













Chemical characterization of *Musa acuminata* AAB Peels (plantain, Blue Java)

Caracterización química de las cáscaras de *Musa acuminata* AAB (plátano, Blue Java)

Caracterização química das cascas de *Musa acuminata* AAB (plátano, Blue Java)

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Abstract

The peels of *Musa acuminata* constitute a by-product of interest due to their richness in metabolites that influence important biological activities. The objective of the study was to evaluate the chemical composition of the peels (maturation stage 1) of *Musa acuminata* AAB (Blue Java), through the application of chemical and physicochemical methods, for their utilization. A comparative study was carried out with a single experimental factor and two independent samples: treatment without antioxidants (ST) and treatment with antioxidants (T). The proximate analysis performed demonstrated the presence of fiber, proteins, and minerals; moreover, it was verified that the application of antioxidants does not alter the nutritional profile. The absence of heavy metals was confirmed, ensuring the safety of the samples. For the identification and structural characterization of specialized metabolites, an HPLC-MS-UV study was carried out, which allowed the identification of 9 flavonoid glycosides in the acetone extracts of the peels. A higher concentration of phenols and tannins (20.21 mg GAE.g⁻¹; 8.36 mg GAE.g⁻¹) was quantified in the samples treated with antioxidants (T) compared to the untreated samples (ST) (6.46 mg GAE.g⁻¹; 1.71 mg GAE.g⁻¹), demonstrating that the application of antioxidants inhibits oxidative degradation and preserves these metabolites. These findings show that the immature peels of *Musa acuminata* AAB of the Blue Java variety constitute a viable and safe source of phytonutrients and phenols. Additionally, the effectiveness of a treatment with antioxidants was confirmed for preserving these metabolites without compromising their nutritional composition.

Resumen

Las cáscaras de *Musa acuminata* constituyen un subproducto de interés por su riqueza de metabolitos que condicionan actividades biológicas importantes. El objetivo del estudio fue evaluar la composición química de las cáscaras (grado de maduración 1) de *Musa acuminata* AAB (Blue Java), mediante la aplicación de métodos químicos y fisicoquímicos, para su aprovechamiento. Se implementó un estudio comparativo con un único factor experimental y dos muestras independientes: tratamiento sin antioxidantes (ST) y tratamiento con antioxidantes (T). El análisis proximal realizado demostró la presencia de fibra, proteínas y minerales; además, se comprobó que la aplicación de antioxidantes no altera el perfil nutricional. Se verificó la ausencia de metales pesados garantizando la inocuidad de las muestras. Para la identificación y caracterización estructural de metabolitos especializados se realizó un estudio HPLC-MS-UV, el cual permitió identificar nueve glicósidos de flavonoides en los extractos acetónicos de las cáscaras. Se cuantificó una mayor concentración de fenoles y de taninos (20,21 mg EAG.g⁻¹; 8,36 mg EAG.g⁻¹) en las muestras tratadas con antioxidantes (T) en contraste con las muestras no tratadas (ST) (6,46 mg EAG.g⁻¹; 1,71 mg EAG.g⁻¹), demostrando que la aplicación de antioxidantes inhibe la degradación oxidativa y preserva estos metabolitos. Estos hallazgos evidencian que las cáscaras inmaduras de *Musa acuminata* AAB de la variedad Blue Java constituyen una fuente viable y segura de fitonutrientes y de fenoles, además, se constató la eficacia de un tratamiento con antioxidantes para la preservación de estos metabolitos sin comprometer su composición nutricional.

Palabras clave: *Musa acuminata*, fitoquímica, análisis proximal, fenoles, taninos.

