 0.0^{b}	/	65.5 ± 6.7^{a}	
Boiled with peel	225 ± 15^{ab}	502 ± 5.4^{b}	53.4 ± 5.1^{a}	51.3 ± 1.5^{a}	2.9 ± 0.2^{a}	9.3 ± 0.3^{a}	4.6 ± 0.0^{b}	5.1 ± 0.0^{b}	78 ± 7.5^{a}	659 ± 170^{b}
F568										
Raw	241 ± 5.4^{a}	516 ± 34.4^{a}	54.7 ± 4.6^{a}	58.8 ± 5.8^{a}	2.5 ± 0.1^{b}	9.9 ± 0.5^{a}	4.8 ± 0.1^{a}	5.5 ± 0.1^{a}	49.8 ± 6^{a}	766 ± 176^{a}
Boiled without peel	351 ± 30.8^{a}	**	45.3 ± 10.9^{a}	**	3 ± 0.1^{a}	**	4.8 ± 0.1^{a}	**	41.6 ± 11.9^{a}	
Boiled with peel	303 ± 80.3^{a}	490 ± 96.4^{a}	49.6 ± 2.6^{a}	50.9 ± 0.7^{a}	3.5 ± 0.3^{a}	8.7 ± 0.1^{b}	4.8 ± 0.1^{a}	5.2 ± 0.1^{b}	57.4 ± 3.5^{a}	481 ± 160^{a}

a, b, and c are letters resulting from ANOVA followed by the Tukey's test for the pulp and Student's test for the peel performed to compare the treatments. Mean values connected by the same letter are not statistically different ($p \leq 0.05$).
* Total soluble solids are expressed in sucrose equivalent.
** The peel was not concerned by the corresponding treatment.

phenolics of the pulp and peel of five plantain cultivars and the newly created hybrid. Samples were boiled either with or without peel. The results of the two-way ANOVA performed for each variable are presented in the Supplementary materials (Table S1).

Generally, boiling significantly affected the pulp and peel water contents ($p < 0.01$). The boiling treatment induced an increase of the pulp water content, but not in all the cases (Table 1). This suggests that the presence of the peel during boiling limits the water uptake by the pulp. The average water uptake during boiling without peel found in our work (+30%) is in good agreement with literature data (Baiyeri et al., 2013). At the same time, a general decrease of the peel water content was observed (−17%), which could be partly due to a transfer of the water to the pulp during boiling.

Pulp and peel total ash contents were significantly affected by the boiling process in a cultivar-dependent manner ($p < 0.01$) (Table S1). In most cultivars, the pulp total ash content decreased after boiling without peel, whereas it remained stable or increased after boiling with peel (Table 1). The peel total ash content mostly decreased, suggesting a possible leaching into the boiling water or a transfer into the pulp. The loss of minerals after the boiling of plantain pulp has also been reported by Baiyeri et al. (2013). This result is in agreement with the reported loss of minerals during the boiling of vegetables and tubers including carrot, bamboo shoot, broccoli, potato, and cocoyam (Dugo et al., 2005; Lewu et al., 2010). The preventing effect of the peel against mineral loss during the boiling process has also been described for carrot and potato (Burgos et al., 2007; Dugo et al., 2005).

Cooking is known to release organic acids from the cell walls to the cytoplasm and thus to lower the pH (Vaclavik and Christian, 2014). In our study, the cultivars Red Yade and Mbouroukou N°1 showed a significant decrease of the pulp pH after boiling with (−2%) or without peel (−3%), although no other cultivar presented a significant change. For all the cultivars, boiling the whole fruit significantly lowered the pH of the peel by about 13%. This decrease which is higher than those observed in the pulp suggests that the increase of organic acids in the peel was markedly more intense than in the pulp.

Boiling plantains did not affect the pulp and peel total soluble solids in almost all the cultivars (Table 1). As in plantain the total soluble solids are related to the soluble sugars (Dadzie et al., 1997), this result suggests that the boiling may not significantly affect the pulp and peel soluble sugar contents.

Total phenolics were obtained by summing the amount of individual phenolics as quantified by HPLC-DAD. Interestingly, the peel total phenolics presented an average loss of 34.3%, but this loss was only significant for the cultivars Red Yade, Moto Ebanga, and Mbouroukou N°1. Similarly, Ranilla et al. (2009) and Siah et al. (2014) showed that a part of the phenolic compounds of the beans was leached into the boiling water. No significant change was observed for most pulp samples, except for the cultivar Moto Ebanga, which increased. The pulp total phenolic contents of Moto Ebanga were 57 ± 0.8 μg/g DW, 60.9 ± 3.7 μg/g DW and 64.5 ± 2.4 μg/g DW for the raw sample, the boiled without peel sample and the boiled with peel sample, respectively (Table 1). These values reveal a more important increase of total phenolics in the pulp when boiling with peel than when boiling without peel. This increase of total phenolics in the pulp boiled with peel (although not significant) is also observed for all samples, except for Mbeta 1 cultivar. Ebun and Santosh (2011) obtained similar results for the total phenolic compounds after boiling unripe and ripe plantains with and without peel. These increases have as well been noticed in other vegetables such as eggplant (Chumyam et al., 2013) and potato (Burgos et al., 2013). It has been shown that heat treatments applied during cooking processes weaken the cell wall and may facilitate the release of phenolic compounds (Chávez-Reyes et al., 2013).

