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# Phenolic and glycidic profiling of bananas *Musa* sp associated with maturation stage and cancer chemoprevention activities

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ABSTRACT

Banana, *Musa* sp, is widely consumed all over the world, being popularly known as a natural antioxidant. The glycidic profile obtained by negative-ion mode electrospray Fourier transform ion cyclotron resonance mass spectrometry of the Pacovan, Ambrosia, Japira, Prata Comum, Vitória and Tropical cultivars, in four stages of maturation, showed that the Prata-type cultivar presented higher monosaccharide content than the disaccharides. On the other hand, Gros Michel-type (Ambrosia) and Maça-type (Tropical) cultivars presented the opposite behavior. The phenolic profile showed that, in all cultivars, the most abundant phytochemical class was flavonoids, such as myricetindeoxyhexose-hexoside, followed by cinnamic acids, such as caffeic acid. All samples evaluated showed high inhibition of the nitric oxide production. Furthermore, the Pacovan-type cultivar, at maturity stage 2, showed high inhibition of NF-κB; and extracts of cultivar Japira-type, maturity stages 2, 4 and 7, and Ambrosia, stage 4, showed greater aromatase inhibition, i.e., higher than 50%.

## 1. Introduction

Banana is a widely consumed fruit throughout the world. In 2015, world production reached about 16 million tons [1]. The main banana exporters are the Caribbean and Latin America and main consumers of the fruit are the United States, and the European Union, which together consume approximately 58% of all bananas exported [2].

Because of the great variety of metabolites, this fruit has a pleasant taste, provided by sugars, acids and volatile compounds. Banana is known to have antioxidant activity, being attributed to several classes of metabolites, especially to phenolic compounds [3]. Banana pulp has known antioxidant compounds such as catechin, epicatechin, lignins, tannins and anthocyanins [4]. Studies have shown that flavonoids have beneficial effects on human health that are linked to their antioxidant, chelating, antimutagenic and antitumor properties [5]. They act as antioxidants because of polyphenols that preferably oxidize while preserving the body's natural antioxidants. Flavonoids also inhibit some enzyme systems, such as prostaglandin synthase, interfering with carcinogenesis [6].

Carcinogenesis is divided into three stages, which often overlap, namely: initiation, promotion and progression [7]. Natural or synthetic pharmaceutical agents, or components of the diet, such as polyphenols that act in preventing, retarding or reversing the carcinogenesis process are described as cancer chemopreventive agents [7,8]. These are classified into three classes: (i) inhibitors of carcinogenic formation, which act to prevent formation of nitrosamines in an acidic environment; (ii) blocking agents, capable of inhibiting the initiation step by preventing carcinogenic activation (i.e., inducers of quinone reductase); and (iii) suppressive agents, which hinder the proliferation of malignant cells (i.e., inhibitors of NF-κB, inhibitors of aromatase, and inhibitors of nitric oxide production) [7]. One of the main goals for cancer chemoprevention continues to be the discovery of new effective agents with low side effects and no toxicity [7]. For the screening of natural products with potential anticarcinogenic activity, a panel of *in vitro* bioassays has been established that allows monitoring the inhibition of carcinogenesis in several stages [7].

The cancer chemopreventive potential of fruits of *Musa* × *paradisiaca* L. cultivar was evidenced using a bioassay based on the induction of quinone reductase (QR) *in vitro* [9] and an *in vitro* bacterial mutagenicity bioassay [10].

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The search for higher quality and productivity resulted in the development of new banana cultivars. These cultivars showed greater resistance to diseases and tolerance to drought and cold [11]. Studies that investigate, at a molecular level, the phenolic and glycidic profile associated with maturation and chemopreventive activities, mainly of these cultivars, are scarce. Therefore, this work has as its goal to investigate the chemical profile of six banana cultivars in four maturation stages, using the negative-ion mode electro spray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI(–)FT-ICR MS) and the results were associated with physicochemical analyses such as pH, titratable acidity and total soluble solids (TSS). In addition, their potential cancer chemopreventive effect was investigated by employing *in vitro assays* (cytotoxicity by the MTT method, QR induction, NF-κB inhibition, aromatase inhibitionand inhibition of nitric oxide production (iNOS)).

## 2. Materials and methods

### 2.1. Samples

Six *Musa* sp banana cultivars, namely: Prata Comum (AAB), Pacovan (AAB), Japira (AAAB), Ambrosia (AAAA), Vitória (AAAB) and Tropical (AAAB), in four maturation stages, were harvested from experimental farms of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER). Samples were harvested at maturation stage 1 and allowed to mature naturally at approximately 20 °C. For the choice of four stages of maturation, a visual criterion was used according to the color chart developed in previous works by Chitarra [12], being the scale of variation described as: T1 - totally green; T2 - green with yellow traces; T3 - greener than yellow; T4 - more yellow than green; T5 - yellow with green tip; T6 - all yellow and T7 - yellow with coffee tone areas. In this study, the stages T1, T2, T4 and T7 (Figure 1S) were chosen to be studied. All chemical, physicochemical and biological analyses were performed in triplicate.

### 2.2. Physicochemical analysis

Analyses of pH, total acid (TA, in terms of malic acid) and total soluble solids content (expressed in the form of°Brix) were performed. Analyses of pH and TSS were measured with a pH meter (Metrohm® 827 pH Lab) and benchtop refractometer (ABBE®2 WAJ, with a refractive scale of 1.300 – 1.72 nDwith 0 – 95°Brix), respectively. Values of TA were determined by titration using a standard solution of NaOH at 0.1 $molL^{-1}$ (Sigma Aldrich®) (results were expressed in grams of malic acid by 100 g of pulp) [13].

### 2.3. Preparation of glycidic extracts

For the sugar content analysis, 10 g of banana pulp was homogenized in a commercial blender with 15 mL solution of methanol / water (50% v/v). The homogenate was filtered to extract the fibrous part. Subsequently, a new filtration was performed using filter paper (Unifil® black stripe 125 mm) to extract possible non solubilized materials.

### 2.4. Preparation of polyphenolic extracts

To extract and concentrate phenolic compounds, 10 g of pulp were ground in a commercial blender, placed in an amber recipient with 20 mL solution of methanol, ethanol and acetone (1:1:1, in volume) and left in an ultrasonic bath at a frequency of 40 kHz for 1 h. Subsequently, the extract was filtered with filter paper (Unifil® black stripe 125 mm).This procedure was performed two times, using the material retained on the paper and volumes of 30 and 20 mL of methanol, ethanol and acetone (1:1:1, in volume), consecutively. The filtrates were concentrated in a single solution and the solvent evaporated in a rotary evaporator (RV 10, IKA-Werke GmbH & Co. KG, Staufen, Germany) at a temperature below 40 °C. Then, 20 mL of methanol was added to the extract and kept in the freezer for 24 h.