Resumo

As cascas de *Musa acuminata* constituem um subproduto de interesse devido à sua riqueza em metabólitos que influenciam atividades biológicas importantes. O objetivo do estudo foi avaliar a composição química das cascas (grau de maturação 1) de *Musa acuminata* AAB (Blue Java), por meio da aplicação de métodos químicos e físico-químicos, para seu aproveitamento. Foi realizado um estudo comparativo com um único fator experimental e duas amostras independentes: tratamento sem antioxidantes (ST) e tratamento com antioxidantes (T). A análise proximal realizada demonstrou a presença de fibras, proteínas e minerais; além disso, verificou-se que a aplicação de antioxidantes não altera o perfil nutricional. Foi confirmada a ausência de metais pesados, garantindo a segurança das amostras. Para a identificação e caracterização estrutural de metabólitos especializados, foi realizado um estudo HPLC-MS-UV, o qual permitiu identificar: 9 glicosídeos de flavonoides nos extratos acetônicos das cascas. Foi quantificada uma maior concentração de fenóis e taninos (20,21 mg EAG.g⁻¹; 8,36 mg EAG.g⁻¹) nas amostras tratadas com antioxidantes (T) em contraste com as amostras não tratadas (ST) (6,46 mg EAG.g⁻¹; 1,71 mg EAG.g⁻¹), demonstrando que a aplicação de antioxidantes inibe a degradação oxidativa e preserva esses metabólitos. Esses achados evidenciam que as cascas imaturas de *Musa acuminata* AAB da variedade Blue Java constituem uma fonte viável e segura de fitonutrientes e fenóis. Além disso, constatou-se a eficácia de um pré-tratamento com antioxidantes para a preservação desses metabólitos sem comprometer sua composição nutricional.

Palavras-chave: *Musa acuminata*, fitoquímica, análise proximal, fenóis, taninos.

Introduction

Plantain production is one of the most important agro-productive activities in the province of El Oro, playing a strategic role both in the regional economy and national food security. According to data from El Instituto Nacional de Estadística y Censos (2024), around 8,968 tons of plantain (*Musa* AAB) were produced in this province. However, this production system generates a significant amount of waste during primary processing and consumption, considering that the peel represents between 40 % of the fruit's weight. This residual biomass is often discarded without proper management, which generates negative impacts on the environment and the loss of potentially useful resources (Álvarez Morales *et al.*, 2020).

It has been reported that *Musa acuminata* peels have important chemical constituents such as fibers, proteins, essential amino acids, fatty acids, minerals, among others; this nutritional profile has allowed for their incorporation as additives in the development of food formulations (Pilco *et al.*, 2018). Additionally, a variety of chemical compounds, called secondary metabolites, have been determined. Among the metabolites identified, phenolic compounds stand out, known for their health benefits associated with their antioxidant capacity. Research shows that immature peels have a higher phenolic content compared to mature ones; This behavior is attributed to metabolic changes during maturation that condition the oxidation of this metabolite (Espinosa and Santacruz, 2019; J. Zhang *et al.*, 2022)

Studies carried out on *Musa acuminata* peel extracts show that the concentration of phenolic compounds is significantly related to bioactive functions such as antioxidant, anti-inflammatory, anticancer, antihypertensive, antimicrobial, lipid-lowering, hepatoprotective, and gastroprotective properties of high interest in the area of pharmacognosy (Anupama, 2021; Chauhan *et al.*, 2025)

As far as is known, there are no analytical studies on the chemical profile of the immature *Musa acuminata* AAB peels of the Blue Java variety; this constitutes a scientific gap that limits opportunities for the valorization of this byproduct. The Blue Java variety is planted in a cultivation area of the Faculty of Agricultural Sciences, intended to supply the cafeterias at UTMACH. As a product of the consumption chain of this variety, waste corresponding to its peels is generated, which is discarded without proper management.

In this context, the objective of this study was to determine the chemical composition of *Musa acuminata* AAB (Blue Java) peels, using chemical and physicochemical methods, generating relevant information that contributes to the valorization and potential use of this by-product at the local level.

Materials and methods

Study area

The plantains analyzed came from a cultivation area of the Faculty of Agricultural Sciences of the Technical University of Machala, located in the province of El Oro, Ecuador (3°11'36" south latitude, 79°91'38" west latitude at 12 m.a.s.l.). The area has a tropical climate with an average temperature of 25°C, 500 mm of annual precipitation, and 2 to 3 hours of sunshine duration per day. The plantation supplies the institutional cafeterias, in which waste corresponding to the peels of this variety is generated.

