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Original Research Article

Identification and quantification of the native carotenoid composition in fruits from the Brazilian Amazon by HPLC–DAD–APCI/MS

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ABSTRACT

In this study the native carotenoid composition of previously uninvestigated fruits from the Brazilian Amazon was determined; marirana (*Couepia subcordata* Benth.), inajá (*Maximiliana maripa*) and caranã (*Mauritiella armata*) were analyzed by an HPLC–DAD–APCI–MS methodology. Three carotenoids were identified in marirana pulp, all-*trans*-α-carotene (6407 ± 380 μg 100 g^{-1}, fresh weight) and all-*trans*-β-carotene (6331 ± 410 μg 100 g^{-1}, fresh weight) being the two major carotenoids, followed by zeaxanthin dilaurate (1061 ± 80 μg 100 g^{-1}, fresh weight). Only β-carotene was found in inajá pulp (1371 ± 370 μg 100 g^{-1}, fresh weight). In caranã pulp, four carotenoids were identified, all-*trans*-β-carotene being the most predominant (373 ± 80 μg 100 g^{-1}, fresh matter), followed by all-*trans*-α-carotene (230 ± 60 μg 100 g^{-1}, fresh weight), lutein (198 ± 40 μg 100 g^{-1}, fresh weight) and 9-*cis*-β-carotene (111 ± 30 μg 100 g^{-1}, fresh weight). The cultivation and consumption of these fruits should be encouraged, since they could contribute to the intake of important carotenoids that could have beneficial effects on human health.

1. Introduction

Brazil boasts various underexploited native and exotic fruit species of potential interest to the agroindustry and a possible future source of income for the local population (Rufino et al., 2010). The Amazon region in Brazil is considered a valuable natural reserve of food and medicinal plants worldwide. It is estimated that 44% of native fruits are located in this region (Neves et al., 2015). Most of these native fruits are wild or cultivated only for the local markets, due to the lack of studies related to the conditions of crop growth, lack of data on the nutritional value of fruits, lack of knowledge about the presence of bioactive compounds and the possibilities of their commercialization (Neves et al., 2015). These fruits are considered a potential source of micronutrients, such as minerals and vitamins, in addition to dietary fiber and phenolic compounds, with importance for human health (Rufino et al., 2010).

Among these native fruits are marirana, inajá and caranã. The marirana (*Couepia subcordata* Benth.) is a drupe fruit, with a yellow-orange epicarp and mesocarp, and with a strong and peculiar aroma surrounding the endocarp, which contains a seed inside. It is a little-known fruit from the Amazon, and is usually sold in open markets (Cardoso et al., 2003). The fruit pulp can be consumed fresh. Inajá is a fruit of inajazeiro (*Maximiliana maripa* (Aubl.) Drude), a palm tree of the family Arecaceae (Palmae) found throughout the Amazon region (Bezerra, 2011). Flowering occurs from October to November and fruit production on the eastern side of the region is concentrated between January and March. In the western Amazon, flowering takes place in mid-July with fruits in early November (Bezerra, 2011). The industrial potential of inajá relies on the edible oil obtained from the fruit kernel. Both kernel and fruit pulp can be used in the cosmetics, soaps and food industries. Inajá (whole fruit) is considered a source of phosphorus, magnesium and fatty acids. The fruit pulp can be consumed fresh or cooked (Bezerra, 2011; Shanley et al., 2005). Caranã (*Mauritiella armata*) also belongs to the family Arecaceae and is well distributed throughout the Amazon region. The immature fruits are globose to oblong-ellipsoid, with imbricated brown-red scales (Hiura and Rocha, 2018). When they are ripe, the fruits fall on the ground. To be processed the fruits are softened in hot water for about 1 h and only then is the

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Fig. 1. Marirana (*Couepia subcordata* Benth.) (A), inajá (*Maximiliana maripa*) (B) and caranã (*Mauritiella armata*) (C) from Brazilian Amazon.

peel removed, and the pulp scraped from the core. The pulp of the caranã fruit can be eaten as a sweet or after fermentation and heating it is used in the preparation of a "wine", which is flavored with sugar and flour or tapioca.

