

Acylated anthocyanins from organic purple-fleshed sweet potato (*Ipomoea batatas* (L.) Lam) produced in Brazil

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ABSTRACT: Acylated anthocyanins from a purple-fleshed sweet potato (PFSP), obtained by organic cultivation in Brazil, were characterized after separation by a high performance liquid chromatography-diode array detector (HPLC-PDA). These anthocyanins were manually collected at the detector output, concentrated and injected into a high resolution mass spectrometer (ESI-QTOF-MS²). Twenty-two acylated anthocyanins were detected. Among them, sixteen had been reported in the literature and six, derived from peonidin were reported for the first time in sweet potato roots in this study. These compounds showed molecular ions with accurate mass/charge ratios (*m/z*) of 909.2081, 961.3010, 961.2571, 963.3345, 1123.2932 and 1179.3862. Although anthocyanins in PFSP have already been extensively studied, the variety studied in this work is probably genetically different from all varieties and cultivars already researched, which would explain why these anthocyanins have not been observed in the previously studied varieties.

Keywords: phenolic, mass spectrometry, natural dye, antioxidant

Introduction

Sweet potato, the tuberous root of *Ipomoea batatas* L., native to the Andes (Shan et al., 2012) is a nutritionally valuable food with high antioxidant activity (Kim et al., 2011). It is a high-yielding industrially important crop used in hunger relief in Asia and Africa, with a range of different colors and amounts of carotenoids, anthocyanins and phenolic acids (Zhao et al., 2014; Wang et al., 2016).

Consumption of anthocyanins is associated with reduced risk of cardiovascular and degenerative metabolic diseases, visual and brain function improvement, cancer chemoprevention, anti-inflammatory, hepatoprotective and antihyperglycemic activities even when bioavailability is low (Hou, 2003; Oliveira et al., 2019c; Smeriglio et al., 2016; Tsuda, 2012). Since acylated anthocyanins have higher processing stability, bioprotective capacity and resistance to overall simulated digestions than non-acylated anthocyanins they are desirable for their provision of color and health benefits (Luo et al., 2018; Oliveira et al., 2019b; Yang et al., 2019).

The purple-fleshed sweet potato (PFSP) has a high content of acylated anthocyanins that provide greater color stability when compared to other vegetables as in the case of red fruits which have a high content of anthocyanins with low levels of acylation. Studies have shown a wide-ranging physiological functionality for these anthocyanins, as the majority have cyanidin or peonidin, or are mono- or di-acylated with at least one caffeic acid, which confers greater thermal and ultraviolet resistance, and antioxidant capacity. They degrade only partially when subjected to heating (Kim et al., 2015) and have been used in industrialized

foodstuffs (Wang et al., 2012) such as juices, alcoholic beverages, pasta, flour, breads and others as natural dye and antioxidant. It may be recommended in healthy food for the prevention of chronic diseases related to certain lifestyles.

A PFSP has been cultivated for many years by family farmers in the organic production system in the state of Rio de Janeiro, Brazil. However, the anthocyanin profile of this plant has never been studied under these conditions. The objective of this study was to characterize the profile of the anthocyanins present in this PFSP variety and compare it with the profile of the varieties already studied in the works available in the literature. For this purpose the PFSP was cultivated in different places and seasons, for the same total period of cultivation, and the anthocyanins were separated by HPLC-DAD and characterized by ESI-QTOF-MS².

Materials and Methods

Chemicals

The acetonitrile, methanol and formic acid used were HPLC grade, the water was ultrapure, and 0.5 g C₁₈ reverse phase cartridges and C₁₈ 60A carbon 17 % particle size 40-63 µm for solid phase extraction (SPE) were used.

Plant material

The branches of a PFSP variety with purple peel, of unknown origin, commonly cultivated in Brazil by organic farming, were grown for the same total cultivation period of five months (from 27 Apr 2016 to 5 Oct 2016) at Cachoeiras de Macacu-RJ (geographic coordinates: 22°37'56.88" S, 42°48'31.24" W, altitude of 59 m) and again for five months, although in different

seasons and years (from 8 Nov 2017 to 3 Apr 2018) at an integrated agroecological production system, Seropédica-RJ (geographic coordinates: 22°45'13.18" S, 43°40'25.25" W, altitude of 36 m). The PFSP branches (approximately 0.3 m in length) were linearly distributed, separated by 0.3 m at the center of three parallel rows 1 m wide by 5 m long, separated by 1 m intervals. Only on the first day was fertilization implemented with 200 g m⁻² fermented organic compound based of wheat bran (60 %) and castor bran (40 %) inoculated with compost accelerator, 22 g m⁻² thermophosphate and 4 kg m⁻² potassium sulfate. A drip irrigation system was also used. After five months the tuberous roots were harvested, peeled, cut, crushed, freeze dried, ground and frozen until use.

Sample preparation

The samples of PFSP tuberous root previously prepared and frozen until use as described above (30.0 g of each farm), after reaching room temperature (24 °C) were individually extracted for 30 min under stirring at 40 °C, with 300 mL of ultrapure water containing 0.068 mol L⁻¹ formic acid, followed by filtration of the supernatant. The procedure was repeated three times until no more intense pink color was observed in the solvent. The total volume (900 mL) of each extract obtained was concentrated by SPE using manually prepared 10 g reverse phase C₁₈ 60A cartridges.

The manually prepared cartridges were pre-conditioned with 100 mL of methanol, equilibrated with 100 mL of ultrapure water, loaded with the extract and washed with 100 mL of ultrapure water. The retained anthocyanins were eluted with 30 mL of acetonitrile with 5 % formic acid, collected and reserved for HPLC separation.

