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Carotenoids and Phenolic Compounds from *Solanum sessiliflorum*, an Unexploited Amazonian Fruit, and Their Scavenging Capacities against Reactive Oxygen and Nitrogen Species

Eliseu Rodrigues, Lilian R. B. Mariutti, and Adriana Z. Mercadante*

Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), 13083-862 Campinas-SP, Brazil

Supporting Information

ABSTRACT: The composition of carotenoids and phenolic compounds from mana-cubiu (*Solanum sessiliflorum*), a fruit native to Amazonia, was determined by high-performance liquid chromatography coupled to diode array and mass spectrometry detectors (HPLC−DAD−MS^n). The antioxidant capacities of the hydrophilic and carotenoid extracts against some reactive oxygen ($ROO^•$, H_2O_2, HOCl, and $HO^•$) and nitrogen ($ONOO^-$) species were also determined. Seventeen carotenoids and three phenolic compounds were found in mana-cubiu. The major carotenoids were (all-*E*)-*β*-carotene (7.15 *μ*g/g of dry weight) and (all-*E*)-lutein (2.41 *μ*g/g of dry weight). The 5-caffeoylquinic acid (1351 *μ*g/g of dry weight) was the major phenolic compound, representing more than 78% (w/w) of the total phenolic compounds. Moreover, two dihydrocaffeoyl spermidines were found in the hydrophilic extract. Both mana-cubiu extracts were able to scavenge all the tested reactive species. The carotenoid extract was shown to be a potent scavenger of peroxyl radical, while the hydrophilic extract was a potent hydrogen peroxide and hypochlorous acid scavenger.

KEYWORDS: *mass spectrometry, chlorogenic acid, antioxidant capacity, Solanaceae, bioactive compounds, ORAC*

INTRODUCTION

Mana-cubiu (*Solanum sessiliflorum*) is a fruit that belongs to the Solanaceae family, the same family to which the potato and tomato belong. This fruit is native to the Amazonian region and widely distributed across the humid equatorial regions of Brazil, Peru, and Colombia.[1] The fruits, also known as topiro/tupiro in Peru, cocona in Venezuela, Indian tomato in northeast Brazil, cubiu in the Brazilian Amazonian region, and oricono or apple/peach tomato in English-speaking countries, are 5−6 cm in diameter and weigh between 30 and 400 g, and their edible fraction represents approximately 91% (w/w) of the total fresh weight [9% (w/w) of the peel]. The fruit consists of ∼90% (w/w) water. The remaining components are basically citric acid (14 g/100 g of dry weight) and carbohydrates (32 g/100 g of dry weight), where glucose and fructose are predominant.[2] The pulp has an acidic pleasant taste; the flavor is similar to that of citrus fruits and peaches, and it is usually consumed as salad, juice, or jelly or can be added to cakes. The color of the peel goes from green to orange during ripening, and the pulp is light yellow because of the presence of carotenoids.[2]

Epidemiological evidence has associated fruit intake with a decrease in the incidence of cardiovascular diseases and certain types of cancer.[3,4] Moreover, plants with therapeutic components are commonly used, particularly in phytogeographic regions, such as Amazonia, and are usually known in folklore medicine for their therapeutic potential. For instance, mana-cubiu has been used against snake bites, scorpion stings, and skin infections, exhibited antihypertensive activity, and reduced cholesterol, glucose, and uric acid levels in the blood.[5−7]

The antioxidant properties of the bioactive compounds present in mana-cubiu probably contribute to the beneficial health effects attributed to its consumption. However, no data about the carotenoid and phenolic compositions of mana-cubiu have been reported. Thus, the aim of this work was to identify and quantify the carotenoids and phenolic compounds from mana-cubiu by high-performance liquid chromatography coupled to photodiode array and mass spectrometry detectors (HPLC−DAD−MS^n). Moreover, the scavenging capacities of mana-cubiu extracts were evaluated against reactive oxygen (ROS) and nitrogen (RNS) species, namely, peroxyl radical ($ROO^•$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^•$), and peroxynitrite anion ($ONOO^-$).

MATERIALS AND METHODS

Chemicals. Standards of (all-*E*)-*β*-carotene, caffeic acid, catechin, epicatechin, quinic acid, rutin, naringin, hesperidin, neohesperidin, luteolin, quercetin, taxifolin, naringenin, apigenin, myricetin, kaempferol, rhamnetin, hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, gallic acid, and 5-caffeoylquinic acid were purchased from Sigma-Aldrich (St. Louis, MO). (all-*E*)-Lutein, (all-*E*)-*α*-carotene, (9*Z*)-*β*-carotene, (13*Z*)-*β*-carotene, (15*Z*)-*β*-carotene, and (all-*E*)-*β*-cryptoxanthin were donated by DSM Nutritional Products (Basel, Switzerland), and standards of (9′*Z*)-neoxanthin and (all-*E*)-violaxanthin were acquired from CaroteNature (Lupsingen, Switzerland). All standards were at least 93% pure, as determined by HPLC−DAD. Methyl *tert*-butyl ether (MTBE) was acquired from J. T. Baker (Phillipsburg, NJ), and the other HPLC grade solvents were obtained from Merck (Darmstadt, Germany) or Mallinckrodt Baker (Philipsburg, NJ). Dihydrorhodamine 123 (DHR), a 30% hydrogen peroxide solution, a

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sodium hypochlorite solution with 4% available chlorine, α,α′-azodiisobutyramidine dihydrochloride (AAPH), dimethyl sulfoxide (DMSO), luminol, lucigenin, fluorescein sodium salt, and 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) were obtained from Sigma-Aldrich. Azobisisobutyronitrile (AIBN) was donated by Mig Quimica (São Paulo, Brazil). The fluorescent probe 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-undecanoic acid (C_{11}-BODIPY$^{581/591}$, MW = 504.43 g/mol) was purchased from Invitrogen (Carlsbad, CA). Sodium carbonate, sodium phosphate monobasic monohydrate, sodium phosphate dibasic, sodium hydroxide, sodium nitrite, sodium chloride, potassium chloride, and sodium bicarbonate were obtained from Synth (São Paulo, Brazil). Water was purified by a Milli-Q (Billerica, MA) system. The samples and solvents were filtered through Millipore 0.22 and 0.45 μm membranes, respectively.