Information on the carotenoids composition of fruits from the Amazonian region is limited (Cardoso et al., 2003; Rosso and Mercadante, 2007; Santos et al., 2015) and more studies are needed to add value to these species by characterizing their carotenoid profiles and content. Carotenoids are classified into carotenes, composed only by atoms of carbon and hydrogen, and xanthophylls, which besides carbon and hydrogen, also have oxygen in the structure, as part of a functional group (Mercadante et al., 2017). Carotenoids are natural pigments widely present in vegetables and fruits such as carrots, tomatoes, watermelon, and are also used as additives, being responsible for the yellow-reddish color of many foods (Mortensen, 2006; Rosso and Mercadante, 2007). Studies on the composition of carotenoids in fruits are important because carotenoids have been associated with the reduction of the risks of various degenerative conditions, including cardiovascular diseases, chronic liver disease, diabetes, cancer, and macular degeneration (Britton and Khachik, 2009; Fiedor and Burda, 2014; Krinsky and Johnson, 2005; Leoncini et al., 2015; Bahonar et al., 2017; Yahia et al., 2017).

To date, there are no studies available evaluating the qualitative profile and content of the carotenoid composition of the marirana, inajá and caranã fruits. Thus, this study aimed to determine the qualitative and quantitative profile of the carotenoid composition of these three Amazonian fruits by a high-performance liquid chromatography–diode array detection–atmospheric pressure chemical ionization mass spectrometry (HPLC–DAD–APCI–MS) methodology.

2. Material and methods

2.1. Chemicals

For the carotenoid extractions, analytical grade reagents purchased from VETEC (São Paulo, Brazil) were used. LC–MS grade solvents and general reagents were purchased from Sigma-Aldrich (Milan, Italy). Carotenoids standards, namely β-carotene, β-cryptoxanthin, lutein, zeaxanthin and physalein, were purchased from Extrasynthèse (Genay, France).

The purity of the standards was calculated by spectrophotometry. The wavelengths and absorption coefficients used for calculation were, respectively: β-carotene: 449 nm and 2592 in petroleum ether (PE); β-cryptoxanthin: 449 nm and 2386 in PE; lutein: 445 nm and 2550 in ethanol; lycopene: 470 nm and 3450 in PE; zeaxanthin: 449 nm and 2348 in PE.

2.2. Collection and preparation of the samples

The Amazonian fruits marirana (*Couepia subcordata*), inajá (*Maximiliana maripa*) and caranã (*Mauritiella armata*) (Fig. 1) were collected in December 2017, in the municipality of Mazagão (00°06′54″ S latitude and 51°17′20″ W longitude), Amapá, Brazil. Samples of each fruit from different rural locations were collected by harvest resulting in one 1.5 kg composite sample for each fruit.

The fruit maturation was determined according to Donadio (2007) and defined by the peel color and the characteristic smell. The marirana fruit showed a yellow-orange epicarp and mesocarp. Inajá had a thin bark and a yellowish, juicy, and oily pulp. Caranã pulp showed an orange color, which involves a single very hard white seed. In addition, ripe fruits were considered as those obtained after their natural fall from the trees or fall after being lightly touched by hand.

The samples were transported from the harvest site to the laboratory (Food Analysis Laboratory, Embrapa Amapá, Brazil) protected in Styrofoam boxes with ice packs, within two hours of collection. In the laboratory, the samples were selected for appearance, excluding those with any epidermis injury or mechanical damage due to transport. Next, the fruits were peeled, the seeds and kernels were removed, and the fruit flesh was homogenized to a pulp in a food processor (Mallory, FAET 400 W), and stored in plastic containers with screw caps, covered with aluminum foil in freezer at -18 ± 1 °C for three hours.

Subsequently, the samples were packed in Styrofoam boxes with ice and transported to the Federal University of Viçosa, Minas Gerais, Brazil.