Thereafter, the extracts were centrifuged at 2500 × *g* for 10 min for pectin precipitation. After centrifugation, the procedure was repeated twice. Methanol was totally evaporated in a rotary evaporator at temperatures below 40 °C and 50 mL of water were added to the samples.

The extracts were washed with 30 mL of water in a cartridge (MACHEREY-NAGEL, Germany) composed of 360 mg of silica $C_{18}$-modified, to remove sugars and concentrate phenolic compounds. The phenolic compounds were extracted from the cartridge using 20 mL of 25% of methanolic solution (v/v) and, thereafter, were evaporated and brought up to 2 mL. The cartridges had previously been conditioned by the injection of 30 mL of methanol and then with 30 mL of water. [14,15]

### 2.5. ESI(–)FT - ICR MS and ESI(–)FT - ICR MS/MS

For the injection of extracts rich in sugar, 300 μL of the homogenate, 300 μL of deuterated glucose (D-Glucose-1, 2, 3,4, 5, 6, 6-$d_7$,SIGMA) ($5 \times 10^{-3}$ $molL^{-1}$) and 4 μL of $NH_4OH$ PA (Vetec Química Fina Ltda, Brazil) were added to 1.5 mL of methanol. For the injection of extracts rich in polyphenols, 1 mL of the extracts was basified with 4 μL of $NH_4OH$ PA.

Samples were directly injected into an FT-ICR MS (9.4 T Solarix, Bruker Daltonics®, Bremen, Germany). ESI(–) mass spectra were obtained in a mass range of *m/z* 200 – 2000, and source conditions were: nebulizer gas pressure of 2 bar, capillary voltage of 2.5 kV and capillary transfer temperature of 180 °C. Accumulation time of ions was 0.10 s. Each spectrum was acquired by the accumulation of 100 scans. Mass spectra were obtained with high resolution ($m/\Delta m_{50\%}$ = 400,000, where $\Delta m_{50\%}$corresponds to the full width of the peak at half its maximum height at *m/z* 400), providing unambiguous molecular formulas for single charge molecular ions ($C_cH_hN_nO_oS_s$ species). Mass spectra were acquired and processed with *Compass DataAnalysis* (BrukerDaltonics, Bremen, Germany) software. The structural formulas of the compounds were obtained through the ChemSpiderdatabase software (www.chemspider.com).

For the MS/MS experiments, the quadrupole window was closed in a range of 1 Da, providing isolation of the ion of interest. Subsequently, the ion is conducted to the interior of a collision cell (hexapole) with collision energy varying between 3 and 20 eV, with argon being the collision gas used. To increase the number of ions in the ICR cell, ion accumulation time in the hexapole was 0.1 s. Each mass spectrum was acquired from the accumulation of 100 scans.

### 2.6. Quantification of total polyphenols

The total concentration of polyphenols was determined for the polyphenolic extracts using a Folin–Ciocalteu reagent. The spectrophotometer absorbance was registered at a wave length of 715 nm. A five-point calibration curve was constructed ($R^2$ = 0.999), using anhydrous gallic acid standard solutions (VETEC®). The results were expressed as μg gallic acid equivalent per 100 g pulp *in natura* [16].

### 2.7. In vitro bioassays of cancer chemoprevention

For the evaluation of cancer chemopreventive potential, the cell viability test, QR induction, NF-κB inhibition, inhibition of iNOS production and inhibition of aromatase were used.

The cytotoxicity of the extracts was evaluated using the Sulforhodamine B (SRB) method [16]. Cells of Hepa 1c1c7 (murine hepatoma cells, ATCC CRL-2026), cells 293 – NF-κB, transfected with gene NF-κB-luciferase and macrophages of RAW 264.7(ATCC® TIB-71™) line were seeded at the same concentration used for each chemoprevention assay in Dulbecco'smodified-Eagle's Medium (DMEM) medium

**Table 1**
Physicochemical results for four maturation stages of six banana cultivars. Different letters mean that for Tukey's test with $p < 0.05$, the relative intensity values are significantly different.

