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QUANTIFICATION OF ASCORBIC ACID IN AMAZON FRUITS

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RESUMO – Os alimentos funcionais naturais, com destaque para os frutos, tornaram-se bastante populares por seus efeitos benéficos a saúde humana e no tratamento de doenças. A família Myrtaceae possui frutos muito apreciados por suas propriedades organolépticas e por ser ricos em compostos antioxidantes naturais. Foram selecionados frutos de Myrtaceae e quantificado o ácido ascórbico pelo método analítico de HPLC-DAD. É o primeiro relato sobre a determinação do ácido ascórbico em polpas frescas dessas frutas consideradas Plantas Alimentícias não Convencionais (PANC) endêmicas na Amazônia, e os resultados revelam que elas podem ser consideradas como alimentos funcionais devido aos altos potenciais antioxidantes e nutricionais atribuídos à vitamina C.

ABSTRACT – Natural functional foods, especially fruits, have become quite popular for their beneficial effects on human health and in the treatment of diseases. The Myrtaceae family has fruits that are highly appreciated for their organoleptic properties and for being rich in natural antioxidant compounds. Myrtaceae fruits were selected and ascorbic acid was quantified by the HPLC-DAD analytical method. It is the first report on the determination of ascorbic acid in fresh pulps of these fruits considered non-conventional food plants (PANC) endemic in the Amazon, and the results reveal that they can be considered as functional foods due to the high antioxidant and nutritional potentials attributed to vitamin C.

PALAVRAS-CHAVE: Myrtaceae; alimentos funcionais; HPLC-DAD; alimentos saudáveis; prevenção de doenças.

KEYWORDS: Myrtaceae; functional foods; HPLC-DAD; healthy food; disease prevention.

1. INTRODUCTION

Functional foods have become quite popular in the treatment of diseases. There are several scientific studies that prove that unconventional tropical fruits can be considered as functional foods (Dantas et al. 2019), because they have high antioxidant and nutritional potentials (Ramos et al. 2015a; Candido et al. 2015; Neri-Numa et al. 2013; Borges et al. 2011; Azevedo et al. 2019). These substances are also responsible for their pharmacological potential (Agarwal et al. 2019; Azevedo et al. 2019), for example, camu-camu (*Myrciaria dubia* McVaugh), considered the Brazilian fruit with the highest amount of vitamin C (1 to 3 grams / 100g of fresh fruit) (Justi et al. 2000; Fracassetti et al. 2013), another examples are the species of araçá-boi (*Eugenia stipitata* McVaugh) e araçá (*Psidium cattleianum* Sabine) (Medina et al. 2011; Astrid Garzon et al. 2012), and araçá-pera (*Psidium acutangulum*) (Ramos et al. 2015a).

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Therefore, functional or nutraceutical food can be classified as a safe ingredient for consumption such as dietary fiber, polyunsaturated fatty acid, protein, peptide, amino acid, mineral, vitamin, antioxidant and bioactive phenolic substance (ANVISA 1999). In this context, the demand of several countries for fruits rich in vitamin C increased due to their potential in the prevention and treatment of chronic diseases (Belwal et al. 2018; Prakash and Baskaran 2018). Several fruits rich in vitamin C, whose levels were recently quantified, stood out in reducing oxidative stress, such as powder of bergamot whole-fruit (66.93 ± 0.05 mg AAE/100 g DW) (Gabriele et al. 2017), powder of chilto (*Solanum betaceum*) (117.0 ± 10.2 mg AAE/100 g) (Orqueda et al. 2017), the Indian Himalayan region fruits *Phyllanthus emblica* (33.15 mg/g_{FW}) and *Morus alba* (29.53 mg/g_{FW}) (Bhatt et al. 2017), blackcurrant (162.73 mg/100 g_{FW}), black-berry (45.07 mg/100 g_{FW}) and strawberry (57.95 mg/100 g_{FW}) (Donno et al. 2015). As oxidative stress plays a crucial role in Alzheimer's disease (AD) (Betteridge 2000), and antioxidative supplement such as vitamin C, that present antioxidant capacity, can be used to reduce the oxidative stress.