Samples. Three batches of mana-cubiu (~7 kg each, ~40 fruits per batch) were acquired at CEAGESP (São Paulo General Warehousing and Centers Co., São Paulo, Brazil). The fruits were harvested in the Cajati City region (24°44′9″ South, 48°7′22″ West, São Paulo, Brazil), and all of them were ripe and selected by visual inspection (orange peel). The fresh fruits exhibited the following characteristics: water content,[8] 88.9 ± 0.1 g/100 g (n = 9); weight, 152 ± 31 g (n = 120); diameter, 5.95 ± 0.52 cm; height, 7.09 ± 0.71 cm (n = 120); pH of pulp, 3.4 ± 0.0 (n = 9).[8] The fruits were washed; the peel was manually discarded, and the pulp (with small seeds) was cut into small pieces and immediately frozen in liquid nitrogen. The frozen fruit pieces were lyophilized for 96 h at −60 °C below 40 μmHg (Liobras, São Paulo, Brazil). The freeze-dried fruit was ground into powder in a domestic food mixer (Black & Decker, São Paulo, Brazil), and the three batches were homogenized to compose a single composite sample. The freeze-dried composite sample was vacuum-packed (Jumbo Plus, Selovac, São Paulo, Brazil) in polyethylene bags containing 50 g portions and stored at −37 °C in the dark until analysis.

Extraction of the Bioactive Compounds. The carotenoids were exhaustively extracted from the freeze-dried composite sample (5.0 ± 0.5 g) with acetone, transferred to a petroleum ether/diethyl ether mixture [1:1 (v/v)], and saponified with 10% (w/v) methanolic KOH overnight (~16 h) at room temperature.[9] The alkali was removed by washing the extract with distilled water, and the solvent was evaporated in a rotary evaporator (T < 30 °C). The dry extract was stored at −80 °C under a nitrogen atmosphere (99.9% purity) in the dark until analysis. The carotenoid extraction was conducted in triplicate.

The carotenoid extract was dissolved in petroleum ether (stock solution), and the total carotenoid concentration was spectrophotometrically determined using the specific absorption coefficient ($A^{1\%}_{1\ cm}$ = 2396) of β-carotene.[10] To determine the carotenoid composition, an aliquot of the stock solution was evaporated under a N_2 flow, redissolved in a MeOH/MTBE mixture [70:30 (v/v)], and analyzed by HPLC−DAD−MS^n.[9] To determine the ROO$^\bullet$ scavenging capacity of the carotenoid extract, appropriate aliquots were taken from the stock solution to prepare working solutions at five concentrations (30, 40, 77, 153, and 227 μM), evaporated under a N_2 flow, redissolved in a DMSO/MTBE mixture [10:1 (v/v)], and sonicated for 30 s. To avoid carotenoid degradation during analyses, the manipulation of the samples and extracts was conducted in dim light at a controlled room temperature (22 ± 3 °C).

The phenolic compounds were exhaustively extracted from the freeze-dried composite sample (0.10 g) in 10 mL Teflon tubes with 5 mL of a methanol/water mixture [8:2 (v/v)] by being vortexed (Phoenix Luferco, São Paulo, Brazil) for 5 min at ambient temperature (22 ± 3 °C). After centrifugation (model Allegra 64R, Beckman Coulter, Palo Alto, CA) at 3864g for 5 min at 20 °C, the supernatant was transferred to a 25 mL volumetric flask. The extraction was repeated five times, and the supernatants were combined to obtain a final volume of 25 mL (extract of phenolic compounds) and immediately injected into the HPLC−DAD−MS^n apparatus. The extraction procedure was conducted in triplicate. The absence of phenolic compounds in the supernatant was verified using the Folin-Ciocalteau reagent.[11]

The extract of phenolic compounds was lyophilized and stored at −37 °C. The freeze-dried extract of phenolic compounds was suspended in water (10 mg of freeze-dried extract/mL of water) and centrifuged at 37000g for 10 min at 10 °C, and the supernatant (hydrophilic extract) was used for the ROS and RNS scavenging capacity analyses. The hydrophilic extract was also injected into the HPLC−DAD apparatus to determine the phenolic compound content (Table S1 of the Supporting Information) because the complete dissolution of the extract of phenolic compounds in water was not observed.

HPLC−DAD−MS^n Analysis. A Shimadzu (Kyoto, Japan) HPLC apparatus equipped with quaternary pumps (LC-20AD), an online degasser, and a Rheodyne (Rheodyne LCC, Robert Park, LA) injection valve with a 20 μL loop, connected in series to a DAD detector (Shimadzu) and a mass spectrometer with an ion trap analyzer and atmospheric-pressure chemical ionization (APCI) and electrospray ionization (ESI) sources (model Esquire 4000, Bruker Daltonics, Bremen, Germany), was used to assess the carotenoids and phenolic compounds.

The carotenoids were separated on a C_{30} YMC column [5 μm, 250 mm × 4.6 mm (inside diameter)] (Waters, Wilmington, DE) using as the mobile phase a linear gradient of a methanol/MTBE mixture from 95:5 (v/v) to 70:30 (v/v) over 30 min, followed by a 50:50 (v/v) ratio for 20 min, and maintaining this proportion for 15 min,[9] at 0.9 mL/min and with the column temperature set to 29 °C. The HPLC−DAD−MS^n parameters were set using the same conditions previously described in detail by De Rosso and Mercadante.[9] The identification of the carotenoids was performed considering the combination of the following parameters: elution order on the C_{30} column, UV−vis spectral features [maximal absorption wavelength (λ_{max}), spectral fine structure (%III/II), and peak *cis* intensity (%A_B/A_{II})], and MS spectrum characteristics as compared to standards analyzed under the same conditions and data available in the literature. The carotenoids were quantified by HPLC−DAD, using seven-point analytical curves of (9*Z*)-neoxanthin (0.9−17.1 μg/mL), (all-*E*)-violaxanthin (0.7−13.6 μg/mL), (all-*E*)-lutein (1.0−59.5 μg/mL), and (all-*E*)-β-carotene (1.1−30.2 μg/mL). All other carotenoid contents were estimated using the curve of (all-*E*)-β-carotene, and the (*Z*)-isomers were estimated using the curve of the corresponding (all-*E*)-carotenoid. All analytical curves were linear (r^2 = 0.99); the limit of detection was 0.1 μg/mL, and the limit of quantification was 0.5 μg/mL. The NAS-IOM[12] conversion factor was used to calculate the vitamin A value, with 12 μg of dietary (all-*E*)-β-carotene corresponding to 1 μg of retinol activity equivalent (RAE), and the activity used was 100% for (all-*E*)-β-carotene.

