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Article

Use of Paper Spray Mass Spectrometry for Determining the Chemical Profile of Green Cavendish Banana (*Musa* AAA) Peel and Pulp Flours and Evaluation of Its Functional Potential

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Green Cavendish banana peel and pulp flours were obtained by three drying methods: oven dryer at 70 °C; air fryer at 180 °C and domestic oven at 180 °C, being the latter two new possibilities. Bioactive constituents using paper spray ionization mass spectrometry (PS-MS), phenolic identification and quantification by ultra-performance liquid chromatography with UV-Visible detection (UPLC/UV-Vis) and antioxidant capacity were evaluated. Phenolic acids showed distinct thermal stability between the treatments. Gallic acid was the predominant compound, ranging from (29.56 to 1211.74 mg 100 g^{-1}) and had higher concentration than that found in other bananas described in literature. Green Cavendish banana flour is an advantageous source of bioactive compounds and antioxidant capacity, especially its peel. 26 compounds were identified by PS-MS: phenolics, organic acids, sugars, amino acid, phytosterol, iridoid and coumarin derivatives. Green Cavendish banana flour has great functional potential, and the air fryer can be a promising alternative for drying.

Keywords: green banana, functional potential, drying, mass spectrometry, paper spray

Introduction

Banana (*Musa* sp.) is one of the most cultivated fruits in the world and has great economic and social importance. The production of different banana varieties represents about 110 million tons *per* year. *Musa* Cavendish is the most traded (about 45% of the world banana market) because of its high productivity and because it is less prone to damage caused by environmental changes.[1]

Banana has attracted a great deal of attention because it is an important accessible source of phenolic compounds, especially its peels, including flavonols (quercetin, myricetin, rhamnetin, rutin and kaempferol), flavanones (naringenin), flavones (luteolin, apigenin), flavanols (epicatechin, gallocatechin, catechin and procyanidins) and phenolic acids such as gallic acid, chlorogenic acid, ellagic acid, coumaric acid, caffeic acid and ferulic acid.[2-4] However, few studies have been done with green banana and a more thorough investigation of the phenolic compounds profile in peel and pulp has not been performed.

Bioactive constituents are found in various vegetables, and their contents differ according to variety, growing region and maturity stage. Regarding the content of phenolic compounds, responsible for the astringency of green bananas, it decreases with fruit ripening, while the content of carotenoids increases at the ripe stage.[2-5]

Several biological actions have been attributed to phenolic compounds, and the main ones are anti-inflammatory and antioxidant. Their molecular structure allows effective neutralization of radicals. Thus, phenolic compounds have been the fundamental link between diet and decreased risk of chronic diseases.[6]

Due to its typical hardness and high astringency, green banana is commonly used to obtain flour for bakery

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products due to its gelling and thickening properties, as well as for increasing phenolic content, antioxidant activity and reducing glycemic index by increasing the undigested starch fraction or through the complexation of carbohydrates with condensed tannins.[7,8]

Peels can also be used as an alternative source of nutrients and add nutritional value to flours. In addition, they can act as bacteriostatic or fungistatic agents in the pharmaceutical industry, natural preservative in foods and play an important role in reducing the risk of degenerative diseases.[4]

Post-harvest processing of green bananas is important to ensure the functional quality of flours, especially drying. The effects of drying on the phenolic content and antioxidant activity of foods depend on the drying conditions, food matrix and genetic factors.[9] Phenolic compounds and the antioxidant capacity of foods are either degraded or modified when prolonged drying periods and/or high temperatures are applied.[10]

Oven dryer with forced air is the most commonly used method for drying bananas with a temperature range from 50 to 70 °C.[11,12] However, drying methods such as domestic oven and air fryer can be employed for the same purpose.

According to Guiné *et al*.,[13] lower total phenolic degradation in *Musa* Cavendish banana pulp was observed in oven dryer with forced air at 70 °C when compared to the temperature of 50 °C. On the other hand, Gálvez *et al*.[9] identified in red pepper, lower levels of vitamin C and total phenolics in oven dried samples at 80 and 90 °C in comparison to lower temperatures (50 and 70 °C).

Tian *et al*.[14] observed higher antioxidant activity and lower total phenolic losses in purple sweet potato using the air fryer treatment for drying (180 °C for 18 min) compared to domestic oven (210 °C for 30 min); however, there was a higher amount of total carotenoids and anthocyanins in sweet potato dried in the oven when compared to air fryer.

The antioxidant activity of phenolic compounds is due to their ability to eliminate free radicals, donate hydrogen atoms or electrons, or chelate with metal cations. Antioxidant activity is higher with the increase in the degree of hydroxylation, so the different antioxidant capacities of phenolic compounds depend on the number and position of OH– groups present in their structure.[6]

Antioxidant activity can be expressed through various parameters. It is recommended that at least two assays be combined to provide a more reliable result of the total antioxidant capacity of a food. The ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) method is generally indicated for hydrophilic compounds such as vitamin C and most phenolic compounds. DPPH (2,2-diphenyl-1-picrylhydrazil) can be used for both hydrophilic and lipophilic compounds.[15]

Paper spray ionization mass spectrometry (PS-MS) is an ambient ionization technique that has received attention in recent years and has been quite efficient for analyzing substances in complex matrices. The process by which ions are generated is similar to the electrospray ionization (ESI) technique.[16,17] However, the PS-MS is an ionization technique which obtains fast fingerprints in wide ranges of mass, with minimal or no sample preparation.[18,19] PS-MS has been used in the food area to detect frauds, pesticide analysis in vegetables and fruits, as well as in the phytochemical characterization of foods such as wine, cagaita (*Eugenia dysenterica*), olive oil, beer, coffee, sorghum and peel of ripe banana (*Musa sapientum* AAB).[16,18,19]

Thus, the aim of this study is to evaluate the *in vitro* antioxidant activity of green banana, peel and pulp flours, identify and quantify the phenolic compounds responsible for such activity, identify other bioactive constituents using PS-MS, as well as investigate the impact of drying conditions on the stability of these compounds.

