

## $\alpha$ -Tocopherol in Amazon Fruits

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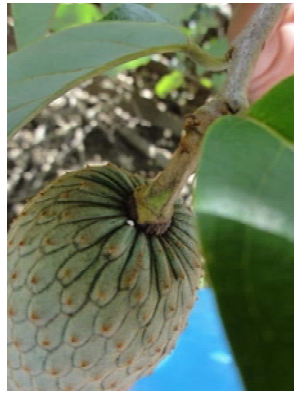
The aim of this work was to perform analysis of the  $\alpha$ -tocopherol content by HPLC in Amazonian fruits consumed in Roraima state, Brazil, namely: *ata brava*, *açaí*, *bacaba*, *buriti*, *buritirana*, yellow *murici*, red *murici*, *piçaba braba*, *pupunha* varieties red and yellow and *tucumzinho*. A high concentration of the vitamin in the *açaí* pulp (1101.11 mg L<sup>-1</sup>) and of the *ata brava* seeds (644.27 mg L<sup>-1</sup>) was observed, but other fruits such as *buritirana* pulp (166.34 mg L<sup>-1</sup>), red *murici* (179.52 mg L<sup>-1</sup>), and *piçaba braba* (8.77 mg L<sup>-1</sup>) presented low amounts of  $\alpha$ -tocopherol. As for the yellow and red varieties of *pupunha* and the *tucumzinho*, no presence of the isomer was detected. Identification and quantification of  $\alpha$ -tocopherol cited in this work are unpublished for the *ata brava*, *buritirana* and red *murici*, as for the other fruits studied there were variations. Amazonian fruits may present  $\alpha$ -tocopherol in its lipid composition, which is one of the most bioactive isomers of vitamin E, for this reason.

### 1. Introduction

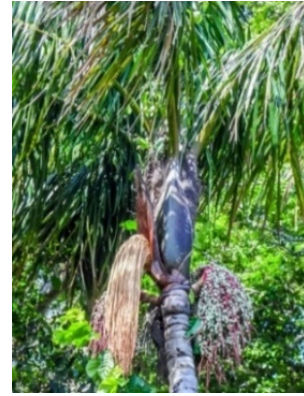
The Brazilian Amazon presents the greatest biodiversity on the planet. The plants of this region present notably the medicinal and / or food potential, for example: *açaí*, *ata brava*, *bacaba*, *buriti*, *buritirana*, *cupuaçu murici*, *piçaba braba*, *pupunha*, *tucumã*, *tucumzinho*, among many others. As an example, the *açaí* is a fruit, well known in Brazil and, in the exterior, it is called superfruit, because it has a bioactive potential as neuroprotection (Peixoto et al., 2016), antioxidant (Pala et al., 2017), antimicrobial (Belda-Galbis et al., 2015), among several other bio-activities, according to Yamaguchi et al. (2015). Fibigr et al. (2017) affirm the presence of anthocyanins, which is confirmed by Yamaguchi et al. (2015), according to the authors in addition to the aforementioned substance there are other bioactives also, such as: flavonoids, lignoids, phenolic compounds, fatty acids, quinones, terpenes and norisoprenoids. Still, it has nutritional and energetic value, which makes this fruit as a functional food. The plant species present in their seeds amounts of oils or fats (lipids) as energy source for their germination, but other plant species may also present lipid source in the pulps (Sasaki, 2008). Lipids include fats, waxes, steroids, liposoluble vitamins, mono-, di- and triglycerides, phospholipids, among others (Solomons and Fryhle, 2005). Vitamin E, for example, has several isomers, such as:  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols and  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ - Tocotrienols (Craft, 2016; Mangialasche et al., 2012; Colombo, 2010). According to Gutierrez-Gonzalez and Garvin (2016),  $\alpha$ -tocopherol is the most bioactive of the tocopherols and tocotrienols groups. For this reason, the aim of the study was to verify the  $\alpha$ -tocopherol content in oils and fats of Amazonian fruits in Roraima state, such as *açaí*, *ata brava*, *bacaba*, *buriti*, *buritirana*, yellow *murici*, red *murici*, *piçaba braba*, *pupunha* varieties yellow and red and *tucumzinho* (Figure 1).



