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Phenolic Compounds and Carotenoids from Four Fruits Native from the Brazilian Atlantic Forest

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Supporting Information

ABSTRACT: Fruits from the Atlantic Forest have received increasing interest because they contain high levels of bioactive compounds with notable functional properties. The composition of carotenoids and phenolic compounds from fruits found in the Atlantic Forest (jussara, uvaia, araça, and grumixama) was determined by high-performance liquid chromatography coupled to diode array and mass spectrometry detectors. Uvaia showed the highest levels of carotenoids (1306.6 μg/100 g fresh matter (f.m.)). Gallic acid was the major phenolic compound in araça (12.2 mg GAE/100 g f.m.) and uvaia (27.5 mg GAE/100 g f.m.). In grumixama, eight quercetin derivatives were found; the main carotenoids included all-*trans*-β-cryptoxanthin (286.7 μg/100 g f.m.) and all-*trans*-lutein (55.5 μg/100 g f.m.). Uvaia and grumixama contain high amounts of carotenoids, while jussara showed greater levels of phenolic compounds (415 mg GAE/100 g f.m.), particularly anthocyanins (cyanidin 3-rutinoside: 179.60 mg/100 g f.m.; cyanidin 3-glucoside: 47.93 mg/100 g f.m.).

KEYWORDS: *carotenoids, anthocyanins, phenolic compounds, LC-MS, Euterpe edulis Mart., Eugenia brasiliensis Lam., Psidium cattleianum Sabine, Eugenia pyriformis Cambess*

INTRODUCTION

The Atlantic Forest is a large Brazilian biome present in 17 states, but currently occupies only 4% of its original territory.[1] Many well-known fruit species, such as guava (*Psidium guajava*) and jabuticaba (*Plinia trunciflora* O. Berg), are native to the Atlantic Forest. Other species are less popular and presently unexplored, including grumixama, araça, and uvaia. In addition, some species, such as palm jussara, are threatened by extinction due to deforestation and illegal trade.[2] Several public initiatives have been undertaken to improve fruit commercialization from the reforested Atlantic Forest; for example, fruits would be used in school meals, to provide economic and social development in the regions neighboring the forest.[1,2]

Jussara (*Euterpe edulis* Mart.) is a palm tree distributed throughout the Atlantic Forest and belongs to the Arecaceae family.[3] The fruit from this palm has a rounded shape and is dark purple due to its anthocyanin content.[3] It is similar to the açai berry in the context of its sensorial and nutritional properties, as well as ways of human consumption. Grumixama (*Eugenia brasiliensis* Lam.), araça (*Psidium cattleianum* Sabine), and uvaia (*Eugenia pyriformis* Cambess) belong to the family Myrtaceae; all of them are small, round fruits approximately 3.0 cm in diameter. Grumixama is commonly known as the Brazilian cherry; its peel is smooth and shiny, and its coloration ranges from to yellow to dark purple according to its stage of maturation due to the presence of carotenoids and anthocyanins.[4,5] Uvaia is yellowish-gold in color due to the presence of carotenoids, and its pulp has a pleasant aroma and flavor, making it suitable for preparing juices, jams, and jellies.[5] Araça has various common names, such as true araça, araçai, araça of the field, araça of Brazil, and others. It shows a smooth and shiny yellowish peel, and its fleshy pulp is whitish and surrounded by many small seeds.[4,5]

The search for exotic fruit, such as jussara, grumixama, uvaia, and araça, that taste good and have high levels of bioactive compounds has been intensified due to the inverse association between dietary patterns and the risk of developing chronic diseases, such as cancer, diabetes, coronary heart disease, and Alzheimer's.[6−9] Under normal circumstances, the human metabolism maintains the balance between oxidants and antioxidants to attain proper physiological homeostasis.[7,10] Overproducing reactive oxygen (ROS) and nitrogen species (RNS) can cause an imbalance, leading to oxidative and nitrosative stress; this situation is implicated in the development of chronic and degenerative diseases.[9,10] The inverse association is hypothetically attributed to the antioxidant properties of the bioactive compounds, which minimize the *in vivo* oxidative damages induced by ROS and RNS.[9,10]

Previous studies have revealed the presence of bioactive compounds in these fruits.[11−15] However, none of these studies identified or quantified the carotenoids individually. Furthermore, the identification of phenolic compounds by HPLC-DAD-MS^n was restricted to the anthocyanins in most cases. Thus, the aim of this work was to identify and quantify the carotenoids and phenolic compounds from four Atlantic Forest fruits (grumixama, jussara, araça, and uvaia) by high-perform-

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Table 1. Characterization and Proximate Composition of Atlantic Forest Fruit Pulps

fruit	pH	soluble solids (°Bx)	moisture (g/100g)	proteins[a] (g/100g)	lipids[a] (g/100g)	carbohydrates[a] (g/100g)	fibers[a] (g/100g)	ashes[a] (g/100g)	energetic values[a] (kcal/100g)
araça	4.3 ± 0.1	9.8 ± 0.1	85.5 ± 3.2	6.9 ± 0.1	1.4 ± 0.2	55.1 ± 3.4	31.0 ± 0.7	5.5 ± 0.2	260.7 ± 15.8
grumixama	4.2 ± 0.3	9.4 ± 0.1	90.8 ± 2.4	0.5 ± 0.1	n.d.[b]	82.6 ± 1.4	10.8 ± 0.5	5.4 ± 0.1	332.6 ± 8.7
jussara	5.6 ± 0.4	11.6 ± 0.1	88.7 ± 3.8	6.0 ± 0.3	29.2 ± 0.9	28.3 ± 3.5	28.3 ± 0.3	8.8 ± 0.8	400.0 ± 23.9
uvaia	3.7 ± 0.3	9.2 ± 0.2	91.0 ± 4.2	5.5 ± 0.8	2.2 ± 0.2	44.4 ± 5.5	42.2 ± 2.2	5.5 ± 0.9	213.3 ± 26.6

[a]Calculated values for dry basis. n = 3 samples. [b]n.d.: not detected.

ance liquid chromatography coupled to photodiode array and mass spectrometry detectors (HPLC-DAD-MS^n).

MATERIALS AND METHODS

Chemicals. HPLC-grade methanol, methyl *tert*-butyl ether (MTBE), acetonitrile, and formic acid were obtained from Merck (Darmstadt, Germany). The other reagents were all analytical grade and obtained from Labsynth (Diadema, Brazil). The samples and solvents were filtered through Millipore (Billerica, MA, USA) membranes (0.22 and 0.45 μm) before the HPLC analyses. The standards for all-*trans*-β-carotene (99.5%), all-*trans*-β-cryptoxanthin (99.0%), all-*trans*-lutein (98.0%), cyanidin 3-glucoside (98.9%), cyanidin 3-rutinoside (98.0%), and gallic acid (99%) were purchased from Sigma-Aldrich (Darmstadt, Germany). Standards of 9-*cis*- (99%), 13-*cis*- (98%), and 15-*cis*-β-carotene (97%) were provided by DSM Nutritional Products (Basel, Switzerland). The cyanidin 3-rhamnoside chloride (98%), cyanidin 3-sambubioside chloride (97%), and pelargonidin 3-glucoside chloride (98%) standards were obtained from Extrasynthése (Genay, France). The purity of these standards was determined by HPLC-DAD, and they were used as received.

Samples. The fruits were collected from the Atlantic Forest (Figure 1S). The samples of grumixama (*Eugenia brasiliensis* Lam.), jussara (*Euterpe edulis* Mart.), araça (*Psidium cattleianum* Sabine), and uvaia (*Eugenia pyriformis* Cambess) were obtained from Bello's farm, located in Paraibuna (latitude 23°23′10″ S and longitude 45°39′44″ W) in São Paulo State, Brazil. The fruits were harvested at ripe stage. They were part of batches sent to the fresh fruit market and were also used to obtain frozen pulp (farm produced). The fruits endured a selection and washing process before being pulped to remove any seeds or other inedible parts. The pulps were immediately frozen in farm and sent to the laboratory. The samples were stored at −40 °C until analysis. The harvest and sampling of the fruits were conducted between April and November of 2011. The fruit pulps were analyzed from a 3.0 kg single batch.

Characterization and Proximate Composition of Pulp. Each fruit pulp was thawed, homogenized, and characterized regarding pH and total soluble solids. The analyses of the moisture, protein (nitrogen conversion factor of 6.25 total protein), lipids, fiber, and ash contents were performed according to the official methods of AOAC.[16] The carbohydrate content of the samples was obtained using the difference between the total percentage of moisture, protein, lipids, fiber, and ash. The energy value was expressed in kcal/100 g and was calculated using the conversion factors of nutrients into energy (1 g of protein = 4 kcal, 1 g carbohydrate = 4 kcal, and 1 g of fat = 9 kcal). All analyses were performed in triplicate.

HPLC-DAD-MS^n Analysis of the Carotenoids, Anthocyanins, and Other Phenolic Compounds. The carotenoids, anthocyanins, and phenolic compounds were determined with a Shimadzu HPLC (Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), a DAD detector (Shimadzu, model SPD-M20A), a degasser unit (DGU-20A5), and a Rheodyne injection valve with a 20 μL loop. The equipment was also connected in series to a mass spectrometer (Esquire 4000 model) from Bruker Daltonics (Bremen, Germany) with an ion-trap analyzer (MS^n), as well as APCI (atmospheric pressure chemical ionization) and ESI (electrospray ionization) sources.