The phenolic compounds were separated on a C_{18} Synergi Hydro-RP column (4 μm, 250 mm × 4.6 mm, Phenomenex, Torrance, CA) at a flow rate of 0.9 mL/min and a column temperature of 29 °C, using a mobile phase consisting of a water/formic acid mixture [99.5:0.5 (v/v)] (solvent A) and an acetonitrile/formic acid mixture [99.5:0.5 (v/v)] (solvent B) in a linear gradient from 99:1 (v/v) A/B to 50:50 (v/v) A/B over 50 min and then from 50:50 (v/v) A/B to 1:99 (v/v) A/B over 5 min. The former ratio [1:99 (v/v)] was maintained for an additional 5 min.[13] The column eluate was split to allow only around 0.15 mL/min entering the ESI interface. The UV−vis spectra were obtained between 200 and 600 nm, and the chromatograms were processed at 280 and 320 nm. The mass spectra were acquired with a scan range from m/z 100 to 800. The MS parameters were set as follows: ESI source in positive and negative ion modes; capillary voltage, 3400 V (positive) or −2500 V (negative); end plate offset, −500 V; capillary exit, −82 V (negative) or 82 V (positive); dry gas (N_2) temperature, 310 °C; flow rate, 8 L/min; nebulizer gas, 30 psi. MS^2 and MS^3 were set in manual mode applying a fragmentation energy of 1.6 V. The phenolic compounds were identified on the basis of the following information: elution order and retention time in the reversed phase column, UV−vis and MS spectra features as compared to standards analyzed under the same conditions, and data available in the literature. The phenolic compounds were quantified by HPLC−DAD, using a six-point analytical curve of 5-caffeoylquinic acid (5−200

μg/mL). The analytical curve was linear (r^2 = 0.997); the limit of detection was 2 μg/mL, and the limit of quantification was 7 μg/mL.

ROS and RNS Scavenging Capacity. The assays were conducted in a microplate reader (Synergy Mx, BioTek, Winooski, VT) for fluorescence, absorbance, and luminescence measurements, equipped with a thermostat and a dual-reagent dispenser. Two control assays were conducted in all microplates, one of them to verify the interaction between the probe and the extract, without addition of radical generator or reactive species, and the other as an analytical quality control (positive control), adding a compound with known capacity to scavenge the specific reactive species. No interaction between the probes and the extract compounds was observed, and the maximal variation in the response of the positive controls during the assays was less than 15%. Each ROS or RNS scavenging assay corresponds to two independent experiments, performed in triplicate. Except for ROO• scavenging capacity, the results are presented as IC_{50} values calculated by four-parameter nonlinear regression using GraphPad Prism version 5.03 (GraphPad Software Inc., San Diego, CA).

Peroxyl Radical Scavenging Assay for Carotenoid Extract. The ROO• scavenging capacity was measured by monitoring the effect of the carotenoid extract or α-tocopherol standard on the fluorescence decay resulting from ROO•-induced oxidation of C_{11}-BODIPY$^{581/591}$.[14] The ROO• was generated by thermodecomposition of AIBN at 41 °C. Reaction mixtures in the wells contained the following reagents at the indicated final concentrations (final volume of 225 μL): 0.18 μM C_{11}-BODIPY$^{581/591}$ in DMSO, 195 mM AIBN in a DMSO/MTBE mixture [10:1 (v/v)], and the carotenoid extract (2.4, 3.2, 6.1, 12.1, and 15.8 μM). The fluorescence was measured until 120 min had elapsed. The ROO• scavenging capacity was calculated according to the method of Rodrigues et al.[14] and was expressed as an undimensional value that represents how many times that extract or compound is more efficient than α-tocopherol. α-Tocopherol was also used as a positive control [net area (56 μM) of 8.02 ± 0.13].

Peroxyl Radical Scavenging Assay for Hydrophilic Extract. The ROO• scavenging capacity was measured by monitoring the effect of the hydrophilic extract or standard (5-caffeoylquinic acid and trolox) on the fluorescence decay resulting from ROO•-induced oxidation of fluorescein, using the ORAC method.[15] The ROO• was generated by thermodecomposition of AAPH at 37 °C. Reaction mixtures in the wells contained the following reagents at the indicated final concentrations (final volume of 200 μL): fluorescein (61 nM), AAPH solution (19 mM), and hydrophilic extract (1.25, 6.25, 12.5, 25, and 50 μg/mL), 5-caffeoylquinic acid (0.35, 0.53, 0.7, and 1.4 μg/mL), or trolox (2, 4, 8, 12, 16, and 24 μg/mL) in 75 mM phosphate buffer (pH 7.4). The ROO• scavenging capacity was calculated according to the method of Ou et al.[15] and expressed as micromoles of trolox equivalent per milligram of extract. Trolox was used as a positive control [net area (2 μM) of 7.4 ± 0.9, net area (4 μM) of 13.1 ± 1.1, and net area (8 μM) of 22.6 ± 1.4].[14]

Hydrogen Peroxide Scavenging Assay. The H_2O_2 scavenging capacity was measured by monitoring the effect of the hydrophilic extract or standard (5-caffeoylquinic acid and ascorbic acid) on the increase in luminescence resulting from H_2O_2-induced oxidation of lucigenin.[16] Reaction mixtures contained the following reagents at final concentrations (final volume of 300 μL): 50 mM Tris-HCl buffer (pH 7.4), 0.8 mM lucigenin in Tris-HCl buffer, 1% (w/w) H_2O_2, and hydrophilic extract (10, 40, 80, 120, 160, 200, 400, and 800 μg/mL) or 5-caffeoylquinic acid (43, 86, 173, 346, 691, and 1000 μg/mL) dissolved in Tris-HCl buffer. The chemiluminescence signal was measured in the microplate reader after incubation for 5 min at 37 °C. Ascorbic acid was used as a positive control (IC_{50} = 155 ± 18 μg/mL).