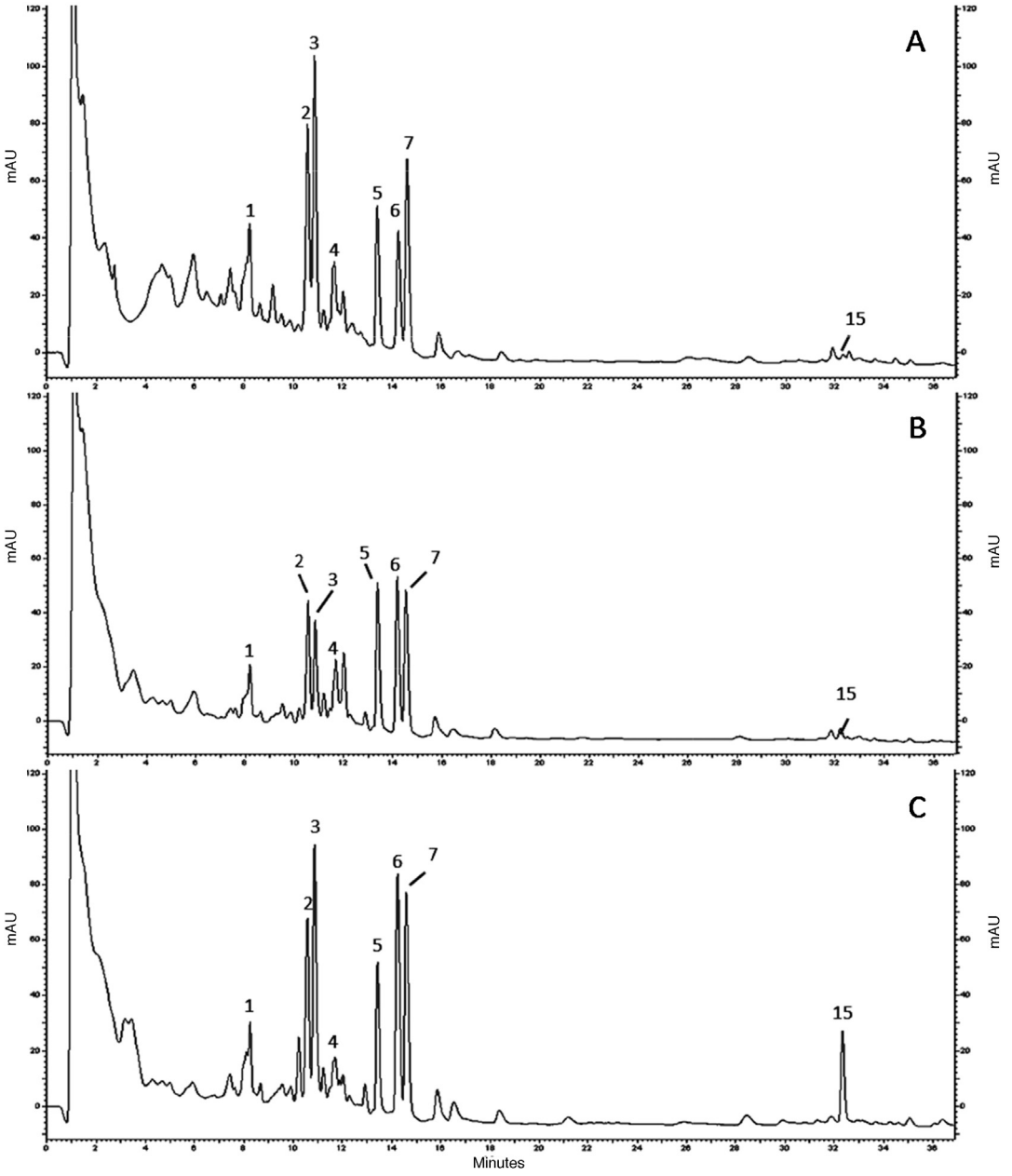

Fig. 1. Pulp phenolic profiles of raw (A), boiled without peel (B) and boiled with peel (C) Red Yade plantain (*Musa sp*) obtained by HPLC-DAD-HR-MS and recorded at 320 nm. Peak names: 1, caffeic acid-hexoside; 2, ferulic acid-hexoside; 3, sinapic acid-hexoside; 4, ferulic acid-dihexoside; 5, myricetin-deoxyhexose-hexoside; 6, ferulic acid; 7, sinapic acid; 15, vomifoliol-conjugate-2.

3.2. Changes in individual phenolic compounds of plantain pulp and peel induced by the boiling treatments

Figs. 1 and 2 illustrate the changes in the phenolic profile of the pulp and peel extracts as the result of the boiling treatments by comparing the chromatograms of raw pulp, pulp boiled without peel and pulp boiled with peel for the cultivar Red Yade (Fig. 1A–C), as well as for the corresponding peels (Fig. 2A and B). The fragmentation patterns of the phenolic compounds are reported in Table 2.

Major changes in the pulp phenolic profile concerned peaks 2, 3, 6, 7, and 15. In our previous work (Passo Tsamo et al., 2015) the identities of peaks 2 and 3 were tentatively attributed to ferulic acid-hexoside and sinapic acid-hexoside, respectively, and those of peaks 6 and 7 were confirmed as ferulic acid and sinapic acid, respectively, by authentic standards. Peak 15, which has never been previously identified, showed an increase upon boiling with or without peel, but this increase is much higher after boiling with peel. It presented a λ_{max} at 315 nm and a molecular ion $[M-H]^-$ at m/z 267.1249 ($C_{14}H_{20}O_5$). This molecular ion underwent successive neutral losses of carbon dioxide (44 amu, CO_2) and water (18 amu, H_2O) to give fragment ions at m/z 223.1344 ($C_{13}H_{20}O_3$) and 205.1236 ($C_{13}H_{18}O_2$) (Table 2). The fragment ions at m/z 223.1344 and 205.1236 and their related molecular formulas are characteristic of vomifoliol or an isomer (hydroxy-oxo-ionone), a natural volatile organic compound derived from the degradation of neoxantine (Tan et al., 2012) and recently reported to exhibit antigonococcal activity (Nair et al., 2013). The increase of this vomifoliol-derivative (vomifoliol-conjugate-2) might reflect the degradation of carotenoids, which have been reported to occur during cooking (Vaclavik and Christian, 2014). Interestingly, a vomifoliol-glycoside has recently been identified in plantain flower as vomifoliol-glucoside or roseoside ($C_{19}H_{30}O_8$) (Martin et al., 2000).