2.3. Extraction of carotenoids

Carotenoids were extracted, in three repetitions, in accordance with Rodriguez-Amaya (2001), with minor modifications. Portions of 4 g of fruit pulp of each sample were homogenized with 20 mL of acetone for approximately 2 min in a micro grinder (IKA T 18® Ultra Turrax; IKA Werke GmbH & Co. KG, Staufen, Germany) and were then vacuum filtered using a Buchner funnel with filter paper. The extraction steps were repeated until samples became colorless (three times). For caranã, the extract was centrifuged for 2 min, because the extract still had pulp residue, differing from the other fruits.

Then, the filtrate was transferred in three fractions to a separatory funnel containing 50 mL of petroleum ether to transfer the pigments from acetone to petroleum ether; distilled water being added after each addition of the extract and the lower aqueous phase discarded, to

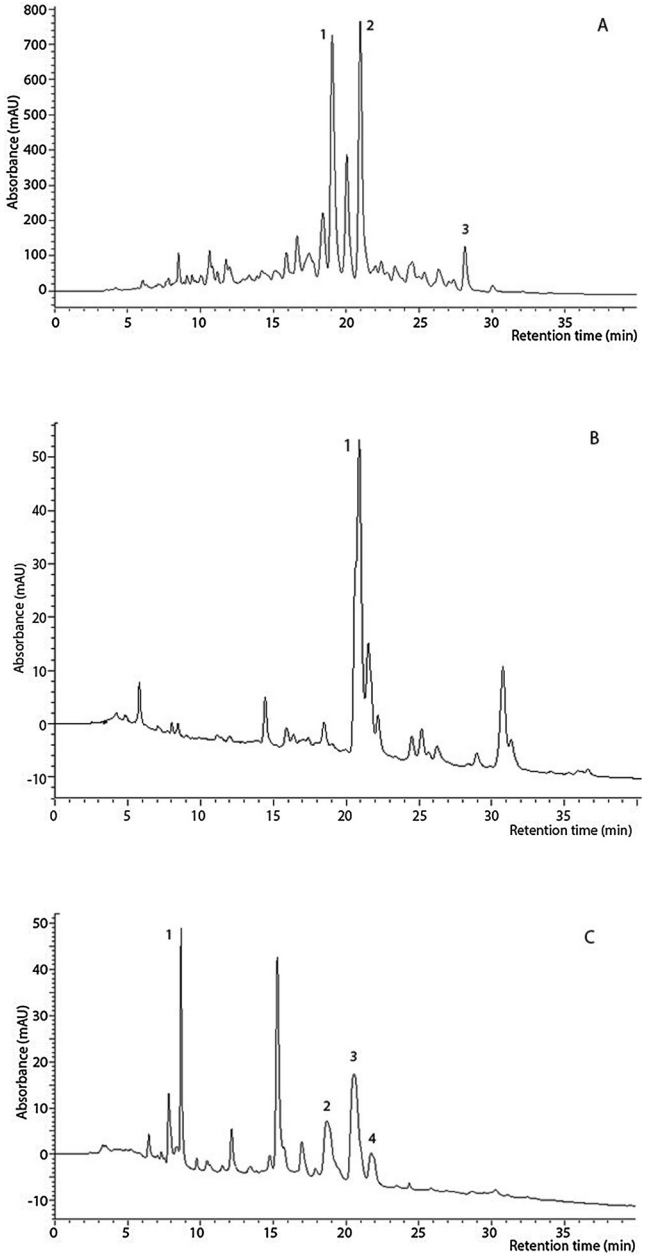

Fig. 2. HPLC–DAD–APCI–MS analysis of carotenoids in marirana (*Couepia subcordata* Benth.) (A), inajá (*Maximiliana maripa*) (B) and caranã (*Mauritiella armata*) (C).
Chromatogram A, peak numbers: 1 = all-*trans*-α-carotene, 2 = all-*trans*-β-carotene, 3 = zeaxanthin dilaurate. Chromatogram B, peak numbers: 1 = β-carotene. Chromatogram C, peak numbers: 1 = lutein, 2 = all-*trans*-α-carotene, 3 = all-*trans*-β-carotene, 4 = 9-*cis*-β-carotene.

remove all acetone. Anhydrous sodium sulfate was added to the ether extract to remove any residual water that could impair evaporation of the material. Then the extract in ether was transferred to an amber glass vial, evaporated under nitrogen gas flow and stored at −18 ± 1 °C for five weeks.