Hydroxyl Radical Scavenging Assay. The HO• scavenging capacity was measured by monitoring the effect of the hydrophilic extract or standard (5-caffeoylquinic acid and gallic acid) on the increase in luminescence resulting from HO•-induced oxidation of luminol.[16] The HO• was generated by a Fenton system ($FeCl_2$/EDTA/H_2O_2). Reaction mixtures contained the following reactants at the indicated final concentrations (final volume of 250 μL): luminol (20 mM), $FeCl_2$-EDTA (25 and 100 μM), H_2O_2 (3.5 mM), and hydrophilic extract (5, 10, 20, 40, and 60 μg/mL) or 5-caffeoylquinic acid (0.06, 0.13, 0.3, 0.5, 0.9, and 1.8 μg/mL). The chemiluminescence signal was measured in the microplate reader after incubation for 5 min at 37 °C. Gallic acid was used as a positive control (IC_{50} = 0.16 ± 0.01 μg/mL).

Hypochlorous Acid Scavenging Assay. The HOCl scavenging capacity was measured by monitoring the effect of the hydrophilic extract or standard (5-caffeoylquinic acid and trolox) on the fluorescence increase resulting from HOCl-induced oxidation of DHR to rhodamine 123.[16] HOCl was prepared by adjusting the pH of a 1% (w/v) solution of NaOCl to 6.2, with 10% (v/v) H_2SO_4. The concentration of HOCl was determined spectrophotometrically at 235 nm using a molar absorption coefficient of 100 M^{-1} cm^{-1}, and further dilutions were made in 100 mM phosphate buffer (pH 7.4). Reaction mixtures contained the following reactants at the indicated final concentrations (final volume of 300 μL): DHR (5 μM), HOCl (5 μM), and hydrophilic extract (1.7, 4.2, 8.3, 16.7, 33.3, 66.7, and 100 μg/mL) or 5-caffeoylquinic acid (12, 24, 48, 96, 192, and 384 μg/mL) dissolved in phosphate buffer (pH 7.4). The fluorescence signal (excitation at 485 ± 20 nm, emission at 528 ± 20 nm) was assessed immediately after addition of HOCl (5 μM). Trolox was used as a positive control (IC_{50} = 134 ± 18 μg/mL).

Peroxynitrite Anion Scavenging Assay. The $ONOO^-$ scavenging capacity was measured by monitoring the effect of the hydrophilic extract or standard (5-caffeoylquinic acid and ascorbic acid) on the increase in fluorescence resulting from the $ONOO^-$-induced oxidation of the nonfluorescent DHR to fluorescent rhodamine.[16] $ONOO^-$ was synthesized as previously described.[17] Reaction mixtures contained the following reactants at the indicated final concentrations (final volume of 300 μL): DHR (5 μM), $ONOO^-$ (600 nM), and hydrophilic extract (4.2, 16.7, 33.3, 66.7, 133.7, and 333.3 μg/mL) or 5-caffeoylquinic acid (0.4, 0.8, 1.5, 3, and 6 μg/mL). The fluorescence signal was measured in the microplate reader after incubation for 5 min at 37 °C, with wavelengths of excitation of 485 ± 20 nm and emission of 528 ± 20 nm. In a parallel set of experiments, the assays were performed in the presence of 25 mM $NaHCO_3$ to simulate the physiological CO_2 concentration. Ascorbic acid was used as a positive control (IC_{50} values of 0.23 ± 0.01 and 0.34 ± 0.01 μg/mL in the absence and presence of $NaHCO_3$, respectively).

Statistical Analysis. The mean and standard deviation (SD) related to the HPLC−DAD analysis and antioxidant capacities of mana-cubiu were calculated using Origin version 8.

RESULTS AND DISCUSSION

Carotenoids and ROO• Scavenging Capacity. Seventeen carotenoids from freeze-dried mana-cubiu were separated by HPLC (Figure 1), and 14 carotenoids were identified or tentatively identified on the basis of the combined information obtained from chromatographic elution on a C_{30} column and characteristics of UV−vis and mass spectra (Table 1). The MS^2 fragments characteristic of the polyenic chain and functional groups allowed the confirmation of the assigned protonated molecules. Considering that a detailed description of carotenoid identification using the information described above was already reported,[9,18] only some of the most important aspects are discussed below.

Peak 4 was tentatively identified as (all-*E*)-luteoxanthin, based on the UV−vis spectral features, protonated molecule $[M + H]^+$ at *m*/*z* 601, and MS^2 mass fragments of *m*/*z* 583 $[M + H - 18]^+$, 565 $[M + H - 18 - 18]^+$, and 509 $[M + H - 92]^+$, corresponding to the loss of one water molecule, two water molecules, and toluene from the polyene chain, respectively. Moreover, the detection of a mass fragment at *m*/*z* 221 in the MS^2 spectrum indicated the presence of an epoxy group in the β-ionone ring with a hydroxyl substituent.

Peak 10 was tentatively identified as (all-*E*)-zeinoxanthin. To differentiate between this carotenoid and its isomer, (all-*E*)-α-cryptoxanthin, the relation between the signal intensity of the

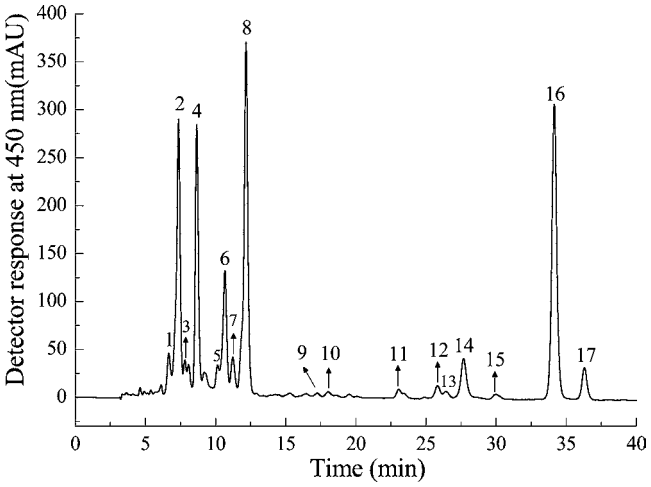

Figure 1. Chromatogram obtained by HPLC–DAD of the carotenoids from mana-cubiu. For chromatographic conditions, see the text. Peak characterization is given in Table 1.

protonated molecule at m/z 553 and the fragment at m/z 535 $[M + H - 18]^+$ was assessed. The zeinoxanthin mass spectrum showed a higher intensity of the protonated molecule (m/z 553) as compared to the mass fragment at m/z 535 $[M + H - 18]^+$, while the contrary was observed for α-cryptoxanthin, as previously reported in the literature.[9,19]

As far as we are concerned, this is the first report on the carotenoid composition of mana-cubiu. The major carotenoids were (all-*E*)-β-carotene and (all-*E*)-lutein (Table 1), which represented 60% (w/w) of the total carotenoid content. A considerable amount of carotenoids possessing epoxy groups [25% (w/w)] was found; thus, the carotenoid extraction was performed with and without addition of $NaHCO_3$ in both fresh and freeze-dried mana-cubiu fruits, and no difference was observed among the obtained carotenoid profiles (data not shown).