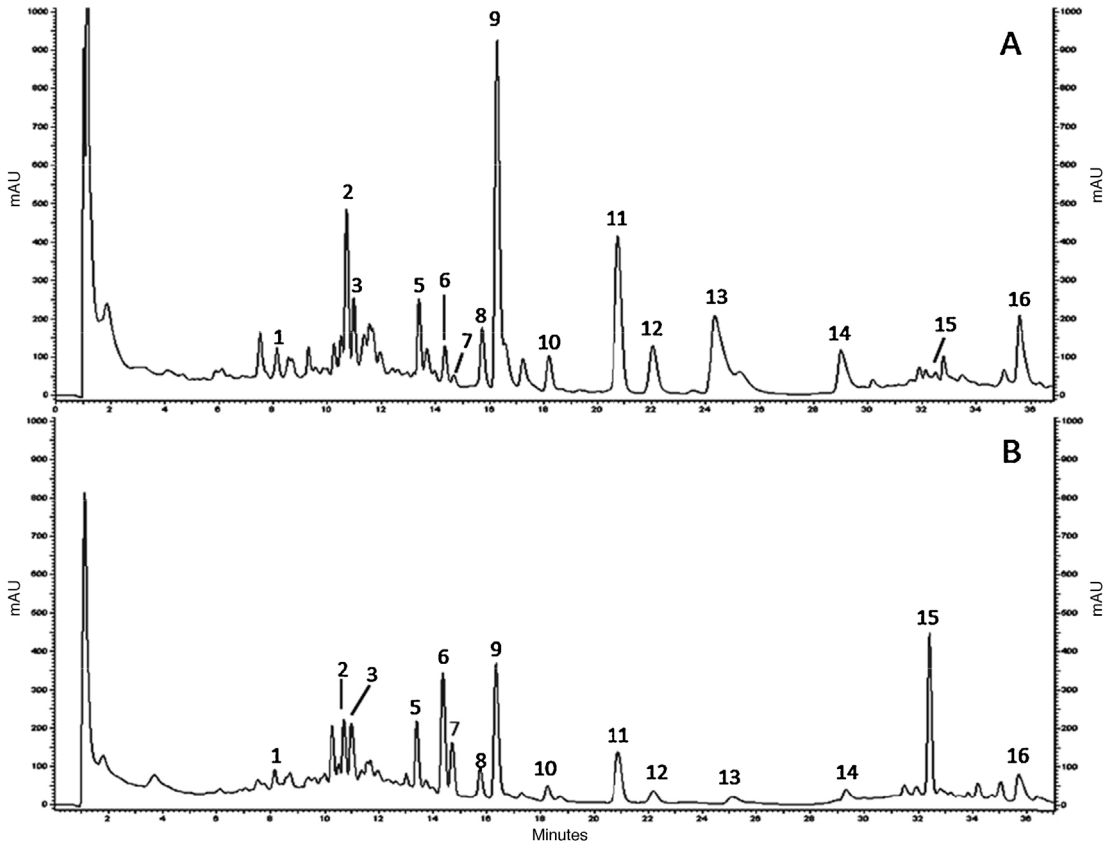

Fig. 2. Peel phenolic profiles of raw (A) and boiled (B) Red Yade plantain (*Musa sp*) obtained by HPLC-DAD-HR-MS and recorded at 320 nm. Peak names: 1, caffeic acid-hexoside; 2, ferulic acid-hexoside; 3, sinapic acid-hexoside; 5, myricetin-deoxyhexose-hexoside; 6, ferulic acid; 7, sinapic acid; 8, quercetin-deoxyhexose-hexoside; 9, rutin; 10, kaempferol-deoxyhexose-hexoside; 11, kaempferol-3-*O*-rutinoside; 12, isorhamnetin-3-*O*-rutinoside; 13, Ferulic acid-conjugate; 14, Vomifoliol-conjugate-1; 15, Vomifoliol-conjugate-2; 16, Triferuloyl-dihexose.

Table 2
Phenolic compounds determined by HPLC-ESI-HR-MS in plantain (cultivar Red Yade) pulp and peel extracts.

Peaks	RT (Min)	λ_{max} (nm)	MS[A] (*m*/*z*)			MS[B] (*m*/*z*)			Putative identification
			$[M-1]^-$	Molecular formula	Error (ppm)	$[M-1]^-$	Molecular formula	Error (ppm)	
Hydroxycinnamic acids									
1	8.23	287sh; 328	341.0876	$C_{15}H_{18}O_9$	−0.631	nd	nd	nd	Caffeic acid-hexoside[C]
2	10.74	291sh; 329	355.1032	$C_{16}H_{20}O_9$	−0.606	193.0506; 175.0402	$C_{10}H_{10}O_4$; $C_{10}H_8O_3$	−0.270; 0.815	Ferulic acid-hexoside[B]
3	11.02	279sh; 329	385.1137	$C_{17}H_{22}O_{10}$	−0.883	223.0611; 205.0507	$C_{11}H_{12}O_5$; $C_{11}H_{10}O_4$	−0.568; 0.185	Sinapic acid-hexoside[B]
4	11.65	282; 326	517.1567	$C_{22}H_{30}O_{14}$	0.892	355.1032; 193.0506	$C_{16}H_{20}O_9$; $C_{10}H_{10}O_4$	−0.775; −0.322	Ferulic acid-dihexoside[B]
6	14.43	281sh; 323	193.0503	$C_{10}H_{10}O_4$	−1.979	nd	nd	nd	Ferulic acid[A]
7	14.70	323	223.0611	$C_{11}H_{12}O_5$	−0.568	nd	nd	nd	Sinapic acid[A]
13	24.93	285sh; 327	379.0671	$C_{17}H_{16}O_{10}$	0.027	193.0507; 185.0092	$C_{10}H_{10}O_4$; $C_7H_6O_6$	0.352; 0.72	Ferulic-acid-conjugate-x[B]
16	35.70	325	869.2499	$C_{42}H_{46}O_{20}$	−1.250	693.2030; 517.1562	$C_{32}H_{38}O_{17}$; $C_{22}H_{30}O_{14}$	−0.898; −0.171	Triferuloyl-dihexose[B,D]
Flavonols									
5	13.39	254; 357	625.1405	$C_{27}H_{30}O_{17}$	−0.836	479.0830; 317.0299	$C_{21}H_{20}O_{13}$; $C_{15}H_{10}O_8$	−0.279; −1.295	Myricetin-deoxyhexose-hexoside[B]
8	15.76	255; 354	609.1458	$C_{27}H_{30}O_{16}$	−0.489	463.0880; 301.0349	$C_{21}H_{20}O_{12}$; $C_{15}H_{10}O_7$	−0.408; −1.647	Quercetin-deoxyhexose-hexoside[B]
9	16.36	254; 350	609.1464	$C_{27}H_{30}O_{16}$	0.414	463.0881; 301.0351	$C_{21}H_{20}O_{12}$; $C_{15}H_{10}O_7$	−0.279; −0.916	Rutin[A]
10	18.28	265; 347	593.1514	$C_{27}H_{30}O_{15}$	0.399	447.0931; 285.0403	$C_{21}H_{20}O_{11}$; $C_{15}H_{10}O_6$	−0.525; −0.741	Kaempferol-deoxyhexose-hexoside[B]
11	20.76	265; 346	593.1516	$C_{27}H_{30}O_{15}$	0.703	447.0931; 285.0405	$C_{21}H_{20}O_{11}$; $C_{15}H_{10}O_6$	0.031; −0.323	Kaempferol-3-*O*-rutinoside[A]
12	22.06	254; 353	623.1616	$C_{28}H_{32}O_{16}$	−0.318	477.1044; 315.0508	$C_{22}H_{22}O_{12}$; $C_{16}H_{12}O_7$	1.154; −0.781	Isorhamnetin-3-*O*-rutinoside[A]
Other									
14	29.30	315	453.1403	$C_{21}H_{26}O_{11}$	0.188	267.1239; 223.1335; 185.008	$C_{14}H_{20}O_5$; $C_{13}H_{20}O_3$; $C_7H_6O_6$	0.386; −2.096; −0.006	Vomifoliol-conjugate-1[B,D]
15	32.40	315	267.1249	$C_{14}H_{20}O_5$	3.830	223.1344; 205.1236	$C_{13}H_{20}O_3$; $C_{13}H_{18}O_2$	1.847; 1.009	Vomifoliol-conjugate-2[B,D]

nd, not detected.
[A] Identity was further confirmed with an authentic standard.
[B] Putative identification based on the UV spectrum, the fragmentation pattern and the molecular formulas of the parent ion and the fragment ions.
[C] Putative identification based on the UV spectrum and the molecular formula of the parent ion.
[D] Indication if compounds which were not identified in our previous work, all the other compounds were previously identified.