Separation and purification of anthocyanins

The solutions obtained in the previous step were injected into an HPLC, consisting of a separations module equipped with a photodiode array detector (PDA), acquiring chromatograms recorded from 200 to 600 nm. The column used was C18 (100 × 4.6 mm, particle size 2.4 µm). The column oven temperature was 35 °C, and the injection volume 30 µL with a mobile phase flow rate of 1.0 mL min⁻¹. The elution gradient was applied according to Lee et al. (2013) with certain modifications. It started with 90 % of ultrapure water with 5 % of formic acid (A) and 10 % of acetonitrile (B) decreasing to 86 % A in 12 min, reaching 80 % A in 3 min, and remaining at that level for 3 min, then rising further to 90 % A in 2 min, and remaining at that level for 2 min. Fourteen peaks with maximum absorption in the range of 518-532 nm and 308-337 nm were detected with the same chromatogram profile for both extracts. They were manually collected at the detector output. Twenty injections were required to ensure a sufficient amount was obtained for the characterization analyses.

The separated anthocyanins in the collected peaks were concentrated by solid phase extraction (SPE) in

0.5 g SEP-PAK C₁₈ reverse phase cartridges, conditioned with 1 mL of acetonitrile, equilibrated with 1 mL of ultrapure water, loaded with the collected peak diluted two times with ultrapure water, washed with 1 mL of ultrapure water and then eluted with 3 mL of acetonitrile containing 5 % formic acid. The samples were dried under air flow, solubilized in 500 µL of acetonitrile and analyzed by ESI-QTOF-MS².

Identification of anthocyanins

The solutions obtained in the previous step were directly injected into the ESI-QTOF-MS² with ESI in positive mode and QTOF under the following conditions: capillary, sampling cone and extraction cone energies of 3.0 kV, 50.0 V and 3.0 V, respectively; source temperature of 80 °C, desolvation gas temperature and flow of 250 °C and 500 L h⁻¹ and gas flow of 25 L h⁻¹ (nitrogen was used as the nebulizing and drying gas). Depending on the analysis, collision energies used in the trap and transfer ranged from 25 to 35 V. The MS² spectral data, maximum absorption wavelength region, elution order and other studies that detailed the characteristic fragmentation of the PFSP anthocyanins were used to identify them (Goda et al., 1997; Grass et al., 2017; He et al., 2016; Hu et al., 2016; Islam et al., 2002; Jie et al., 2013; Lee et al., 2013; Montilla et al., 2010; Otake et al., 1992; Qiu et al., 2009; Terahara et al., 1999; Terahara et al., 2000; Terahara et al., 2004; Tian et al., 2005; Truong et al., 2010; Truong et al., 2012; Wang et al., 2017; Xu et al., 2015; Ying et al., 2011; Zhao et al., 2014; Zhu et al., 2017).

Results and Discussion

HPLC-DAD profile of PFSP

Using the described chromatographic conditions, fourteen peaks were separated (Figure 1) which exhibited typical acylated anthocyanin spectral profiles showing maximum absorbance of approximately 525 nm, characteristic of anthocyanins, and approximately 320 nm, characteristic of acylation with hydroxycinnamic acid derivatives (Giusti and Wrolstad, 2003; Hang and Wrolstad, 1990), for every peak detected in both extracts (from different locations of farms and in a different season and year of cultivation) that have qualitatively identical chromatograms. Genetic, biochemical, physiological, ecological and evolutionary factors may both quantitatively and qualitatively influence the production of secondary metabolites such as anthocyanins, but the most important is the genetic factor (Endt et al., 2002; Figueiredo et al., 2008; Pichersky and Gang, 2000). As both crops used the same genetic material, the other different factors were not enough to alter the gene expression of anthocyanin biosynthesis. The band intensity ratio in the range of 308-337 nm with a band intensity of approximately 518-532 nm ($E_{\text{acyl,max}}/E_{\text{vis,max}}$) (Giusti and Wrolstad, 2003; Hang and Wrolstad, 1990) at the spectrum of each peak showed, in a number

of cases, higher ratios that characterize hydroxycinnamic derived di-acylated compounds and lower ratios that characterize hydroxycinnamic derived mono-acylation (Lee et al., 2013; Otake et al., 1992; Terahara et al., 2004). The $E_{acyl,max}/E_{vis,max}$ ratios were low for peaks 1, 2, 3, 4, and high for peaks 5, 6, 7, 8, 9, 10, 11, 12, 13, 14.

The spectra had no interference from free phenolic acids since the procedure used in this work for anthocyanin extraction was more selective than those used in other studies. In this study, the solvent used to extract anthocyanins was 0.068 mol L⁻¹ formic acid in water, which did not require phenolic acid elimination treatments and facilitated the extraction of anthocyanins compared to the solvents used in other studies, since the phenolic acids in PFSP roots are bound to other molecules and the acid concentration in the extraction solvent does not promote hydrolysis. Organic solvents (methanol, acetonitrile or ethanol) or their mixtures with acidic water, commonly used in other studies to extract anthocyanins from fruits, had difficulty in extracting anthocyanins from these PFSP samples. Due to their high affinity for starch, anthocyanins were preferably adsorbed on starch and did not migrate to the organic solvents tested. That is one reason for starch being a good wall material for microencapsulation of anthocyanins (Fang and Bhandari, 2010). The 0.068 mol

L⁻¹ formic acid in water solvent, more polar than organic solvents or mixtures of these solvents with acid water, was more efficient in extracting PFSP anthocyanins as it competed with starch for anthocyanin affinity. The difficulty in extracting anthocyanins from PFSP, which has high starch contents, through the use of organic solvents in general does not occur in the extraction of anthocyanins from fruits that usually have much lower starch contents. The interfering polar compounds that may have been present in the extract were eliminated by the washing step during SPE as Lee et al. (2013) in their anthocyanin purification procedure.