The carotenoids were exhaustively extracted with acetone from 15 g of fruit pulp and transferred to petroleum ether/diethyl ether (2:1); the solution was saponified overnight at room temperature with 10% methanolic KOH, washed until alkali free, and concentrated to dryness.[17,18] The experimental conditions for the separation, identification, and quantification by HPLC-DAD-APCI-MS^2 were the same as previously described.[17]

The following parameters were utilized to identify the carotenoids: elution order on a C_{30} reversed phase column, cochromatography with standards, UV/visible spectral features (λ_{max}, spectral fine structure (%III/II), and *cis* peak intensity (%A_B/A_{II})), and MS spectrum characteristics compared to standards analyzed under the same conditions and data available in the literature.[18−21] In addition, the protonated molecule ($[M + H]^+$) was assigned and confirmed using second-order MS fragmentation. The carotenoids were quantified by HPLC-DAD using five-point analytical curves of all-*trans*-lutein (1.0−50.0 μg/mL), all-*trans*-β-cryptoxanthin (1.0−60 μg/mL), and all-*trans*-β-carotene (1.0−50 μg/mL). The limit of detection (LOD) was calculated using the parameters of each standard curve: LOD = 3.3 × SD/S, where SD is the standard deviation of the response and S is the slope of the curve. For the three analytical curves of carotenoids, R^2 = 0.99, the limit of detection was 0.1 μg/mL, and the limit of quantification was 0.5 μg/mL. The carotenoid concentration was expressed in μg/100 g of fresh matter (f.m.). All analyses were performed in triplicate.

The anthocyanins were extracted from the grumixama and jussara pulps (20 g) using 100 mL of 0.5% HCl in methanol overnight at 5 °C under darkness. The slurry was filtered, and the solids were washed with an additional 100 mL of 0.5% HCl in methanol at room temperature three times. The acidic methanol extracts were combined and concentrated in a rotary evaporator (T < 38 °C) to yield the crude extract. The crude extract was diluted with water containing 5% formic acid/methanol (85:15, v/v) immediately before analysis by HPLC-DAD-MS^2. The anthocyanin separation was carried out as previously described.[22]

The anthocyanins were identified based on the combined information provided by elution order in the C_{18} reversed phase column and cochromatography with standards, as well as UV−visible and mass spectra in comparison with the literature data.[22−25] The anthocyanins were quantified using the percentage of the area obtained during the HPLC-DAD-ESI-MS^2 analysis and the monomeric anthocyanin content. The monomeric anthocyanin content was evaluated using the pH-differential method, as described by Lee et al.[26] The concentration was expressed in mg cyanidin 3-glucoside/100 g fresh matter or mg cyanidin 3-rutinoside/100 g fresh matter according to the major anthocyanin identified. All analyses were performed in triplicate.

The other phenolic compounds were extracted from 20 g of frozen pulp with 100 mL of methanol/water (8:2 v/v) by agitation provided by a magnetic homogenizer (Tecnal, Piracicaba, Brazil) for 20 min. The slurry was filtered, and the solids were washed with an additional 50 mL of methanol/water (8:2) two times more and concentrated in a rotary evaporator (T < 38 °C) until methanol evaporation. The extract was used to determine the phenolic compounds by HPLC-DAD-ESI-MS^n, as previously described.[27] The phenolic compounds were tentatively identified based on the following information: elution order, retention time, and UV−visible and mass spectral features compared to those of the standards analyzed under the same conditions and the available literature data.[27−29] The phenolic compounds were quantified using a seven-point analytical curve of gallic acid (0.5−60 mg/mL). The analytical curve was linear (r^2 = 0.99); the limit of detection was 0.2 μg/mL, and the limit of

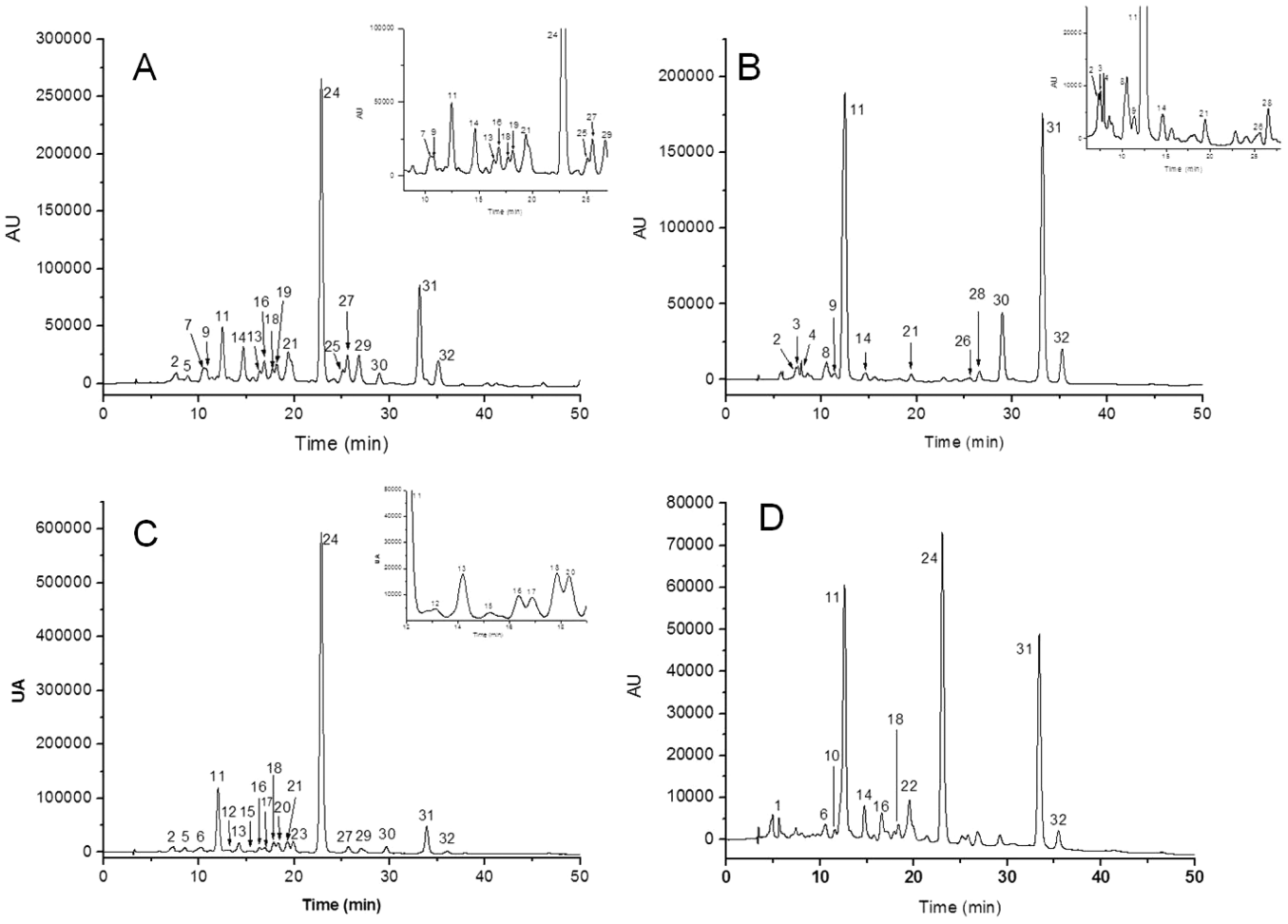

Figure 1. Chromatogram, obtained by HPLC-PDA-MS/MS, of the carotenoids from Atlantic Forest fruits: (A) uvaia, (B) jussara, (C) grumixama, and (D) araça. Chromatographic conditions: see text. Peak identification and characterization are given in Table 2. Processed at 450 nm.

quantification was 0.5 μg/mL as calculated using the parameters of the analytical curves (standard deviation and the slope). All analyses were performed in triplicate.

Statistical Analysis. The mean and standard deviation results related to the proximate composition and carotenoid and phenolic compound levels were calculated using the Microcal Origin 5.0 software.

RESULTS AND DISCUSSION

Characterization and Proximate Composition of the Fruit Pulp. The jussara fruit pulp had the highest pH (Table 1) and showed characteristics and chemical compositions similar to those of the established standards of identity and quality of açai.[30] Using the parameters of the Brazilian legislation, we might classify jussara as medium (type B) because the soluble solid content was between 11 and 14 degrees Brix. The levels of proteins, lipids, and carbohydrates of jussara, by dry weight, are consistent with the established legislation for açai pulp[30] (6.0 g protein/100 g; 20−60 g lipid/100 and 40 g total carbohydrates/100 g) since there is no current legislative regulation for jussara pulp. Moreover, our results show that the proximate composition of jussara from the state of São Paulo falls into the range of jussara samples obtained from five different regions in the state of Santa Catarina, Brazil.[11] However, the differences observed over the chemical composition of the pulp demonstrate that even jussara, which is native to the Atlantic Forest, is influenced by the soil, temperature, sunlight, and precipitation in the geographic location where the palms are grown.

The pulps under study were obtained from exotic fruits that have no current legislative regulation in Brazil, and only a few data for some fruits are available in the literature. The protein content of uvaia collected in the state of Rio Grande do Sul,[31] Brazil, was approximately 2.9 times higher than that measured in the present study, whereas soluble solid content (7.7°Bx)[11] was slightly lower than our results. In addition, the high fiber contents of these fruits are worth highlighting, particularly those from araça and jussara, because the consumption of a 100 g portion of fresh pulp of either fruit provides approximately 20% of the recommended daily intake of fiber, based on a 2000 kcal diet.

Carotenoid Composition. Figure 1 shows the chromatograms of the carotenoids from the four Atlantic Forest fruit pulps. The carotenoids separated from the jussara, grumixama, araça, and uvaia pulps were identified based on the combined information obtained from the chromatographic elution and UV−visible and mass spectral data (Table 2). Considering that a detailed description of carotenoid identification has already been reported,[18,22] only the most important aspects are discussed below.