Selection of plant material

A bunch of *Musa acuminata* Blue Java variety with eight hands and 14 fingers each was collected, selected for its low degree of Sigatoka and health. The harvest was carried out one year from the time of planting, at 13 weeks from flowering, and with a sprinkler irrigation system. Immediately after the fruits were peeled, the peels were transferred to the laboratory, where those that were without signs of deterioration were selected, and their weight was verified using a technical balance (OHAUS brand, B6US, United States).

Sample treatment

Subsequently, the peels were divided into two equal parts to apply two treatments that are detailed in Table 1:

Table 1. Description of treatments.

Treatment	Description
Peels without antioxidant application (ST)	Washing and disinfection by immersion in Star-Bac solution (3 caps in 9 L of water) for 5 min.
Peels with antioxidant mixture application (T)	Washing and disinfection with Star-Bac under the same conditions + immersion for 10 min in an antioxidant mixture (citric acid 1.5 %; ascorbic acid 1 %; tartaric acid 0.5 % m/v) in 5 L of water.

The selection of antioxidants and their concentrations was based on the procedure described by Apintanapong *et al.* (2007) with modifications, which indicates that the combination of these organic acids generates a synergistic activity that favors the inhibition of oxidative processes and conservation of metabolites in *Musa* peels. Subsequently, each fraction was chopped and dried for 24 h at 40 °C in an oven (MEMMERT 30 – 1060, Germany) with a flap and 100 % air ventilation. An electric grinder (NM-8300, China) was used for grinding. A No. 20 sieve was employed to obtain a particle size of 850 µm. Finally, the samples were packed in airtight plastic bags and stored in a desiccator at a temperature of 24 °C.

Degree of maturity

It was carried out by determining the pH and Brix degrees, followed by a correlation with the scale proposed by Von Loesecke (Arrieta *et al.*, 2006). The procedure detailed by Ramirez Céspedes *et al.* (2010) was followed. A pH meter (Bante 900 P, China) and a refractometer (HANNA HI 96801, Italy) were used.

Proximate analysis

Moisture

The thermogravimetric method was employed, a balance with a halogen heat source (OHAUS MB90, China) was used, temperature was maintained at 105 °C up to constant weight was reached. The determination was made in triplicate for each sample.

Fats

The method described by Pilco *et al.* (2018) adapted from the AOAC 991.36 standard (AOAC, 1996) was applied, and it was developed in a Soxhlet equipment using hexane (Fisher Chemical, United States) as the solvent. The extraction took 4 hours. A gravimetric analysis was performed after concentration in a rotary evaporator (Heidolph - Laborata 2001, Germany). The determination was made in triplicate for each sample.

Proteins

The micro-Kjeldahl method was applied according to the AOAC 955.04D standard (AOAC, 1990) proteins were estimated from total nitrogen.

Fiber

The PEEL/LA/16 INEN 522 gravimetric method was applied (NTE INEN, 2013a).

Total ash

The method described by Enriquez Estrella and Ojeda Caiza (2020) adapted to the AOAC 942.05 standard was applied. Incineration was performed at 750 °C for 4 h in a muffle furnace (Nabertherm, L-180, Germany). Three replicates were made for each sample.

Heavy metals

For the determination of arsenic, the Modified Gutzeit method was employed (NTE INEN, 2013b), and for lead the Modified Standard Methods 3111B (NTE INEN, 1984).

Minerals

The technique of wet digestion with nitric and perchloric acid was used to remove organic matter according to the AOAC 985 standards (AOAC, 1988). The minerals Zn, Cu, Fe, K, Ca, Mg, Mn, and Na were determined by atomic absorption spectrophotometry (ThermoScientific SOLAAR, Germany), and the phosphorus content was evaluated by UV spectroscopy at 820 nm.