*Açaí*  
(*Euterpe oleracea*)



*Ata brava*  
(*Annona hypoglauca*)



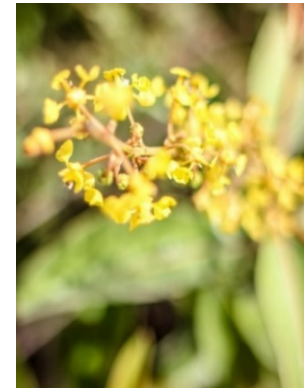
*Bacaba*  
(*Oenocarpus bacaba*)



*Buriti*  
(*Mauritia flexuosa*)



*Buritirana*  
(*Mauritia aculeata*)



*Yellow Murici*  
(*Byrsonima crassifolia*)



*Red Murici*  
(*B. coccolobifolia*)



*Pupunha yellow variety*  
(*Bactris gasipaes*)



*Pupunha red variety*  
(*Bactris gasipaes*)



*Piaçava brava*  
(*Barcella odora*)



*Tucumanzinho*  
(*Astrocaryum acaule*)

*Figure 1: Amazonian fruits in Roraima state, Brazil (Pictures by R.C. Santos)*

## 2. Materials and methods

### 2.1 Sample preparation

The fruits of *açaí* (pulp), *ata atava* (seed), *bacaba* (pulp), *buriti* (pulp), *buritirana* (pulp), yellow *murici* (seed), red *murici* (seed), *piçaba braba* (pulp), *pupunha* varieties red (pulp) and yellow (pulp) and *tucumzinho* (pulp) were obtained at local fairs in the cities of Alto Algre, Caracará, Boa Vista and Mucajá in Roraima state. All fruits collected were mature and good appearance. The seeds and pulps were dried in an air circulating oven at 50 °C for 48 hours, then ground and sieved between 20-40 mesh to obtain homogenized powder, followed by crude oils and fats with hexane in Soxhlet extractor, these were packed in amber flask under nitrogen atmosphere for further analysis. The solvent was reused by rotoevaporator (Santos et al., 2015; Fernández et al., 2016).

### 2.1 Content of $\alpha$ -tocopherol in oils and fats of Amazon Fruits by HPLC

To avoid oxidation of  $\alpha$ -tocopherol, amber and low-light glassware were used, and then the samples were analyzed. Initially the oils and fats were diluted in hexane (1:10), then 200  $\mu$ L were transferred to a screw cap tube, where 600  $\mu$ L of methanol and 200  $\mu$ L of standard internal solution (300  $\mu$ g mL<sup>-1</sup> of  $\alpha$ -tocopherol in ethanol) were added. After being mixed in vortexes and centrifuged at 3000 g for 5 min, the samples were filtered through 0.45  $\mu$ m micropore and injected (50  $\mu$ L) in triplicate into a chromatograph (Gimeno et al., 2000). The analyzes were performed on Shimadzu LC-6AD High Efficiency Liquid Chromatograph (HPLC) equipped with UV-Vis detector. A Supelco column was used at room temperature. 20  $\mu$ L injection loop. The methanol-deionized water mobile phase (91: 9, v/v) and elution was performed at a flow rate at 1 mL min<sup>-1</sup> in isocratic mode. The data acquisition software was LC Solution Shimadzu. To determine  $\alpha$ -tocopherol in the samples, the standard solutions were applied and their calibration curve made on HPLC. The proportions of  $\alpha$ -tocopherol in the samples and the peak areas were calculated according to the external standard method. The UV-Vis detection was done at 290 nm and each run lasted 10 min (Casanova, 2013; Escrivá et al., 2002; Gimeno et al., 2000).

## 3. Results and discussion

The Amazon fruits, commonly consumed in the region, provide favorable yields in the oils, in which good concentrations of  $\alpha$ -Tocopherol are observed, as can be seen in Table 1.

Table 1:  $\alpha$ -Tocopherol in oils and fats of Amazonian fruits

Amazon Fruits	Part Used	Yield of oil (%)	$\alpha$ -tocopherol (mg L <sup>-1</sup> )
<i>Ata braba</i>	Seed	35.4	644.27 (NF)
<i>Açaí</i>	Pulp	15.0	1101.11
<i>Bacaba</i>	Pulp	43.9	7.32
<i>Buriti</i>	Pulp	23.2	156.70
<i>Buritirana</i>	Pulp	26.0	166.34 (NF)
Yellow <i>Murici</i>	Seed	46.5	7.20
Red <i>Murici</i>	Seed	38.0	179.52 (NF)
<i>Piçaba braba</i>	Pulp	17.1	8.77 (NF)
<i>Pupunha</i> var. Yellow	Pulp	27.3	ND
<i>Pupunha</i> var. Red	Pulp	12.0	ND (NF)
<i>Tucumzinho</i>	Pulp	33.1	ND* (NF)

NF: not found literature; ND: not detected; \*: below the detection limit for the method.

The concentration of  $\alpha$ -tocopherol in fruits obtained in Roraima may differ from the concentration of  $\alpha$ -tocopherol in the same fruits of other Brazilian states. Thus, the amount of  $\alpha$ -tocopherol in the oil of the *açaí* pulp ranges from 67 to 97.55% (Darnet et al., 2011a; Lubrano and Robin, 1997). The presence of  $\alpha$ -tocopherol in the *bacaba* is 97% (Lubrano and Robin, 1997), the *buriti* is 37.2% (Darnet et al., 2011b), yellow *murici* 67.25% (Sousa, 2013) and yellow *pupunha* in 96% (Lubrano and Robin, 1997).