The carotenoid composition of mana-cubiu was similar to that of other fruits belonging to the genus *Solanum*, such as naranjilla (*Solanum quitoense* Lam.),[20] whose major carotenoids are (all-*E*)-lutein (45–55.0%) and (all-*E*)-β-carotene (13.0–21%), and potato (*Solanum tuberosum*),[21] which contains a considerable amount of epoxy carotenoids. However, a marked difference can be noticed between mana-cubiu and tomato (*Solanum lycopersicum*), whose major carotenoid is lycopene.[22]

Mana-cubiu (0.08 μg of RAE/g of fresh weight) exhibited lower vitamin A activity than other Amazonian fruits (3.44–36.4 μg of RAE/g of fresh fruit).[9] A carotenoid possesses vitamin A activity when it has at least one unsubstituted β-ionone ring bonded to the polyene chain with at least 11 carbons. Thus, to calculate the vitamin A activity of mana-cubiu, the following carotenoids were considered: (all-*E*)-, (9*Z*)-, (13*Z*)-, and (15*Z*)-β-carotene, α-carotene, and β-cryptoxanthin.

The carotenoid extract from mana-cubiu was able to scavenge $ROO^{\bullet}$, and the net AUC values were linearly dependent on the total carotenoid concentration ($r^2 > 0.99$; $p < 0.05$) (Figure S1 of the Supporting Information). Although mana-cubiu had a lower total carotenoid content (16.1 ± 0.50 μg/g of dry weight) than other Amazonian fruits,[14] such as mamey (318.03 ± 25.90 μg/g of dry weight) and peach palm (52.50 ± 2.22 μg/g of dry weight), the carotenoid extract from mana-cubiu showed almost 1.5 times higher $ROO^{\bullet}$ scavenging capacity (Table 2) than those of the carotenoid extracts from

Table 1. Chromatographic, UV–Vis, and Mass Spectroscopy Characteristics and Carotenoid Content of *S. sessiliflorum* Fruit, Obtained by HPLC–DAD–APCI–MS²

peak[a]	carotenoid[f]	concentration (μg/g dry weight)	tr[b] (min)	λmax (nm)[c]	%III/II	%A_B /A_{II}	$[M+H]^+$ (*m/z*)	fragment ions (*m/z*)
1	(9′*Z*)-neoxanthin[1,g]	0.05 ± 0.01	6.7	330, 415, 440, 468	64	31	601	583[M+H-18]$^+$, 565[M+H-18-18]$^+$, 547[M+H-18-18-18]$^+$, 509[M+H-92]$^+$, 393, 221
2	(all-*E*)-violaxanthin[2,g]	0.98 ± 0.10	7.4	415, 438, 468	92	0	601	583[M+H-18]$^+$, 565[M+H-18-18]$^+$, 509[M+H-92]$^+$, 221, 181
3	mixture 1[4]	0.03 ± 0.00	8.0	328, 422, 440, 462	n.c.[d]	16	601	583[M+H-18]$^+$, 565[M+H-18-18]$^+$, 509[M+H-92]$^+$, 221
4	(all-*E*)-luteoxanthin[2,h]	1.30 ± 0.06	8.7	398, 421, 448	96	0	601	583[M+H-18]$^+$, 565[M+H-18-18]$^+$, 509[M+H-92]$^+$, 221, 181
5	mixture 2[4]	0.35 ± 0.01	10.1	381, 401, 425	175	0	601	583[M+H-18]$^+$, 565[M+H-18-18]$^+$, 509[M+H-92]$^+$, 221
6	(9*Z*)-violaxanthin[2,h]	0.55 ± 0.03	10.6	324, 412, 434, 463	85	10	601	583[M+H-18]$^+$, 565[M+H-18-18]$^+$, 545[M+H-56]$^+$, 509[M+H-92]$^+$, 221, 181
7	(9*Z*)-luteoxanthin[2,h]	1.33 ± 0.06	11.2	302, 396, 417, 443	90	0	601	583[M+H-18]$^+$, 565[M+H-18-18]$^+$, 545[M+H-56]$^+$, 509[M+H-92]$^+$, 221
8	(all-*E*)-lutein[3,g]	2.41 ± 0.09	12.1	422, 444, 472	57	0	569	551[M+H-18]$^+$, 533 [M+H-18-18]$^+$, 477 [M+H-92]$^+$
9	5,8-epoxy-β-cryptoxanthin[2,h]	0.04 ± 0.00	17.3	401, 428, 452	50	0	569	551[M+H-18]$^+$, 459[M+H-18-92]$^+$, 221
10	(all-*E*)-zeinoxanthin[4,h]	0.09 ± 0.00	18.1	420, 445, 472	75	0	553	535[M+H-18]$^+$, 496
11	(all-*E*)-β-criptoxanthin[4,g]	0.08 ± 0.00	23.0	418, 450, 476	33	0	553	535[M+H-18]$^+$, 495, 461 [M+H-92]$^+$
12	mixture 3[4]	0.26 ± 0.09	25.8	405, 427, 452	100	0	553	535[M+H-18]$^+$, 205
13	(15*Z*)-β-carotene[4,g]	0.08 ± 0.00	26.4	337, 422, 449, 473	n.c.[d]	n.c.[d]	537	444 [M-92]$^+$
14	(13*Z*)-β-carotene[4,g]	0.78 ± 0.02	27.7	337, 418, 444, 470	20	47	537	444 [M-92]$^+$
15	(all-*E*)-α-carotene[3,g]	0.05 ± 0.00	29.9	420, 445, 473	64	0	537	481 [M+H-56]$^+$
16	(all-*E*)-β-carotene[4,g]	7.15 ± 0.21	34.1	421, 451, 478	25	0	537	444 [M-92]$^+$
17	(9*Z*)-β-carotene[4,g]	0.57 ± 0.02	36.3	331, 420, 446, 472	33	6	537	457 [M+H-80]$^+$, 445 [M+H-92]$^+$, 399[M -137]$^+$, 400[M+H-137]$^+$, 269,177
Total carotenoids (μg/g dry weight)		16.1± 0.50						
Total carotenoids (μg/g fresh weight)		1.45± 0.05						
Vitamin A value (μg RAE/g dry weight)[e]		0.88 ± 0.03						
Vitamin A value (μg RAE/g fresh weight)[e]		0.08 ± 0.00						