Secondary metabolites

Preparation of extracts

Sample extraction was performed using ultrasonic extraction, employing an acetone:water mixture (8:2 % v/v) as the extraction solvent at a 5 % sample-to-solvent ratio for 30 min, at room temperature, in an ultrasonic bath (Fisher Scientific, 40 KHz, United States). Once the extraction time was completed, the extracts were filtered and concentrated to dryness in a rotary evaporator. Subsequently, they were defatted through solid-phase extraction (C18 reverse-phase column with 85 % methanol (v/v) as the mobile phase). The resulting solution was concentrated to dryness in a rotary evaporator, and a 10 mg.mL⁻¹ solution (methanol:water, 80:20 v/v) was prepared with the solid residue.

Identification of secondary metabolites by HPLC-MS-UV

The identification of the secondary metabolites was carried out in a high-performance chromatographic system (Thermo Scientific, UltiMate 3000, United States), coupled to a mass spectrometer (Thermo Scientific, LTQ XL, United States), equipped with a diode array detector (DAD) and a quaternary pump (Dionex, UltiMate 300 RS). A C18 Accucore RP-MS reversed-phase column (100 x 2.1 mm; 2.6 µm) was employed; at 35 °C, flow rate of 0.4 mL.min⁻¹ and injection volume of 2 µL. The mobile phase corresponded to an acetonitrile gradient: 0.1 % formic acid (A) (0-3 min 97 % A, 3-10 min 97-40 % A, 10-11 min 40-5 % A). The diode array detector was configured to operate at wavelengths of 214 nm, 250 nm, 280 nm, and 330 nm. The mass spectrometer was set to a capillary voltage of -50.00 V, a spray voltage of 5.00 kV, and a capillary temperature of 225 °C. Nitrogen was used as auxiliary and purge gas. The samples were evaluated in negative ion mode, in Full Scan with ranges of 100 – 1000 Da and in Scan dependent mode to obtain MS² spectra.

Quantification of secondary metabolites

Total phenols

The Folin-Ciocalteu method (Singleton *et al.*, 1999) was employed, using acetone extracts from the samples treated and not treated with antioxidants; each sample was analyzed in triplicate. Absorbance measurements were made at a wavelength of 765 nm, using a UV spectrophotometer (Evolution 201, Thermo Scientific, United States). A calibration curve was developed from gallic acid (Fluka Analytical) for quantification. Results were expressed as total gallic acid equivalent (GAE) per g sample.

Total tannins

The procedure of (Mex-Álvarez *et al.*, 2022) was performed. The GAE was determined using the standard curve described above for the quantification of total phenols.

Experimental design and statistical analysis

A comparative study was established with a single experimental factor (application of treatments) and two independent samples (treatments: ST and T). The following studies were performed in each independent sample: proximate analysis, heavy metals, HPLC-MS-UV analysis, and quantification of phenols and tannins to evaluate the influence of antioxidant treatment.

Descriptive statistics were performed using Jamovi software (version 2.4.11), and Student's t-test for independent samples was applied. The Shapiro-Wilk test was carried out to evaluate the normality acceptance criteria, and Levene's test was used to verify homogeneity with a significance level of 0.05. Experimental results were expressed as mean/standard deviation. Correlation coefficients and linear regressions were performed using Excel software.

Results and discussion

Determination of the degree of maturity

The pulp analyses indicated an acidic pH (5.740) and a low concentration of soluble solids (5.33 °Brix), values characteristic of an immature fruit. These parameters were evaluated in the fruit pulp because the main biochemical and physiological transformations of maturation take place in this tissue. The green pigmentation of the peels studied corresponds to a maturation stage 1, according to the Von Loesecke scale (Etienne *et al.*, 2013). Integrally, these results determine the degree of maturity, which is essential in studies of chemical composition and biological activity, as the components of the fruit and its peels vary according to their physiological state.

Proximate analysis

The parameters evaluated did not present statistically significant differences ($p > 0.05$) among the treatments applied. This demonstrates that the application of the antioxidant treatment does not significantly alter the content of the compounds analyzed. Table 2 shows the values obtained in this determination.

Table 2. Proximate analysis of extracts from *Musa acuminata* (Blue Java) peels.