According to Leffa et al. (2015), vitamin C content in acerola (*Malpighia emarginata* DC.) juice contributes to the reduction of oxidative stress under obesogenic conditions. Vitamin C can efficiently attenuate the toxicities of 3R-tau, predominantly deposited in neurons bearing neurofibrillary tangles in AD, causing neurodegeneration (Xu et al. 2020). The consumption of fruits rich in vitamin C or related bioactive may reduce the risk of Alzheimer's dementia risk (Agarwal et al. 2019) and other diseases. Higher dietary intakes of vitamin C is associated with significantly higher indices of fat-free mass and leg explosive power (Welch et al. 2020). Young et al. (2020) characterized endogenous small molecule vascular enhancers and inhibitors of multimerization. The results obtained suggest that multimerization of proteins in the aging brain is not restricted to neuronal molecules and may participate in age-dependent vascular pathology. The vitamin C acted inhibiting catecholamine-dependent multimerization. Other proprieties from vitamin C is the antimicrobial effect against the bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, and a moderate effect against *Salmonella typhimurium*, *Escherichia coli*, *Enterococcus faecium* and *Candida albicans* observed in ethanolic extract of the mature species of 'medronheiro' (*Arbutus unedo* L.), which most abundant component is vitamin C (48 mg/100 g fruit) (Salem et al. 2018). According to Habib et al. (2019), ascorbic acid showed positive antimicrobial effects against the bacteria *Staphylococcus aureus* and *Escherichia coli*, using the grafted low-density polyethylene (LDPE) assay.

A good diet rich in fruits rich in vitamin C makes it an important source for residents of certain regions (Kumari et al. 2017) due to positive correlation between this vitamin with antioxidant activity, and together with the polyphenols present in the fruit, it enhances its health protection activity. Tropical fruits are rich in antioxidant and anticancer phytochemicals (Ullah et al. 2012). According to Olivás-Aguirre et al (2017), mango has ascorbic acid content higher than in papaya or pineapple. In the same study, they verify that freeze-dried mango, papaya and pineapple are ready-to-eat functional food with cancer preventing properties. There are several other unconventional or little-known fruits, which have a high content of vitamin C in their composition, and, therefore, can also be considered functional foods and included in the human diet with the aim of promoting health.

The most known species of Myrtaceae in Brazil are guava (*Psidium* spp.), jaboticaba (*Myrciaria cauliflora*) and pitangueira (*Eugenia uniflora*). The fruits of Myrtaceae are presented in capsular form with a single core, or resemble small rounded olives, or even in the baciform form with several seeds, such as guava (Sobral and De Souza 2015; Caliari et al. 2016; Ribeiro and Silva 1999; Oliveira et al. 2008). Most of the unconventional fruits of the Amazon are generally obtained from nature or grown only for the local market and sold in the natural form, resulting in low added value (Schreckinger et al. 2010; Kuskoski et al. 2006; Kinupp and Lorenzi 2014). The study of the chemical composition, biological properties and nutritional evaluation of fruits can enable local and regional economic growth due to the valuation and nutraceutical value to the fruit species of the Amazon region (Sonia S. Anand et al. 2015; Andrew D. Jones and Ejeta 2016; Djidel et al. 2010).

2. MATERIAL AND METHODS

The fruits were collected at Reserva Florestal Adolpho Ducke (RFAD), Manaus, Amazonas, Brazil. And they were analyzed still fresh.

2.1. SAMPLE PREPARATION FOR EXTRACTION OF ASCORBIC ACID

The extraction was made with a commercial blender (5 min) and ice bath, in triplicate, based on the Ramos et al (2015b) extraction method. Each extract was centrifuged (BioSpin) for 5 min and the supernatant was filtered through a PTFE membrane, 500 μ L was homogenized in 500 μ L of the mobile phase (water acidified with 1% formic acid (pH 3.5) and, 20 mM $(\text{NH}_4)_2\text{HPO}_4$, 0.015% MPA), then it was subjected to analysis by HPLC-DAD.

2.2. ANALYSIS CONDITIONS AND SAMPLE PREPARATION FOR QUANTIFICATION OF L-(+)-ASCORBIC ACID.

Calibration was performed by injecting a standard solution of L-(+)-ascorbic acid (AA) (1 mg mL⁻¹) in triplicate, prepared on each day of analysis with 1% MPA solution and, then, it was diluted to obtain final concentrations of 1, 20, 40, 60, 80, 100 μ g mL⁻¹.