| | | Vitória | Pacovan | Ambrosia | Prata Comum | Tropical | Japira |
|---|---|---|---|---|---|---|---|
| SST | T1 | 2.6 ± 0.2$^{a}$ | 2.4 ± 0.2$^{a}$ | 10.1 ± 1.3$^{a}$ | 2.8 ± 0.2$^{a}$ | 2.5 ± 0.2$^{a}$ | 2.7 ± 0.2$^{a}$ |
| | T2 | 4.9 ± 0.2$^{b}$ | 5.2 ± 0.3$^{b}$ | 17.5 ± 0.9$^{b}$ | 5.0 ± 0.2$^{b}$ | 5.9 ± 0.1$^{b}$ | 3.0 ± 0.2$^{a}$ |
| | T4 | 19.0 ± 0.2$^{c}$ | 13.8 ± 0.1$^{c}$ | 20.4 ± 0.1$^{c}$ | 15.0 ± 0.2$^{c}$ | 15.9 ± 0.5$^{c}$ | 12.5 ± 0.2$^{b}$ |
| | T7 | 21.0 ± 0.2$^{d}$ | 26.8 ± 0.3$^{d}$ | 21.3 ± 0.6$^{c}$ | 25.3 ± 0.3$^{d}$ | 23.0 ± 0.2$^{d}$ | 21.1 ± 0.2$^{c}$ |
| AT | T1 | 0.19 ± 0.01$^{a}$ | 0.16 ± 0.02$^{a}$ | 0.33 ± 0.04$^{a}$ | 0.16 ± 0.02$^{a}$ | 0.22 ± 0.03$^{a}$ | 0.20 ± 0.03$^{a}$ |
| | T2 | 0.21 ± 0.02$^{a}$ | 0.27 ± 0.03$^{b}$ | 0.51 ± 0.03$^{b}$ | 0.16 ± 0.03$^{a}$ | 0.33 ± 0.03$^{b}$ | 0.23 ± 0.03$^{a}$ |
| | T4 | 0.66 ± 0.02$^{b}$ | 0.58 ± 0.03$^{c}$ | 0.53 ± 0.03$^{b}$ | 0.45 ± 0.05$^{b}$ | 0.61 ± 0.04$^{c}$ | 0.77 ± 0.05$^{b}$ |
| | T7 | 0.70 ± 0.02$^{b}$ | 0.67 ± 0.03$^{d}$ | 0.41 ± 0.03$^{a}$ | 0.65 ± 0.04$^{c}$ | 0.72 ± 0.04$^{d}$ | 0.70 ± 0.04$^{b}$ |
| SST/AT | T1 | 13.7 ± 1.6$^{a}$ | 13.7 ± 2.7$^{a}$ | 30.2 ± 2.0$^{a}$ | 17.3 ± 2.8$^{a}$ | 11.1 ± 1.4$^{a}$ | 14.1 ± 2.6$^{a}$ |
| | T2 | 22.9 ± 1.0$^{b}$ | 22.9 ± 1.9$^{ab}$ | 34.2 ± 3.7$^{ab}$ | 31.7 ± 3.7$^{b}$ | 18.0 ± 1.3$^{b}$ | 13.3 ± 1.5$^{a}$ |
| | T4 | 28.9 ± 1.1$^{c}$ | 28.9 ± 1.3$^{b}$ | 38.3 ± 1.7$^{b}$ | 33.6 ± 3.5$^{b}$ | 26.2 ± 1.0$^{c}$ | 16.5 ± 3.1$^{a}$ |
| | T7 | 30.2 ± 0.9$^{c}$ | 30.2 ± 1.9$^{c}$ | 52.2 ± 3.9$^{c}$ | 38.7 ± 2.0$^{b}$ | 31.9 ± 1.4$^{d}$ | 30.4 ± 1.3$^{b}$ |
| pH | T1 | 5.9 ± 0.1$^{a}$ | 5.8 ± 0.1$^{a}$ | 4.7 ± 0.1$^{a}$ | 5.9 ± 0.1$^{a}$ | 5.8 ± 0.3$^{a}$ | 5.7 ± 0.1$^{a}$ |
| | T2 | 6.0 ± 0.1$^{a}$ | 5.8 ± 0.2$^{a}$ | 4.8 ± 0.1$^{ab}$ | 5.7 ± 0.1$^{a}$ | 5.3 ± 0.4$^{a}$ | 5.5 ± 0.3$^{a}$ |
| | T4 | 4.4 ± 0.1$^{b}$ | 4.4 ± 0.1$^{b}$ | 4.6 ± 0.2$^{a}$ | 4.4 ± 0.2$^{b}$ | 4.4 ± 0.2$^{b}$ | 4.3 ± 0.3$^{b}$ |
| | T7 | 4.2 ± 0.1$^{b}$ | 4.5 ± 0.2$^{b}$ | 5.1 ± 0.2$^{b}$ | 4.2 ± 0.2$^{b}$ | 4.3 ± 0.2$^{b}$ | 4.5 ± 0.3$^{b}$ |

added with 100 IUmL$^{-1}$penicillinand 100 gml$^{-1}$streptomycin (Sigma, USA) at 37 °C, controlled humidity, 5% $CO_2$ and plated in a 96-well plate at density of $5 \times 10^4$ cellsmL$^{-1}$. All extracts were tested at the concentration of 20 μgmL$^{-1}$. After 72 h of incubation, the cells were fixed with 50 μL of 20% trichloroacetic acid (TCA) for 30 min at 4 °C. After this time, the plate was washed four times with tap water. The adhered proteins were stained with 50 μL of 0.4% SRBin 1% acetic acid per well and incubated for 30 min at room temperature. Then, it was rinsed carefully four times with 1% acetic acid. After drying for 30 to 60 min or overnight, the crystals were solubilized with 200 μL of base pH 10 for 5 min in a shaker. The optical density was determined at 515 nm. Survival was calculated in relation to the negative control,10% dimethylsulfoxide(DMSO). For the positive control, camptotensin (10 μM) was used [17]. Samples are considered cytotoxic when the survival value is below 90% [19].

Determination of QR activity was performed using Hepa 1c1c7 (murine hepatoma cells, ATCC CRL-2026) seeded at a density of $0.5 \times 10^4$ cells mL$^{-1}$, together with extracts (20 μg mL$^{-1}$) in 96-well plates. Enzyme activity was determined using the aqueous extract as a function of menadiol-mediated reduction of MTT (3-(4,5-dimethylthiazo-2-yl) − 2,5-diphenyltetrazolium bromide). Absorption was determined at 595 nm. Total protein assay using crystal violet staining was performed in parallel [7]. The experiments were performed in triplicate.

The inhibition assay of NF-κB was performed using cell 293 – NF-κB, transfected with gene NF-κB-luciferase. These cells were seeded on a sterile plate at a concentration of $1.5 \times 10^5$ cells mL$^{-1}$ and allowed to reach approximately 80% confluence for 48 h incubation period. After treatment with the samples tested for 10 min, the cells were incubated for a further 6 h with and without TNF-α (5ngmL$^{-1}$). Then, the cells were washed with phosphate buffered saline and the luciferase assay was performed using the Luc assay system from Promega®, according to the manufacturer's instructions. Luciferase activity was monitored using a microplate reader and *N*-tosyl-L-phenylalanine chloromethyl ketone was used as a positive control [20].

The inhibition test for the iNOS production was carried out in the culture of macrophages RAW 264.7(ATCC® TIB-71™) line. The cells were seeded at a density of $2 \times 10^5$ cells mL$^{-1}$ in 96-well plates and cultured in an incubator under a controlled $CO_2$ atmosphere for 24 h. The culture medium was replaced with phenol-red-free medium and the extract (20 μg mL$^{-1}$) was added to the plate. After 15 min, it was stimulated with 1 μg mL$^{-1}$ of lipopolysaccharide (LPS), followed by incubation for 20 h. Next, the NO production was spectrophotometrically determined by the nitrite content, which is an oxidized NO product. The nitrite ion content was determined by the Griess reaction. Absorbance was measured on a microplate reader at 540 nm against a calibration curve with nitrite standards. The 1-NG-monomethyl arginine citrate (1-NMMA) was used as a positive control for the obtaining of the $IC_{50}$ value (that is the half-maximal inhibitory concentration of cell viability) [21].

To evaluate aromatase activity, NADPH regeneration system was preincubated at a concentration of 20 μgmL$^{-1}$of extract. Nangerin (final concentration at 0.57 μM) was used as a positive control and 0.5% DMSO was used as a negative control. After addition of the test compound and controls, incubation was carried out for 10 min at 37 °C. Then, 100μL of enzyme mixture and substrate were added and incubated for another 30 min at 37 °C. Finally, 75 μL of 5 mol L$^{-1}$ NaOH was added, the reaction was stirred for 5 min and incubated for 2 h at 37 °C. Fluorescence was measured at 485 nm (excitation) and 530 nm (emission) [7].