Parameters	Samples without antioxidant treatment	Samples with antioxidant treatment
	Mean/standard deviation	Mean/standard deviation
Moisture (%)	2.570 / 0.69	2.660 / 0.440
Fats (g)	0.090 / 0.005	0.077 / 0.008
Proteins (%)	11.880	11.380
Crude fiber (%)	1.940	1.970
Ashes (%)	6.560 / 0.087	6.600 / 0.101

Moisture

The FAO-OMS (1985) establishes a moisture limit of 15.5 % for vegetable flour; the values obtained are within the established regulatory limits. The control of this parameter prevents the proliferation of microorganisms, fungi, and mold, and oxidative degradation, preserving the stability of the metabolites (Miranda Martínez and Cuéllar, 2014).

Fats

Although plantain peels are not a major source of fat, they contain lipids with the presence of essential fatty acids. The low-fat content obtained is consistent with other studies. Tapia Romero *et al.* (2025) reported 0.109 g of fat in *Musa paradisiaca* (Dominico) peels (maturation stage 1), while Khawas and Deka (2016) recorded 1.96 g of fat in the peel of the culinary banana *Musa ABB* (maturation stage 1). The low-fat content obtained could be conditioned by metabolic or structural factors typical of this variety, so more studies are required to corroborate these results.

Proteins

Protein analysis of the sample without treatment showed a slightly higher percentage compared to the sample treated. It has been reported that ascorbic acid, one of the antioxidants used in the treatment, can interact with proteins, generating structural modifications that favor the formation of glycosylated proteins by non-enzymatic pathways, which can cause a reduction in lysine content. Likewise, it has been described that the products derived from the oxidation of ascorbic acid can condition Maillard-type reactions with proteins, causing changes in their composition (Ortwerth and Olesen, 1988). Although these factors could justify the differences recorded, it is necessary to contrast the findings with other analytical methods.

Crude fiber

No significant differences were recorded among the samples analyzed, suggesting that the integrity of the fibrous matrix is maintained, which is important for cellular structural stability. The result obtained is low compared to other varieties: Handayani *et al.* (2023) obtained 8.81 % crude fiber in peels of *Musa acuminata* Cavendish Subgroup, while Shankar *et al.* (2017) reported 10% crude fiber in peels of *Musa acuminata* Sagor variety.

Ashes

The ash content allows for the detection of inorganic materials that would affect the authenticity of the plant material. Farmacopea Española (2002) states that ash concentration in plant drugs should not exceed 12 %; therefore, the results obtained comply with the established limit.

Minerals

The results indicate that the antioxidant treatment does not considerably affect the concentration of minerals in the variety studied. The values of the quantification of minerals are presented in Table 3.

Potassium was quantified as the main mineral. The other chemical elements quantified in descending order were Ca, Mg and P; in lower concentration Na, Fe, Zn, Mn and Cu. These results are consistent with other studies (Islam *et al.*, 2023; Tapia Rosemary *et al.*, 2025).

The proximate analysis estimates the nutrient content and provides relevant information to establish appropriate drying,

Table 3. Quantification of minerals (mg.g⁻¹) in *Musa acuminata* (Blue Java) peels.

	P	K	Ca	Mg	Zn	Cu	Fe	Mn	Na
ST Samples	1.400	32.600	4.000	1.600	0.016	0.002	0.017	0.014	0.051
T-Samples	1.500	32.100	4.100	1.600	0.017	0.002	0.019	0.016	0.050

storage, and processing conditions. Likewise, the determination of macronutrients and minerals suggests that the Blue Java variety could be incorporated as an input in nutritional formulations. It should be noted that complementary studies are required to evaluate the bioavailability of nutrients, their stability during processing, and their technological feasibility.

Heavy metals

The concentration of lead and arsenic was determined to verify the safety of the samples. The results obtained showed that Pb and As contents were below 0.02 mg.kg⁻¹ in both samples. According to the FAO-OMS (2018), Pb and As contents in food must be lower than 0.1 mg.kg⁻¹, based on this criterion, the Blue Java variety meets the permitted limit.