[a]Numbered according to the chromatogram shown in Figure 1. [b]Retention time on the C_{30} column. [c]Linear gradient of the methanol/MTBE mixture. [d]Not calculated. [e]RAE, retinol activity equivalent. [f]The peaks were quantified ($n = 3$) as being equivalent to (9′*Z*)-neoxanthin,[1] violaxanthin,[2] lutein,[3] and β-carotene.[4] [g]Identified (standard available). [h]Tentatively identified.

Table 2. ROS and RNS Scavenging Capacities of the Hydrophilic and Carotenoid Extracts from Mana-cubiu and Standard Compounds

	IC_{50} (μg/mL)						
				$ONOO^-$		$ROO^•$	
sample	H_2O_2	$HO^•$	HOCl	with $NaHCO_3$	without $NaHCO_3$	hydrophilic[a]	lipophilic[b]
hydrophilic extract	305 ± 17	36 ± 0.3	13 ± 0.8	27 ± 4	20 ± 2	0.32 ± 0.01	nd[c]
carotenoid extract	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	9.80 ± 0.80
5-caffeoylquinic acid standard	544 ± 10	0.54 ± 0.05	56 ± 2.5	0.37 ± 0.05	0.47 ± 0.07	11.95 ± 0.31	nd[c]
ascorbic acid standard	155 ± 18	nd[c]	0.24 ± 0.02	0.21 ± 0.01	0.34 ± 0.04	5.42 ± 0.30	nd[c]
trolox standard	nd[c]	0.17 ± 0.02	134 ± 18	0.20 ± 0.01	0.20 ± 0.01	4.03 ± 0.30	nd[c]
α-tocopherol standard	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	1.00

[a]Micromoles of trolox equivalent per milligram of extract or standard. [b]α-Tocopherol relative. [c]Not determined.

mamey (6.90 ± 0.44) and peach palm (7.83 ± 0.21).[14] Moreover, the carotenoid extract from mana-cubiu was also more potent as a $ROO^•$ scavenger than authentic standards of lycopene (8.67 ± 0.74), β-carotene (3.24 ± 0.22), and lutein (1.90 ± 0.17),[14] suggesting the occurrence of synergy among the compounds present in this fruit extract.

The synergistic effect among carotenoids was previously reported between astaxanthin and β-carotene, astaxanthin and lycopene, β-carotene and lycopene, and also lycopene and lutein.[23−25] The three mechanisms by which the carotenoids can scavenge $ROO^•$ are electron transfer, allylic hydrogen abstraction, and addition of a radical to the conjugated double-bond system,[26,27] generating a carotenoid radical as one of the reaction products. In a carotenoid extract, the regeneration mechanisms of a carotenoid radical by another carotenoid molecule are probably responsible for the synergistic effect on the $ROO^•$ scavenging capacity. An efficiency hierarchy was established to compare the ability of regeneration of carotenoid radical cations by other carotenoids (eq 1) in which astaxanthin was the least efficient, while lycopene, β-carotene, and zeaxanthin were among the most efficient and showed regeneration ability comparable to that of α-tocopherol.[28]

$$CAR1^{•+} + CAR2 \rightarrow CAR1 + CAR2^{•+} \quad (1)$$

Phenolic Compounds and ROS and RNS Scavenging Capacity. The HPLC−DAD chromatograms, processed at 280 and 320 nm, show the separation of three phenolic compounds from mana-cubiu (Figure 2). Table 3 shows the identification or tentative identification of the phenolic compounds, considering the combined results of the following parameters: elution order on the C_{18} column, UV−vis spectral features [maximal absorption wavelength (λ_{max})], spike with standard, MS spectral characteristics compared to those of standards analyzed under the same conditions, and data available in the literature.[20,29] The retention time, UV−vis, and mass spectral data of 20 standards of phenolic compounds were also used to confirm their presence or absence (data not shown). The mass spectra of peaks 1−3 are shown in Figures S2−S7 of the Supporting Information.

Peak 1 was identified as 5-caffeoylquinic acid (MW = 354) (Figures 2 and 3). It presented the same retention time, UV−visible and MS spectra, and MS^2 and MS^3 fragmentation patterns as the 5-caffeoylquinic acid standard, which were also the same as the data previously reported in the literature.[29,30] The identity of this compound was confirmed by co-elution with the 5-caffeoylquinic acid standard.

Peak 2 was tentatively assigned as N^1,N^5- or N^5,N^{10}-bis(dihydrocaffeoyl) spermidine (MW = 473) (Figures 2 and 3). In the positive ionization mode, the mass spectrum showed the protonated molecule $[M + H]^+$ at m/z 474 and the MS^2 spectrum showed a peak at m/z 457 $[M + H - NH_3]^+$ corresponding to the loss of ammonium. Moreover, the MS^2 spectrum showed a base peak at m/z 222 $[M + H - NH_3 - C_{12}H_{16}NO_3]^+$ and a peak at m/z 236 $[M + H - NH_3 - C_{13}H_{17}NO_3]^+$, both arising from cleavage of the bond at N^5. The MS^3 spectrum of the peak at m/z 457 showed fragments at m/z 222 $[M + H - NH_3 - C_{12}H_{16}NO_3]^+$ and a peak at m/z 236 $[M + H - NH_3 - C_{13}H_{17}NO_3]^+$. In the negative ionization mode, the mass spectrum showed the deprotonated molecule