Experimental

Chemical reagents

Reagents for phenolic compounds and analytical standards were purchased from Sigma-Aldrich (St. Louis, USA).

Plant materials and drying methods

Musa acuminata bananas (AAA group), sub-group Cavendish (so called "Nanicão" in Brazil), at maturity stage I (unripe) according to the Von Loesecke scale, cultivated in Ravena-Sabará district (Brazil) and not either subjected to a ripening chamber and or sprayed with pesticides, were acquired. Approximately 300 g of bananas, evenly distributed, were used for each treatment. The bananas were washed and sanitized with a solution of chlorine of 150 mg L^{-1} for 15 min. The peels were manually removed from the pulp, cut into pieces of about 15 cm^2 and the pulps into 5 mm thick slices and subjected to drying at 70 °C in an oven dryer (B) with forced air (Quimis Q-314M242) for 370 min (peel) and 330 min (pulp), air fryer (A) at 180 °C (Mondial S.A, AF-17) for 18 min (peel) and 16 min (pulp) and domestic oven (C) at 180 °C (Esmaltec-6Q) for 210 min (peel) and 180 min (pulp).

The dried peels and pulps were ground and screened through a mesh size 32 (500 μm). The obtained flours, called "green banana peel (GBPe)" and "green banana pulp (GBPu)", were stored at −18 °C in polyethylene bags and used as raw material for all experiments in this study.

Sample preparation

The flour extracts (GBPe and GBPu) of the three drying methods were prepared according to Rufino *et al*.,[15] with adaptations: the amount of flours ranged from 0.5 to 2.0 g and were extracted with 4 mL of methanol/water (50:50, v/v) at room temperature for 60 min. The tubes were centrifuged at 1811 × g for 20 min and the supernatant was recovered. Then, 4 mL of acetone/water (70:30, v/v) was added to the residue, extracted and centrifuged for 60 min. Methanol/acetone extracts were combined and the volume was completed with distilled water up to 10 mL to determine antioxidant capacity, total phenolics, phenolics profile, and bioactive constituents using PS-MS. All analyses were performed in triplicate.

Identification and quantification of phenolics profile of the obtained flours by UPLC/UV-Vis

The phenolic compounds were analyzed by ultra-performance liquid chromatography with UV-Visible detection (UPLC/UV-Vis) according to the chromatographic method described by Chisté *et al*.,[20] with adaptations: the extracts (1 μL) were injected for the analysis after filtration on a reversed-phase column (2.1 × 100 mm; 1.7 μm particle; Acquity UPLC® BEH) at 29 °C. The flow rate was 0.3 mL min^{-1}. The solvents were acetonitrile (A) and water with 0.25% formic acid (B). The isocratic elution mode was 5% A and 95% B from 0 to 17 min. The linear gradient was applied under the following conditions: 8% A and 92% B from 0 to 8 min; 15% A and 85% B from 8 to 14 min; 25% A and 75% B from 14 to 22 min. Gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, rutin and quercetin were identified based on the retention times of commercial standards, UV-Vis spectra, and data reported in the literature. Quantification of each compound was accomplished by comparing the peak areas with that of a calibration curve of each standard and the results were expressed as mg *per* 100 g^{-1} dry weight (d.w).

PS-MS fingerprints

Qualitative analysis of the chemical profile of the flours was done using a mass spectrometer, LCQ Fleet (Thermo Scientific, San Jose, CA, USA), equipped with a paper spray ionization source, according to the method described by Silva *et al*.,[16] with adaptations, in the mass range from *m/z* 100 to 1000 (positive and negative ionization mode). The ions and their fragments were identified based on the data reported in the literature.

Total phenolics determination

Total phenolic compounds were determined according to the Folin-Ciocalteu spectrophotometric method described by Singleton *et al*.,[21] with adaptations: 100 and 200 μL aliquots of flour extracts (GBPe and GBPu, respectively) were mixed with 5 mL of 10% (v/v) Folin-Ciocalteu solution and diluted with water. After 3 min, 4 mL of 7.5% (m/v) sodium carbonate solution was added. The mixture was allowed to rest at room temperature for 60 min and the absorbance at 760 nm was read in the spectrophotometer (Femto Instruments Ltda, Cirrus 80, Brazil). The total phenolic concentration was calculated using a gallic acid standard curve ($y = 0.1196x + 0.0236$, coefficient of determination (R^2) = 0.9998), ranging from 150 to 900 μL mL^{-1}. Results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} (d.w).

Antioxidant capacity

The DPPH assay was carried out according to the Association of Official Analytical Chemists (AOAC) method 2012.4.[22] Aliquots of GBPe and GBPu flour extracts ranged from 10 to 350 μL. The standard curve was constructed using Trolox (20 to 100 μL) solution. The absorbance was read at 517 nm and the results were expressed as μM Trolox 100 g^{-1} (d.w).

The antioxidant capacity by the ABTS method was determined as described in the literature,[23] $ABTS^{\bullet+}$ radical cations were produced by reacting 7 mM ABTS stock solution with 140 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 16 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. After the addition of 30 μL of flour extracts (GBPe and GBPu) or Trolox standard to 3 mL of $ABTS^{\bullet+}$ solution, absorbances were recorded at 6 min after mixing. Ethanolic solutions of Trolox 2 mM concentrations (0.5 to 10 mL) were used for calibration and the results were expressed as μM Trolox 100 g^{-1} (d.w).

Statistical analysis

The results were submitted to analysis of variance (ANOVA) and comparison of means by Tukey's test (5% significance level). To determine if phenolic compounds are associated with antioxidant capacity, Pearson correlation coefficients were calculated. Statistical analyses were performed using the RStudio Team (2015) software.[24]

Results and Discussion

Total phenolic compounds

The amount of phenolic compounds present in peel and pulp flours obtained by three drying methods: (A) 1686.47 ± 84.95 (peel) and 200.23 ± 20.67 mg GAE 100 g^{-1} (pulp); (B) 690.49 ± 17.86 (peel) and 76.77 ± 3.20 mg GAE 100 g^{-1} (pulp); (C) 465.92 ± 22.39 (peel) and 62.53 ± 4.01 mg GAE 100 g^{-1} (pulp), is shown in (Figure 1).