As expected for the reversed phase columns, polar carotenoids with three and two hydroxyl groups, such as 9-*cis*-neoxanthin (peak 2) and all-*trans*-lutein (peak 11), eluted

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Table 2. Chromatographic, UV−Vis, and Mass Spectroscopy Characteristics of Carotenoids from Atlantic Forest Fruit, Obtained from HPLC-DAD-MS/MS

peak[a]	carotenoid[b]	t_R (min)	λ_{max} (nm)[c]	% III/II	% A_B/II	$[M + H]^+$ (m/z)	MS/MS (m/z)
1	apocarotenoid	6.1	441	0	0	n.d.[d]	n.d.
2	9-*cis*-neoxanthin	7.3	327, 415, 438, 468	87	24	601	583, 565, 547, 509, 491, 221
3	mixture	7.6	327, 415, 438, 468	80	21	601	583, 565, 547, 509, 491, 221
4	13-*cis*-violaxanthin	7.9	326, 412, 435, 463	83	23	601	583, 565, 509, 491, 221
5	*all-trans*-neochrome	8.5	399, 421, 447	71	0	601	583, 565, 221
6	mixture	10.2	328, 402, 425, 447	33	50	585	n.d.
7	*cis*-antheraxanthin	10.5	328, 418, 440, 466	68	23	585	567, 549, 493, 221
8	*cis*-lutein	10.5	353, 328, 438, 464	53	34	569	551
9	9-*cis*-violaxanthin	11.4	328, 415, 435, 461	83	35	601	583, 565, 509, 491, 221
10	mixture	12.1	420, 445, 472	67	0	601	n.d.
11	*all-trans*-lutein	12.3	420, 444, 472	57	0	569	551, 533, 477, 459
12	mixture	13.1	402, 427, 448	n.c.[e]	n.c.	545	n.d.
13	5,6-epoxy-β-cryptoxanthin	14.2	421, 448, 474	40	0	569	551, 459, 221
14	*all-trans*-zeaxanthin	14.6	427, 450, 475	30	0	569	551, 533, 463
15	*cis*-lutein	15.2	353, 418, 439, 466	50	n.c.	n.d.	n.d.
16	5,8-epoxy-β-cryptoxanthin	16.3	401, 426, 453	75	0	569	551, 459, 221
17	15-*cis*-β-cryptoxanthin	16.9	335, 422, 447, 470	33	57	553	535, 495, 461
18	13-*cis*-β-cryptoxanthin or 13'-cis-β-cryptoxanthin	17.8	338, 418, 444, 470	23	50	553	535, 495, 461
19	phytoene	18.1	276, 286, 300	0	0	545	489, 435, 339
20	13-*cis*-β-cryptoxanthin or	18.3	336, 420, 444, 469	17	51	553	535, 495, 461
21	*all-trans*-zeinoxanthin	19.3	421, 447, 471	20	0	553	535, 495, 443
22	*all-trans*-α-cryptoxanthin	19.4	420, 444, 472	50	0	553	535, 461
23	not identified	19.9	405, 427, 452	50	0	569	551, 221
24	*all-trans*-β-cryptoxanthin	22.8	421, 450, 477	22	0	553	535, 497, 461
25	5,8-epoxy-β-carotene	25.1	408, 427, 452	66	0	553	535, 461, 205
26	15-*cis*-β-carotene	25.6	337, 420, 448, 471	15	61	537	444
27	9-*cis*-β-cryptoxanthin or 9'-cis-β-cryptoxanthin	25.7	339, 419, 446, 471	40	14	553	535, 497, 461
28	13-*cis*-β-carotene	26.5	335, 420, 444, 471	20	44	537	444
29	9-*cis*-β-cryptoxanthin or 9'-cis-β-cryptoxanthin	27.0	339, 420, 446, 471	37	17	553	535, 497, 461
30	*all-trans*-α-carotene	29.7	420, 445, 472	60	0	537	481, 444
31	*all-trans*-β-carotene	33.9	425, 451, 478	25	0	537	444
32	9-*cis*-β-carotene	36.0	338, 421, 447, 471	66	18	537	444

[a]Numbered according to the chromatograms shown in Figure 1. [b]Tentative identification based on UV−vis and mass spectra as well as relative HPLC retention times and published data. [c]Linear gradient of methanol/MTBE. [d]n.d.: not detected. [e]n.c.: not calculated.

before the monohydroxy carotenoids (all-*trans*-β-cryptoxanthin, peak 24). The carotenes, including the all-*trans*-α-carotene (peak 30) and all-*trans*-β-carotene (peak 31), were the last to elute under these conditions. The carotenoids with 5,6-epoxide and/or 5,8-furanoid groups located on the 3- or 3′-hydroxy β-rings, 9-*cis*-neoxanthin (peak 2), 13-*cis*-violaxanthin (peak 4), and 9-*cis*-violaxanthin (peak 9) showed UV−vis and mass spectra features similar to those reported in the literature.[18−21]

Although the all-*trans*-lutein (peak 11) and all-*trans*-zeaxanthin (peak 14) have the same chemical formula ($C_{40}H_{56}O_2$) and therefore identical protonated molecule $[M + H]^+$ at m/z 569, zeaxanthin possesses two β-rings, while lutein has one β-ring and one ε-ring. Therefore, one of the lutein hydroxyl groups is allylic to the double bond in the ε-ring and not conjugated with the polyene chain, resulting in 10 conjugated double bonds (c.d.b.). However, zeaxanthin has both double bonds in the β-ring conjugated to the polyene chain; consequently, its chromophore displays 11 c.d.b. Therefore, it is possible to distinguish these two compounds using their UV−visible and mass spectra. As expected, zeaxanthin showed λ_{max} values (427, 450, 475 nm) higher than those of lutein (420, 444, 472 nm). The mass spectrum of zeaxanthin showed a more intense protonated molecule peak ($[M + H]^+$) at m/z 569 compared to the fragment at m/z 551 $[M + H - 18]^+$, while the opposite was observed for lutein.[18,21,22]

Peak 24 was identified as all-*trans*-β-cryptoxanthin; the protonated molecule was detected at m/z 553 along with less intense fragments at m/z 535 $[M + H - 18]^+$ and 461 $[M + H - 92]^+$, generated from the loss of a hydroxyl group and toluene, respectively. The isomers 15-*cis*-β-cryptoxanthin (peak 17), 13- or 13′-*cis*-β-cryptoxanthin (peaks 18 and 20), and 9- or 9′-*cis*-β-cryptoxanthin (peaks 27 and 29) were tentatively identified by comparing their elution order and UV−vis characteristics with the literature data.[22] The fine spectral structure (%III/II) decreases and the intensity of the *cis*-peak (%A_B/A_{II}) increases as the *cis*-double bond gets closer to the center of the molecule. However, it was not possible to differentiate between the 13-*cis* and 13′-*cis* or between the 9-*cis* and 9′-*cis* isomers of β-cryptoxanthin because there are no data available in the literature reporting their elution order on a C_{30} column after assignment of the *cis*-double bond positions in the β-cryptoxanthin structure using nuclear magnetic resonance. All-*trans*-β-carotene (peak 31) and its *cis* isomers (15-*cis*- (peak 26), 13-*cis*- (peak 28), and 9-*cis*-β-carotene (peak 32)) were confirmed via coelution with authentic standards, as well as comparison of the UV−vis and mass spectral features with standards and literature data.[18,21,22]

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Table 3. Content of Carotenoids from Four Atlantic Forest Fruits

		concentration (μg/100 g fresh weight)[a]			
peak	carotenoid	grumixama	jussara	araça	uvaia
1	apocarotenoid	n.d.	n.d	1.9 ± 0.01	n.d.
2	9-*cis*-neoxanthin[b]	14.7 ± 8.6	13.2 ± 4.2	n.d.	22.8 ± 0.3
3	mixture[b]	n.d.	4.4 ± 2.9	n.d.	n.d.
4	13-*cis*-violaxanthin[b]	n.d.	6.5 ± 4.3	n.d.	n.d.
5	*all-trans*-neochrome[b]	3.2 ± 1.8	n.d.	n.d.	11.7 ± 1.8
6	mixture[b]	7.3 ± 2.0	n.d.	0.6 ± 0.1	n.d.
7	*cis*-antheraxanthin[b]	n.d.	n.d.	n.d.	22.1 ± 1.8
8	*cis*-lutein[b]	n.d.	12.6 ± 1.3	n.d.	n.d.
9	9-*cis*-violaxanthin[b]	n.d.	5.5 ± 0.4	n.d.	19.4 ± 7.6
10	mixture[b]	n.d.	n.d.	0.7 ± 0.1	n.d.
11	*all-trans*-lutein[b]	55.5 ± 12.89	292.7 ± 3.3	15.7 ± 3.8	86.0 ± 7.8
12	mixture[c]	1.1 ± 1.0	n.d.	n.d.	n.d.
13	5,6-epoxy-β-cryptoxanthin[c]	13.6 ± 1.1	n.d.	n.d.	24.3 ± 1.1
14	all-*trans*-zeaxanthin[c]	n.d.	5.4 ± 2.4	1.7 ± 0.4	56.0 ± 4.4
15	*cis*-lutein[b]	0.2 ± 0.01	n.d.	n.d.	n.d.
16	5,8-epoxyi-β-cryptoxanthin[c]	9.3 ± 1.6	n.d.	3.5 ± 0.9	36.5 ± 5.4
17	15-*cis*-β-cryptoxanthin[c]	14.4 ± 8.7	n.d.	n.d.	n.d.
18	13-*cis*-β-cryptoxanthin[c] or 13′-*cis*-β-cryptoxanthin[c]	26.8 ± 2.4	n.d.	n.d.	22.8 ± 2.4
19	phytoene[d]	n.d.	n.d.	0.6 ± 0.1	5.9 ± 0.5
20	13-*cis*-β-cryptoxanthin[c] or 13′-*cis*-β-cryptoxanthin[c]	9.5 ± 4.1	n.d	n.d	n.d
21	*all-trans*-zeinoxanthin[c]	7.4 ± 5.5	7.7 ± 0.4	n.d	78.4 ± 5.9
22	*all-trans*-α-cryptoxanthin[c]	n.d.	n.d.	3.6 ± 1.0	n.d.
23	not identified[c]	11.6 ± 1.3	n.d.	n.d.	n.d.
24	*all-trans*-β-cryptoxanthin[c]	286.7 ± 123.3	n.d.	26.4 ± 1.9	521.1 ± 18.6
25	5,8-epoxy-β-carotene[d]	n.d.	n.d.	n.d.	32.9 ± 1.9
26	15-*cis*-β-carotene[d]	n.d.	9.2 ± 0.3	n.d.	n.d.
27	9-*cis*-β-cryptoxanthin[c] or 9′-*cis*-β-cryptoxanthin[c]	7.1 ± 2.6	n.d.	n.d.	57.9 ± 1.3
28	13-*cis*-β-carotene[d]	n.d.	15.8 ± 1.9	n.d.	n.d.
29	9-*cis*-β-cryptoxanthin[c] or 9′-*cis*-β-cryptoxanthin[c]	6.2 ± 2.5	n.d.	n.d.	68.1 ± 0.01
30	*all-trans*-α-carotene[d]	4.2 ± 1.3	60.2 ± 6.0	n.d.	14.2 ± 1.2
31	*all-trans*-β-carotene[d]	31.1 ± 15.8	266.5 ± 41.5	20.0 ± 4.6	170.9 ± 11.3
32	9-*cis*-β-carotene[d]	5.3 ± 0.3	37.8 ± 3.5	3.0 ± 2.9	56.5 ± 3.0
	total carotenoids	515.2 ± 195.8	737.5 ± 72.8	77.7 ± 15.7	1306.6 ± 73.3
	vitamin A value (μg RAE/100 g)[e]	18.4 ± 7.7	27.8 ± 4.0	3.2 ± 0.7	53.6 ± 2.3