Identification of secondary metabolites

HPLC-MS-UV Analysis

It can be seen that there is a great similarity between the samples treated and not treated with antioxidants. In particular, three intense signals between 7.8 and 8.47 min are observed, produced by flavonoid glycosides (compounds 2, 3, and 6). From a qualitative approach, there appear to be no significant differences between the two samples, since the same components were identified in both extracts. However, the possibility of quantitative differences is not ruled out. The chromatograms obtained are shown in Figure 1.

The identification made was developed by comparing mass spectrometry and UV spectroscopy data of compounds reported for the species in previous studies (Rodrigues Borges *et al.*, 2021; Silva *et al.*, 2020), the results are summarized in Table 4.

Nine flavonoids characteristic of the species were identified. Rutin (compound 3) showed the most intense chromatographic signal, and its MS² spectrum evidenced the loss of rhamnose (m/z 463) and the rutinoside residue that originates the base peak m/z 301, associated with the aglycone quercetin. Rutin is the most abundant flavonoid of the Plantain cultivar (Vu *et al.*, 2018).

Compounds 4 and 6 are isomers with high structural similarity; both are rutinoides (m/z 447 evidences the loss of rhamnose) and show very similar mass spectra. However, one of the UV spectra (compound 6) showed a slight bathochromic shift in band I, consistent with the absorption of kaempferol; the differentiation was performed on this basis (Cuesta Rubio *et al.*, 2015).

Identified flavonoids, particularly quercetin derivatives, have been reported in plantain peels (Rodrigues Borges *et al.*, 2021). The glycosides of type 3-O-rutinoides and specifically rutin, constitute the group of flavonols predominantly identified in plantain peels of various species (Vu *et al.*, 2018).

The results of this study are consistent with previous reports regarding the presence of flavonols in plantain peels. The chemical identification of plant material is essential to predict its possible pharmaceutical uses and provides the necessary tools for standardization and quality control.

Quantitative determination of metabolites

Both in the quantification of phenols and tannins, statistically significant differences were observed between the two samples (p<0.05), evidencing that the application of antioxidants considerably increases the content of these metabolites. The results obtained are presented in Table 5.

This behavior is attributed to the fact that the application of these organic acids decreases the pH of the medium, inhibiting the enzymatic activity mainly of polyphenol oxidase and peroxidase, avoiding the oxidation of phenols to quinones and their subsequent polymerization; this mechanism maintains the stability of these metabolites, justifying their high concentration in samples with antioxidants (Tilley *et al.*, 2023). In contrast to untreated samples, phenolic compounds are exposed to oxidative degradation by decreasing their content.