Identification of isolated anthocyanins by ESI-QTOF-MS²

The results present three main possibilities: the loss of glycosyl bound to position 5 of anthocyanidin, the loss of sophorosyl in position 3 (higher probability) (Tian et al., 2005), and the loss of both, as in Figure 2. The fragmentation of glycosidic bond within the sophorose and between the aromatic acids and sophoroside was negligible (Tian et al., 2005). Therefore, the fragments with m/z of 287 [C₁₅H₁₁O₆]⁺ and 449 [C₂₁H₂₁O₁₁]⁺ originate from cyanidin and mono glycosylated cyanidin while the fragments with m/z 301 [C₁₆H₁₃O₆]⁺ and 463 [C₂₂H₂₃O₁₁]⁺ originate from peonidin and mono glycosylated peonidin. The loss of acylated sophorosyl, which is the preferred reaction, explains the higher intensity of the resulting fragment peak compared to the peak of glycosyl loss. The glycosyls and acyl moieties in anthocyanins were assumed to occur in positions already confirmed in other studies (Figure 2) through mass spectrometry and nuclear magnetic resonance (NMR) (Goda et al., 1997; Montilla et al., 2010; Otake et al., 1992; Qiu et al., 2009; Terahara et al., 1999; Terahara et al., 2000; Terahara et al., 2004; Ying et al., 2011; Zhang et al., 2018). The data obtained for the anthocyanins detected are presented in an organized listing in Table 1.

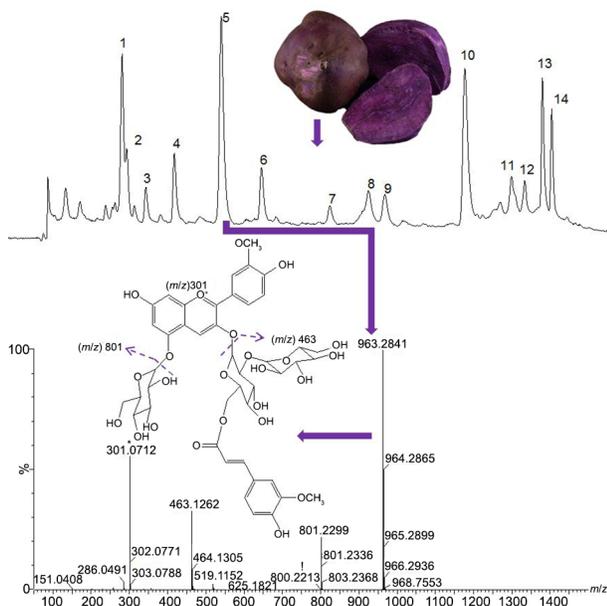


Figure 1 – Scheme of the process performed. The arrows, from top to bottom indicate the order of the analyses performed. Anthocyanins extracted from the purple-fleshed sweet potato (PFSP) were separated by HPLC-DAD to obtain the chromatogram showing fourteen peaks at 525 nm. Peak five was collected manually at the detector output and then analyzed by QTOF-MS² to obtain the mass spectrum for the identification of the molecular structure. This procedure was carried out to identify the anthocyanins present in the fourteen peaks.

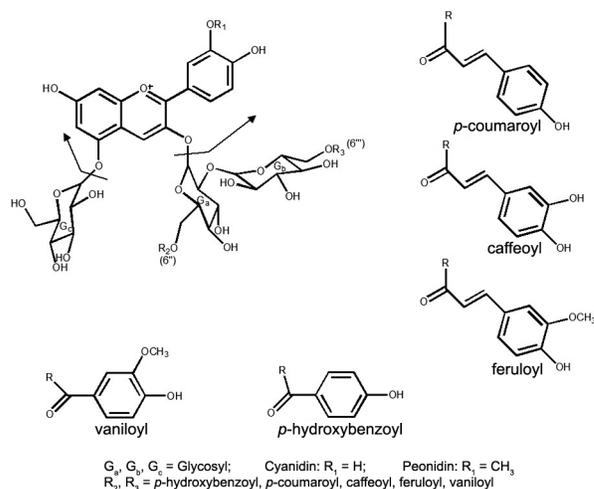


Figure 2 – Molecular structure and fragmentation sites of acylated anthocyanins identified from purple-fleshed sweet potato (PFSP).

Table 1 – Spectral and chromatographic data obtained for the identified acylated anthocyanins from Purple-Fleshed Sweet Potato.