Total phenolic compounds showed significant results for both factors individually: flour (peel/pulp) and treatment. Regardless of the treatment, higher concentration of phenolic compounds was found in the flour of the peel, in comparison to the pulp, with the averages of 947.63 and 113.18 mg GAE 100 g^{-1}, respectively. Regarding the treatments, the highest total phenolic content was found in flours obtained by the treatment A followed by B and C, in increasing order: A > B > C. The highest concentration of total phenolics in flours obtained by treatment A was due to the short drying time and consequently lower exposure of phenolics to the thermal effect.[10]

The values obtained in the pulp flours of the three treatments were higher than those reported by Campuzano *et al.*[8] in green Cavendish banana pulp flours at 2 ripening stages: 16.54 (1st stage) and 29.68 mg GAE 100 g^{-1} (2nd stage). These differences can be attributed to management and cultivation conditions, genetic factors, flour processing and storage.[4]

The value obtained in the flour of the peel in treatment B (690.49 mg GAE 100 g^{-1}) is in the same range as that reported by Fatemeh *et al.*,[5] for the total phenolic content in green Cavendish banana peel flour (685.57 mg GAE 100 g^{-1}) in oven dryer with forced air at 50 °C. This study had a differential, the peel flour when using treatment A obtained the highest value (1686.47 mg GAE 100 g^{-1}).

The total phenolic content of this study for peel + pulp flours obtained with treatment A (943.35 mg GAE 100 g^{-1}) was higher than those found in 4 freeze-dried fruits (peel + pulp) by Rufino *et al.*,[15] expressed as (mg GAE 100 g^{-1}): 579 ± 12.9 for yellow mombin, 830 ± 26.5 for cashew, 830 ± 28.3 for carnauba, 742 ± 19 for umbu, and was similar to that found in mangaba (935 ± 37). The content obtained in the flours (peel + pulp) for treatment B (383.63 mg GAE 100 g^{-1}) and treatment C (264.23 mg GAE 100 g^{-1}) were lower than those of all fruits analyzed.

Phenolics profile by UPLC/UV-Vis

The effects of the three drying methods on the phenolic profile of the obtained flours are shown in Table 1. The results for rutin, caffeic acid and chlorogenic acid were statistically significant only for the treatment, with no significant difference between the flours. For chlorogenic acid, larger reductions were observed in flour using treatments A and B. A significant loss in caffeic acid was observed for treatment A and there was no significant difference for ellagic acid.

Rutin and catechin showed the same behavior for the three equipment, with greater degradation in the flour obtained in treatment C compared to B. Using treatment A, a higher value of gallic acid was obtained. It was also observed that the evaluated compounds were not detected in the pulp flours, except catechin and gallic acid, and catechin was identified only in the pulp obtained in treatment A. However, catechin and gallic acid obtained higher concentrations in the peel flours.

The variations observed for phenolic acids may be attributed to the different stability during drying and it is suggested that the high air velocity (treatment A) may have had a greater impact on caffeic acid as well as the air circulation also present in the treatment B on chlorogenic acid. Caffeic acid and chlorogenic acid appear to be more sensitive to degradation by factors other than time and temperature, such as light, irradiation, and air.[14,25] However, gallic acid was found in higher concentration in flours

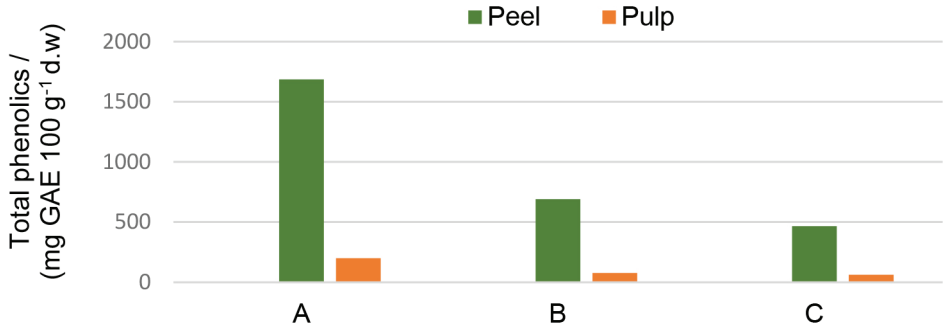

Figure 1. Total phenolic compounds of flours obtained by three drying methods. (A) air fryer at 180 °C; (B) oven dryer with forced air at 70 °C; (C) domestic oven at 180 °C.

Table 1. Phenolics profile of the obtained flours by UPLC/UV-Vis

Compound	Flour	Phenolic content / (mg 100 g^{-1} d.w)			
		Treatment			Mean
		A	B	C	
Gallic acid	peel	1211.74 ± 31.90	337.97 ± 19.82	330.35 ± 21.25	626.68^{a}
	pulp	168.74 ± 46.57	50.69 ± 1.86	29.56 ± 1.54	82.99^{b}
	mean	690.24^{a}	194.33^{b}	179.95^{b}	
Catechin	peel	6.52 ± 0.70	4.63 ± 0.63	2.56 ± 0.04	4.57^{a}
	pulp	0.76 ± 0.01	ND	ND	0.76^{b}
	mean	3.64^{ab}	4.63^{a}	2.56^{b}	
Ellagic acid	peel	0.22 ± 0.12	0.15 ± 0.01	0.19 ± 0.07	
	pulp	ND	ND	ND	
Rutin	peel	1.06 ± 0.20^{ab}	1.71 ± 0.28^{a}	0.66 ± 0.08^{b}	
	pulp	ND	ND	ND	
Caffeic acid	peel	0.03 ± 0.02^{a}	0.27 ± 0.05^{b}	0.19 ± 0.04^{b}	
	pulp	0.05 ± 0.02^{a}	ND	ND	
Chlorogenic acid	peel	0.12 ± 0.01^{a}	0.10 ± 0.01^{a}	0.20 ± 0.02^{b}	
	pulp	0.09 ± 0.03^{a}	ND	ND	
Quercetin	peel	ND	ND	ND	
	pulp	ND	ND	ND	

Equal lowercase letters in the columns are not significantly different by Tukey's test at 5% significance level. d.w: dry weight; A: air fryer at 180 °C; B: oven dryer with forced air at 70 °C; C: domestic oven at 180 °C; ND: not detected.

obtained with treatment A, and this phenolic acid seems to be more stable to drying conditions in this treatment.