Major changes in the peel phenolic profile appeared for the peaks corresponding to ferulic acid-hexoside (peak 2), ferulic acid (peak 6), sinapic acid (peak 7), vomifoliol-conjugate-2 (peak 15) and peaks 9, 11, 12, 13, 14, and 16. Peaks 9, 11 and 12 were previously identified as rutin, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside, and peak 13 was putatively identified as an hydroxycinnamic derivative. Peak 13 could be assigned as ferulic acid-conjugate since its fragmentation pattern investigated in this work revealed a fragment ion at *m/z* 193.0507 ($C_{10}H_{10}O_4$) which could correspond to ferulic acid (Table 2). According to its λ_{max} (315 nm) and its fragmentation pattern characterised by a molecular ion $[M-H]^-$ at *m/z* 453.1403 ($C_{21}H_{26}O_{11}$) and fragment ions at *m/z* 223.1335 ($C_{13}H_{20}O_3$), *m/z* 267.1239 ($C_{14}H_{20}O_5$) and *m/z* 185.0091 ($C_7H_6O_6$), peak 14 appears related to peak 15 and was therefore assigned as a vomifoliol derivative or isomer (vomifoliol-conjugate-1, Table 2). After boiling, the peak area of peel vomifoliol-conjugate-2 was on average 15-fold higher than the pulp one. We also observed a 7-fold increase of vomifoliol-conjugate-2 in the peel after the boiling process. This compound could be partly released from vomifoliol-conjugate-1, which considerably decreased (−50%) (Fig. 2A and B). Peak 16 showed a ferulic acid-based structure. Its molecular ion $[M-H]^-$ at *m/z* 869.2499 ($C_{42}H_{46}O_{20}$) gave fragments at *m/z* 693.2030 ($C_{32}H_{38}O_{17}$) and *m/z* 517.1562 ($C_{22}H_{30}O_{14}$) corresponding to two successive losses of feruloyl units (176 amu, $C_{10}H_8O_3$). The remaining fragment at *m/z* 517.1562 ($C_{22}H_{30}O_{14}$) could correspond to a ferulic acid-dihexoside (or isomer), previously identified (peak 4) (Table 2). Therefore, peak 16 could be putatively identified as a triferuloyl-dihexose, whose molecular formula is similar to the one of triferuloylgentiobiose which has been identified in broccoli (Vallejo et al., 2003). The mass spectrum illustrated by the ion structures and suggesting the fragmentation pathways of the putative triferuloyl-dihexose is presented in Fig. 3.

Major phenolic compound contents of plantain cultivars raw, boiled without peel and boiled with peel are presented in Tables 3 and 4 for the pulp and the peel, respectively. For each of the two phenolic classes (hydroxycinnamic acids and flavonols), the values of total phenolics obtained by summing individual compounds are also presented. The results of the two-way ANOVA statistical analysis of the effects of the boiling process on these compounds is reported in the Supplementary materials (Table S2).

Pulp total hydroxycinnamic acid content was not affected by the boiling process except for the hybrid F568 for which an increase of 38.1% was obtained after boiling with peel (Table 3). Among the major phenolic compounds of the pulp, only ferulic acid and sinapic acid were found to be significantly affected by the boiling treatment ($p < 0.01$). However, it can be noticed that, at a *p*-value < 0.06, ferulic acid-hexoside was affected by the boiling treatment and cultivar behaviours were found significantly different ($p < 0.05$) (Table S2). Table 3 shows that ferulic acid-hexoside significantly decreased for the cultivar Moto-Ebanga, while ferulic acid significantly increased for the cultivars Red Yade, Moto Ebanga, and F568. Sinapic acid significantly increased for the cultivar Moto Ebanga.

The decrease in ferulic acid-hexoside and the increase in ferulic acid observed for the pulp of the cultivar Moto Ebanga support the release of free ferulic acid from its glycosylated forms. This trend, though not significant, was also observed for other cultivars. The increase in ferulic acid was higher after the boiling with peel than after the boiling without peel (+63.7% and +33.2%, respectively for Moto Ebanga). This could be partly due to the known protecting effect of the peel against the leaching of compounds into the boiling water, as reported earlier in potato (Danesi and Bordoni, 2008). Moreover, the lower reduction of ferulic acid-hexoside as compared to the increase of ferulic acid suggests that other conjugated ferulic acid which were not detected by our method of analysis, largely contributed to this increase. Chan et al. (2013) reported that boiling may release organic acids bound to flavonoids. This could have contributed to the increase of phenolic acid. A hydrolysis of glycosylated forms of ferulic acid has also been observed after boiling millet and sorghum by N'Dri et al. (2013) and also during the processing of maize in tortillas by Gutiérrez-Uribe et al. (2010).

Generally, peel total hydrocycinnamic acid content was not significantly affected by the boiling, except for the cultivars Red Yade and Moto Ebanga for which the contents decreased of 45.4% and 40.5%, respectively (Table 4). The boiling process significantly affected peel hydroxycinnamic acids ($p < 0.05$) in a cultivar-dependent manner for caffeic acid-hexoside ($p < 0.01$), ferulic acid-hexoside ($p < 0.05$), ferulic acid ($p < 0.01$), and triferuloyl-dihexose ($p < 0.01$) (Table S2). Caffeic acid-hexoside, ferulic acid-hexoside, and triferuloyl-dihexose significantly decreased or remained stable depending on the cultivar, while ferulic acid-conjugate (peak 13) decreased in all cultivars. Sinapic acid-hexoside was not significantly affected by the boiling in all the cultivars except in the cultivar Essang for which a significant decrease was observed. On the other hand, a significant increase in ferulic acid was observed in all the cultivars, while sinapic acid significantly increased in all the cultivars except in cultivars Mbeta 1 and Moto Ebanga for which no significant effect was observed (Table 4). In quantitative terms, the decrease of conjugated ferulic acids and increase of its free form appeared to be more pronounced in the peel than in the pulp. Average decreases of 51.7% for ferulic acid-hexoside, 86.1% for ferulic acid-conjugate (peak 13) and 55.2% for triferuloyl-dihexose were observed, whereas ferulic acid increased on average by 72.1%. The net loss of ferulic acid-based compounds was calculated at 41.4% on average, which could be attributed to the degradation by the heat, the migration into the boiling water or a transfer into the pulp. After the boiling treatment, ferulic acid became the most abundant free phenolic acid in peel with the contents varying between 30 ± 2.9 μg/g DW for the cultivar Mbouroukou N°1 and 56.6 ± 5.1 μg/g DW for the cultivar Essang. Increases of sinapic acid were observed for the cultivars Red Yade (304%), Essang (218%), Mbouroukou N°1 (338%), and the hybrid F568 (326%), although changes in sinapic acid-hexoside were not significant (for the cultivars Red Yade and Mbouroukou N°1) or low (31.1%, for the cultivar Essang). This observation supports the large contribution of other conjugated sinapic acid derivatives to these increases.