[a] $n = 3$ samples. n.d.: not detected. [b] The peaks were quantified as equivalent to all-*trans*-lutein. [c] The peaks were quantified as equivalent to all-*trans*-β-cryptoxanthin. [d] The peaks were quantified as equivalent to all-*trans*-β-carotene. [e] RAE: retinol activity equivalent.

According to Table 3, the total carotenoid content was highest in uvaia, followed by jussara and grumixama, and finally araça. The HPLC chromatogram separating the 18 carotenoids from uvaia is shown in Figure 1A. The all-*trans*-β-cryptoxanthin was the major carotenoid, representing 40% (m/m) of the total carotenoids content in this fruit pulp, followed by all-*trans*-β-carotene, which contributed 13% (m/m), in agreement with those reported in the literature for uvaia harvested in Rio Grande do Sul (South Brazil).[31] The levels of total carotenoids found in this study were lower than those previously reported (1700 μg/100 g fresh weight[32] for uvaia collected in Ceará (northwest region of Brazil)).

A typical HPLC-DAD chromatogram depicting the carotenoids from jussara is presented in Figure 1B, demonstrating the separation of 13 carotenoids on the C_{30} column. All-*trans*-lutein was the major carotenoid (39%, m/m) followed by all-*trans*-β-carotene (36%, m/m). The total carotenoid content found in the present study (737.5 μg/100 g) was 2.6 times lower than that found previously in jussara (1900 μg/100 g fresh matter) collected in Santa Catarina (South Brazil).[32] As far as we are concerned, this is the first report on the carotenoid profile of jussara.

Figure 1C shows the chromatogram of the carotenoids from grumixama, with 19 separated carotenoids, the principal ones being all-*trans*-β-cryptoxanthin (56%, m/m) and all-*trans*-lutein (11%). Studies regarding the carotenoid composition in grumixama were not found in the literature.

As observed in Figure 1D, 11 carotenoids from araça were separated on a C_{30} column, and nine were identified. The all-*trans*-β-cryptoxanthin was the major carotenoid, representing 34% of the total carotenoid content in this fruit, followed by all-*trans*-β-carotene and all-*trans*-lutein, corresponding to 26% and 20% of the total content, respectively. The other six minor carotenoids identified corresponded to 18.4% of the total content. This is the first report of the carotenoid composition of araça using mass spectrometry. In another study lutein has

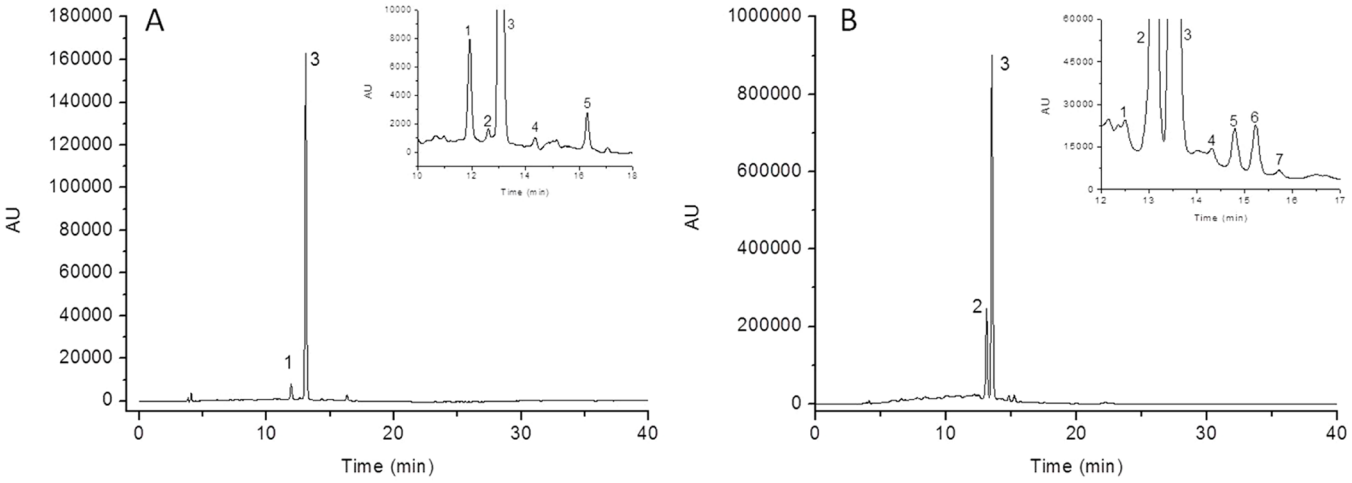

Figure 2. Chromatogram, obtained by HPLC-PDA-MS, of the anthocyanins from Atlantic Forest fruits: (A) grumixama and (B) jussara. Chromatographic conditions: see text. Peak identification and characterization are given in Table 4. Processed at 520 nm.

Table 4. Chromatographic and Spectroscopic Characteristics and Composition of Anthocyanins from Grumixama and Jussara, Obtained by HPLC-DAD-MS/MS

peak[a]	t_R (min)	λ_{max} (nm)[b]	$[M]^+$ (m/z)	MS/MS (m/z)	compound	concentration[c] (mg C3G/100 g)
Grumixama (*Eugenia brasiliensis*)						
1	11.9	228, 526	465	303 $[M + H - 162]^+$	delphinidin 3-glucoside	0.10 ± 0.01
2	12.6	270, 508	449	287 $[M + H - 162]^+$	cyanidin 3-galactoside	0.01 ± 0.001
3	13.1	280, 520	449	287 $[M + H - 162]^+$	cyanidin 3-glucoside	2.73 ± 0.02
4	14.3	271, 520	n.d.[d]	n.d.	n.i.[e]	0.01 ± 0.001
5	16.3	270, 518	491	287 $[M + H - 204]^+$	cyanidin 3-acetyl-hexoside	0.05 ± 0.004
monomeric anthocyanins (mg C3G/100 g)						2.90 ± 0.1
Jussara (*Euterpe edulis*)						
1	12.6	279, 521	581	449 $[M + H - 132]^+$, 287 $[M + H - 132 - 162]^+$	cyanidin 3,5-hexose pentose	1.43 ± 0.05
2	13.1	280, 520	449	287 $[M + H - 162]^+$	cyanidin 3-glucoside	47.93 ± 1.52
3	13.5	280, 522	595	449 $[M + H - 146]^+$, 287 $[M + H - 146 - 162]^+$	cyanidin 3-rutinoside	179.60 ± 5.77
4	14.5	278, 515	433	271 $[M + H - 162]^+$	pelargonidin 3-glucoside	1.66 ± 0.05
5	14.8	280, 509	579	433 $[M + H - 146]^+$, 271 $[M + H - 146 - 162]^+$	pelargonidin 3-rutinoside	2.87 ± 0.09
6	15.2	278, 522	609	463 $[M + H - 146]^+$, 301 $[M + H - 146 - 162]^+$	peonidin 3-rutinoside	3.59 ± 0.11
7	15.7	294, 524	433	287 $[M + H - 146]^+$	cyanidin 3-rhamnoside	0.30 ± 0.01
monomeric anthocyanins (mg C3R/100 g)						239.16 ± 7.6

[a]Peaks numbered according to Figure 2. [b]Linear gradient of water 5% v/v formic acid/methanol. C3G, cyanidin 3-glucoside; C3R, cyanidin 3-rutinoside. [c]Calculated using the percentage of the area and the content of monomeric anthocyanins. [d]n.d.: not detected. [e] n.i.: not identified.

been identified as the major carotenoid in araça (26.38 μg/g dry bases),[31] using only HPLC-DAD.