Anthocyanin N°	Peak N°	t _r (min)	M ⁺ (m/z)	Fragment ions (m/z)	λ _{UV/Vis} max (nm)	Compound / Molecular Formula
1	1	3.62	907.2524	745.2003, 463.1253, 301.0712	280, 321, 518	Peonidin 3-O-(6"-O-p-hydroxybenzoyl sophoroside)-5-O-glucoside / C ₄₁ H ₄₇ O ₂₃
2	2	3.76	1179.3862	1119.3651, 973.3016, 841.2552, 737.2061, 601.1586, 505.1393, 301.0712	284, 322, 524	Unidentified Anthocyanin (Peonidin derivative)*
3	3	4.33	949.2623	787.2089, 449.1082, 287.0556	282, 308, 531	Cyanidin 3-O-(6"-O-feruloyl sophoroside)-5-O-glucoside / C ₄₃ H ₄₉ O ₂₄
4	3	4.33	909.2081	729.1376, 627.1047, 567.0831, 393.0292, 367.0496, 349.0400, 301.0712	282, 308, 531	Unidentified Anthocyanin (Peonidin derivative)*
5	4	5.18	961.3010	801.2238, 519.1119, 463.1237, 301.0712	300, 324, 532	Unidentified Anthocyanin (Peonidin derivative)*
6	5	6.62	963.2841	801.2299, 463.1262, 301.0712	280, 327, 520	Peonidin 3-O-(6"-O-feruloyl sophoroside)-5-O-glucoside / C ₄₄ H ₅₁ O ₂₄
7	5	6.62	1097.2838	935.2285, 449.1088, 287.0556	280, 327, 520	Cyanidin 3-O-(6"-6'''-O-dicaffeoyl sophoroside)-5-O-glucoside / C ₅₁ H ₅₃ O ₂₇
8	6	7.86	961.2571	801.2265, 463.1236, 301.0712	283, 320, 534	Unidentified Anthocyanin (Peonidin derivative)*
9	6	7.86	1055.2709	893.2127, 449.1088, 287.0556	283, 320, 534	Cyanidin 3-O-(6-O-caffeoyl-6-O-p-hydroxybenzoyl sophoroside)-5-O-glucoside / C ₄₉ H ₅₁ O ₂₆
10	7	9.92	1111.2947	949.2388, 449.1076, 287.0556	280, 321, 534	Cyanidin 3-O-(6-O-caffeoyl-6-O-feruloyl sophoroside)-5-O-glucoside / C ₅₂ H ₅₅ O ₂₇
11	7	9.92	1125.3098	963.2556, 463.1238, 301.0712	280, 321, 534	Peonidin 3-O-(6"-O-caffeoyl-6'''-O-feruloyl sophoroside)-5-O-glucoside / C ₅₃ H ₅₇ O ₂₇
12	8	11.11	1111.3011	949.2415, 463.1253, 301.0712	281, 319, 526	Peonidin 3-O-(6"-6'''-O-dicaffeoyl sophoroside)-5-O-glucoside / C ₅₂ H ₅₅ O ₂₇
13	8	11.11	1099.3037	937.2398, 463.1247, 301.0712	281, 319, 526	Peonidin 3-O-(6-O-caffeoyl-6-O-vaniloyl sophoroside)-5-O-glucoside / C ₅₁ H ₅₃ O ₂₇
14	8	11.11	949.2988	787.2139, 463.1235, 301.0712	281, 319, 526	Peonidin 3-O-(6"-O-caffeoyl sophoroside)-5-O-glucoside / C ₄₃ H ₄₉ O ₂₄
15	8	11.11	963.3345	801.2732, 639.2275, 579.2079, 513.1323, 301.0712	281, 319, 526	Unidentified Anthocyanin (Peonidin derivative)*
16	9	11.66	1069.2830	907.2289, 463.1239, 301.0712	282, 324, 531	Peonidin 3-O-(6-O-caffeoyl-6-O-p-hydroxybenzoyl sophoroside)-5-O-glucoside / C ₅₀ H ₅₃ O ₂₆
17	10	14.12	1123.2932	961.2393, 943.2283, 605.1298, 479.0979, 301.0712	285, 326, 531	Unidentified Anthocyanin (Peonidin derivative)*
18	10	14.20	1125.3123	963.2538, 463.1229, 301.0712	285, 326, 531	Peonidin 3-O-(6"-O-feruloyl-6'''-O-caffeoyl sophoroside)-5-O-glucoside / C ₅₃ H ₅₇ O ₂₇
19	11	15.59	1053.2903	891.2363, 463.1245, 301.0712	280, 325, 532	Peonidin 3-O-(6-O-coumaroyl-6-O-p-hydroxybenzoyl sophoroside)-5-O-glucoside / C ₅₀ H ₅₃ O ₂₅
20	12	16.00	1083.3026	921.2470, 463.1245, 301.0712	285, 324, 531	Peonidin 3-O-(6-O-p-hydroxybenzoyl-6-O-feruloyl sophoroside)-5-O-glucoside / C ₅₁ H ₅₅ O ₂₆
21	13	16.56	1109.3230	947.2606, 463.1236, 301.0712	280, 337, 528	Peonidin 3-O-(6-O-feruloyl-6-O-coumaroyl sophoroside)-5-O-glucoside / C ₅₃ H ₅₇ O ₂₆
22	14	16.84	1139.3292	977.2733, 463.1246, 301.0712	287, 330, 532	Peonidin 3-O-(6"-6'''-O-diferuloyl sophoroside)-5-O-glucoside / C ₅₄ H ₅₉ O ₂₇

t_r = retention time; * Detected for the first time in PFSP.

An anthocyanin was observed at peak one, M⁺ m/z 907.2524, which corresponds to M⁺ = [C₄₁H₄₇O₂₃]⁺. Although that peak was not completely separate from peak 2 and it was not possible to differentiate them by their UV/Vis spectra only (Table 1), it was possible to tell them apart by selecting that precursor ion (m/z 907) through MS². Its UV/Vis spectra was typical of mono-

acylation with both maximum absorption bands of the anthocyanin and the acid derivative very intense. It was the most polar anthocyanin in the extract since it was the first in elution order and due to the presence of the fragment m/z 301.0712 [Peonidin]⁺, it was a peonidin derivative. Fragments m/z 745.2003 (C₃₅H₃₇O₁₈) resulting from the loss of glucosyl [M-glucosyl]⁺ were detected