### 2.8. Statistical analyses

Chemical quantification, physicochemical and cancer chemoprevention data are presented as mean and standard deviation. For the presentation of mass data of the polyphenols, bubble plots were constructed using the signals detected in the FT-ICRMS. In the abscissa, the *m/z* values were plotted and in the ordinates the maturation stages. The relative intensities of the signals of each polyphenol were represented by the size of the bubbles, that is, the larger the signal intensity, the larger the size of the bubble. To analyze the glycidic profile of the six banana varieties at the four maturation stages, using the ESI(–)FT-ICR MS data, graphs were constructed using the ratio of the sum of the intensities of all sugar signals detected (*m/z* 179, 215, 239, 269, 297, 313, 327, 341, 359, 377, 431, 521, 683, 719 and 1025), divided by the sum of the intensities of the deuterated glucose signals (*m/z* 186, 222, 366, 373 and 528) (red color bar). All data were analyzed using analysis of variance and the Tukey test for comparison of means between the four stages of maturation at 5% significance.

## 3. Results and discussion

### 3.1. Physicochemical analysis

The Vitória, Pacovan, Prata Comum and Tropical cultivars showed an increase in TSS values as a function of maturation degree (T1→T7) (Table 1). Except for Ambrosia cultivar, the TSS contents had significantly increased from maturation stage T4 in the Vitória, Pacovan, Japira, Tropical and Prata Comum cultivars. Conversely, fruits of Ambrosia cultivar, showed an increase of TSS contents until the stage T4, being maintained constant in the last stage, i.e., stage T7 (Table 1). The increase of the TSS content during fruit ripening occurs mainly due to the formation of soluble carbohydrates such as sucrose, glucose and

fructose, which can be caused due to the degradation of the starch by the amylase enzyme [22].

Similar to TSS, for the four maturation stages studied, TA values increased from stage T1 to stage T7 for the Tropical and Pacovan cultivars. In the Vitória variety, the TA values can be grouped into two groups (stages T1 and T2, and stages T4 and T7). The last two stages are much higher than the first (i.e., 300%). For the Ambrosia variety, there was an increase in the TA content between stages T1 and T2, remaining constant between stages T2 and T4 and increasing between stages T4 and T7. In the Prata Comum variety, the TA content for stages T1 and T2 are almost identical and increase from stages T2 to T7. In the Japira variety, the TA content remained constant in the first two stages increasing after stage T4 (Table 1). In previous works using the Caipira, ThapMaeo and Tropical cultivars, generally, the variation of the TA contents corresponds to that reported in the literature, in which they increased during the maturation of the fruits [23].

Another property monitored is pH. It is expected that their values decrease during fruit maturation. These results corroborate with the results found in the TA assays because the increase of the organic acids content causes a pH decrease of the samples. The results for the Vitória, Pacovan, Japira, Tropical and Prata Comum cultivars showed that the first two stages of maturation had similar pH values, decreasing until stageT4, remaining constant until stageT7. For the Ambrosia variety, pH values remained constant up to stage 4 and from this stage showed a slight increase to stage T7 (Table 1).

The TSS/TA ratio is an important factor for determining the degree of fruit maturation. Fruits with advanced maturation stages have high TSS/TA ratio values. The Tropical variety presented an increase in the TSS/TA ratio and the statistical analysis showed that there is a significant difference between all the stages studied. For the Vitória variety, there was an increase in the values of TSS/TA until maturation stage T4, remaining constant thereafter. The Japira, Ambrosia and Pacovan cultivars, presented constancy in the values of TSS/TA up to stage T4, increasing their value in the last stage. For the Prata Comum variety, TSS/TA values increased until stage T2 and from this phase remained constant (Table 1).

In general, physicochemical results found for banana samples as a function of the maturation stage are consistent with the results reported in the literature [24].

### 3.2. Glycidic profile

ESI(–)FT-ICR mass spectra were obtained from all banana samples. To correlate the glycidic profile of the different banana cultivars in their maturation stages, an internal standard (glucose-1,2,3,4,5,6,6-$d_7$) was added to the samples. In this way, it was possible to observe (Fig. 1) that the intensities of the deuterated glucose signals (values in red) decrease with the increase of the maturation stage. The increasing of TSS values as a function of the maturation stage increases the intensities of the sugar signals (blue values) as the fruit matures.

The high resolution and accuracy of FT-ICR MS, as well as collision-induced dissociation (CID) experiments (i.e., ESI(–)MS/MS), allowed the structural identification of 20 compounds, where their molecular formulas, *m/z* values, double bound equivalents (DBEs), and mass errors (ppm) are shown in Table 1S.

Compounds 1 and 2, $[C_6H_{11}O_6]^-$ and $[C_6H_4D_7O_6]^-$ ions, of *m/z* 179 and 186, were identified as glucose and deuterated glucose, respectively. The glucose molecule, as well as its deuterated form, were also identified as chlorine adducts, producing the compounds 3, $[C_6H_{12}O_6 + Cl]^-$ ion, of *m/z* 215; and 4, $[C_6H_5D_7O_6 + Cl]^-$ ion of *m/z* 222. The fragmentation profile of compound 3 showed a fragment of *m/z* 179, relative to the loss of chlorine (Supplementary Material, Figure 2Sa). Compound 5, $[C_8H_{15}O_8]^-$ ion of *m/z* 239, was identified as an octapyranose, having its chemical connectivity identified from the CID experimentswhere the fragment produced (*m/z* 179) is relative to the removal of the hexoside ring substituent (Figure 2Sb).

Compounds 6–8, $[C_8H_{13}O_{10}]^-$, *m/z* 269, $[C_{11}H_{21}O_9]^-$, *m/z* 297 and $[C_{10}H_{17}O_{11}]^-$, of *m/z* 313, were identified as adducts of galactopyranose/ethanedioicacid, trihydroxy-2-methylbutyl-gulopyranoside and glucose/malic acid, respectively. Compound 9, $[C_{12}H_{23}O_{10}]^-$ ion of *m/z* 327, 3-(2,3-dihydroxypropoxy) − 2-hydroxypropyl-galactopyranoside, was identified and its ESI(–)MS/MS spectrum generated fragments of *m/z* 179, resulting from the loss of the hexoside ring substituent (Figure 2Sc). Compound 10, $[C_{12}H_{21}O_{11}]^-$ ion of *m/z* 341, was identified as sucrose, and its fragmentation resulted in the ion of *m/z* 179, related to glucose loss and in the ion of *m/z* 161, due to fructose loss (Figure 2Sd).