According to the classifications of good sources of carotenoids (low: 0−100 μg/100 g; moderate: 100−500 μg/100; high: 500−2000 μg/100 g; and very high: >2000 μg/100 g) proposed by Britton and Khachik,[33] uvaia and grumixama are high sources of carotenoids, particularly of β-cryptoxanthin (all-*trans* + *cis* isomers), while jussara is a moderate source of all-*trans*-lutein. However, araça is not a good source of carotenoids. Among the Atlantic Forest fruits evaluated in this study, the provitamin A activity value was higher in uvaia and jussara (Table 3). However, none of the fruits studied can be considered a good source of provitamin A compared with the Amazonian fruits[18] and acerola cultivar Olivier.[17]

Anthocyanin Composition. A typical chromatogram of the anthocyanins from grumixama is shown in Figure 2A, and their characteristics are presented in Table 4. The analysis performed by HPLC-DAD-MS/MS separated five anthocyanins. Peak 1 was identified as delphinidin 3-glucoside. This anthocyanin had a molecular ion at m/z 465 and a 303 u fragment related to the loss of a hexose; the identity of the hexose was assumed to be glucose according to the elution order.[24,25] Peaks 2 and 3 were identified as cyanidin 3-galactoside and cyanidin 3-glucoside, respectively. Both anthocyanins have a molecular ion at m/z 449 and a fragment at m/z 287 that is related to the loss of a hexose. This identification was confirmed using the elution order[24] and the coelution of peak 3 with a cyanidin 3-glucoside standard. The molecular ion and MS/MS fragments were not detected in peak 4, and thus this peak was not identified. Peak 5 exhibited a molecular ion at m/z 491, and in the MS/MS data, only a 287 u fragment ion was detected, indicating the presence of cyanidin after the loss of either an acetyl hexose or an oxalyl pentose moiety (204 u). Considering the occurrence of acylated anthocyanins in foods,[24,25] peak 5 was tentatively identified as cyanidin (acetyl)hexoside.

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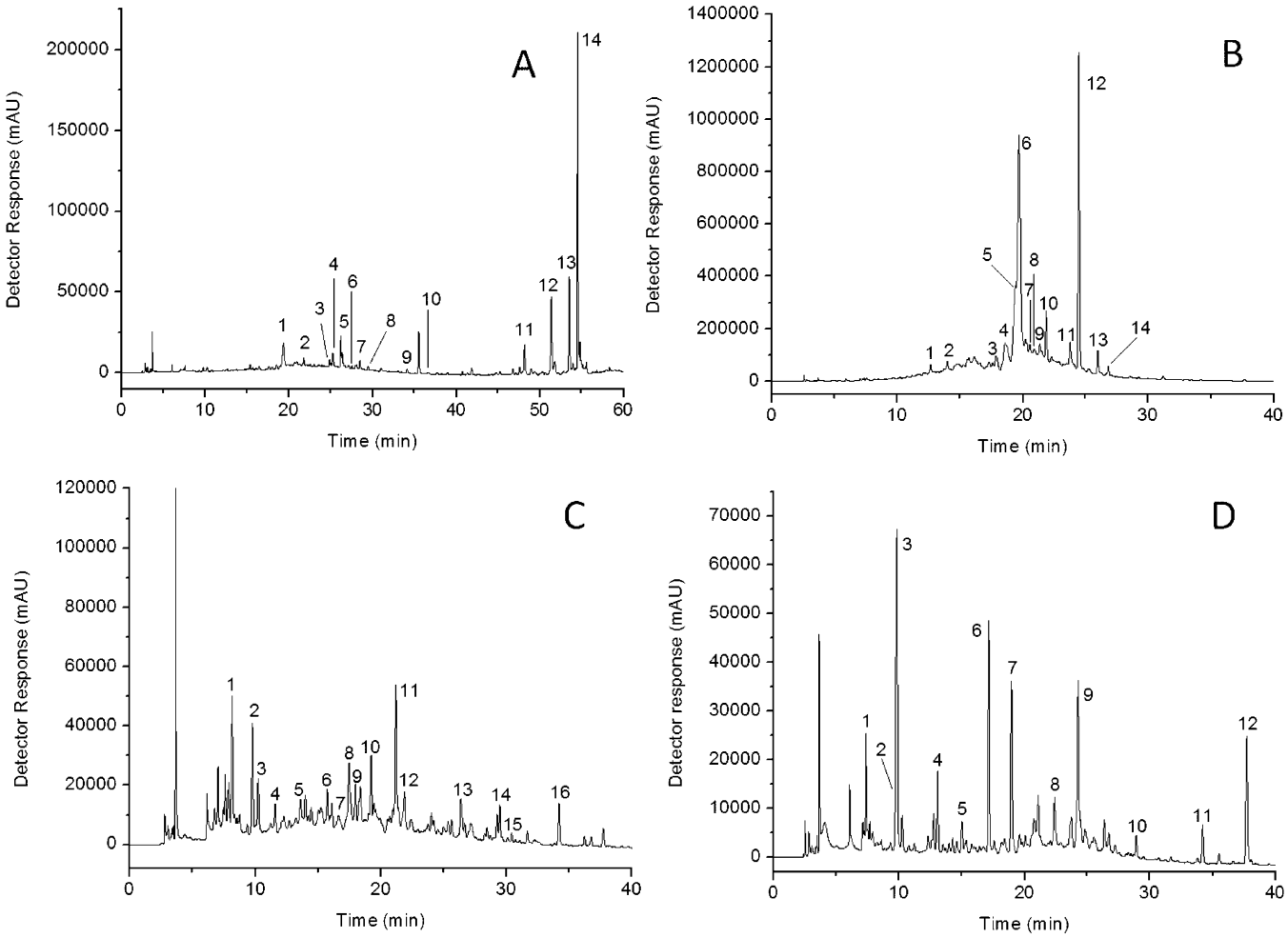

Figure 3. Chromatogram, obtained by HPLC-PDA-MS, of the phenolic compounds from Atlantic Forest fruits: (A) grumixama, (B) jussara, (C) araça, and (D) uvaia. Chromatographic conditions: see text. Peak identification and characterization are given in Tables 5 and 6. Processed at 280 nm.

The major anthocyanins found in grumixama were cyanidin 3-glucoside (94%) and delphinidin 3-glucoside (3.5%); the other anthocyanins (peaks 2, 4, and 5) were detected in amounts below 1.5%. These two major anthocyanins and cyanidin 3-galactoside are coincident with those reported in the literature;[13] however, cyanidin 3-acetylhexoside was not found by Flores et al.[13] and is therefore reported in grumixama for the first time. Cyanidin 3-arabinoside, delphinidin 3-pentoside, cyanidin 3-xiloside, malvidin 3-glucoside, and anthocyanidins delphinidin and cyanidin were detected in small amounts in grumixama by Flores et al.[13] but were not found in the present study. The monomeric anthocyanin level found in the present study was lower than that reported by Haminiuk et al.[14] for grumixama fruits collected in Brazil (266.34 ± 2.92 mg C3G/100 g).

The typical chromatogram of jussara shows the separation of seven anthocyanins (Figure 2B), and their characteristics are presented in Table 4. Peak 1 had a molecular ion peak at m/z 581, and two mass fragments were detected: one at m/z 449 related to the loss of one molecule of pentose ($[M - 132]^+$) and another at m/z 287 generated by elimination of one molecule of pentose plus one hexose ($[M - 132 - 162]^+$), furnishing a cyanidin aglycone with 287 u. Due to the presence of the fragment with 449 u, anthocyanin 1 was characterized as cyanidin 3,5-diglycosilated with a hexose and a pentose as the monosaccharides. However, the exact position of these two sugars could not be determined by MS. The identification as cyanidin 3-sambubioside could be excluded because two MS/MS fragments at m/z 287 and 449 were detected.[23]

In the mass spectrum of peak 2, the molecular ion was observed at m/z 449 with a fragment ion with 287 u ($[M - 162]^+$) by second-order MS, corresponding to the cyanidin moiety after hexose loss (162 u). The sugar moiety was confirmed to be glucose by coelution of peak 2 with the cyanidin 3-glucoside standard. Peak 3 showed a molecular ion at m/z 595 and two mass fragments with 287 and 449 u; the first corresponded to the cyanidin moiety after rutinose loss (308 u), and the fragment at m/z 449 resulted from the loss of deoxyhexose $[M - 146]^+$. The only disaccharide that showed fragmentation by MS/MS due to cleavage of the glycosidic bonds between the monosaccharides is rutinose, as previously described in the literature.[23−25] Cyanidin 3-rutinoside was definitively identified by coelution with a standard.