and the fragment m/z 463.1253 was more intense than the last, resulting from the loss of sophorosyl bonded to acyl moiety. When the acylation occurs in the carbon at the 6'' position of the sophorosyl (Figure 2), as in this case, the intensity of the [M-acyl-sophorosyl]⁺ peak is much lower than the M⁺ and the [anthocyanidin]⁺ peaks, although it is always more intense than the peak of the [M-glycosyl]⁺ fragment, showing that the reaction of the loss of the sophorosyl occurs in a greater proportion than the loss of glycosyl as evidenced by Lee et al. (2013). When the acylation occurs in the carbon at the 6''' position of the sophorosyl (Figure 2), the peak the [M-acyl-sophorosyl]⁺ fragment becomes much more intense, surpassing or equaling intensity of the peaks of M⁺, [M-glycosyl]⁺ and [anthocyanidin]⁺ (Lee et al., 2013). The acyl moiety matched the molecular formula of *p*-hydroxybenzoyl, [M-*p*-hydroxybenzoyl-sophorosyl]⁺ with m/z 463.1253. Thus, it was possible to identify this compound as peonidin 3-*O*-(6''-*O*-*p*-hydroxybenzoyl sophoroside)-5-*O*-glucoside, as previously identified in other studies (He et al., 2016; Hu et al., 2016; Islam et al., 2002; Jie et al., 2013; Lee et al., 2013; Montilla et al., 2010; Tian et al., 2005; Truong et al., 2010; Truong et al., 2012; Wang et al., 2017; Xu et al., 2015; Zhu et al., 2017).

At peak two, an unidentified anthocyanin was detected for the first time in PFSP, with M⁺ m/z 1179.3862 (MS² precursor ion m/z 1179) and fragments m/z 1119.3651, 973.3016, 841.2552, 737.2061, 605.1586, 505.1393 and 301.0712 [Peonidin]⁺.

Two compounds were observed at peak three with typical UV/Vis spectrum of mono-acylates with hydroxycinnamic acid anthocyanins (Table 1). It was only possible to separate them by selecting their precursor ion through MS². An unidentified anthocyanin detected for the first time in PFSP with M⁺ m/z 909.2081 (MS² precursor ion m/z 909), with fragments 729.1376, 627.1047, 567.0831, 393.0292, 367.0496, 349.0400, 301.0712 [Peonidin]⁺. The other compound from peak 3 was the anthocyanin with M⁺ m/z 949.2623 (MS² precursor ion m/z 949), related to M⁺ = [C₄₃H₄₉O₂₄]⁺, fragments m/z 787.2089 [M-glycosyl]⁺ (C₃₇H₃₉O₁₉), m/z 449.1082 [M-feruloyl-sophorosyl]⁺ and m/z 287.0556 [Cyanidin]⁺. It was cyanidin 3-*O*-(6''-*O*-feruloyl sophoroside)-5-*O*-glucoside, studied previously (He et al., 2016; Hu et al., 2016; Islam et al., 2002; Jie et al., 2013; Lee et al., 2013; Montilla et al., 2010; Tian et al., 2005; Truong et al., 2010; Truong et al., 2012; Wang et al., 2017; Xu et al., 2015; Zhu et al., 2017), with the ferulic acylation at the 6'' position since the intensity of m/z 449 [M-feruloyl-sophorosyl]⁺ is only higher than the m/z 787.2089 (Lee et al., 2013).

At peaks four and six an anthocyanin was detected for each peak, both with the similar M⁺ and fragments (MS² precursor ions m/z 961). The first with M⁺ m/z 961.3010 and fragments m/z 801.2238, 519.1119, 463.1236 and 301.0712 [Peonidin]⁺ (Figure 3A). The second with M⁺ m/z 961.2571 and fragments m/z

801.2265, 463.1237 and 301.0712 [Peonidin]⁺ (Figure 3B). Both peonidin derivative isomers were detected for the first time in PFSP.

At peak five two anthocyanins were detected with an intense hydroxycinnamic acid derivative band at UV/Vis spectrum and MS² precursor ions m/z 963 and 1097 (Table 1). One with M⁺ m/z 963.2841 and fragments m/z 801.2299 [M-glycosyl]⁺ (C₃₈H₄₁O₁₉), 463.1262 [M-feruloyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺ was observed, identified as M⁺ = [C₄₄H₅₁O₂₄]⁺, peonidin 3-*O*-(6''-*O*-feruloyl sophoroside)-5-*O*-glucoside, previously characterized (Hu et al., 2016; Jie et al., 2013; Lee et al., 2013; Montilla et al., 2010; Tian et al., 2005; Truong et al., 2010; Wang et al., 2017; Xu et al., 2005; Zhu et al., 2017), where the feruloyl moiety, according to the intensity of m/z 463.1262 [M-feruloyl-sophorosyl]⁺, more intense only than the [M-glycosyl]⁺ fragment, was at the 6'' position (Figure 2) (Lee et al., 2013). The other with M⁺ m/z 1097.2838, which corresponded to M⁺ = [C₅₁H₅₃O₂₇]⁺, with fragments m/z 935.2285 [M-glycosyl]⁺ (C₄₅H₄₃O₂₂), 449.1088 [M-dicaffeoyl-sophorosyl]⁺ and 287.0556 [Cyanidin]⁺, identified as cyanidin 3-*O*-(6''-6'''-*O*-dicaffeoyl sophoroside)-5-*O*-glucoside, as has already been identified in other studies (Grass et al., 2017; He et al., 2016; Islam et al., 2002; Lee et al., 2013; Montilla et al., 2010; Terahara et al., 1999; Tian et al., 2005; Truong et al., 2010; Truong et al., 2012; Wang et al., 2017).