For the majority of the cultivars, the boiling significantly lowered the peel total flavonol content and the most important decrease was observed for the cultivar Red Yade (−54.8%) (Table 4). Except for myricetin-deoxyhexose-hexoside, all the flavonols from the peel were affected by the boiling treatment ($p < 0.01$). The *p*-values of the interaction between the cultivar and the boiling treatment ($p > 0.05$) indicate that, except for kaempferol-deoxyhexose-hexoside, a decrease was generally observed for all the flavonols (Table S2). Rutin and kaempferol-3-*O*-rutinoside contents, for instance, decreased by 59.8% and 61.3%, respectively, for the Red Yade cultivar (Table 4). Similar losses of flavonols including rutin, quercetin and kaempferol have been observed during boiling of beans (Ranilla et al., 2009), cauliflower (Ahmed and Ali, 2013), and onion (Rodrigues et al., 2009), and have been attributed to their running off in the boiling water and their breakdown due to their sensibility to heat. Oxidation and polymerisation reactions may also take place (Ahmed and Ali, 2013). Interestingly, in this study, myricetin-deoxyhexose-hexoside was not affected by the boiling in contrast to the other flavonols, probably indicating better stability under boiling conditions. Ioannou et al. (2012) reported that flavonoids show different sensitivities to the heat treatment depending on their structures. Moreover, Zhang et al. (2005) found that the dissociation of the

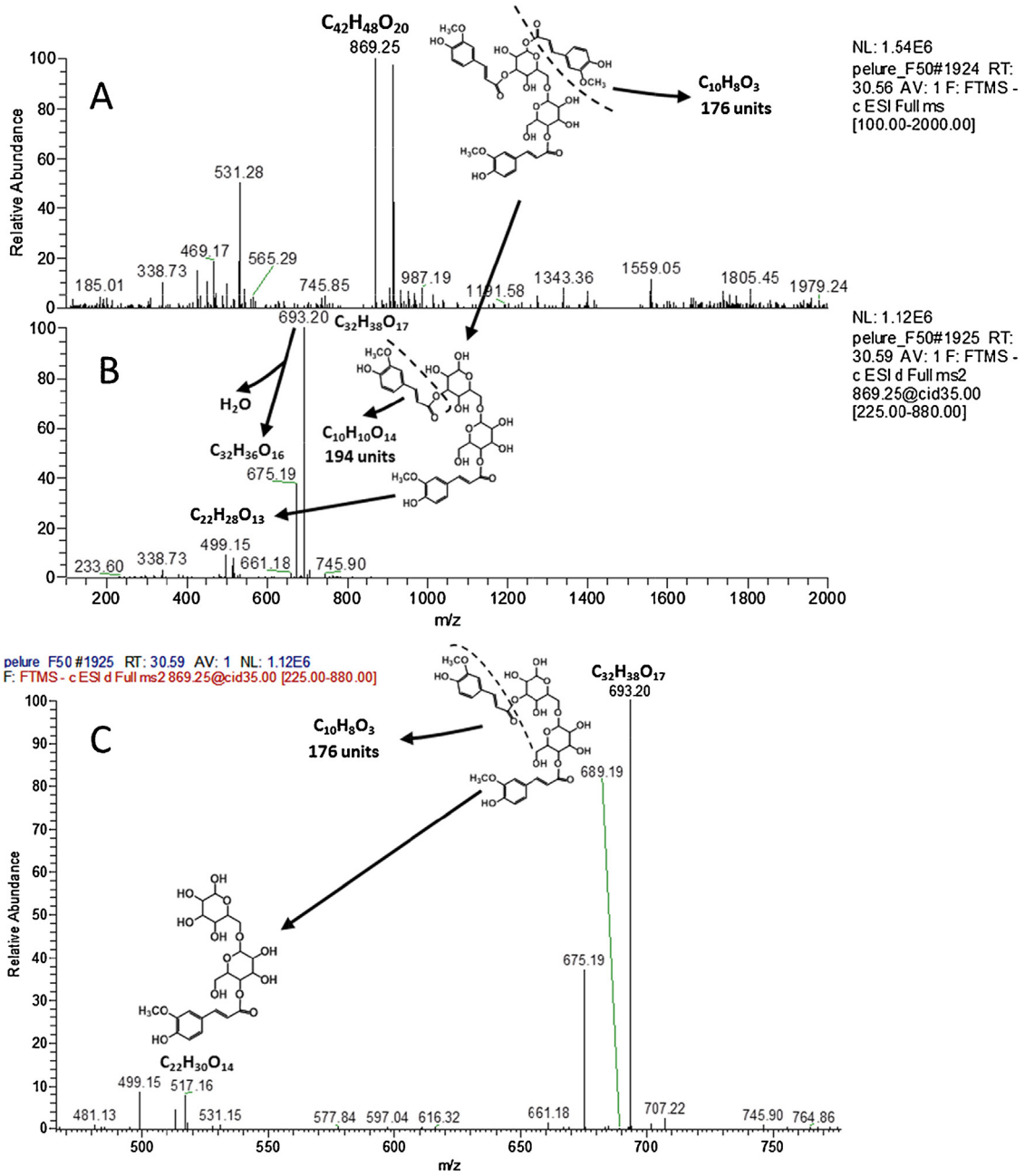

Fig. 3. Mass spectra of the putatively identified triferuloyl-dihexose illustrated by the structures of the ions and the suggested cleavage pathways. The MS^1 (A), the MS^2 (B) and a zoom in on the MS^2 spectra picture. The successive loss of two feruloyl units (176, $C_{10}H_8O_3$) from the precursor ion (*m/z* 869. 2499, $C_{42}H_{46}O_{20}$) (A) to give the fragment ions of the putative diferuloyl-dihexose (*m/z* 693.2030, $C_{32}H_{38}O_{17}$) (B) and ferulic acid dihexoside (*m/z* 517.1562, $C_{22}H_{30}O_{14}$) (C) could have occurred through the cleavage of the -*O*- linkage on the side of the ferulic acid group. The fragment ion at *m/z* 499.1455 ($C_{22}H_{28}O_{13}$) could have resulted from the loss of a 194 units ($C_{10}H_{10}O_4$), which could be ferulic acid or an isomer, from the putative diferuloyl-dihexose through the cleavage of the -*O*- linkage on the side of the hexose group.

glycoside moiety, which is involved in the degradation of the whole compound, had threshold energies depending on the glycosylation pattern. They revealed that to break down, the rutinose form (with 1 → 6 inter-disaccharide linkage) requires more energy than the neohesperidose form (with 1 → 2 inter-disaccharide linkage), and that the glycosylation at the 3-*O*-position needs lower energy than the 7-*O* or the 4′-*O*-position. The effect of cooking on myricetin and its related conjugated forms is poorly addressed in the literature.