The amount of gallic acid obtained in the flours of this study was higher than those obtained by Borges *et al.*,[26] in 9 different genomes of ripe banana pulp, ranging from 0.61 ± 0.06 to 10.2 ± 0.40 μg 100 g^{-1} using HPLC (high-performance liquid chromatography) analyses with UV-Visible detection. Anyasi *et al.*,[11] using HPLC with diode array detection, electrospray ionization and mass spectrometry (DAD-ESI-MS), did not detect gallic acid in the green banana pulp flours of 4 cultivars at maturity stage 2 (Luvhele; Mabonde; Muomva-red and Williams).

The gallic acid obtained in the flours of this study was also higher than that of green Cavendish banana pulp (maturity stage 2) flours obtained by oven drying, freeze-drying and extrusion analyzed by Pico *et al.*,[27] ranging from 0.008 to 0.669 mg 100 g^{-1} using UPLC-MS.

It is suggested that the identification and quantification of gallic acid are more associated with genetic and edaphoclimatic conditions.

PS-MS fingerprints

The fingerprints of peel and pulp flours obtained by three drying methods using PS-MS in negative and positive ionization mode with 26 tentatively identified compounds are shown in Table 2.

Phenolic acids

In the present study, 6 phenolic acids were characterized: *m/z* 325, 179, 353, 193, 169 and 301 in negative ionization mode, with one hexoside (*m/z* 325), proposed as coumaryl-hexoside, which resulted in the loss of a hexose portion, *m/z* 163.[28,29]

Flavonoids

The signal of *m/z* 447 was suggested as luteolin-7-glycoside and *m/z* 463 as diosmetin-8-*C*-glucoside or chrysoeriol-8-*C*-glucoside, according to the fragmentations reported in the literature.[31,34]

The ion *m/z* 289 corresponds to the flavan-3-ol catechin monomer, based in its fragments *m/z* 245 $[M - H - C_3H_8]^-$ and 217 $[M - H - C_3H_8 - CO]^-$ according to Wang *et al.*,[31] and the ion *m/z* 577 was designated as procyanidin B3 (condensed tannin).[29,32] Three flavonol derivatives were characterized as kaempferol-3-*O*-rutinoside (*m/z* 593),[35] syringetin-3-glucoside (*m/z* 653),[36] and myricetin-3-*O*-rhamnoside (*m/z* 465) based on the obtained

Table 2. Compounds determined by paper spray ionization mass spectrometry (PS(–/+)-MS) in extracts of peel and pulp flours obtained by three drying methods

Tentative identification	MS (*m/z*); ID	Molecular formula	MS^2 (*m/z*)	Reference
Hydroxycinnamic acids				
Coumaryl-hexoside	325; $[M - H]^-$	$C_{15}H_{18}O_8$	163	Abu-Reidah *et al.*[28] Aaby *et al.*[29]
Caffeic acid	179; $[M - H]^-$	$C_9H_8O_4$	135	Ncube *et al.*[30]
Chlorogenic acid	353; $[M - H]^-$	$C_{16}H_{18}O_9$	191, 179	Ncube *et al.*[30] Wang *et al.*[31]
Ferulic acid	193; $[M - H]^-$	$C_{10}H_{10}O_4$	178, 149, 134	Wang *et al.*[31]
Hydroxybenzoic acids				
Gallic acid	169; $[M - H]^-$	$C_7H_6O_5$	125	Zhang *et al.*[32]
Ellagic acid	301; $[M - H]^-$	$C_{14}H_60_8$	257, 229	Wyrepkowski *et al.*[33]
Flavones				
Luteolin-7-glycoside	447; $[M - H]^-$	$C_{21}H_{20}O_{11}$	284, 255, 227	Wang *et al.*[31]
Diosmetin-8-*C*-glucoside/chrysoeriol-8-*C*-glucoside	463; $[M + H]^+$	$C_{22}H_{22}O_{11}$	445, 343	Zheng *et al.*[34]
Flavanols				
Catechin	289; $[M - H]^-$	$C_{15}H_{14}O_6$	245, 217	Wang *et al.*[31]
Procyanidin B3	577; $[M - H]^-$	$C_{30}H_{26}O_{12}$	451, 425, 407, 289	Zhang *et al.*[32] Aaby *et al.*[29]
Flavonols				
Kaempferol-3-*O*-rutinoside	593; $[M - H]^-$	$C_{27}H_{30}O_{15}$	285	Tsamo *et al.*[35]
Syringetin-3-glucoside	653; $[M - H]^-$	$C_{29}H_{34}O_{17}$	345	Pérez-Navarro *et al.*[36]
Rutin	609; $[M - H]^-$	$C_{27}H_{30}O_{16}$	301	Tsamo *et al.*[35] Wang *et al.*[31]
Myricetin-3-*O*-rhamnoside	465; $[M + H]^+$	$C_{21}H_{20}O_{12}$	319	Abu-Reidah *et al.*[28]
Rhamnetin	317; $[M + H]^+$	$C_{16}H_{12}O_7$	300, 165, 154	Abu-Reidah *et al.*[28]
Others				
L-Arginine	175; $[M + H]^+$	$C_6H_{14}N_4O_2$	129	Silva *et al.*[16]
Umbelliferone	163; $[M + H]^+$	$C_9H_6O_3$	133, 117	Abu-Reidah *et al.*[28]
Sucrose	381; $[2Hex + K - H_2O]^+$	$C_{12}H_{22}O_{11}$	201, 219	Silva *et al.*[16]
β-Sitosterol	397; $[M + H - H_2O]^+$	$C_{29}H_{50}O$	243	Wang *et al.*[31]
Morroniside	429; $[M + Na]^+$	$C_{17}H_{26}O_{11}$	267	Zhao *et al.*[37]
Malic acid	133; $[M - H]^-$	$C_4H_6O_5$	115	Abu-Reidah *et al.*[28] Wang *et al.*[31]
Citric acid	191; $[M - H]^-$	$C_6H_8O_7$	173, 111, 87	Zhang *et al.*[32] Wang *et al.*[31]
Hexose	215; $[Hex + 2H_2O - H]^-$	$C_6H_{12}O_6$	71, 89, 179	Silva *et al.*[16]
Digalloylglucose	483; $[M - H]^-$	$C_{20}H_{20}O_{14}$	193, 169, 271, 423	Wyrepkowski *et al.*[33]
Trigalloylglucose	635; $[M - H]^-$	$C_{27}H_{24}O_{18}$	483, 465	Wyrepkowski *et al.*[33] Abu-Reidah *et al.*[28]
Tetragalloylglucose	787; $[M - H]^-$	$C_{34}H_{28}O_{22}$	635	Wyrepkowski *et al.*[33] Abu-Reidah *et al.*[28]