The dried samples were then redissolved in 1 mL of MeOH/MTBE (1:1, *v/v*) and filtered (nylon 0.2 µm filter) before HPLC–DAD–APCI–MS analysis, which occurred immediately.

2.4. Analysis of carotenoids by HPLC–DAD–APCI–MS

For the carotenoids determination, the method described by Dugo et al. (2008) was used, including a liquid chromatography system (Shimadzu, Kyoto, Japan), consisting of a CBM-20A controller, two LC-20AD pumps, a DGU-20 A3R degasser, a CTO-20AC column oven, a SIL-20AC autosampler, and a SPD-M20A diode array detector. The LC system was coupled to an LCMS-2020 mass spectrometer through an APCI (atmospheric pressure chemical ionization) source (Shimadzu, Kyoto, Japan).

Separation of carotenoids was carried out on a 250 mm × 4.6 mm i.d., 5 µm, YMC C_{30} column. Mobile phase A (MeOH/MTBE/H_2O, 81:15:4) and phase B (MeOH/MTBE/H_2O, 16:80.4:3.6) were applied in a linear gradient changing from 99 to 66% A in 30 min, maintaining this condition for 5 min, changing from 66 to 44% A in 15 min, keeping this condition for 5 min, changing from 44 to 22% A in 15 min and from 22 to 0% A in 5 min, returning to the initial conditions (99% A) in 5 min, and keeping this condition for 5 min. The flow rate was set at 0.8 mL/min, the column temperature was maintained at 35 °C, the UV/vis spectra were acquired between 220 and 700 nm, and the chromatograms were processed at 450 nm.

The LCMS-2020 detection was achieved through an APCI interface operated in positive and negative mode; detector voltage, 1.05 kV; interface temperature: 350 °C; DL temperature, 300 °C; heat block temperature, 300 °C; nebulizing gas flow (N_2), 2.0 L/min; drying gas flow (N_2), 5.0 L/min; full scan range (positive and negative mode), *m/z* 300–1200; event time, 0.2 s. The analyses were carried out in three repetitions.

2.5. Identification and quantification of carotenoids

The carotenoids were identified considering combined information of: elution order on the C_{30} column, co-chromatography with authentic standards, UV–vis and mass spectra, and comparison with data available in the literature.

Quantitative data were obtained by HPLC–DAD using external calibration curves from carotenoid standards, in a concentration range from 0.01 to 100 µg/mL at six concentration levels, considering the purity of standards), calculated by spectrophotometry. The results were obtained from an average of three injections of each standard and the CV% was below 8% in all the LC measurements. Standard purity was above 98% and the *R* coefficient for the calibration curves was always above 0.9962, with LOD and LOQ values of respectively for β-carotene 0.07 and 0.22, for β-cryptoxanthin of 0.10 and 0.33, for lutein of 0.06 and 0.18, for zeaxanthin of 0.08 and 0.30, and for physalein of 0.12 and 0.24 µg/mL. The alpha-carotene was quantified using the beta-carotene standard calibration curve.

The carotenoid concentrations are expressed in µg 100 g^{-1} of fresh weight, and the results are represented as mean values (± SD).

3. Results and discussion

3.1. Qualitative profile of carotenoids in Amazonian fruits

Fig. 2 shows the chromatograms obtained by HPLC–DAD–APCI–MS of marirana (A), inajá (B) and caranã (C), and the chromatographic, UV–Vis and MS characteristics of the detected compounds are shown in Table 1.

Three carotenoids were identified in marirana pulp (Fig. 2A). The predominant carotenoids were all-*trans*-α-carotene and all-*trans*-β-carotene, representing 37.3% and 36.8% of total carotenoids, respectively, followed by a smaller amount of the xanthophyll diester, zeaxanthin dilaurate, contributing 6.2% to the total carotenoid content (Table 1). All-*trans*-α-carotene and zeaxanthin were also detected in others Amazonian fruits, like mamey, marimari, physalis and tucuma (Rosso and Mercadante, 2007). To the best of the authors' knowledge, this is the first report on the carotenoids composition in marirana fruits.