3.3. Contribution of the individual phenolics to the differentiation between the raw and the cooked fruits taken as a whole

In order to investigate the relationships between all the different phenolics and the physico-chemical parameters such as pH, water content, total ash content, and total soluble solids following the cooking process, a principal component analysis was performed. Fig. 4A presents the scatter plot obtained for the raw or cooked plantain cultivars, the boiling process having been performed with the peel. Fig. 4B presents the loading plot of the phenolics and the physico-chemical attributes.

The first two principal components accounted for 60.3% of the total variability observed: PC1 32.1% and PC2 28.2% (Fig. 4). The scatter plot (Fig. 4A) showed a separation of cultivars along PC1 and a clear separation of the raw samples from the boiled samples along PC2. It can be observed that the relative separations between genotypes did not change after the boiling treatment. This suggests that taken together, the attribute changes occurred in the same way in all the genotypes. Fig. 4B shows that in general, all the

Table 3
Phenolic compounds of plantain cultivar pulps (mean value ± standard deviation in μg/g dry weight) resulting from the analyses of three experimental replicates ($n = 3$) each analysed in duplicate by HPLC-DAD.

Cultivar and treatment	Hydroxycinnamic-acid							Flavonol
	Caffeic acid-hexoside	Ferulic acid-hexoside	Sinapic acid-hexoside	Ferulic acid-dihexoside	Ferulic acid	Sinapic acid	Total hydroxycinnamic acids	Myricetin-deoxyhexose-hexoside
Mbeta 1								
Raw	15 ± 1.5[a]	72.5 ± 11.7[a]	nd	7 ± 0.8[a]	25 ± 5.9[a]	nd	119.5 ± 19.8[a]	43.7 ± 6.2[a]
Boiled without peel	17.7 ± 6.6[a]	67.7 ± 16.1[a]	nd	6.9 ± 1.5[a]	39.3 ± 9.1[a]	nd	131.6 ± 28.6[a]	45.1 ± 4.9[a]
Boiled with peel	14.4 ± 2[a]	48.8 ± 5.6[a]	nd	5.8 ± 2.2[a]	30.6 ± 5.6[a]	nd	99.7 ± 11.4[a]	50.47 ± 3.73[a]
Red Yade								
Raw	2.6 ± 1.1[a]	4.3 ± 0.8[a]	5.2 ± 1.2[a]	1.4 ± 0.4[a]	2.9 ± 0.8[b]	4.3 ± 0.7[a]	20.7 ± 1.4[a]	33 ± 6[a]
Boiled without peel	2.2 ± 0.3[a]	4.3 ± 1.6[a]	5.8 ± 3.3[a]	1.2 ± 0.1[a]	3.3 ± 0.3[b]	4.8 ± 1.3[a]	21.4 ± 5.5[a]	31.8 ± 6.5[a]
Boiled with peel	2.8 ± 0.7[a]	5.5 ± 1.7[a]	8.14 ± 2.56[a]	1.4 ± 0.1[a]	5.9 ± 0.2[a]	6.3 ± 0.6[a]	30.1 ± 4.8[a]	33.7 ± 6.6[a]
Essang								
Raw	3.5 ± 0.7[a]	31.4 ± 2.8[a]	8.1 ± 1.7[a]	11.1 ± 1.4[a]	13.6 ± 4.7[a]	3.8 ± 0.2[a]	71.4 ± 6.3[a]	37.5 ± 5.2[a]
Boiled without peel	3.4 ± 0.4[a]	32.7 ± 3.6[a]	7.4 ± 2[a]	9.2 ± 1.7[a]	20.1 ± 4.2[a]	4.1 ± 1.1[a]	76.7 ± 7.5[a]	39.3 ± 6.6[a]
Boiled with peel	3.2 ± 1[a]	30 ± 11.3[a]	5.4 ± 2.5[a]	9.9 ± 3.7[a]	24.8 ± 7.2[a]	4 ± 1.6[a]	77.3 ± 26.9[a]	42 ± 5.1[a]
Moto Ebanga								
Raw	nd	9.8 ± 1[a]	2.6 ± 1.7[a]	2.8 ± 0.3[a]	6 ± 0.4[b]	2.7 ± 1.4[b]	23.8 ± 3.8[a]	33.3 ± 4.6[a]
Boiled without peel	nd	7.6 ± 1.3[ab]	3.3 ± 1.3[a]	4 ± 1.0[a]	8 ± 1.2[ab]	4.8 ± 1[ab]	27.7 ± 5[a]	33.3 ± 4.5[a]
Boiled with peel	nd	6.5 ± 0.7[b]	3.6 ± 0.8[a]	3.2 ± 0.7[a]	9.8 ± 2.1[a]	5.4 ± 0.1[a]	28.5 ± 2.8[a]	36 ± 3.4[a]
Mbouroukou N°1								
Raw	3.3 ± 1[a]	12.1 ± 1.9[a]	2.3 ± 0.8[a]	4.1 ± 0.6[a]	12.7 ± 1.9[a]	2.7 ± 0.5[a]	37.3 ± 5[a]	28.8 ± 3.8[a]
Boiled without peel	2.9 ± 0.5[a]	10.3 ± 3[a]	3.1 ± 2.7[a]	4.1 ± 0.4[a]	15.6 ± 6.7[a]	4.9 ± 4.2[a]	40.8 ± 2[a]	24.7 ± 7[a]
Boiled with peel	3.5 ± 0.2[a]	11.5 ± 3.4[a]	4.4 ± 2.9[a]	4.9 ± 0.8[a]	17.1 ± 6.1[a]	8.1 ± 4.1[a]	49.5 ± 8[a]	28.5 ± 4.8[a]
F568								
Raw	1.3 ± 0.2[a]	2 ± 0.9[a]	2.4 ± 0.7[a]	0.9 ± 0.3[a]	2.9 ± 1.9[b]	4.6 ± 1.2[a]	14.1 ± 1.4[b]	35.7 ± 4.7[a]
Boiled without peel	1.6 ± 0.4[a]	1.2 ± 0.7[a]	1.2 ± 0.5[a]	1.2 ± 0.8[a]	3.3 ± 2.12[ab]	3.3 ± 1.5[a]	11.8 ± 2[b]	29.9 ± 9.9[a]
Boiled with peel	1.6 ± 0.8[a]	1.5 ± 0.2[a]	2.1 ± 0.4[a]	0.9 ± 0.2[a]	7.6 ± 1.3[a]	5.9 ± 1.2[a]	19.5 ± 2.2[a]	37.9 ± 5.1[a]

a, b, and c are letters resulting from ANOVA followed by Tukey's test performed to compare treatments. Mean values connected by the same letter are not statistically different ($p \leq 0.05$).
nd: not detected.
Caffeic acid-hexoside is expressed in caffeic acid equivalent. Ferulic acid-hexoside, Ferulic acid-dihexoside, Ferulic acid, Sinapic acid-hexoside and Sinapic acid are expressed in Ferulic acid equivalent. Myricetin-deoxyhexose-hexoside is expressed in Myricetin equivalent.
LOD: caffeic acid 0.02 μg/mL, ferulic acid 0.01 μg/mL, and myricetin 0.16 μg/mL.

variables whose vectors are directed above the PC1 axis decreased and those whose vectors are directed below the PC1 axis increased during the boiling treatment.