fragments.[28] The ions *m/z* 609 and 317 were suggested as flavonols: rutin and rhamnetin, respectively.[28,31,35]

Other compounds

Signals of *m/z* 191 and 133 were suggested as citric acid and malic acid, respectively, based on the ions obtained and described in the literature.[28,31,32] According to the fragmentation profile, the signals of *m/z* 215, 381 and 175 were recognized as hexose, sucrose and the amino acid L-arginine, respectively.[16]

The ions of *m/z* 483, 635 and 787 represent a homologous series of galloylglucose and were identified as digalloylglucose (*m/z* 483), trigalloylglucose (*m/z* 635) and tetragalloylglucose (*m/z* 787) in negative ionization mode.[28,33] When the glucose number is esterified with five

or less galloyl groups, the resulting compounds are defined as precursors of gallotannin.[38]

A phytosterol was proposed as β-sitosterol (*m/z* 397) based on the fragmentation obtained *m/z* 243 $[C_{18}H_{27}]^+$ as reported by Wang *et al*.[31]

Iridoid and coumarin derivatives were proposed in this study and characterized as morroniside *m/z* 429 and umbelliferone *m/z* 163, respectively, based on the fragmentation pattern obtained in comparison with data in the literature.[28,37] Morroniside and umbelliferone have been associated with anti-inflammatory, antioxidant and antimicrobial effects. Morroniside is a more abundant iridoid glycoside in *Cornus officinalis*, whereas umbelliferone belongs to coumarins and is found in many plants, more predominantly in the Rutaceae, Apiaceae and Asteraceae families.[39,40] The identification of the compounds using PS-MS by type of flour, peel or pulp, in each treatment, is shown in Table 3.

The quercetin not determined in this study by UPLC/UV-Vis and PS-MS may be associated with

Table 3. Compounds identified by paper spray ionization mass spectrometry (PS(−/+)-MS) in extracts of peel and pulp flours obtained by three drying methods

Compound	[+][−]	A		B		C	
		Peel	Pulp	Peel	Pulp	Peel	Pulp
Hydroxycinnamic acids							
Coumaryl-hexoside	−	×	×	×	×	×	×
Caffeic acid	−	×	×	×	×	×	×
Chlorogenic acid	−	×	×	×	×	×	×
Ferulic acid	−	×	×	×	×	×	×
Hydroxybenzoic acids							
Gallic acid	−	×	×	×	×	×	×
Ellagic acid	−	×	×	×	×	×	×
Flavones							
Diosmetin/chrysoeriol-8-*C*-glucoside	+	×					
Luteolin-7-glycoside	−	×	×				
Flavanols							
Catechin	−	×	×	×	×	×	×
Procyanidin B3	−	×	×	×	×		
Flavonols							
Kaempferol-3-*O*-rutinoside	−	×					
Syringetin-3-glusoside	−	×	×	×	×	×	×
Rutin	−	×		×		×	
Rhamnetin	+	×	×	×	×	×	×
Myricetin-3-*O*-rhamnoside	+	×	×				
Others							
Malic acid	−	×	×	×	×	×	×
Citric acid	−	×	×	×	×	×	×
Hexose	−	×	×	×	×	×	×
Digalloylglucose	−	×	×	×	×	×	
Trigalloylglucose	−	×	×	×	×	×	
Tetragalloylglucose	−	×	×	×		×	
L-Arginine	+		×		×	×	×
Umbelliferone	+	×					
Sucrose	+	×	×	×	×	×	×
β-Sitosterol	+	×					
Morroniside	+	×	×	×	×	×	×

A: air fryer at 180 °C; B: oven dryer with forced air at 70 °C; C: domestic oven at 180 °C.

the maturity stage of the banana used, since higher concentrations were observed in ripe bananas.[3]

Morais *et al*.[12] also found malic acid in pulp and peel of ripe banana using UPLC-ESI-MS, cultivar not mentioned, and citric acid only in the pulp. In this work citric acid was also identified in green Cavendish banana peels.

The compounds diosmetin-8-*C*-glucoside/chrysoeriol-8-*C*-glucoside, kaempferol-3-*O*-rutinoside, rutin, umbelliferone and β-sitosterol were identified only in peel flours. Oliveira *et al*.[41] observed that β-sitosterol was more prevalent in peel than in pulp of Dwarf Cavendish green banana. Tsamo *et al*.[35] also identified kaempferol and rutin only in the peel of freeze-dried ripe banana, cv. Red Yade, using HPLC-DAD-ESI-MS and observed ferulic acid, caffeic acid and myricetin also in peels and pulps. The results for rutin in this study corroborate those found by Kanazawa and Sakakibara,[42] who did not identify rutin in pulps of green Cavendish banana at maturity stage 1. However, the compounds umbelliferone, β-sitosterol, myricetin-3-*O*-rhamnoside, kaempferol-3-*O*-rutinoside, diosmetin-8-*C*-glucoside/chrysoeriol-8-*C*-glucoside and luteolin-7-glycoside were detected only in flour obtained by drying in the air fryer, and these compounds may have been less degraded in this treatment. In addition, procyanidin B3 was not detected in the domestic oven, which may have caused greater degradation.