At peak six it was possible to observe two distinct anthocyanins separated by selecting their MS² precursor ions, the previously reported m/z 961 and the m/z 1055. That peak showed an intense hydroxycinnamic acid derivative band typical of di-acylation at UV/Vis spectrum (Table 1). The anthocyanin with M⁺ m/z 1055.2709 M⁺ = [C₄₉H₅₁O₂₆]⁺, with fragments m/z 893.2127 [M-glycosyl]⁺ (C₄₃H₄₁O₂₁), 449.1088 [M-*p*-hydroxybenzoyl-caffeoyl-sophorosyl]⁺ and 287.0556 [Cyanidin]⁺, was identified as cyanidin 3-*O*-(6-*O*-*p*-hydroxybenzoyl-6-*O*-caffeoyl sophoroside)-5-*O*-glucoside, as in other studies (He et al., 2016; Hu et al., 2016; Islam et al., 2002; Jie et al., 2013; Lee et al., 2013; Montilla et al., 2010; Terahara et al., 1999; Truong et al., 2010; Wang et al., 2017; Xu et al., 2015; Zhu et al., 2017).

At the separated peak seven, with intense hydroxycinnamic acid derivative bands at UV/Vis spectrum as in di-acylated anthocyanins, two anthocyanins were observed (Table 1). One with M⁺ m/z 1111.2947, related to M⁺ = [C₅₂H₅₅O₂₇]⁺ (MS² precursor ion m/z 1111), fragments 949.2388 [M-glycosyl]⁺ (C₄₆H₄₅O₂₂), 463.1076 [M-caffeoyl-feruloyl-sophorosyl]⁺ and 287.0556 [Cyanidin]⁺ was identified as cyanidin 3-*O*-(6-*O*-caffeoyl-6-*O*-feruloyl sophoroside)-5-*O*-glucosyl as other authors have done (Hu et al., 2016; Islam et al., 2002; Jie et al., 2013; Lee et al., 2013; Montilla et al., 2010; Terahara et al., 1999; Tian et al., 2005; Truong et al., 2010; Xu et al., 2015; Zhu et al., 2017). The other with M⁺ m/z 1125.3098 which corresponds to M⁺ = [C₅₃H₅₇O₂₇]⁺, and fragments m/z 963.2556

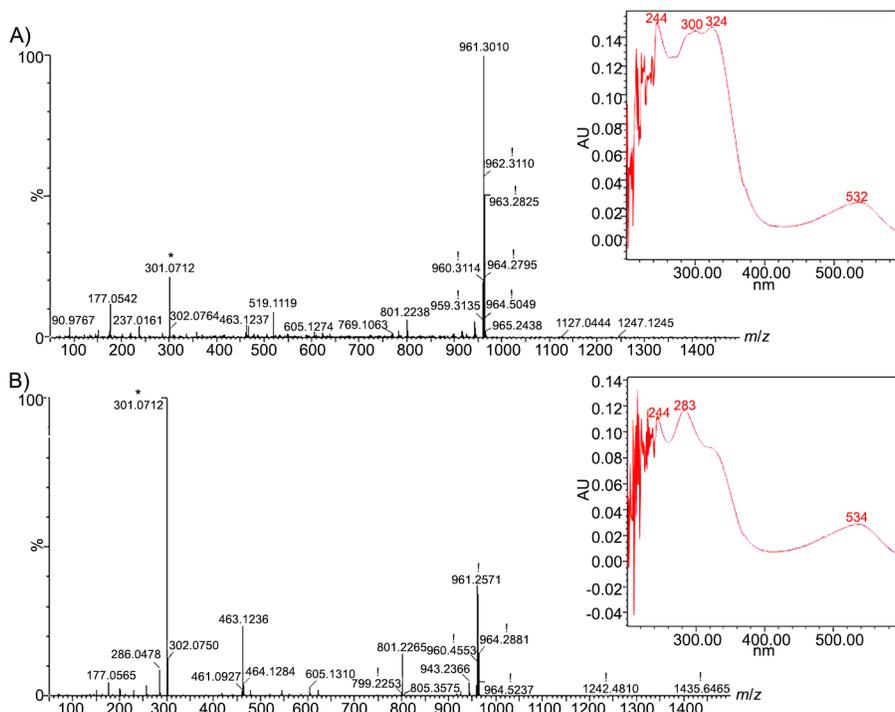


Figure 3 – High-resolution MS² (precursor ion *m/z* 961) and UV/Vis spectrum of two first time detected anthocyanins in PFSP: A) unknown anthocyanin (peonidin derivative), collected from peak 4; B) unknown anthocyanin (peonidin derivative), collected from peak 6.

[M-glycosyl]⁺ (C₄₇H₄₇O₂₂), 463.1238 [M-caffeoyl-feruloyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺ was identified as peonidin 3-*O*-(6''-*O*-caffeoyl-6'''-*O*-feruloyl sophoroside)-5-*O*-glucoside as in previous studies (Hu et al., 2016; Islam et al., 2002; Jie et al., 2013; Montilla et al., 2010; Otake et al., 1992; Terahara et al., 1999; Truong et al., 2010; Truong et al., 2012; Wang et al., 2017; Xu et al., 2015; Ying et al., 2011; Zhang et al., 2018; Zhu et al., 2017).