The mass spectrum of peak 4 showed a molecular ion at m/z 433 and an MS/MS fragment at m/z 271, corresponding to pelargonidin after the loss of a hexose. The identification of this anthocyanin was confirmed as pelargonidin 3-glucoside by coeluting peak 4 with the pelargonidin 3-glucoside standard. Peaks 5 and 6 were identified as pelargonidin 3-rutinoside and peonidin 3-rutinoside, respectively. The mass spectra of both

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Table 5. Chromatographic and Spectroscopic Characteristics and Content of Phenolic Compounds from Grumixama and Jussara, Obtained from HPLC-DAD-ESI-MS/MS

peak	t_R[a] (min)	phenolic compound[b]	λ_{max}[c] (nm)	$[M - H]^-$ (m/z)	MS/MS (m/z)	concentration[d] (mg GAE/100 g)
Grumixama						
1	19.3	cyanidin 3-glucoside	279, 518	447	285 $[M - H\text{-}162]^-$	5.0 ± 0.2
2	21.8	quercetin glucorunide	360	477	301 $[M - H - 176]^-$	1.3 ± 0.04
3	24.8	quercetin galloyl-hexoside	360	615	463 $[M - H - 152]^-$, 301 $[M - H - 152 - 162]^-$	1.6 ± 0.02
4	25.2	quercetin deoxy-hexoside	361	447	301 $[M - H - 146]^-$	1.2 ± 0.04
5	26.2	quercetin hexoside	355	463	301 $[M - H - 162]^-$	6.7 ± 0.4
6	27.5	quercetin pentoside	353	433	301 $[M - H - 132]^-$	2.8 ± 0.9
7	28.4	quercetin pentoside	352	433	301 $[M - H - 132]^-$	6.6 ± 0.9
8	29.4	eriodictyol hexoside	285, 360	449	287 $[M - H - 162]^-$, 151	0.8 ± 0.04
9	34.1	quercetin deoxyhexosyl-hexoside	353	609	463 $[M - H - 146]^-$, 301$[M - H - 162]^-$	1.4 ± 0.02
10	36.6	quercetin	365	301	273 $[M - H - 28]^-$, 257 $[M - H - 44]^-$, 229, 179, 151	10.7 ± 1.1
11	48.2	synapic acid dihexoside hydroxy benzoyl	277, 340	685	667 $[M - H - 18]^-$, 461 $[M - H - 224]^-$, 299 $[M - H - 224 - 162]^-$, 137 $[M - H - 224 - 162 - 162]^-$	7.2 ± 0.3
12	51.4	not identified	280, 335	699	681 $[M - H - 18]^-$, 461 $[M - H - 18 - 220]^-$, 299 $[M - H - 18 - 220 - 162]^-$, 137 $[M - H - 18 - 220 - 162 - 162]^-$	17.6 ± 1.0
13	53.5	gallic acid dihexoside sinapoyl	280, 330	699	493 $[M - H - 206]^-$, 331 $[M - H - 206 - 162]^-$, 169 $[M - H - 206 - 162 - 162]^-$	19.6 ± 1.1
14	54.5	sinapic acid dihexoside benzoyl	282, 335	651	633 $[M - H - 18]^-$, 427 $[M - H - 224]^-$, 265 $[M - H - 224 - 162]^-$, 103 $[M - H - 224 - 162 - 162]^-$	62.8 ± 2.5
total phenolic						145.2 ± 8.6
Jussara						
1	12.6	gallic acid hexoside	280	331	169 $[M - H - 162]^-$	1.7 ± 0.04
2	14.0	not identified	281	359	197 $[M - H - 162]^-$	8.8 ± 0.4
3	17.8	apigenin dihexoside	279, 323	593	417 $[M - H - 162]^-$, 269 $[M - H - 162]^-$	11.06 ± 0.9
4	18.6	luteolin deoxyhexosyl-hexoside	281, 310	593	285 $[M - H - 308]^-$	37.6 ± 1.9
5	19.4	cyanidin 3-glucoside	280, 517	447	285 $[M - H - 162]^-$	51.4 ± 3.1
6	19.7	cyanidina 3-rutinoside	280, 518	593	575, 467, 299, 285 $[M - H - 308]^-$	141.0 ± 8.5
7	20.6	apigenin hexoside	279, 325	431	269 $[M - H - 162]^-$, 253, 225	13.2 ± 1.0
8	20.9	apigenin deoxyhexoside-hexoside	280, 328	577	269$[M\text{-}308]^-$, 253, 225	25.4 ± 1.5
9	21.3	chrysoeriol deoxyhexosyl-hexoside	280, 320	607	299 $[M - H - 308]^-$, 285, 255, 239	22.5 ± 0.7
10	21.9	taxifolin hexoside	289, 330 (sh)	465	339, 303 $[M - H - 162]^-$, 285, 151	13.3 ± 0.4
11	23.8	dihydroluteolin deoxyhexosyl-hexoside	281, 313	595	449 $[M - H - 146]^-$, 287 $[M - H - 308]^-$	12.7 ± 0.5
12	24.4	dihydrokaempferol hexoside	294, 335 (sh)	449	287 $[M - H - 162]^-$, 269 $[M - H - 162 - 18]^-$, 169, 151	66.4 ± 2.6
13	26.0	kaempferol deoxyhexosyl-hexoside	294, 335 (sh)	593	447 $[M - H - 146]^-$, 285 $[M - H - 308]^-$, 269	7.21 ± 0.9
14	26.8	dihydrokaempferol acetyl-hexoside	292, 333 (sh)	491	287 $[M - H - 204]^-$, 269 $[M - H - 204 - 18]^-$, 151	2.8 ± 0.01
total phenolic						415.1 ± 22.3

[a]Retention time on the C_{18} Synergi Hydro (4 μm) column. [b]Tentative identification based on UV−vis and mass spectra as well as relative HPLC retention times and published data. [c]Solvent: gradient of 0.5% formic acid in water and acetonitrile with 0.5% formic acid. [d]Peaks were quantified as equivalent to gallic acid.

anthocyanins showed the corresponding expected molecular ions (m/z 579 and 609, respectively) and two mass fragments after the loss of rhamnose and rutinose units, as verified in the MS/MS spectra. The molecular ion of peak 7 was found at m/z 433 with a fragment at m/z 287, indicating that the aglycone cyanidin was glycosylated with a deoxyhexose due to the loss of 146 u; this peak was identified as cyanidin 3-rhamnoside by coelution with the standard.

Cyanidin 3-glucoside (peak 2), cyanidin 3-rutinoside (peak 3), pelargonidin 3-glucoside (peak 4), pelargonidin 3-rutinoside (peak 5), and cyanidin 3-rhamnoside (peak 7) were also previously detected in jussara fruit.[12] In the present study cyanidin 3,5-diglycosilated with a hexose and a pentose was found instead of cyanidin 3-sambubioside;[12] our identification was confirmed by UV−vis and MS and the lack of coelution with the cyanidin 3-sambubioside standard. Peonidin 3-

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rutinoside (peak 6) was identified in jussara for the first time in the present study.

The monomeric anthocyanin content (239.16 ± 7.6 mg C3R/100 g) found in our jussara pulp was similar to the literature ranges for jussara pulps (192 mg/100 g f.m.[32] and 207 mg/100 g f.m. in jussara collected in region C in Santa Catarina State).[11] When compared to other fruits from the *Euterpe* genus, jussara showed a lower content of monomeric anthocyanins than those reported for açai (*Euterpe oleracea*) (282−303 mg C3G/100 g[23]). Similar to the results for jussara with 20% cyanidin 3-glucoside and 76% cyanidin 3-rutinoside, açai also shows large fractions of cyanidin 3-glucoside (12.5−13.2%) and cyanidin 3-rutinoside (86.8−87.5%).[23]

Profile of Phenolic Compounds. The HPLC-DAD-ESI$^-$-MS/MS method allowed for the separation and tentative identification of 14 phenolic compounds in the grumixama pulp (Figure 3A). Peak 1 was positively identified as cyanidin 3-glucoside based on coelution and comparison of the UV and mass spectral data with an authentic standard. Peaks 2−7, 9, and 10 belong to a series of quercetin derivatives (Table 5). All peaks exhibited λ_{max} values characteristic of flavonols, at 352−365 nm. Peaks 4, 5, 6, and 7 presented deprotonated molecules at m/z 447, 463, 433, and 433, respectively. In addition, the MS/MS fragmentation pattern obtained from these ions revealed the same fragment at m/z 301 corresponding to quercetin, suggesting neutral losses of the sugar moiety, deoxyhexose (loss of 146 u from $[M - H]^-$ in peak 4), hexose (loss of 162 u from $[M - H]^-$ in peak 5), and pentose (loss of 132 u from $[M - H]^-$ in peaks 6 and 7). Therefore, these peaks were identified as quercetin deoxyhexoside (peak 4), quercetin hexoside (peak 5), and quercetin pentoside (peaks 6 and 7). Peaks 2 and 3 show deprotonated molecules at m/z 477 and 615, respectively, and they showed MS/MS fragments at m/z 301 (loss of 176 u from $[M - H]^-$ in peak 2), as well as at m/z 463 and 301 (losses of 152 and 162 u from $[M - H]^-$ in peak 3). Consequently, these peaks were tentatively identified as quercetin glucuronide (peak 2) and quercetin galloylhexoside (peak 3). Due to the MS (m/z 609) and MS^2 (m/z 463, 301) data, quercetin deoxyhexosylhexose is presumptively identified as peak 9. Peak 10 was identified as quercetin (aglycone) based on coelution and comparison of the UV−vis and mass spectral data with an authentic standard. This peak presented a $[M - H]^-$ peak at m/z 301 and typical MS/MS fragmentation ions for quercetin at m/z 273 (loss of CO), 257 (loss of CO_2), and 229 (loss of CO + CO_2); the most interesting fragments concern the base peaks at m/z 179 and 151, due to a specific retro-Diels−Alder pathway leading to the $^{1,2}A^-$ and $^{1,3}A$ − CO.

Peaks 11, 13, and 14 were tentatively identified as sinapic acid derivatives, and peak 12 was not identified. Peak 11 was identified as sinapic acid dihexoside hydroxybenzoyl because of the deprotonated molecule at m/z 685 and MS/MS fragments at m/z 461 due to synapic acid loss (224 u) and at m/z 299 and 137 (loss of two hexose molecule). Peak 13 shows a $[M - H]^-$ peak at m/z 699 and MS/MS fragments at m/z 493, 331, and 169 after losing a sinapoyl group (206 u) and two hexose molecules, respectively. In addition the fragment at m/z 169 corresponded to gallic acid; consequently, these peaks were tentatively identified as gallic acid dihexoside sinapoyl. Sinapic acid dihexoside benzoyl (peak 14) was tentatively identified due to a deprotonated molecule at m/z 651 and MS/MS fragments at m/z 427, 265, and 103, suggesting losses of sinapic acid (224 u) and two hexose molecules.