Compound 11, $[C_{12}H_{23}O_{12}]^-$ ion of *m/z* 359, was identified as the adduct of two glucose molecules, where its fragmentation profile produced ions of *m/z* 179, relative to the separation of the two glucose molecules (Figure 2Se). Compound 12, $[C_{12}H_{16}D_7O_{12}]^-$of *m/z* 366, was identified as glucose/deuterated glucose adduct and its fragmentation profile generated ions of *m/z* 179, relative to the loss of deuterated glucose molecule (Figure 2Sf). Compound 13, $[C_{12}H_9D_{14}O_{12}]^-$ of *m/z* 373, was identified as an adduct composed by two deuterated glucose molecules and its fragmentation profile produced ions of *m/z* 186, again related to adduct separation (Figure 2Sg). Compound 14, $[C_{12}H_{22}O_{11} + Cl]^-$ of *m/z* 377, which corresponds to the sucrose molecule, was identified as a chlorine adduct, where its fragmentation profile generated ions of *m/z* 341, related to the loss of chlorine and *m/z* 179, resulting from the fragmentation of the glycosidic bond (Figure 2Sh). Compound 15, $[C_{14}H_{23}O_{15}]^-$ of *m/z* 431, was identified as an adduct of fructofuranosyl-glucopyranoside/ethanedioicacid, and its fragmentation generated the ion of *m/z* 341 from dissociation of the adduct (Figure 2Si). Compound 16, $[C_{18}H_{33}O_{17}]^-$ of *m/z* 521, was identified as an adduct of sucrose and glucose and its fragmentation profile is shown in Figure 2Sj. Compound 17, $[C_{18}H_{26}D_7O_{17}]^-$ of *m/z* 528, was identified as sucrose/glucose-D7 adduct. Compounds 18 and 19, $[C_{24}H_{43}O_{22}]^-$ and $[C_{24}H_{44}O_{22} + Cl]^-$ ions of *m/z* 683 and 719, were identified as adducts of two sucrose molecules, in their deprotonated and chlorinated forms, respectively. The fragmentation profiles of the compounds 17–19 are shown in Figure 2Sk-m. Compound 20, $[C_{36}H_{65}O_{33}]^-$ ion of *m/z* 1025, was identified as a sucrose cluster and its fragmentation profile generated ions of *m/z* 683 and 341, related to the loss of one and two sucrose molecules, respectively (Figure 2Sn).

To evaluate the glucose profile using the ESI(–) FT-ICR MS data of the six banana cultivars as a function of the four maturation stages studied, graphs were constructed using the ratio of the sum of intensity of all sugars signals detected (*m/z* 179, 215, 239, 269, 279, 313, 327, 341, 359, 377, 431, 521, 683, 719 and 1025), divided by the sum of the intensities of the deuterated glucose signals (*m/z* 186, 222, 366, 373, and 528) (blue bar, Figure 3S). In general, the sugar content increased during the ripening process. The Ambrosia variety showed the highest sugar content in the first two maturation stages (T1 and T2). These results are consistent with the TSS measurements and show that this variety, although presenting visual aspects of a fruit at the initial stage of maturation (Stage 1 - totally green; Stage 2 - green with yellow traces), presents a behavior, in which, an early conversion of starch into carbohydrates occurs.

Another way of interpreting the ESI(–) data obtained for the glucose fractions is to analyze the ratio of the signals of monosaccharide and disaccharide compounds. The ratio of the absolute intensities of the monosaccharide signals (*m/z* 179, 215, 239, 269, 297, 313, 327, 359 and 521) by the sum of the intensities of deuterated glucose signals (*m/z* 186, 366, 373 and 528) was represented by the red bars and the ratio of the absolute intensities of the disaccharide signals (*m/z* 341, 377, 431, 521, 683, 719 and 1025) by the sum of the intensities of deuterated glucose signals (*m/z* 186, 222, 366, 373 and 528) was represented by green bars (Figure 3S).

Bananas of the Prata-type cultivar (Japira, Vitória, Prata Comum and Pacovan) presented monosaccharide content higher than the content of disaccharides. However, bananas of Gros Michel (Ambrosia) and Maçã-types (Tropical) cultivars showed higher disaccharide content