Among the phenolics identified, as well as other compounds, all have already been reported[2,4,12,18,26,35] in banana peel and/or pulp in different cultivars and degrees of maturity, except one flavone glycoside diosmetin/chrysoeriol and umbelliferone, which are found for the first time in banana.

The following compounds are reported in different green banana cultivars: kaempferol, myricetin, quercetin, epicatechin, catechin, luteoline, apigenin, gallic acid, caffeic acid, in pulps, ferulic acid, coumaric, and naringin in peels, β-sitosterol and rutin in peel and pulp, as well as the presence of tannins, sugars and organic acids.[2-4,11,27,41,42] However, few studies have simultaneously evaluated the bioactive constituents in green banana peel and pulp, with only two studies[41,42] identifying the compounds rutin and naringin in peel and β-sitosterol in green Cavendish banana peel and pulp. Thus, the other compounds identified by PS-MS and UPLC/UV-Vis in this study were characterized for the first time in green Cavendish banana peel and pulp flours.

Antioxidant capacity

The antioxidant capacity of flours obtained by three different drying methods is shown in Table 4.

The antioxidant capacity by DPPH radical scavenging showed significant results for both factors individually: flour (peel/pulp) and treatment. Higher antioxidant capacity was obtained in the flours of the peels, compared to the pulps, and there was higher antioxidant capacity in flours obtained with treatment A, followed by B and C. The ABTS assay, on the other hand, showed significant results for both factors combined. Peel flour obtained using treatment A had a higher antioxidant capacity compared to the other flours, and there was no statistically significant difference between pulp flour obtained with treatment A and peel flour obtained with treatment B and between pulp flour obtained with treatment B and the flours obtained with treatment C. When the flours of the peel and pulp are evaluated considering the same treatment, higher antioxidant capacity is observed in the peel.

Although the total phenolic concentrations in the flour using different treatments were obtained in this order: A > B > C, the type of phenolic compound and the presence of other non-phenolic antioxidants, as observed

Table 4. Antioxidant capacity of obtained flours

Treatment		DPPH / (μM Trolox 100 g^{-1} d.w)	Mean / (μM Trolox 100 g^{-1} d.w)	ABTS / (μM Trolox 100 g^{-1} d.w)
A	peel	12597.83 ± 690.13	6745.88^{a}	3655.05 ± 203.51^{d}
	pulp	893.93 ± 153.90		1565.45 ± 307.74^{a}
B	peel	6661.92 ± 961.78	3642.67^{b}	2084.09 ± 205.67^{a}
	pulp	623.41 ± 68.52		347.11 ± 18.80^{bc}
C	peel	4182.05 ± 279.93	2251.96^{c}	890.03 ± 146.55^{c}
	pulp	321.87 ± 17.79		268.01 ± 13.29^{b}
Mean	peel	7813.93^{a}		
	pulp	613.07^{b}		

Equal lowercase letters in the columns are not significantly different by Tukey's test at 5% significance level. DPPH: 2,2-diphenyl-1-picrylhydrazil; d.w: dry weight; ABTS: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); A: air fryer at 180 °C; B: oven dryer with forced air at 70 °C; C: domestic oven at 180 °C.

by PS-MS, as well as the observed differences of the compounds quantified by UPLC/UV-Vis between the treatments, besides other unquantified compounds, may have contributed to variations in the ABTS method.[11-15]

Fatemeh *et al*.[5] also observed higher antioxidant capacity in the peel flour compared to the pulp flour of green Cavendish banana, expressed as percentage of DPPH radical scavenging, being 52.66% for the peel and 35.21% for the pulp.

The ABTS antioxidant capacity for pulp flour obtained with treatment B (347.11 μM Trolox 100 g^{-1}) is close to that reported by Guiné *et al*.,[13] for the Cavendish banana pulp flour produced at 70 °C in a similar time (300 min) (380 μM Trolox 100 g^{-1}); however, the banana ripening stage was not reported in this study. Compared to the treatment B, the pulp obtained with treatment C had lower antioxidant capacity (268.01 μM Trolox 100 g^{-1}) and the pulp obtained with treatment A showed higher value (1565.45 μM Trolox 100 g^{-1}).

The obtained ABTS antioxidant capacity values in the peel flours of the treatments A (3655.05 μM Trolox 100 g^{-1}) and B (2084.09 μM Trolox 100 g^{-1}), and the pulp flour obtained with treatment A (1565.45 μM Trolox 100 g^{-1}) were higher than those reported by Silva *et al*.[16] in cagaitas from Paraopeba (934 μM Trolox 100 g^{-1}).

Correlation between antioxidant capacity, total phenolics and profile of phenolic compounds

The contribution of total phenolic compounds to the antioxidant activity of the obtained flours was significant and is strongly and positively correlated for both assays (DPPH: correlation coefficient (r^2) = 0.985; $p < 0.05$ and ABTS: $r^2 = 0.944$; $p < 0.05$). A high positive and significant correlation was also observed between two phenolic compounds (catechin and gallic acid) and antioxidant capacity by DPPH and ABTS, with r^2 values ranging from 0.799 to 0.962 ($p < 0.05$). In addition, there were strong and positive correlations between the methods, indicating that the antioxidant assays are consistent with each other ($r^2 = 0.933$; $p < 0.05$).

Sarawong *et al*.[43] obtained a similar correlation between ABTS and total phenolics ($r^2 = 0.916$, $p < 0.01$) for green banana flour. However, the cultivar, maturity stage and flour composition (with or without peel) were not mentioned.

Anyasi *et al*.[11] obtained lower correlations (r^2) between DPPH and total phenolics in the pulp flours of 3 cultivars (Luvhele; Mabonde; Muomva-red) of green banana at maturity stage 2, which varied from 0.352 to 0.898 ($p < 0.01$), indicating that total phenolic compounds were not the only contributors to antioxidant activity.