The equipment used was a Thermo Scientific high-performance liquid chromatograph (HPLC), Acella® with automatic injector and quaternary pump, coupled to the Thermo® mass spectrometer, triple quadrupole (TQS) (San Jose, CA, USA), electrospray ionizer operating in positive modes (+) and negative (-), equipped with a photodiode matrix detector (DAD). Column used: Phenomenex Hydro-RP (150 mm x 4.5 mm, 4 μ m); flow: 600 μ L/min; injection volume: 10 μ L, oven temperature: 25°C and shelf temperature containing the samples: 4°C; UV range: 200 to 600 nm. XCalibur® software were used for data acquisition.

3. RESULTS AND DISCUSSION

3.1. QUANTIFICATION OF ASCORBIC ACID IN AMAZONIAN FRUITS OF MYRTACEAE

Table 1 shows the concentration values of ascorbic acid (AA) determined in the fruits of Myrtaceae. The adopted method has good linearity in the concentration range of 1-100 μ g mL⁻¹ and good resolution for AA (0.4 <Rs <1.3). The lowest detectable concentration - (Limit of detection - LD) for AA was calculated as the signal to noise ratio (S/N) of 3: 1 and the limit of quantification (LQ) determined was obtained from the lowest concentration whose S/N value was greater than 10: 1 (ICH 1996; Snyder and Kirkland 1979). The LD and LQ for AA determined in this study are similar to those reported in the literature when it comes to vitamin C and fruits and vegetables. Therefore, this AA quantification method can be considered sensitive based on the linearity and the LD and LQ values.

Table 1 Ascorbic acid (AA) content (n=3) in Amazonian fresh fruits (FF) of Myrtaceae.

Specie	AA (mg/100g FF)	Parameters
<i>Myrcia minutiflora</i> Sagot.	<LQ	LD: 0.0221 μ AU
<i>Myrcia bracteata</i> DC	<LQ	
<i>Myrcia fenestrata</i> DC	109.08 \pm 19.69	LQ: 0.0737 μ AU
<i>Myrcia sylvatica</i> (G. Mey.) DC	<LQ	
<i>Myrcia magnoliifolia</i> DC.	616.46 \pm 75.63	Linear Eq: y = 7406.1x + 2364.9
<i>Syzygium cumini</i> L.	71.65 \pm 19.12	
<i>Calyptanthus spruceana</i> O. Berg.	<LQ	
<i>Eugenia stipitata</i> McVaugh	45.91 \pm 15.23	
<i>Eugenia punicifolia</i> (Kunth) DC	<LQ	R ² : 0.9993
<i>Eugenia uniflora</i> L.	56.18 \pm 13.0	



Among all samples of exotic fruits analyzed, *M. magnoliifolia* has the highest AA value (616.46 ± 75.63 mg / 100 g FF). In Brazil, this fruit is still unknown and is not yet cultivated, being found only in the wild form, as well as *M. fenestrata*, which has the second highest AA result.

Chemical studies with Myrtaceae fruits reveal the presence of phenolic substances and vitamins (A, C and E), which are responsible for the anti-inflammatory, antioxidant and hypocholesterolemic (De Souza Schmidt Goncalves et al. 2010; Peralta-Bohorquez et al. 2010; Seraglio et al. 2018). However, this is the first report on the determination of ascorbic acid in fresh pulps of these unconventional Amazonian fruits.

The physiological states of ripeness and fruit appearance are related to climatic conditions and the type of region. These factors can influence the color, flavor and quantity of the chemical constituents contained in these fruits. Thus, the levels of ascorbic acid can vary in the pulps during maturation, influencing the antioxidant responses (Brandao et al. 2011).

3. CONCLUSION

The results obtained reveals the importance of chemical and nutritional studies of fruits. The inclusion of functional natural foods can be an alternative for populations that cannot consume industrialized supplements. Thus, through healthy eating, it would be possible to prevent and even combat certain diseases related to oxidative processes, such as diabetes, Alzheimer and cancer.

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