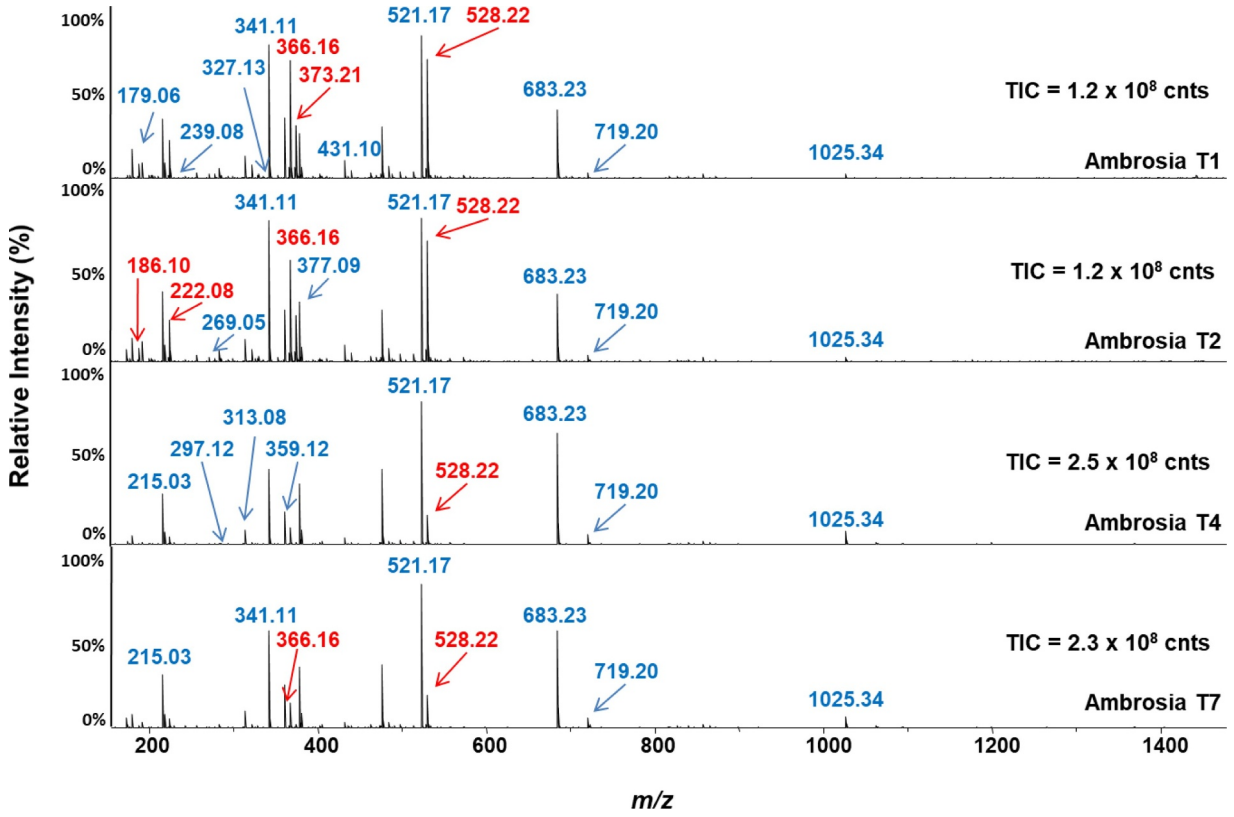


**Fig. 1.** ESI(–)FT-ICR mass spectra of the Ambrosia variety in four maturity stages. The values in red represent the *m/z* signals of deuterated glucose and the blue represent the *m/z* signals of sugars.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

than monosaccharide content (Figure 3S). The literature shows that cultivars Gros Michel-type presented, in the first days of maturation, a higher content of sucrose in relation to the contents of monosaccharides. But, in the final stages of maturation, the content of monosaccharides increased, exceeding the content of sucrose [25].

Sugars can be classified as reducing and nonreducing sugars. The monosaccharides, because they have aldehyde groups or free ketones, are reducing sugars. Some disaccharides, such as sucrose, have glycosidic bonds between their anomeric carbons, and they do not have aldehyde groups or free ketones. Therefore, they are classified as nonreducing sugars. The nonenzymatic browning via the Maillard reaction is an important degradation reaction of reducing sugars that occurs in fruit. The products of this reaction are compounds that interfere in the aroma, flavor and color of the fruits and, in addition, may generate undesirable sensorial characteristics to the fruits, mainly during the processing of these fruits [26]. Thus, the relation between the types of sugars present in the fruit (Figure 3S) is important information. The fruits of the Ambrosia and Tropical cultivars showed a disaccharide content higher than the monosaccharide content (reducing sugars). The disaccharides mostly found in these cultivars are classified as nonreducing sugars and, therefore, do not contribute to nonenzymatic browning via the Maillard reaction, thus making these two cultivars less susceptible to nonenzymatic browning [27].

### 3.3. Analysis. of total polyphenols

Banana samples analyzed presented levels of total polyphenols ranging from 302.58 to 1323.70 µg Eq AG / 100 g of pulp *in natura* (0.3% to 1.3%, Fig. 2). For the Prata Comum variety, total polyphenol contents were not different between stages T1, T2 and T4, but stage T7 had the highest polyphenol content observed (Fig. 2). For the Vitória variety, the polyphenol content increased from stage T1 to stage T4 and remained constant up to stage T7. In the Pacovan variety, the phenol content was higher at the maturation stage T4 and in the Ambrosia variety, it was larger at the stage T2. In the Tropical variety, maturation stage T7 was richer in polyphenols. The total polyphenol content of these cultivars is lower than those reported in the literature for the Terra, Mysore, Figo, Pacovan and Cavendish (cv. Nanicão) cultivars [28]. However, the literature data are related to dry pulp samples and the results of this work are relative to the pulp *in natura*.

### 3.4. Phenolic profile by ESI(–) FT-ICR MS

ESI(–)FT-ICR mass spectra were obtained from the polyphenolic extracts of the six banana cultivars at four maturation stages and the results for stage T7, for example, are shown in Figure 4S.

Table 2S shows the values of *m/z*, DBE, mass errors (in ppm) of the 20 identified polyphenolic compounds, where 13 of them are not reported in the literature for fruits such as bananas.

Compound 1, $[C_{15}H_{13}O_6]^-$ ion of *m/z* 289, was identified as epicatechin. Its fragmentation profile showed a fragment of *m/z* 245, relative to fraction loss of $C_2H_4O$ (–44 Da, Figure S5a). Compound 2, $[C_{15}H_{13}O_7]^-$ ion of *m/z* 305,was identified as gallocatechin. Compound 3, $[C_{15}H_9O_8]^-$ ion of *m/z* 317, was identified as a flavone. Their fragmentation profile showed fragments of *m/z* 289, relative to CO loss (–28 Da) and *m/z* 273, relative to $CO_2$ loss (–44 Da, Figure S5b). Compound 4, $[C_{16}H_{19}O_9]^-$ ion of *m/z* 355, was identified as ferulic acid-hexoside. Their fragmentation profile showed fragments of *m/z* 309 from the loss of $CH_2O_2$ (–46 Da), *m/z* 193 (–162 Da), and *m/z* 175 (–180 Da) relative to fragmentation in the hexoside fractions (Figure S5c). Compound 5, $[C_{16}H_{21}O_9]^-$ ion of *m/z* 357,was assigned as the cinnamicacid-hexoside derivative. ESI(–)MS/MS generated ion of *m/z* 283 (–74 Da), resulting from the loss of hexoside ring fraction ($C_3H_6O_2$) (Figure S5d). Compound 6, $[C_{15}H_{19}O_{10}]^-$ ion of *m/z* 359 was identified as gallic acid-hexoside derivative. Their fragmentation profile showed fragments of *m/z* 284 (–75 Da), *m/z* 239 (–120 Da) and *m/z* 197