Just all-*trans*-β-carotene was identified in the inajá pulp (Fig. 2B, Table 1) in this study. In another investigation on inajá oil, β-carotene

Table 1
Chromatographic, UV/Vis and mass spectroscopy characteristics and quantification of major carotenoids from marirana, inajá and caranã fruits obtained from HPLC–DAD–APCI/MS.

Fruit	Compound	t_R (min)	PDA λ_{max} (nm)	MS-APCI $[M + H]^+$ (*m/z*)	MS-APCI $[M]\cdot$ (*m/z*)	Content (μg.100 g^{-1})[a]	Carotenoid composition (%)
Marirana	all-*trans*-α-carotene	19	425, 447, 474	537	536	6407(± 380)	37.26
	n.i.	20.1	401, 423, 450	n.i.	536	3397(± 180)	19.75
	all-*trans*-β-carotene	21	430, 451, 478	537	536	6331(± 410)	36.82
	zeaxanthin dilaurate	28.1	422, 447, 475	733, 533	932	1061(± 80)	6.17
	Total carotenoids					17,196	
Inajá	all-*trans*-β-carotene	20.9	426, 452, 475	537	536	1371(± 370)	100
	Total carotenoids					1371	
Caranã	all-*trans*-lutein	8.7	414, 445, 474	551	568	198(± 40)	21.71
	all-*trans*-α-carotene	18.7	420, 447, 475	537	536	230(± 60)	25.22
	all-*trans*-β-carotene	20.5	424, 452, 478	537	536	373(± 80)	40.90
	9-*cis*-β-carotene	21.7	339, 423, 448, 473	537	536	111(± 30)	12.17
	Total carotenoids					912	

n.i.: not identified.
[a] Mean of 3 repetitions ± standard deviation (SD), fresh weight.

was the most predominant carotenoid as well, followed by lycopene (Santos et al., 2015).

Four carotenoids were identified in caranã pulp (Fig. 2C), all-*trans*-β-carotene being the predominant one (representing 40.90% of total carotenoids), followed by all-*trans*-α-carotene and all-*trans*-lutein (Table 1). The all-*trans*-lutein has also been identified in other Amazonian fruits, like physalis, tucuma, buriti, marimari and palm oil (Rosso and Mercadante, 2007). Moreover, five types of chlorophylls were detected in this fruit (Fig. 2), since as already discussed, the chlorophylls are commonly present in non-saponified samples (Petry and Mercadante, 2016). To the best of the authors' knowledge, no other reports are available in the literature on caranã fruits.

The extracts of carotenoids from marirana, inajá and caranã were not saponified, which means that the present study evaluated the native carotenoid composition of these Amazonian fruits. Data about the native carotenoids composition of foods are often not available, since a saponification step is usually applied to make carotenoid analysis simpler by releasing the fatty acids bound to carotenoids and eliminating interfering compounds such as chlorophylls and lipids (Petry and Mercadante, 2016). Moreover, the saponification process may cause some carotenoid degradation and modifications, such as isomerization. So, the investigation regarding the native composition of carotenoids is of great importance, especially for foods that are not yet reported in the literature, as in this case.

On the other hand, the presence of high contents of lipids, as triacylglycerols impairs the separation and identification in non-saponified extracts, mainly of carotenoid esters, as well as causing high background noise in APCI positive mode during LC–MS analysis (Breithaupt, 2000; Breithaupt and Bamedi, 2002; Rodrigues et al., 2016).