At peak eight, with intense caffeic acid bands at UV/Vis spectrum as is common in di-acylated anthocyanins, four compounds were observed and distinguished by MS² precursor ions *m/z* 1111, 1099, 949 and 963 (Table 1). The first presented M⁺ *m/z* 1111.3011, which corresponded to M⁺ = [C₅₂H₅₅O₂₇]⁺, with fragments *m/z* 949.2415 [M-glycosyl]⁺ (C₄₆H₄₅O₂₂), 463.1253 [M-dicaffeoyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺, identified as peonidin 3-*O*-(6''-6'''-*O*-dicaffeoyl sophoroside)-5-*O*-glucoside as in other works (Grass et al., 2017; He et al., 2016; Hu et al., 2016; Islam et al., 2002; Lee et al., 2013; Montilla et al., 2010; Terahara et al., 1999; Tian et al., 2005; Truong et al., 2010; Truong et al., 2012; Wang et al., 2017). The second compound with M⁺ *m/z* 1099.3037, related to M⁺ = [C₅₁H₅₅O₂₇]⁺, with fragments 937.2398 [M-glycosyl]⁺ (C₄₅H₄₅O₂₂), 463.1247 [M-caffeoyl-vaniloyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺, was peonidin 3-*O*-(6-*O*-caffeoyl-6-*O*-vaniloyl sophoroside)-5-*O*-glucoside, first reported by Truong et al. (2010) when the same fragmentation was detected and tentatively

identified by He et al. (2016). The third one with M⁺ *m/z* 949.2988, which corresponds to M⁺ = [C₄₃H₄₉O₂₄]⁺, with fragments 787.2139 [M-glycosyl]⁺ (C₃₇H₃₉O₁₉), 463.1235 [M-caffeoyl-sophorosyl]⁺ and *m/z* 301.0712 [Peonidin]⁺, was identified as peonidin 3-*O*-(6''-*O*-caffeoyl sophoroside)-5-*O*-glucoside, with the caffeoyl on carbon 6'' as in other studies (Goda et al., 1997; Hu et al., 2016; Islam et al., 2002; Jie et al., 2013; Lee et al., 2013; Montilla et al., 2010; Qiu et al., 2009; Tian et al., 2005; Truong et al., 2010; Truong et al., 2012; Wang et al., 2017; Xu et al., 2015; Zhu et al., 2017). The fourth presented M⁺ *m/z* 963.3345, that corresponds to M⁺ = [C₄₄H₅₁O₂₄]⁺, with fragments *m/z* 801.2732 [M-glycosyl]⁺, 639.2275, 579.2079, 513.1323 and 301.0712 [Peonidin]⁺, as an unidentified anthocyanin with fragmentation different from the one of the same M⁺ *m/z* reported in other works (Hu et al., 2016; Jie et al., 2013; Lee et al., 2013; Tian et al., 2005; Truong et al., 2010; Truong et al., 2012; Wang et al., 2017; Xu et al., 2015; Zhu et al., 2017).

At the separated peak nine, with intense caffeic and *p*-hydroxybenzoic acid bands at UV/Vis spectrum as in di-acylated anthocyanins (Table 1), an anthocyanin with M⁺ *m/z* 1069.2830, related to M⁺ = [C₅₀H₅₃O₂₆]⁺ (MS² precursor ion *m/z* 1069), fragments 907.2289 [M-glycosyl]⁺ (C₄₄H₄₃O₂₁) which corresponds to the loss of a glucose unit (in anthocyanin carbon at the 5 position) as is characteristic in PFSP anthocyanins and not to the loss of a caffeic acid also with -162 amu as explained

by Oliveira et al. (2019a), 463.1239 [M-caffeoyl-*p*-hydroxybenzoyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺ was observed and identified as peonidin 3-*O*-(6-*O*-*p*-hydroxybenzoyl-6-*O*-caffeoyl sophoroside)-5-*O*-glucoside (Grass et al., 2017; Hu et al., 2016; Islam et al., 2002; Jie et al., 2013; Montilla et al., 2010; Terahara et al., 1999; Tian et al., 2005; Truong et al., 2010; Truong et al., 2012; Xu et al., 2015; Ying et al., 2011; Zhang et al., 2018; Zhu et al., 2017).

At peak ten, with intense hydroxycinnamic acid derivative UV/Vis spectrum bands (Table 1), as is characteristic of di-acylated anthocyanins, two anthocyanins were detected (MS² precursor ions *m/z* 1123 and 1125). One with M⁺ *m/z* 1125.3123 which corresponds to M⁺ = [C₅₃H₅₇O₂₇]⁺, and fragments *m/z* 963.2538 [M-glycosyl]⁺ (C₄₇H₄₇O₂₂), 463.1229 [M-feruloyl-caffeoyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺ was identified as peonidin 3-*O*-(6-*O*-feruloyl-6-*O*-caffeoyl sophoroside)-5-*O*-glucoside, similar to the anthocyanin found at peak seven, probably because the caffeoyl and feruloyl moiety switched their positions. The other anthocyanin was detected for the first time in PFSP with M⁺ *m/z* 1123.2932 with fragments *m/z* 961.2393 [M-glycosyl]⁺, 943.2283, 605.1298, 479.0979, 301.0712 [Peonidin]⁺ (Figure 4). Zhu et al. (2017) detected a similar M⁺ *m/z* 1123 with some similar fragments, but they did not detect the fragment [Peonidin]⁺, consequently, it is not possible to state that it is the same compound.

At peak eleven, an anthocyanin was observed with the coumaroyl and hydroxybenzoyl UV/Vis bands and a low intensity band of anthocyanin that characterized di-acylation (Table 1). It had M⁺ *m/z* 1053.2903 (MS² precursor ion *m/z* 1053), that corresponds to M⁺ = [C₅₀H₅₃O₂₅]⁺, with fragments 891.2363 [M-glycosyl]⁺ (C₄₄H₄₃O₂₀), 463.1245 [M-coumaroyl-*p*-hydroxybenzoyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺, identified as peonidin 3-*O*-(6-*O*-coumaroyl-6-*O*-*p*-hydroxybenzoyl sophoroside)-5-*O*-glucoside. It was recently described by Wang et al. (2017).