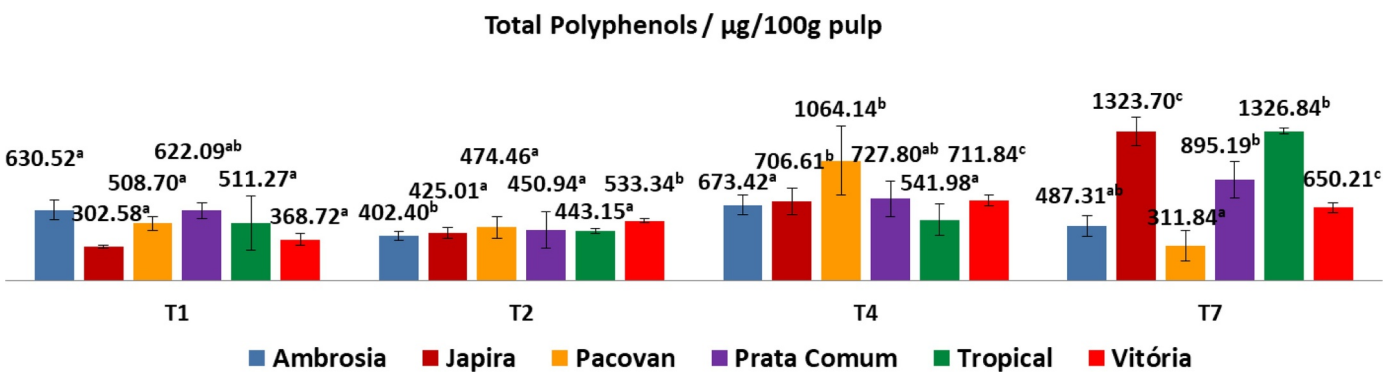


**Fig. 2.** The concentration of total polyphenols, in µg gallic acid / 100 g of pulp *in natura*.

(–164 Da), relative to the fragments in the hexoside ring (Figure S5e). Compound 7, $[C_{19}H_{25}O_{12}]^-$ ion of *m/z* 445, was identified as acetyl-hydroxyphenylhexoside-deoxyhexosidederivative. The CID experiment generated the fragment of *m/z* 265 (–180 Da), relative to the loss of hexoside fraction (Figure S5f).Compound 8, $[C_{21}H_{21}O_{11}]^-$ ion of *m/z* 449, was identified as caffeic acid derivative. Its fragmentation profile showed fragments of *m/z* 287 (–162 Da), *m/z* 269 (–180 Da), from the fragments in the hexoside ring (Figure S5g). Compound 9, $[C_{21}H_{19}O_{13}]^-$ ion of *m/z* 479, was identified as a flavonol hexoside. Their fragmentation profile showed fragments of *m/z* 317 (–162 Da), *m/z* 273 (–44 Da), resulting from the loss of the hexoside ring and $CO_2$, respectively (Figure S5h). Compound 10, $[C_{23}H_{29}O_{12}]^-$ ion of *m/z* 497,was identified as a gallic acid derivative. ESI(–)MS/MS experiment generated a fragment of *m/z* 453 (–44 Da) relative to the $CO_2$ loss of the carboxylic fraction (Figure S5i). Compound 11, $[C_{22}H_{33}O_{13}]^-$ ion of *m/z* 505, was identified as a dihydroxyphenyl-dihexosidederivative. Their fragmentation profile showed fragments of *m/z* 325 (–180 Da), resulting from the loss of the hexoside fraction (Figure S5j). Compound 12, $[C_{23}H_{25}O_{13}]^-$ ion of *m/z* 509, was identified as a flavonolhexosidehydrate. The CID experiment generated the fragment of *m/z* 347 (–162 Da) and *m/z* 329 (–180 Da) relative to the fragments on the hexoside ring (Figure S6k). Compound 13, $[C_{22}H_{29}O_{14}]^-$ ion of *m/z* 517, was identified as ferulicacid-dihexoside. Compound 14, $[C_{24}H_{27}O_{14}]^-$ ion of *m/z* 539, was identified as a flavonolhexosidehydrate. Their fragmentation profile showed fragments of *m/z* 449 (–90 Da), *m/z* 435 (–104 Da) and *m/z* 359 (–180 Da), resulting from the loss of hexoside ring fraction, and fragment of *m/z* 287 (–252 Da), relative to the fragmentation of hydrated flavonol (Figure S5l). Compound 15, $[C_{30}H_{25}O_{12}]^-$ ion of *m/z* 577, was identified as a dimeric B-typeproanthocyanidin. Compound 16, $[C_{25}H_{27}O_{16}]^-$ ion of *m/z* 583, was identified as a xanthonedihexoside. Their fragmentation profile showed fragments of *m/z* 421 (–162 Da), resulting from the loss of hexoside ring fraction (Figure S5m). Compound 17, $[C_{29}H_{43}O_{13}]^-$ ion of *m/z* 599, was identified as substituted hydroxymethyl-phenyl. ESI(–) MS/MS experiment generated the *m/z* 581 (–18 Da) fragment, resulting from the loss of $H_2O$. In addition, fragments at *m/z* 567 (–32 Da), *m/z* 579 (–120 Da) and *m/z* 437 (–162 Da) correspond to fragments on the hexoside ring. Further, the fragment of *m/z* 419 (–180 Da) is produced from the loss of the hexoside fraction (Figure S5n). Compound 18, $[C_{27}H_{29}O_{16}]^-$ ion of *m/z* 609, was identified as quercetin-deoxyhexose-hexoside. Their fragmentation profile showed fragments of *m/z* 300 (–309 Da), resulting from loss of the hexoside rings, and *m/z* 279 (–330 Da) relative to fragmentation in the fraction of deoxyhexose (Figure S5o). Compound 19, $[C_{27}H_{29}O_{17}]^-$ ion of *m/z* 625, was identified as myricetindeoxyhexose-hexoside. The CID experiment generated ion of *m/z* 316 (–309 Da), resulting from loss of hexoside rings (Figure S5p). Compound 20, $[C_{27}H_{33}O_{17}]^-$ ion of *m/z* 629, was identified as leucodelphinidin deoxyhexopyranosyl-hexopyranose. Its fragmentation profile showed fragments of *m/z* 449 (–180 Da), resulting from the loss of a glycolic ring (Figure S5q).

The compounds epicatechin and galocatechin had already been reported in bananas of the Bluggoe, Cavendish (cv. Nanicão), Terra, Mysore and Pacovan cultivars [28]. In addition, galocatechin has already been identified in the Cavendish cultivar [4]. The compounds ferulic acid-hexoside, ferulic acid-dihexoside, quercetin-deoxyhexose-hexoside and myricetindeoxyhexose-hexoside have already been identified in bananas of the RedYade [29], Mbeta 1, Essang, Moto Ebanga, Mbouroukou N°1 and a hybrid F568 cultivar [30]. The compound Dimeric B-type proanthocyanidin has already been identified in banana in the literature [31].

To plot the phenolic profile of the six banana cultivars at four stages of maturation, bubble graphs were constructed using the 20 polyphenol signals detected in the FT-ICRMS. Polyphenols were classified according to their phytochemical classes, using different colors as shown in the legend in Fig. 3.

In all samples, flavonoids were the most abundant compounds, both in amount and abundance of identified compounds (red bubbles) (Fig. 3). The most abundant flavonoid was myricetin deoxyhexose-hexoside, of *m/z* 625, which is a powerful antioxidant and anti-inflammatory. In addition, studies have proven its ability to inhibit the major enzymes involved in cancer initiation and progression [32].

The cinnamic acids class was the second class identified with higher relative intensity signals in all samples (blue bubbles). Among the cinnamic acids found, the most intense was the ion of *m/z* 449, which was identified as a caffeic acid derivative. Previous work has shown that caffeic acid had good antioxidant and anticarcinogenic activities [33].