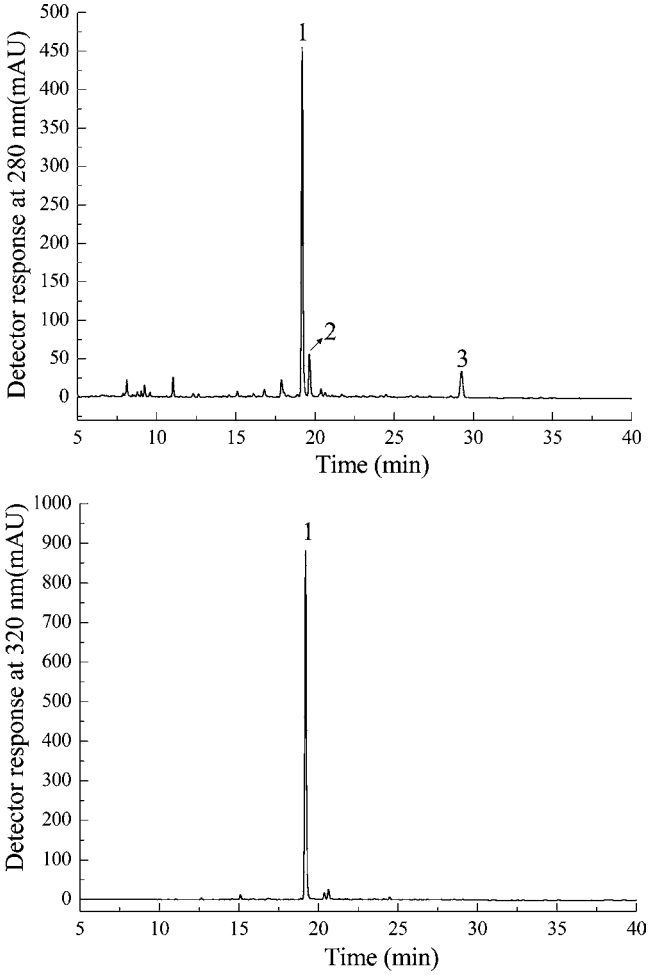

Figure 2. Chromatograms obtained by HPLC−DAD of the phenolic compounds from mana-cubiu. For chromatographic conditions, see the text. Peak characterization is given in Table 3.

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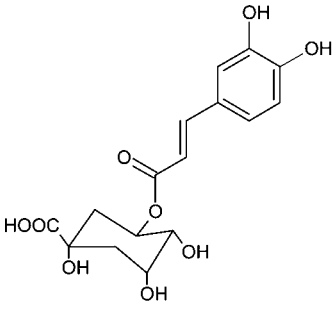
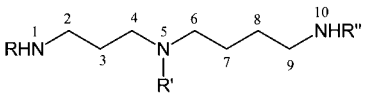
Table 3. Chromatographic and Spectroscopic Characteristics and Phenolic Compound Content of *S. sessiliflorum* Fruit, Obtained by HPLC−DAD−ESI−MS^n

peak[a]	compound	concn (μg/g of dry weight)[b]	tr[c] (min)	λ_{max} (nm)[d]	$[M + H]^+$ (m/z)	fragment ions (m/z) from MS^n (+)	$[M - H]^-$ (m/z)	fragment ions (m/z) from MS^n (−)
1	5-caffeoylquinic acid[f]	1351 ± 36	18.9	300sh,[e] 326	355	MS^2 [355]: 163, 145	353	MS^2 [353]: 191, 179
						MS^3 [355 → 163]: 145, 135, 117, 107		MS^3 [355 → 191]: 173, 171, 127, 111
2	N^1,N^5- or N^5,N^{10}-bis(dihydrocaffeoyl) spermidine[g]	199 ± 6	19.6	280	474	MS^2 [474]: 457, 236, 222, 165	472	MS^2 [472]: 350, 308, 186
						MS^3 [474 → 457]: 236, 222, 165		MS^3 [472 → 308]: 186
3	N^1,N^5,N^{10}-tris(dihydrocaffeoyl) spermidine[g]	168 ± 5	29.1	281	638	MS^2 [638]: 474, 457	636	MS^2 [636]: 472, 350, 308
						MS^3 [638 → 474]: 457, 236, 222, 165		MS^3 [636 → 472]: 350, 308
	total phenolic compounds (μg/g of dry weight)	1718 ± 36						
	total phenolic compounds (μg/g of fresh weight)	155 ± 3						

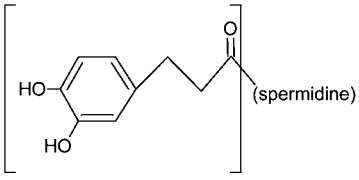
[a]Numbered according to the chromatogram shown in Figure 2. [b]The phenolic compounds were quantified as 5-caffeoylquinic acid equivalents (n = 3). [c]Retention time on the C_{18} column. [d]Solvent, linear gradient of water and acetonitrile both with 0.5% formic acid. [e]sh, shoulder. [f]Identified (standard available). [g]Tentatively identified.



5-caffeoylquinic acid



spermidine



dihydrocaffeoyl-(DHC)

Compound	R	R'	R''
spermidine	-H	-H	-H
N^1,N^5-bis(dihydrocaffeoyl) spermidine	-DHC	-DHC	-H
N^5,N^{10}-bis(dihydrocaffeoyl) spermidine	-H	-DHC	-DHC
N^1,N^5,N^{10}-tris(dihydrocaffeoyl) spermidine	-DHC	-DHC	-DHC

Figure 3. Structures of 5-caffeoylquinic acid and dihydrocaffeoyl spermidines found in mana-cubiu.

$[M - H]^-$ at m/z 472 and the MS^2 spectrum showed a base peak at m/z 308 $[M - H - 164]^-$, arising from cleavage of the amide bond between the caffeic acid residue and spermidine moiety. The MS^3 spectrum of the base peak (m/z 308) showed fragments at m/z 185. Moreover, peak 2 showed MS spectrum and MS^2 and MS^3 fragmentation patterns similar to data previously reported in the literature.[20,31]