Considering the length of the vector and the correlation with the PC2 axis, the most important parameters of the pulp, which contributed to the differentiation between the raw and the boiled samples were water content, sinapic acid and ferulic acid, which increased after the boiling process. Myricetin-deoxyhexose-hexoside was highly correlated with ferulic acid, indicating a tendency to increase during boiling.

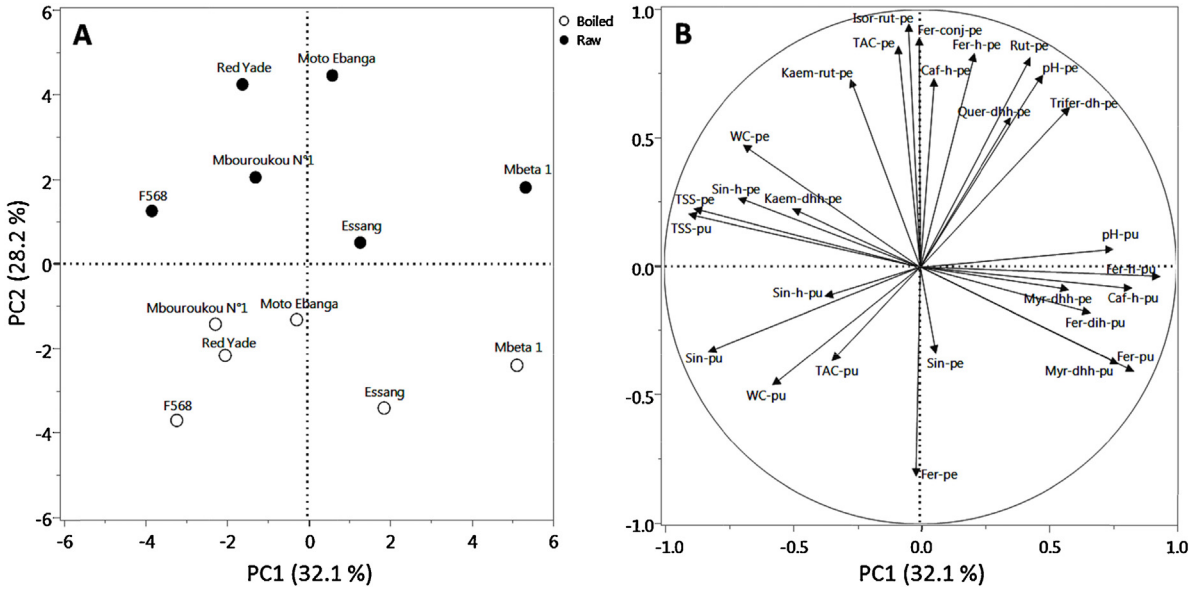

Fig. 4. Principal component analysis resulting from the phenolic compound and physico-chemical profiling of raw or cooked plantain pulps and peels (boiling with peel). (A) Scatter plot of varieties and (B) Loading plot of the phenolic and physico-chemical attributes. Pu (pulp), pe (peel), Caf-h (caffeic acid-hexoside), Fer-h (ferulic acid-hexoside), Fer-dh (ferulic acid-dihexoside), Fer (ferulic acid), Fer-conj-ind (ferulic acid-conjugate), Trifer-dh (Triferuloyl-dihexose), Sin-h (sinapic acid-hexoside), Sin (sinapic acid), Myr-dhh (myricetin-deoxyhexose-hexoside), Quer-dhh (quercetin-deoxyhexose-hexoside), Rut (rutin), Metmyr-dhh (methylmyricetin-deoxyhexose-hexoside), Kaem-dhh (kaempferol-deoxyhexose-hexoside), Kaem-rut (kaempferol-3-*O*-rutinoside), Isor-rut (isorhamnetin-3-*O*-rutinoside), TSS (total soluble solids), WC (water content), TAC (total ash content).

Table 4
Phenolic compounds of plantain cultivar peels (mean value ± standard deviation in μg/g dry weight) resulting from the analyses of three experimental replicates ($n = 3$) each analysed in duplicate by HPLC-DAD.