### 3.5. Cancer chemopreventive activity

Results of the QR induction assays, inhibition of iNOS production, inhibition of NF-κB and aromatase activity are shown in Table 3S.

The reduction of electrophilic quinones by the action of QR enzymes is an important detoxification pathway that reduces the oxidative cycle [18,19]. The samples were evaluated in terms of their QR enzyme induction capacity. Of the samples tested, only the Tropical variety at maturation stage T2 showed QR induction (IR = 2). QR inhibition assays had been carried out on samples of wild bananas of *Musa balbisiana*. Some compounds extracted from these fruits showed activity in the induction of QR [9].

Some studies demonstrate that NF-κB is over expressed and activated in several types of cancer [34], and was identified as an important marker in Hodgkin's tumor cells [35] and breast cancer [36]. The extract of Pacovan cultivar, maturation stage T2, presented 87% inhibition of NF-κB, the other extracts presented a low inhibition rate (Table 3S).

The iNOs have been associated with many tumors and progression [37], such as prostate cancer [38] and breast cancer [39]. In all the extracts tested, there was high inhibition of the iNOs, which shows high chemoprevention capacity.

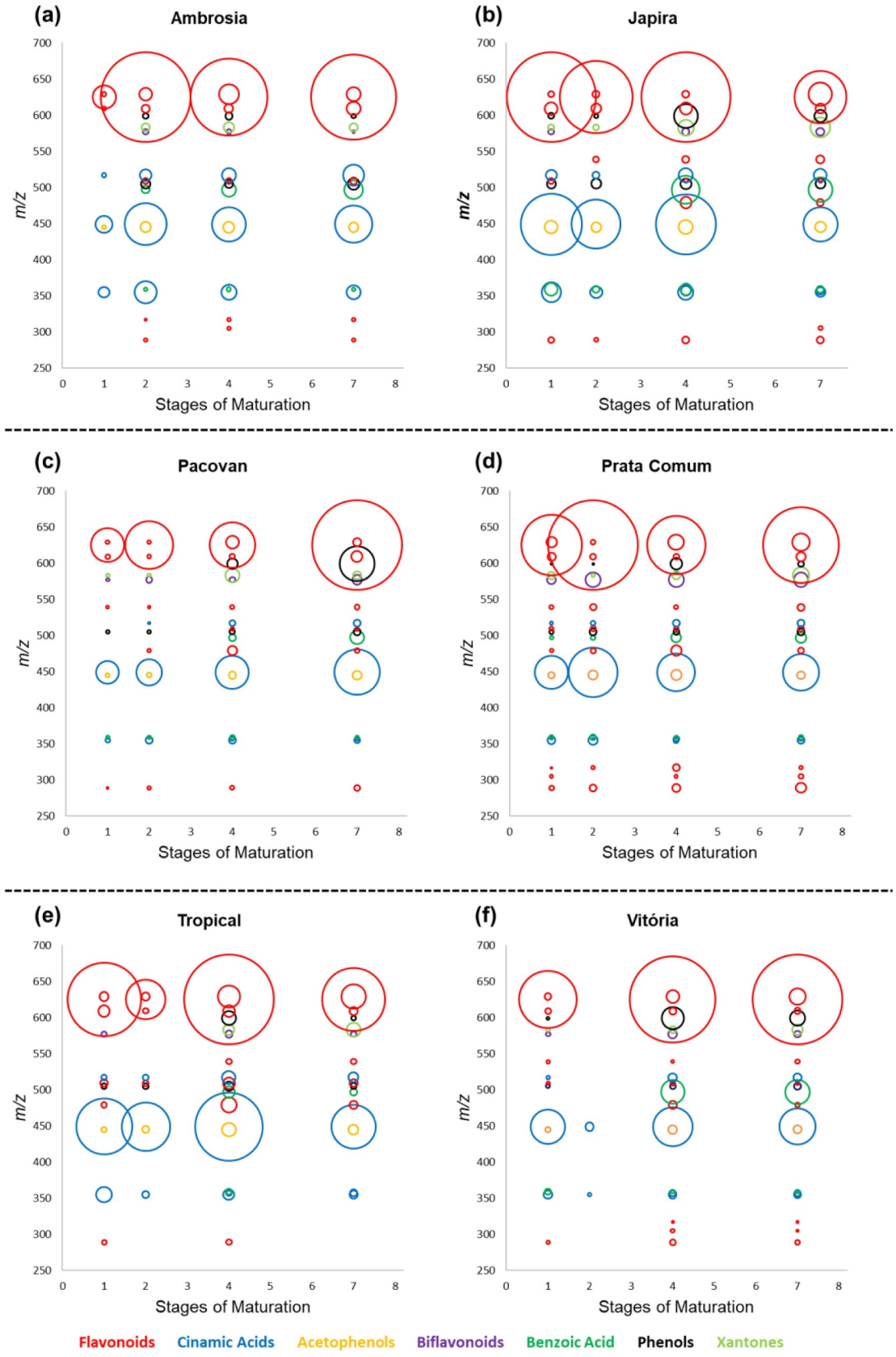


**Fig. 3.** Classes of polyphenols from six banana cultivars in four maturation stages. The size of the bubbles is directly proportional to the relative intensity of the peaks in the spectrum.

Breast cancer tissues express aromatase and produce higher levels of estrogens than healthy cells [40]. In this way, the inhibition of aromatase is important to human health. Extracts of Japira bananas, at maturation stages T2, T4 and T7 and Ambrosia, stage T4, showed aromatase inhibition higher than 50%; however, in an $IC_{50}$ assay (data not shown), no sample showed concentration-dependent inhibition.

## 4. Conclusion

Information on the molecular level of phenolic and glycidic profiles associated with the maturation of the banana cultivars studied is scarce. From the data of this work, it was possible to observe that the increase of the carbohydrate content in the fruits during maturation occurs differently depending on the banana variety. Bananas of the Prata-type cultivar (Japira, Vitória, PrataComum and Pacovan) presented higher monosaccharide contents than the disaccharide content and Gros Michel (Ambrosia) and Maçã (Tropical) cultivars, which had higher disaccharides contents. The polyphenolic profile of these samples indicated a predominance of flavonoids and cinnamic acids, of which 20 polyphenols were detected. All the samples evaluated showed cancer chemopreventive potential, associated with the capacity to inhibit the production of nitric oxide. In addition, Pacovan cultivar, maturation stage T2, showed high inhibition of NF-κB, where as Japira cultivar, at maturation stages T2, T4 and T7 and Ambrosia, stage T4, showed aromatase inhibition higher than 50%.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.microc.2019.104391.

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