The correlation between these two variables is widely studied; however, there are other compounds present in foods that contribute to this functionality, such as vitamins, carotenoids, biogenic amines and synthesized products of the Maillard reaction, which are also influenced by different varieties, maturation, cultivation and processing conditions.[6,11,14,43]

Gallic acid was reported by Dludla *et al*.[44] as a potent antioxidant and an efficient cancer cell apoptosis-inducing agent, so studies on the mechanism of action of gallic acid have received much attention recently. It is widely distributed in vegetables and fruits, however, some foods with greater occurrence have been highlighted, such as: avocado: 198.57 mg 100 g^{-1}; grape pomace extract: 86.70 mg 100 g^{-1}; guava with peel: 681.12 mg 100 g^{-1}; Ceylon cinnamon: 214 mg 100 g^{-1}; *Camellia sinensis* tea: 74 to 547 mg 100 g^{-1}; mulberry leaves: 2262 mg 100 g^{-1}; jaboticaba peel 49.86 mg 100 g^{-1}; grapefruit pulp: 34.37 mg 100 g^{-1} and pomegranate peel: 891.70 mg 100 g^{-1}.

Thus, the gallic acid found in the peel and pulp flours of green Cavendish banana cv. Nanicão in this study, with concentrations ranging from 29.56 ± 1.54 to 1211.74 ± 31.90 mg 100 g^{-1}, could fulfill this role.

Despite the small space for the food in the air fryer treatment, the dried peels and pulps yielded approximately 10 (peel) and 40 g (pulp) of flour, which can be used in preparations such as cakes, cookies, breads and pasta in general,[7] or for therapeutic purposes, since Sardá *et al*.,[45] when offering 8 g of pulp flour of green banana, *Musa acuminata* (AAA), Cavendish group (cv. Nanicão), at maturity stage 1, in a soup vehicle, observed that non-daily consumption of flour led to lower release of ghrelin and higher release of YY peptide, consequently greater satiety and reduced energy consumption, besides lower insulin levels after flour consumption.

Conclusions

The green Cavendish banana cv. Nanicão has been shown to be a potential source of bioactive constituents with high antioxidant activity, especially in its peel.

The PS-MS analysis proved to be a simple technique to obtain fingerprint in green Cavendish banana pulp and peel flours, identifying several phenolic compounds, organic acids, sugars, amino acid, phytosterol, iridoid and coumarin derivatives.

The present study showed that the antioxidant activity and the amount and profile of phenolics were affected by the different drying methods employed and fruit structure (peel and pulp). Flours obtained using the air fryer treatment had greater loss of caffeic acid; however, they obtained a

higher amount of total phenolics and higher antioxidant capacity by the DPPH method (peel and pulp), and by the ABTS method (peel flour). Therefore, air fryer at 180 °C can be used as an alternative method of drying. The use of oven dryer with forced air at 70 °C for the production of green Cavendish banana flour can also be advantageous in terms of phenolic compounds and antioxidant activity.

Supplementary Information

Supplementary information (spectral type PS-MS) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Anna Cláudia F. e Loyola was responsible for the formal analysis, investigation, methodology, project administration and writing original draft; Viviane D. M. Silva for the formal analysis and methodology; Mauro R. Silva for the methodology, software and supervision; Camila G. Rodrigues for the methodology; Amanda N. dos Santos for the methodology; Júlio O. F. Melo for the writing review and editing; Rodinei Augusti for the supervision; Camila A. Fante for the writing review and editing, supervision and project administration.