The majority of the phenolic compounds in grumixama were sinapic acid derivatives (peaks 14 (43.2%) and 13 (13.5%)). The total phenolic compounds in grumixama comprised 145.2 ± 8.6 mg GAE/100 g fresh weight, while Haminuik et al.[14] reported a value of 25.98 ± 1.43 mg GAE/g dry basis. As far as we are concerned, this is the first report on the phenolic composition (except anthocyanins) from grumixama.

The HPLC chromatogram of the jussara extract is shown in Figure 3B. According to Table 5, 14 phenolic compounds were tentatively identified. The major phenolic compounds found in jussara include cyanidin 3-glucoside (peak 5), cyanidin 3-rutinoside (peak 6), and dihydrokaempferol hexoside (peak 12). Peaks 5 and 6 show their deprotonated molecule $[M - H]^-$ peaks at m/z 447 and 593, respectively. The MS/MS spectra of these compounds show fragments at m/z 285 for both peaks due to neutral hexose (peak 5) and rutinose losses (peak 6). This identification was confirmed in ESI positive mode during the anthocyanin analyses and by coelution with an authentic standard. Peak 12 was tentatively identified as dihydrokaempferol hexoside because the deprotonated molecule showed a $[M - H]^-$ peak at m/z 449 and MS/MS fragments at m/z 287 (loss of hexose molecule), corresponding to the dihydrokaempferol moiety, and at m/z 269 (loss of hexose molecule + H_2O). The fragment at m/z 269 (100%) was more intense than the fragment at m/z 287 (4%). The MS^3 spectra from the ion at m/z 287 showed typical flavanonol fragmentation. The fragments were detected at m/z 269 $[M - H - 18]^-$, m/z 243 $[M - H - 44]^-$, and m/z 215 $[M - H - 72]^-$, corresponding to the neutral losses of H_2O, CO_2, and CO + CO_2, respectively, and retro-Diels−Alder fragments were detected at m/z 151 ($^{1,3}A^-$) and 107 ($^{1,3}A^-$ − CO_2). Our MS data agree with those previously reported.[34,35]

Two other kaempferol derivatives are tentatively identified in jussara as peak 13 (kaempferol deoxyhexosylhexoside) and peak 14 (dihydrokaempferol acetylhexoside). Peak 13 shows a $[M - H]^-$ peak at m/z 593 and MS/MS fragments at m/z 447 and 285 after losing a deoxyhexose (146 u) and deoxyhexose + hexose unit (308 u), respectively. An MS^3-specific fragment (257, 243, 213, and 151) confirms the identity as kaempferol.[40] Peak 14 has a $[M - H]^-$ ion at m/z 491 and MS^2 ions at m/z 287 and 269, suggesting neutral losses of acetyl-hexose (204 u) and acetyl-hexose + H_2O (222 u). Peak 1 was tentatively identified as monogalloyl hexoside in jussara because of the deprotonated molecule at m/z 331, as well as the two MS^2 fragments at m/z 169, corresponding to the gallic acid moiety (loss of hexose) and m/z 125, corresponding to the consecutive losses of hexoside (162 u) and the CO_2 molecule (44 u). This fragmentation has been reported in *Caryocar villosum*[27] and jambolão.[36] Peak 2 was not identified; the deprotonated molecule had a m/z of 359, and the MS/MS fragment was at m/z 197, resulting from the loss of a hexose.

Five flavones were identified in jussara, three apigenin derivatives (peaks 3, 7, and 8), one luteolin derivative (peak 4), and one chrysoeriol derivative (peak 9). The flavones and their glycosides present two λ_{max} values at 320−370 and 250−280 nm due to their B-rings (cinnamoyl structure) and A-rings (benzoyl structure), respectively. The UV−vis spectra of the apigenin derivatives have λ_{max} values at 279 and 323−328 nm. Peaks 3, 7, and 8 were tentatively identified as apigenin dihexoside ($[M - H]^-$ at m/z 593), apigenin hexoside ($[M - H]^-$ at m/z 431), and apigenin deoxyhexosylhexoside ($[M - H]^-$ at m/z 577), respectively. All three compounds showed an MS^2 fragment at m/z 269 (apigenin moiety) as the most

Table 6. Chromatographic and Spectroscopic Characteristics and Content of Phenolic Compounds from Araça and Uvaia, Obtained from HPLC-DAD-ESI-MS/MS

peak	t_R[a] (min)	phenolic compound[b]	λ_{max}[c] (nm)	$[M-H]^-$ (m/z)	MS/MS (m/z)	concentration[d] (mg/100g)
Araça						
1	8.1	not identified	268	187	n.d.[e]	12.1 ± 0.6
2	9.8	gallic acid	271	169	125 $[M-H-44]^-$	12.2 ± 0.9
3	10.2	galloyl hexoside	277	331	271 $[M-H-60]^-$, 169 $[M-H-162]^-$, 125 $[M-H-44]^-$	7.4 ± 0.4
4	11.6	digalloyl hexoside	273	483	331 $[M-H-152]^-$, 313 $[M-H-170]^-$, 169	2.9 ± 0.2
5	13.6	di-HHDP hexoside	260	783	481 $[M-H-302]^-$, 301 $[M-H-482]^-$, 257	4.0 ± 0.7
6	15.7	di-HHDP hexoside isomer	260	783	481 $[M-H-302]^-$, 301 $[M-H-482]^-$, 257	3.9 ± 0.9
7	16.6	HHDP digalloyl hexoside	280	785	633 $[M-H-152]^-$, 615 $[M-H-169]^-$, 483 $[M-H-302]^-$, 301, 275, 249	2.3 ± 0.1
8	17.5	vanillic acid hexoside	260, 296	329	167$[M-H-162]^-$	8.1 ± 0.2
9	18.0	epicatechin epicatechin	278	579	289 $[M-H-290]^-$ MS^2 [289]: 271 $[M-H-18]^-$, 245 $[M-H-44]^-$, 205, 179	5.4 ± 0.1
10	19.2	HHDP digalloyl hexoside isomer	280	785	633 $[M-H-152]^-$, 615 $[M-H-169]^-$, 483 $[M-H-302]^-$, 301, 275, 249	5.9 ± 0.4
11	21.2	taxifolin hexoside	291, 347	465	303 $[M-H-162]^-$	11.7 ± 0.8
12	21.9	HHDP hexoside	279	481	301 $[M-H-180]^-$	5.7 ± 0.2
13	26.4	quercetin hexoside	353	463	301 $[M-H-162]^-$ MS^2 [301]: 273 $[M-H-28]^-$, 257 $[M-H-44]^-$, 229 $[M-H-72]^-$, 179, 151	6.4 ± 0.5
14	29.5	eriodictyol hexoside	289, 350	449	287 $[M-H-162]^-$, 181	4.0 ± 0.2
15	30.4	methyl ellagic acid hexoside	257, 373	491	329 $[M-H-162]^-$, 315 $[M-H-176]^-$, 301 $[M-H-190]^-$	5.2 ± 0.2
16	34.2	methyl ellagic acid pentoside	250, 370	461	315 $[M-H-146]^-$, 301$[M-H-160]^-$, MS^2[301]: 257 $[M-H-44]^-$, 229 $[M-H-72]^-$, 185	5.6 ± 0.1
total phenolics						103.1 ± 6.5
Uvaia						
1	7.3	galloyl hexoside	274	331	169 $[M-H-162]^-$, 125	5.1 ± 0.1
2	9.8	gallic acid	271	169	125 $[M-H-44]^-$	27.5 ± 1.2
3	10.2	galloyl hexoside isomer	277	331	169 $[M-H-162]^-$	1.8 ± 0.05
4	13.1	not identified	287	565	367 MS^2[367]: 247, 119	6.0 ± 0.4
5	15.0	dicaffeic acid	328	341	179 $[M-H-162]^-$, 281, 251 MS^2 [179]: 161 $[M-H-18]^-$, 135 $[M-H-44]^-$	2.0 ± 0.3
6	17.1	gallic acid derivative	292	415	367, 345, 331, 313, 169	14.8 ± 1.1
7	18.9	trigalloyl acid lactonized	292	451	313 $[M-H-138]^-$, 282 $[M-H-169]^-$, 169	13.8 ± 0.9
8	22.4	not identified	280	439	393, 379, 315	7.0 ± 0.3
9	24.3	galloyl-bis-HDDP hexoside	274	935	783 $[M-H-152]^-$, 633 $[M-H-302]^-$, 301	19.3 ± 1.5
10	28.9	quercetin deoxyhexoside	352	447	301 $[M-H-146]^-$	4.8 ± 0.1
11	34.2	not identified	276	263	219, 203, 153	2.8 ± 0.2
12	37.7	gallic acid derivative	276	347	329, 201, 169	11.0 ± 0.9
total phenolics						115.9 ± 7.1

[a]Retention time on the C_{18} Synergi Hydro (4 μm) column. [b]Tentative identification based on UV−vis and mass spectra as well as relative HPLC retention times and published data. [c]Solvent: gradient of 0.5% formic acid in water and acetonitrile with 0.5% formic acid. [d]Peaks were quantified as equivalent to gallic acid. [e]n.d.: not detected.

abundant ion, suggesting neutral losses of the sugar units. The MS^3 fragments from the ion at m/z 269 for peaks 3, 7, and 8 were not detected. Peak 4 was tentatively identified as luteolin deoxyhexosylhexoside according to the UV−vis spectra (λ_{max} at 273 nm (band II) and 323 nm (band I)) and MS fragmentation, leading to the luteolin aglycone at m/z 285 after loss of 146 u + 162 u (deoxyhexose and hexose moiety). The deprotonated molecule in peak 9 was observed at m/z 607, and the MS^2 fragment was detected at m/z 299, suggesting the loss of a rutinose unit. Chrysoeriol rutinoside (peak 9) and dihydroluteolin rutinoside (peak 11) were tentatively identified in jussara; both compounds showed a neutral loss of rutinose (308 u) from the deprotonated molecule at m/z 607 for peak 9 and at 595 for peak 11.