3.2. Carotenoids content of Amazonian fruits

The total carotenoids content ranged from 912 μg 100 g^{-1} (caranã pulp) to 17,196 μg 100 g^{-1} (marirana pulp), both fresh weight. According to the classification proposed by Britton and Khachik (2009), the total carotenoid content in fruits and vegetable can be regarded as low (0–100 μg 100 g^{-1}), moderate (100–500 μg 100 g^{-1}), high (500–2000 μg 100 g^{-1}) and very high (more than 2000 μg 100 g^{-1}) fresh weight. Thus, marirana showed a very high content of carotenoids, and inajá and caranã had a high content of carotenoids. The carotenoid content of marirana was higher than that found in other Amazonian fruit pulps, such as mamey (6253 μg 100 g^{-1}), marimari (3798 μg 100 g^{-1}), commercial palm oil (*Elaeis guineensis*) (12,903 μg 100 g^{-1}), physalis (8089 μg 100 g^{-1}) and tucuma (6265 μg 100 g^{-1}) and lower than that found in buriti (51,387 μg 100 g^{-1}) and peach palm (19,766 μg 100 g^{-1}) (Rosso and Mercadante, 2007).

In marirana pulp, all-*trans*-α-carotene (6407 ± 380 μg 100 g^{-1}, fresh weight) and all-*trans*-β-carotene (6331 ± 410 μg 100 g^{-1}, fresh weight) were the carotenoids found in the highest content (Table 1). Zeaxanthin was found in smaller amounts (1061 ± 80 μg 100 g^{-1}, fresh weight). Other Amazonian fruits (buriti, mamey, marimari, commercial palm oil, peach palm, physalis and tucuma) showed lower content of all-*trans*-α-carotene (Rosso and Mercadante, 2007).

Only β-carotene was found in inajá pulp (1371 ± 370 μg 100 g^{-1}, fresh weight), as shown in Table 1. Only two reports were available in the literature on carotenoids in inajá oil (Dos Santos et al., 2015; Santos et al., 2015), but not in inajá pulp, which makes it difficult to compare our results; Santos et al., 2015 reported a content of 2303 ± 159 μg.100 g^{-1} of β-carotene in inajá oil sample. In another study (dos Santos et al., 2015) on inajá oil a content of 400 ± 159 μg 100 g^{-1} of total carotenoids was reported. The difference in results can be attributed to the different food matrices and to the different methods used for the separation and determination of carotenoids, since in the study of dos Santos et al. (2015), the total carotenoids were determined by spectrophotometry and in our study we analyzed carotenoids by HPLC.

In the present study, all-*trans*-β-carotene and all-*trans*-α-carotene were found in highest amounts in caranã pulp (373 ± 80 μg 100g^{-1} and 230 ± 60 μg 100 g^{-1}, fresh weight, respectively), followed by all-*trans*-lutein (198 ± 40 μg 100g^{-1}, fresh weight) (Table 1).

In general, the palm fruits found in the Brazilian Amazon are rich in β-carotene (Rosso and Mercadante, 2007; Santos et al., 2015), similar to our results. However, some studies analyzed the carotenoid composition of the oils extracted from the mesocarp of palm fruits (Santos et al., 2015), which makes it difficult to compare with our results. In addition, studies suggest that the consumption of supplements containing lutein and zeaxanthin or food products that contain these xanthophylls in high concentrations may reduce the risk of macular degeneration (Landrum et al., 1997) and cataract formation (Stringham et al., 2010; Zhao and Sweet, 2008).

Although the consumption of Amazonian fruits has a regional predominance, these fruits present great possibilities for their use in agroindustry. Also, scientific research that seeks to identify functional properties in fruits, such as their sensory characteristics and their nutritional value, also promotes their commercial value and, as a consequence, can benefit family farming and could contribute to a sustainable development of the region, for the exploration and commercialization of new fruit species (Massing et al., 2018).

Thus, the consumption of the fruits analyzed in the present study should be encouraged, due to the health benefits of their carotenoid constituents and especially considering the very high content here reported for the first time in marirana fruits.

4. Conclusion

The carotenoid contents of Amazonian fruits (marirana, inajá and caranã) were successfully identified and quantified by HPLC–DAD–APCI–MS. In addition, the carotenoid compositions of marirana and caranã fruits have not been previously reported. The consumption of these fruits, especially marirana, should be encouraged, since they can contribute to the intake of important bioactive phytochemicals; moreover, their consumption could provide benefits to family farms and could contribute to the sustainable development of the Amazon region.

Declaration of Competing Interest

None.

Acknowledgments

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