References

1. Falcomer, A. L.; Riquette, R. F. R.; Lima, B. R.; Ginani, V. C.; Zandonadi, R. P.; *Nutrients* **2019**, *11*, 1222.
2. Singh, B.; Singh, J. P.; Kaur, A.; Singh, N.; *Food Chem.* **2016**, *206*, 1.
3. Siriamornpun, S.; Kaewseejan, N.; *Sci. Hortic.* **2017**, *221*, 33.
4. Vu, H. T.; Scarlett, C. J.; Vuong, Q. V.; *J. Funct. Foods* **2018**, *40*, 238.
5. Fatemeh, R. S.; Saifullah, R.; Abbas, F. M.; Azhar, M. E.; *Int. Food Res. J.* **2012**, *19*, 1041.
6. Balasundram, N.; Sundram, K.; Samman, S.; *Food Chem.* **2006**, *99*, 191.
7. Zheng, Z.; Stanley, R. J.; Gidley, M.; Dhital, S.; *Food Funct.* **2016**, *7*, 771.
8. Campuzano, A.; Rosell, C. M.; Cornejo, F.; *Food Chem.* **2018**, *256*, 11.
9. Gálvez, A. V.; Scala, K. D.; Rodríguez, K.; Mondaca, R. L.; Miranda, M.; López, J.; Won, M. P.; *Food Chem.* **2009**, *117*, 647.
10. Garau, M. C.; Simal, S.; Rosselló, C.; Femenia, A.; *Food Chem.* **2007**, *104*, 1014.
11. Anyasi, T. A.; Jideani, A. I. O.; Machau, G. A.; *Food Res. Int.* **2018**, *104*, 100.
12. Morais, D. R.; Motta, E. M.; Sargi, S. C.; Schmidt, E. M.; Bonafe, E. G.; Eberlin, M. N.; Sawaya, A. C. H. F.; Visentainer, J. V.; *Food Res. Int.* **2015**, *77*, 392.
13. Guiné, R. P.; Barroca, M. J.; Gonçalves, F. L.; Alves, M.; Oliveira, S.; Mendes, M.; *Food Chem.* **2015**, *168*, 454.
14. Tian, J.; Chen, J.; Lv, F.; Chen, S.; Chen, J.; Liu, D.; Ye, X.; *Food Chem*. **2016**, *197*, 1264.
15. Rufino, M. S. M.; Alves, R. E.; Brito, E. S.; Pérez-Jiménez, J.; Saura-Calixto, F.; Filho, J. M.; *Food Chem.* **2010**, *121*, 996.
16. Silva, M. R.; Freitas, L. G.; Souza, A. G.; Araújo, R. L. B.; Lacerda, I. C. A.; Pereira, H. V.; Augusti, R.; Melo, J. O. F.; *J. Braz. Chem. Soc.* **2019**, *30*, 1034.
17. Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z.; *Angew. Chem., Int. Ed. Engl.* **2010**, *49*, 877.
18. Silva, V. D. M.; Arquelau, P. B. F.; Silva, M. R.; Augusti, R.; Melo, J. O. F.; Fante, C. A.; *Quim. Nova* **2020**, *43*, 579.
19. Campelo, F. A.; Henriques, G. S.; Simeone, M. L. F.; Queiroz, V. A. V.; Silva, M. R.; Augusti, R.; Melo, J. O. F.; Lacerda, I. C. A.; Araujo, R. L. B.; *J. Braz. Chem. Soc*. **2020**, *31*, 788.
20. Chisté, R. C.; Freitas, M.; Mercadante, A. Z.; Fernandes, E.; *Food Chem.* **2012**, *135*, 1740.
21. Singleton, V. L.; Orthofer, R.; Lamuela-Ravento, S. E. M.; *Methods Enzymol.* **1999**, *299*, 152.
22. Plank, D. W.; Szpylka, J.; Sapirstein, H.; Wolllard, D.; Zapf, C. M.; Lee, V.; Chen, C. Y. O.; Liu, R. H.; Tsao, R.; Dusterloh, A.; Baugh, S.; *J. AOAC Int.* **2012**, *95*, 1562.
23. https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPAT/10225/1/Cot_128.pdf, accessed in December 2020.
24. RStudio Team; *RStudio: Integrated Development for R*; RStudio, Boston, MA, USA, 2015.
25. Xue, M.; Shi, H.; Zhang, J.; Liu, Q. Q.; Guan, J.; Zhang, J. Y.; Ma, Q.; *Molecules* **2016**, *21*, 948.
26. Borges, C. V.; Amorim, V. B. O.; Ramlov, F.; Ledo, C. A. S.; Donato, M.; Maraschin, M.; Amorim, E. P.; *Food Chem.* **2014**, *145*, 496.
27. Pico, J.; Xu, K.; Guo, M.; Mohamedshah, Z.; Ferruzzi, M. G.; Martinez, M. M.; *Food Chem.* **2019**, *297*, 124.
28. Abu-Reidah, I. M.; Ali-Shtayeh, M. S.; Jamous, R. A.; Arráez-Román, D.; Segura-Carretero, A.; *Food Chem.* **2015**, *166*, 179.
29. Aaby, K.; Ekeberg, D.; Skrede, G.; *J. Agric. Food Chem.* **2007**, *55*, 4395.
30. Ncube, E. N.; Mhlongo, M.; Piater, L. A.; Steenkamp, P. A.; Dubery, I. A.; Madala, N. E.; *Chem. Cent. J.* **2014**, *8*, 66.
31. Wang, J.; Jia, Z.; Zhang, Z.; Wang, Y.; Liu, X.; Wang, L.; Lin, R.; *Molecules* **2017**, *22*, 476.
32. Zhang, L.; Zhu, M. T.; Shi, T.; Guo, C.; Huang, Y. S.; Chen, Y.; Xie, M. Y.; *Food Funct.* **2017**, *8*, 341.

33. Wyrepkowski, C. C.; Costa, D. L. M. G.; Sinhorin, A. P.; Vilegas, W.; Grandis, R. A.; Resende, F. A.; Varanda, E. A.; Santos, L. C.; *Molecules* **2014**, *19*, 16039.
34. Zheng, G.-D.; Zhou, P.; Yang, H.; Li, Y.-s.; Li, P.; Liu, E.-H.; *Food Chem.* **2013**, *136*, 604.
35. Tsamo, C. V. P.; Herent, M. F.; Tomekpe, K.; Emaga, T. H.; Leclercq, J. Q.; Rogez, H.; Larondelle, Y.; Andre, C.; *Food Chem.* **2015**, *167*, 197.
36. Pérez Navarro, J.; Canas Izquierdo, P. M.; Mena Morales, A.; Martínez Gascuena, J.; Chacón Vozmediano, L.; García Romero, E.; Hermosín Gutiérrez, I.; Gómez Alonso, S.; *Food Chem.* **2019**, *295*, 350.
37. Zhao, M.; Du, L.; Tao, J.; Qian, D.; Guo, J.; Jiang, S.; Shang, E. X.; Duan, J. A.; Wu, C.; *J. Chromatogr. B* **2015**, *976*, 61.
38. Salminen, J. P.; Ossipov, V.; Loponen, J.; Haukioja, E.; Pihlaja, K.; *J. Chromatogr. A* **1999**, *864*, 283.
39. Xu, M.; Wu, H.-Y.; Liu, H.; Gong, N.; Wang, Y.-R.; Wang, Y.-X.; *Br. J. Pharmacol.* **2017**, *174*, 580.
40. Mazimba, O.; *Bull. Fac. Pharm. (Cairo Univ.)* **2017**, *55*, 223.
41. Oliveira, L.; Freire, C. S. R.; Silvestre, A. J. D.; Cordeiro, N.; *J. Agric. Food Chem.* **2008**, *56*, 9520.
42. Kanazawa, K.; Sakakibara, H.; *J. Agric. Food Chem.* **2000**, *48*, 844.
43. Sarawong, C.; Schoenlechner, R.; Sekiguchi, K.; Berghofer, E.; Ng, P. K.; *Food Chem.* **2014**, *143*, 33.
44. Dludla, P. V.; Nkambule, B. B.; Jack, B.; Mkandla, Z.; Mutize, T.; Silvestri, S.; Orlando, P.; Tiano, L.; Louw, J.; Mbeje, S. E. M.; *Nutrients* **2019**, *11*, 23.
45. Sardá, F. A. H.; Giuntini, E. B.; Gomez, M. L. P. A.; Lui, M. C. Y.; Negrini, J. A. E.; Tadini, C. C.; Lajolo, F. M.; Menezes, E. W.; *J. Funct. Foods* **2016**, *24*, 63.

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