The three major phenolic compounds (peaks 5, 6, and 12) identified in jussara in this study were not reported in previous studies, but these compounds have been already identified in açai.[35,37] Previous studies have reported the presence of ferulic, gallic, protocatechuic, and coumaric acids, as well as catechin, epicatechin, and quercetin in jussara collected from the state of Santa Catarina, Brazil.[11] However, these compounds were not identified using mass spectrometry, which is currently the most suitable technique coupled to HPLC for identifying phenolic compounds in complex matrixes, such as fruit. Furthermore, we

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did not find the phenolic compounds reported by Da Silva Campelo Borges et al.[11] in our study.

The chromatograms of the phenolic compounds from araça and uvaia are shown in Figure 3C and D, respectively. Table 6 shows the chromatographic, UV−vis, and mass spectral data used to identify these compounds. Peak 2 from the phenolic compounds in araça (Figure 3C) and uvaia (Figure 3D) was identified as gallic acid because, in addition to presenting a maximum absorption in the UV−vis spectrum of 271 nm, the deprotonated molecule was detected at m/z 169 with a fragment in the MS^2 spectrum at m/z 125 due to neutral loss of 44 u (CO_2). The identification was confirmed by coelution with an authentic standard. Peaks 3 for araça and 1 and 3 of uvaia were identified as monogalloyl hexoside after they presented a deprotonated molecule at m/z 331 and three fragments in the MS^2 spectrum at m/z 271 after cross-ring fragmentation of the hexoside ring (M − H − 60) at m/z 169, corresponding to the gallic acid moiety, and at m/z 125 due to the decarboxylation of gallic acid (44 u). Notably, the existence and intensity of each fragment ion may be different for each galloyl hexoside peak, as well as for the other hydrolyzable tannins due to the presence of different isomeric forms in the same fruit.[15,38]

Peak 4 for araça showed a deprotonated molecule at m/z 483, as well as MS^2 fragments at m/z 331 due to loss of a galloyl group (152 u) and at m/z 313 due to loss of gallic acid (170 u). Furthermore, another isomer of digalloyl hexoside may have fragments at m/z 439 and 287; these signals are the result of the deprotonated molecule and the decarboxylation of a gallic acid linked to a hexoside (331 u). These fragmentation mechanisms were demonstrated by Meyers et al.[38] Peaks 5 and 6 for araça showed a UV−vis spectrum with a λ_{max} at 260 nm that is characteristic of dihexahydroxydiphenoyl hexoside (diHHDP-hexoside); furthermore, the two peaks showed a deprotonated molecule at m/z 783, yielding fragment ions at m/z 481 after the loss of a HHDP group, at m/z 301 after the loss of HHDP-hexoside group, and at m/z 257 after the decarboxylation of HHDP. Peaks 7 and 10 for araça showed UV−vis spectra with a λ_{max} at 280 nm and a deprotonated molecule at m/z 785, indicating the presence of HHDP-digalloyl-hexoside. In addition, the MS^2 spectra of these peaks showed fragments at m/z 633 after the loss of a galloyl residue (152 u), at m/z 615 after the loss of a gallic acid molecule, at m/z 483 due to loss of one HHDP group, and at m/z 301 after the loss of an HHDP hexoside molecule. Peak 12 for the araça sample was identified as HHDP-hexoside using the deprotonated molecule at m/z 481 and the MS^2 fragment at m/z 301, indicating the presence of HHDP. The gallic acid derivatives were identified based on literature data.[15,27,38]

In addition to the gallic acid derivatives, araça also contained quercetin hexoside (peak 13) and taxifolin hexoside (peak 11) in its phenolic fraction, presenting deprotonated molecules at m/z 463 and 465, respectively. The MS/MS spectra of these peaks showed fragments at m/z 301 (quercetin) and at m/z 303 (taxifolin), suggesting the loss of hexose units (162 u). Quercetin aglycone was identified and was confirmed using the MS^3 spectra: 273 $[M - H - CO]^-$, 257 $[M - H - CO_2]^-$, 229 $[M - H - CO - CO_2]^-$, 179 $[^{1,2}A]^-$, and 151 $[^{1,3}A]^-$. It was not possible to obtain an MS^3 spectrum from taxifolin, but the UV−vis absorption maxima at 291 and 347 nm helped to tentatively identify this compound. Peak 8 was tentatively identified as vanillic acid hexoside due to the presence of the deprotonated molecule at m/z 329 and the MS^2 fragment at m/z 167 after the loss of a neutral hexose. The UV−vis spectrum of this compound had λ_{max} at 260 and 296 nm, as well as the characteristic features of vanillic acid.

Peak 9 of araça was tentatively identified as the epicatechin−epicatechin dimer due to the protonated molecule at m/z 579 and the MS^2 fragment at m/z 289; the MS^3 spectrum of this fragment showed the characteristic configuration of an aglycone catechin with fragments at m/z 271 $[M - H - H_2O]^-$, 245 $[M - H - CO_2]^-$, 205 $[M - H - CO - CO_2]^-$, 179 $[^{1,2}A]^-$, and 151 $[^{1,3}A]^-$. Araça contained eriodictyol hexoside (peak 14) with a deprotonated molecular ion at m/z 449 and an MS^2 fragment at m/z 287 due to loss of a hexose. The MS^3 of the fragment at 287 was not obtained. Therefore, the identification of the aglycone was attempted. Peaks 15 and 16 were tentatively identified as ellagic acid derivatives (dimethyl ellagic acid hexoside and dimethyl ellagic acid pentoside) because both peaks showed characteristic fragments of methylated ellagic acid at m/z 315 and 313, respectively; at m/z 301, the ellagic acid was confirmed by the MS^3 spectrum of the fragment at 301 from peak 16.

Aside from peaks 1, 2, and 3, uvaia (Figure 3D) showed other derivatives of gallic acid in its phenolic composition. Peaks 6, 7, and 12 are gallic acid derivatives, but they have not been definitively identified. Peaks 7 and 9 were identified as lactonized trigalloyl acid and galloyl HHDP bis-hexoside, respectively. Peak 7 showed a deprotonated molecule at m/z 451 and MS^2 fragments at m/z 313, 282, and 169 that were attributed to galloyl and lactonized galloyl losses. Peak 9 was identified by the deprotonated molecule at m/z 935 and the MS^2 fragment at m/z 783 assigned to the loss of a galloyl group, as well as at m/z 633 due to loss of HHDP and at m/z 301 due to loss of HHDP-hexoside. The identification of this compound has already been reported in the phenolic composition of blackberries[39] and strawberries from Macedonia.[38]

Peak 5 in uvaia was identified as dicaffeic acid, with the deprotonated molecule at m/z 341 and an MS^2 fragment at m/z 179 due to the caffeoyl loss. Furthermore, the MS^3 spectrum at 179 u revealed fragments at m/z 161 and 135 attributed to water loss and decarboxylated caffeic acid, in accordance with the fragmentation pattern for dicaffeic acid proposed by Plazonić et al.[40] Peak 10 of uvaia presented a deprotonated molecule at m/z 447 and an MS^2 fragment at m/z 301 after the neutral loss of 146 u; this material was tentatively identified as quercetin deoxyhexoside. Peaks 4, 8, and 11 could not be identified.

The majority of the phenolic compounds in araça were gallic acid (peak 2, 12.24 ± 0.9 mg GAE/100 g fresh weight) and taxifolin hexoside (peak 12, 11.73 ± 0.8 mg GAE/100 g fresh weight). The total phenolic compounds in araça comprised 103.10 ± 6.5 mg GAE/100 g fresh weight, while Gordon et al.[15] reported a value of 754.4 ± 12.5 mg GAE/100 g dry weight. Although these authors have not quantified the compounds individually, the chromatogram revealed that the major compound was HHDP-digalloylglucoside.

Uvaia presented gallic acid (peak 2, 27.50 ± 1.2 mg GAE/100 g fresh weight) and galloyl HHDP-bis-hexoside (peak 7, 19.32 ± 1.5 mg GAE/100 g weight fresh) as the major phenolic compounds in the total 115.96 ± 7.15 mg GAE/100 g fresh weight. No data were found in the literature identifying the phenolic compounds in uvaia.

In conclusion, carotenoids and phenolic compounds were identified and quantified in four fruits from the Brazilian Atlantic Forest. In addition, jussara showed high levels of

phenolic compounds, primarily consisting of cyanidin 3-rutinoside, dihydrokaempferol hexoside, and cyanidin 3-glucoside. While the phenolic composition of jussara and grumixama was composed of several flavonoid glycosides, uvaia and araça presented mostly gallic acid derivatives. For the carotenoids, uvaia contained the highest concentrations. All-*trans*-β-Cryptoxanthin was the major carotenoid found in uvaia, grumixama, and araça, while all-*trans*-lutein was the major component in jussara. Therefore, these fruits are important as promising sources of bioactive compounds. Additionally, due to their nutritional value, high fiber content, and sweet taste, these four fruits from the Atlantic Forest can be used to supplement the diet of groups such as schoolchildren.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figure 1S shows the photographs of the fruits studied in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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