Peak 3 was tentatively assigned as N^1,N^5,N^{10}-tris-(dihydrocaffeoyl) spermidine. In the positive ionization mode, the mass spectrum showed the protonated molecule $[M + H]^+$ at m/z 638 and the MS^2 spectrum showed a peak at m/z 474 $[M + H - 164]^+$, resulting from the cleavage of the amide bond between the caffeic acid residue (164 units) and the spermidine moiety, and a base peak at m/z 457 $[M + H - 164 - NH_3]^+$. The MS^3 spectrum of the peak at m/z 474 showed fragment peaks at m/z 457 $[M + H - 164 - NH_3]^+$ and m/z 222 $[M + H - 164 - NH_3 - C_{12}H_{16}NO_3]^+$. In the negative ionization mode, the mass spectrum showed the deprotonated molecule $[M - H]^-$ at m/z 636 and the MS^2 spectrum showed a base peak at m/z 472 $[M - H - 164]^-$, arising from cleavage of the amide bond between the caffeic acid residue and spermidine moiety. The MS^3 spectrum of the base peak (m/z 472) showed a fragment at m/z 308 $[M - H - 164 - 164]^-$. Moreover, peak 3 showed an MS spectrum and MS^2 and MS^3 fragmentation patterns similar to data previously reported in the literature.[20,31,32]

The spermidine hydroxycinnamic acid conjugates found in mana-cubiu are usually present in food, especially in breast milk and meat,[33,34] and are also found in plants from the *Solanum* genus, such as narinjilla[20] and potato.[35] These compounds

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exhibit biological activities, such as immunologic system cell differentiation and regulation of inflammatory reactions.[34,36]

The hydrophilic extract of mana-cubiu was able to scavenge $ROO^{\bullet}$, HOCl, H_2O_2, $HO^{\bullet}$, and $ONOO^-$ in a dose-dependent manner (Table 2 and Figures S8 and S9 of the Supporting Information). The hydrophilic extract of mana-cubiu was shown to be a very potent scavenger of H_2O_2 and HOCl. The capacity of the mana-cubiu extract to scavenge H_2O_2 was almost twice higher than that of 5-caffeoylquinic acid. The high capacity of the mana-cubiu extract to scavenge H_2O_2 can also be verified by comparing its IC_{50} value (305 ± 17 μg/mL) with the IC_{50} values obtained for plant extracts with well-recognized antioxidant capacity, such as walnut (*Juglans regia*) (IC_{50} = 383 μg/mL)[37] and oak (*Quercus robur*) (IC_{50} = 251 μg/mL).[38] The hydrophilic extract of mana-cubiu (13 ± 0.8 μg/mL) was a 4- and 15-fold more potent HOCl scavenger than 5-caffeoylquinic acid standard (56 ± 2.5 μg/mL) and an ethanolic extract of piquiá (199 μg/mL), respectively.[39] However, the $ROO^{\bullet}$, $HO^{\bullet}$, and $ONOO^-$ scavenging capacities of the hydrophilic extract from mana-cubiu represented <3% of the scavenging capacity of 5-caffeoylquinic acid against these reactive species. The capacity of the mana-cubiu extract to scavenge $ONOO^-$ was similar in the presence and absence of $NaHCO_3$. This evaluation is important because, under physiological conditions, the reaction between $ONOO^-$ and bicarbonate is predominant ($k = 3-5.8 \times 10^4\ M^{-1}\ s^{-1}$), generating nitrogen dioxide ($^{\bullet}NO_2$) and carbonate radical anion ($CO_3^{\bullet-}$). Thus, possibly, the hydrophilic extract is also able to scavenge $^{\bullet}NO_2$ and $CO_3^{\bullet-}$.

The main mechanism of the phenolic compounds to scavenge $ROO^{\bullet}$ involves the transfer of one H atom (HAT); however, the transfer of one electron (SET) has already been reported, and more recently, the formation of adducts between the reactive species and the phenolic compound molecule was also reported.[40] The 5-caffeoylquinic acid represented more than 78% (w/w) of the phenolic compounds of the mana-cubiu hydrophilic extract, and it is highly possible that it was the main component responsible for its capacity to scavenge $ROO^{\bullet}$. Despite this fact, the extract exhibited less capacity to scavenge $ROO^{\bullet}$ than 5-caffeoylquinic acid (Table 2), possibly because this compound represents only ∼0.4% (w/w) of the extract total weight (Table S1 of the Supporting Information). The $ROO^{\bullet}$ scavenging capacity of this extract (0.32 μmol of trolox equivalent/mg of extract), measured by the ORAC method, was also inferior to that of other fruit extracts, such as bilberry (2.6 μmol of trolox equivalent/mg of extract) and elderberry (2.2 μmol of trolox equivalent/mg of extract).[15]

The mechanisms of scavenging H_2O_2 and HOCl involve the transfer of two electrons,[41] and it has already been reported that the 5-caffeoylquinic acid scavenges the HOCl by donating two electrons, generating an *o*-quinone.[42] The higher efficiency of the hydrophilic extract in scavenging H_2O_2 and HOCl as compared with that of the 5-caffeoylquinic acid indicated that the other compounds present in the extract, including the spermidines conjugated to caffeic acid, probably exhibit a strong capacity to scavenge these two ROS. Carbohydrates, proteins, and amino acids are also components of the hydrophilic extract and therefore can have a great role in the extract's scavenging capacity against H_2O_2 and HOCl, because these compounds have been previously reported to be efficient scavengers of these two ROS.[43]

The phenolic compound structure influences the mechanism of $ONOO^-$ scavenging, which can occur via nitration or electron donation. Monohydroxylated phenolic compounds, such as *p*-coumaric and ferulic acids, scavenge the $ONOO^-$ by nitration, while the phenolic compounds possessing catechol structures, such as 5-caffeoylquinic acid, scavenge the $ONOO^-$ by electron donation, generating the corresponding quinone.[44]

The carotenoid and phenolic compound compositions of mana-cubiu were successfully determined by HPLC–DAD–MS^n for the first time. As opposed to the case in most fruits,[13,45] only three phenolic compounds were found in manu-cubiu, i.e., 5-caffeoylquinic acid and two different dihydrocaffeoyl spermidines. Despite the low carotenoid content and vitamin A activity, the carotenoid extract from mana-cubiu was a potent $ROO^{\bullet}$ scavenger. The hydrophilic extract of mana-cubiu presented a strong capacity to scavenge H_2O_2 and HOCl, possibly because of the presence of the spermidine caffeic acid conjugates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Contents of phenolic compounds of the hydrophilic extract from mana-cubiu, MS, MS^2, and MS^3 spectra of the phenolic compounds identified in mana-cubiu, and graphic data of the antioxidant capacities of the mana-cubiu extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +55-19-35212160. Fax: +55-19-35212153. E-mail: azm@fea.unicamp.br.

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Notes

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