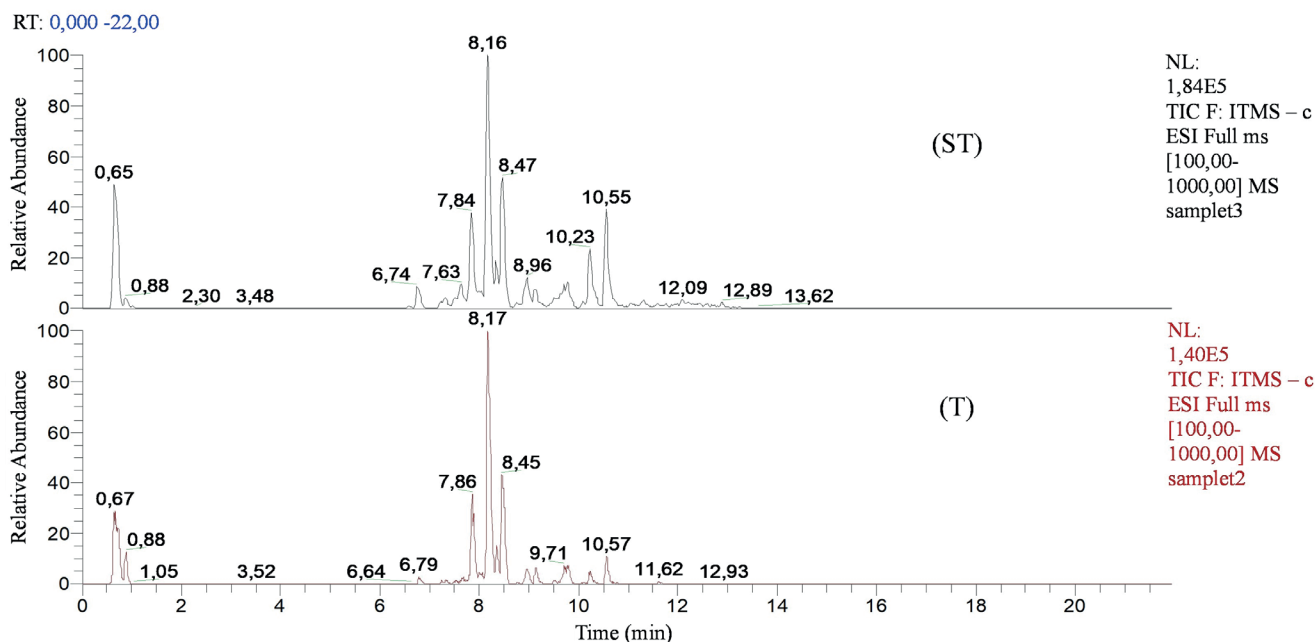


Figure 1. CLAE-EM chromatograms of the extracts studied. (ST) Sample without antioxidant treatment. (T) Sample with antioxidant treatment.

Table 4. Compounds identified in the analyzed extracts.

Nº	Retention Time (min)	Name	(M-H) m/z	MS ² m/z	UV (λ_{max} /nm)
1	7.50	Quercetin 3-O-Rhamnosyl (1-2)-Glucoside-7-O-Rhamnoside	755	593*	-
2	7.84	Myricetin 3-O-rutinoside	625	607, 463, 317, 316*, 301, 271	-
3	8.16	Rutin (quercetin 3-O-rutinoside)	609	463, 343, 301*, 271, 255	204, 255, 356
4	8.34	Luteolin 7-O-rutinoside	593	447, 357, 327, 285*, 284, 255	264, 356
5	8.40	Isoquercetin	463	445, 343, 301*, 179, 151	-
6	8.47	Kaempferol 3-O-rutinoside	593	447, 357, 327, 285*, 257	267, 365
7	8.63	Isorhamnetin 3-O-glucoside 7-O-rhamnoside	623	357, 315*, 300, 271, 255	-
8	8.70	Cynaroside (luteolin 7-O-glucoside)	447	327, 284*, 255, 179, 151	-
9	8.74	Isorhamnetin-3-O-glucoside	477	357, 315, 314*	-

* Base peak

Table 5. Quantification of specialized metabolites in the samples studied.

Parameters mg GAE in 1 g	Samples without antioxidant treatment	Samples with antioxidant treatment
	Mean/standard deviation	Mean/standard deviation
Total phenols	6.46 ^a / 0.48	20.21 ^b / 0.14
Total tannins	1.71 ^a / 0.54	8.36 ^b / 0.62

Values accompanied by different letters in the same row indicate significant differences (p < 0.05).

Conclusions

The results of the study show that the application of antioxidant treatment does not significantly alter the proximate composition (moisture, fats, proteins, crude fiber, ashes, and minerals) of the immature peels of *Musa acuminata* AAB, Blue Java variety. Likewise, the absence of heavy metals confirms the safety of the samples. The analysis using HPLC-MS-UV allowed for the identification of nine flavonoid glycosides in the acetone extracts, evidencing the phytochemical richness of this by-product. Additionally, the treatment with antioxidants significantly increased the concentration of phenols and tannins, demonstrating their efficacy in mitigating oxidative degradation processes and favoring metabolite preservation. Overall, the results obtained demonstrate that the immature peels of *Musa acuminata* AAB (Blue Java) constitute a promising source of phytonutrients and phenolic compounds and show that the application of an antioxidant treatment conditions the preservation of secondary metabolites without altering the nutritional content in this variety.

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