Cultivar and treatment	Hydroxycinnamic acid								Flavonol						
	Caffeic acid-hexoside	Ferulic acid-hexoside	Sinapic acid-hexoside	Ferulic acid	Sinapic acid	Ferulic acid-conjugate	Triferuloyl-dihexosose	Total hydrocycinnamic acids	Myricetin-deoxyhexose-hexoside	Quercetin-deoxyhexose-hexoside	Rutin	Kaempferol-deoxyhexose-hexoside	Kaempferol-3-*O*-rutinoside	Isorhamnetin-3-*O*-rutinoside	Total flavonols
Mbeta 1															
Raw	7.4 ± 1.9[a]	23.9 ± 9.9[a]	6.4 ± 3.4[a]	9.3 ± 4.3[b]	8 ± 1[a]	52.7 ± 25.9[a]	31 ± 10.5[a]	139 ± 53.2[a]	110 ± 27.2[a]	86.8 ± 25[a]	450 ± 156[a]	35.2 ± 10[a]	155 ± 58.5[a]	79.6 ± 32.1[a]	917 ± 293[a]
Boiled	4.2 ± 1.2[b]	11.6 ± 1.9[a]	3.5 ± 1.6[a]	30.5 ± 6.6[a]	12 ± 2.6[a]	5.6 ± 0.9[b]	14 ± 5.9[b]	81.4 ± 2.9[a]	124 ± 12.9[a]	74.4 ± 16[a]	290 ± 111[a]	27 ± 3.0[b]	79.9 ± 7.6[a]	47.6 ± 17.4[a]	643 ± 157[a]
Red Yade															
Raw	7.1 ± 1.6[a]	29.9 ± 7.6[a]	16 ± 4.4[a]	11.9 ± 1.2[b]	3.7 ± 0.4[b]	75.5 ± 21.1[a]	30.9 ± 3.4[a]	175 ± 34.3[a]	114 ± 27.4[a]	75.2 ± 14.1[a]	482 ± 206[a]	35.5 ± 4[a]	173.9 ± 49.7[a]	139 ± 73.1[a]	1020 ± 363[a]
Boiled	4.5 ± 0.7[b]	10.6 ± 2.4[b]	12.8 ± 2.3[a]	34.3 ± 3.3[a]	14.9 ± 1.4[a]	5.7 ± 0.9[b]	12.8 ± 1.8[b]	95.5 ± 7.5[b]	90.7 ± 10.8[a]	36.7 ± 3.4[b]	194 ± 7.5[b]	19.2 ± 2.5[b]	67.3 ± 10.3[b]	52.7 ± 5.1[a]	461 ± 21.3[b]
Essang															
Raw	3.0 ± 0.2[a]	26.2 ± 4.7[a]	10.9 ± 1.6[a]	12 ± 0.3[b]	5.4 ± 2[b]	67.5 ± 12.3[a]	14.6 ± 1.3[a]	140 ± 17.8[a]	90.9 ± 2.5[a]	35.9 ± 5.1[a]	251 ± 115[a]	19.5 ± 1.6[a]	61.5 ± 3.7[a]	81.8 ± 52.1[a]	541 ± 169[a]
Boiled	2.5 ± 1.4[a]	17.6 ± 1.5[b]	7.5 ± 1.6[b]	56.6 ± 5.1[a]	17.2 ± 2.6[a]	14 ± 2.7[b]	14.8 ± 2.1[a]	130 ± 13.2[a]	107.3 ± 16[a]	36.7 ± 4.7[a]	192 ± 40.7[a]	19.9 ± 0.4[a]	52.1 ± 1.7[b]	40.1 ± 7.9[a]	448 ± 40.5[a]
Moto Ebanga															
Raw	7.5 ± 1[a]	29.3 ± 2.5[a]	11.1 ± 4.2[a]	16.2 ± 5.2[b]	23 ± 22.8[a]	102 ± 23.4[a]	24.2 ± 6.1[a]	213 ± 61.4[a]	93.7 ± 14.6[a]	87.1 ± 5.9[a]	476 ± 12.7[a]	36.4 ± 3.3[a]	167 ± 30.9[a]	116 ± 24.3[a]	978 ± 41.3[a]
Boiled	3.5 ± 0.9[b]	10.8 ± 3.1[b]	8.4 ± 1.3[a]	52.6 ± 9.8[a]	18.8 ± 1.5[a]	17.3 ± 6.5[b]	15.7 ± 2.6[a]	127 ± 18.8[b]	90.6 ± 10.1[a]	60.5 ± 13.7[b]	324 ± 135[a]	25.1 ± 5.5[b]	109 ± 37.9[a]	73.9 ± 32.4[a]	683 ± 210[b]
Mbouroukou N°1															
Raw	5.2 ± 0.7[a]	14.3 ± 2.6[a]	7 ± 1.6[a]	10.1 ± 2.7[b]	4.8 ± 0.6[b]	32.8 ± 9.5[a]	13.6 ± 3.2[a]	87.8 ± 18.7[a]	56.5 ± 3[a]	107.4 ± 14.1[a]	368 ± 46[a]	39.6 ± 4.2[a]	140 ± 15.3[a]	106 ± 3[a]	817 ± 33.6[a]
Boiled	8.0 ± 4.9[a]	11.3 ± 1.2[a]	8.7 ± 3.3[a]	30 ± 2.9[a]	21 ± 3.8[a]	4.8 ± 1.3[b]	6.5 ± 1.1[b]	90.2 ± 12.8[a]	69.1 ± 8.7[a]	83 ± 18.3[a]	242 ± 103[a]	28.2 ± 5.1[b]	81.1 ± 20.9[b]	65.8 ± 31.6[b]	569 ± 167[b]
F568															
Raw	6.0 ± 1.7[a]	20.3 ± 5[a]	26.4 ± 11.2[a]	7 ± 2.6[b]	2.9 ± 2[b]	73.5 ± 33.3[a]	nd	138 ± 55.2[a]	89.7 ± 8.9[a]	29.7 ± 12.5[a]	151 ± 50.3[a]	87.1 ± 11.6[a]	208 ± 27.2[a]	64 ± 18.9[a]	630 ± 125[a]
Boiled	<0.1[b]	9 ± 1.1[b]	13.4 ± 4.8[a]	44.3 ± 14.9[a]	12.5 ± 6.9[a]	9.2 ± 3.3[b]	nd	88.5 ± 30.8[a]	96.6 ± 11.7[a]	13.8 ± 9.1[a]	71.5 ± 46.2[a]	54.7 ± 15.3[b]	122 ± 33.7[b]	34.1 ± 17.4[a]	392 ± 130[b]

a and b are letters resulting from Student's test performed to compare treatments. Mean values connected by the same letter are not statistically different ($p \leq 0.05$).
nd: not detected.
Caffeic acid-hexoside is expressed in caffeic acid equivalent. Ferulic acid-hexoside, Ferulic acid, Ferulic acid-conjugate, Triferuloyl-dihexosose, Sinapic acid-hexoside and Sinapic acid are expressed in Ferulic acid equivalent. Myricetin-deoxyhexose-hexoside is expressed in Myricetin equivalent. Rutin and Quercetin-deoxyhexose-hexoside are expressed in Rutin equivalent. Kaempferol-3-*O*-rutinoside and Kaempferol-deoxyhexose-hexoside are expressed in Kaempferol-3-*O*-rutinoside equivalent. Isorhamnetin-3-*O*-rutinoside is expressed in Isorhamnetin-3-*O*-rutinoside equivalent.
LOD – caffeic acid: 0.02 μg/mL; ferulic acid: 0.01 μg/mL; rutin: 0.07 μg/mL; kaempferol-3-*O* rutinoside: 0.11 μg/mL; isorhamnetin-3-*O*-rutinoside: 0.10 μg/mL; myricetin: 0.16 μg/mL.

The most important parameters of the peel contributing to the differentiation between the raw sample and the boiled samples were total ash content, pH, ferulic acid, conjugated ferulic acids as well as all the flavonols except kaempferol-deoxyhexose-hexoside and myricetin-deoxyhexose-hexoside. These parameters appeared to be correlated with each other and their vector directions indicate a decrease during the boiling process, except for ferulic acid. According to the direction of their vectors, ferulic-acid-conjugate-x appeared to be highly inversely correlated with ferulic acid, suggesting a certain contribution of this conjugated compound to the release of ferulic acid.

4. Conclusion

This study investigated the behaviour of phenolic compounds in plantain pulp and peel during the boiling process by identifying and quantifying phenolics using HPLC-ESI-HR-MS in negative mode and HPLC-DAD analysis. These analyses were performed on raw fruits, fruits boiled without peel and fruits boiled with peel. The pulp matrix appeared to have a protective effect against phenolic compound losses whereas the peel experienced considerable losses of compounds. The most important change in the pulp was the increase of ferulic acid. Changes in the peel were marked by the release of ferulic acid from its conjugated forms and the loss of the total ferulic acid based compounds. A possible transfer of these compounds from the peel to the pulp could be considered. Losses of most flavonols were observed in the peel. In conclusion, boiling with peel should be preferred to reduce the loss in ferulic acid. However, further studies on the bioavailability of this compound and its conjugated forms should be carried out to confirm that boiling with peel is more advantageous than boiling without peel. It will be worth studying other cooking processes such as steam boiling, roasting and frying to determine which of these cooking processes best preserves plantain phenolic compounds. In addition, this study showed that it will be very interesting to study the breakdown products of carotenoids during the cooking processes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2015.08.012.

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