At peak twelve, an anthocyanin was observed with intense ferulic and *p*-hydroxybenzoyl acids UV/Vis bands (Table 1) as in di-acylated anthocyanins, with M⁺ *m/z* 1083.3026 (MS² precursor ion *m/z*

1083), which corresponds to M⁺ = [C₅₁H₅₅O₂₆]⁺, with fragments 921.2470 [M-glycosyl]⁺ (C₄₅H₄₅O₂₁), 463.1245 [M-*p*-hydroxybenzoyl-feruloyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺, identified as peonidin 3-*O*-(6-*O*-*p*-hydroxybenzoyl-6-*O*-feruloyl sophoroside)-5-*O*-glucoside. It was first reported by Truong et al. (2010), identified by Wang et al. (2017) and recently by Zhu et al. (2017).

At peak thirteen an anthocyanin was observed (Table 1), with the intense coumaroyl and feruloyl UV/Vis bands characteristic of di-acylated, M⁺ *m/z* 1109.3230 (MS² precursor ion *m/z* 1109), which corresponds to M⁺ = [C₅₃H₅₇O₂₆]⁺, fragments 947.2606 [M-glycosyl]⁺ (C₄₇H₄₇O₂₁), 463.1236 [M-coumaroyl-feruloyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺ identified as peonidin 3-*O*-(6-feruloyl-6-*p*-coumaroyl sophoroside)-5-*O*-glucoside like Tian et al. (2005) had identified.

Finally, at peak fourteen an anthocyanin was observed with the intense feruloyl UV/Vis bands (Table 1), characteristic of di-acylated, M⁺ *m/z* 1139.3292 (MS² precursor ion *m/z* 1139), which corresponds to M⁺ = [C₅₄H₅₉O₂₇]⁺, with fragments 977.2733 [M-glycosyl]⁺ (C₄₈H₄₉O₂₂), 463.1246 [M-diferuloyl-sophorosyl]⁺ and 301.0712 [Peonidin]⁺ identified as Peonidin 3-*O*-(6'',6''-*O*-diferuloyl sophoroside)-5-*O*-glucoside as identified by Wang (2017).

In certain cases it was not possible to indicate the positions of a number of radicals on carbons 6'' or 6''' in the sophorosyl of the anthocyanins (Figure 2) because no structural NMR analyses were performed and this information was not presented in the available literature.

It was possible to verify in the studied PFSP that the majority of the anthocyanins are the peonidin derivatives peonidin 3-*O*-(6''-*O*-feruloyl sophoroside)-*O*-5-glucoside followed peonidin 3-*O*-(6-*O*-feruloyl-6-*O*-caffeoyl sophoroside)-5-*O*-glucoside and peonidin 3-*O*-(6''-*p*-hydroxybenzoyl sophoroside)-5-*O*-glucoside. The anthocyanins first detected in this work should be investigated by other structural identification techniques that allow their molecular structures to be elucidated. The absence of non-acylated anthocyanins was noted, unlike any previously studied PFSP variety. The first reported anthocyanins in sweet potatoes in this study are

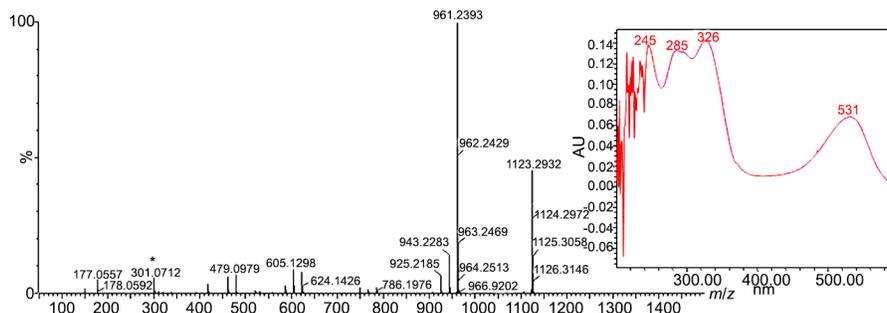


Figure 4 – High-resolution MS² (precursor ion *m/z* 1123) and UV/Vis spectrum of a first time detected anthocyanin (peonidin derivative) in purple-fleshed sweet potato (PFSP) collected from peak 10.

evidence that there are, as yet, an insufficient number of studies to describe all the PFSP anthocyanins. The PFSP variety studied in this work should be genetically different from those investigated in the available literature, which justifies a very differentiated profile of anthocyanins, including the presence of unknown anthocyanins. The organic cultivation had no influence on the biosynthesis of anthocyanins since it was detected the same major anthocyanins found in the references that used conventional cultivation.

Authors' Contributions

Conceptualization: Jesus, M.S.C.; Santiago, M.C.P.A.; Pacheco, S.; Gouvêa, A.C.M.S.; Nascimento, L.S.M.; Borguini, R.G.; Guerra, J.G.M.; Espindola, J.A.A.; Godoy, R.L.O. **Data acquisition:** Jesus, M.S.C.; Santiago, M.C.P.A.; Pacheco, S.; Nascimento, L.S.M.; Borguini, R.G. **Design of methodology:** Jesus, M.S.C.; Guerra, J.G.M.; Espindola, J.A.A.; Godoy, R.L.O. **Writing and editing:** Jesus, M.S.C.; Santiago, M.C.P.A.; Pacheco, S.; Gouvêa, A.C.M.S.; Nascimento, L.S.M.; Borguini, R.G.; Guerra, J.G.M.; Espindola, J.A.A.; Godoy, R.L.O.

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