Compared with the literature, it can be observed that  $\alpha$ -tocopherols are mostly present in Amazonian oils, except for *buriti* (37.2%), where  $\beta$ - and  $\gamma$ -tocopherols stand out with 57% (Darnet et al., 2011b). In Table 1 we present unpublished results on the  $\alpha$ -tocopherol content in the oils of *ata atava*, *bacaba*, *buritirana*, red *murici* and *piçaba brava*. Such results are satisfactory as they will contribute with essential information that can be applied to better feed local consumers, for the benefits of vitamin E.

Vitamin E (Vit E) is generally found in plants, ie it is obtained through food and has essential activity on the human body and its deficiency or insufficiency can generate serious health problems (Mangialasche et al., 2012). Thus, fruits such as *açaí*, *bacaba*, *buriti*, yellow *murici* and red *murici*, *pupunhas* of varieties yellow and red are commonly consumed in the Amazon region *in natura* or in products, such as cakes, ice cream, biscuits, juices, etc. The Vit E is composed of  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols and  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocotrienols (Figure 2a, b), which are obtained by feeding naturally into vegetable oils, green leaves, dairy products, etc. (Colombo, 2010; Craft, 2016; Mangialasche et al., 2012).

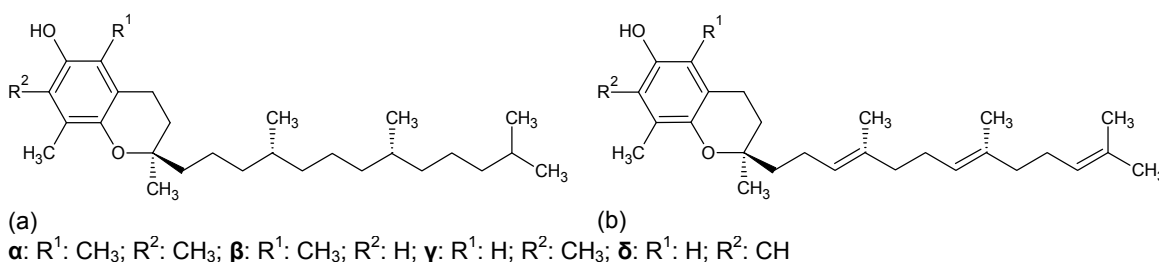


Figure 2: Chemical structures of  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  tocopherol (a) and tocotrienol (b)

According to Colombo (2010), vitamin E has several benefits to the cardiovascular system and low concentrations of cholesterol in the blood. Vitamin E reaches the tissues through LDL cholesterol, because of its strong receptor affinity (Craft, 2016; Kaempf and Linderkamp, 1998) and one of the major functions of vitamin E in the human body is its antioxidant capacity, as it protects cells from free radicals (Azzi et al., 2016; Järvinen and Erkkilä, 2016; Kaempf and Linderkamp, 1998).

Azzi et al. (2016) report that with the use of natural vitamin E could prevent or even cure diseases, supposedly generated by oxidative stress, such as cardiovascular, cancer, cataracts, neurodegenerative and others.  $\alpha$ -tocopherol may have an effect on Alzheimer's disease and other brain problems when taking small doses of the vitamin in a daily diet rather than in the form of supplementation (Morris et al., 2015). Such benefits are confirmed by Järvinen and Erkkilä (2016) and Mangialasche et al. (2012). However, it was observed that the early use of extra virgin olive oil with fatty acids and  $\alpha$ -tocopherol in the long term presented a protective effect against Alzheimer's disease and cerebral amyloid angiopathy in rats (Qosa et al., 2015). Thus, substances (vitamins, minerals, phenolic compounds and carotenoids) present in foods can stimulate the protective effect by their antioxidant activity (Almeida et al., 2011). Frequent consumption of fruits and vegetables is associated with a lower risk of diseases such as stroke, diabetes mellitus, arthritis, Parkinson's, Alzheimer's and cancer (Crowe et al., 2011).

#### 4. Conclusion

As for the presence of  $\alpha$ -tocopherol in oils and fats of the Amazonian fruits, a high concentration of *açaí* pulp oil and of *ata brava* seeds was observed, the latter being an unpublished data in the literature. Other samples such as *buritirana*, red *murici* and *piçaba brava*, presented low concentrations of  $\alpha$ -tocopherol, and this information is unprecedented for these fruits. However, the *pupunha* of yellow and red varieties and the *tucumanzinho* were not identified with  $\alpha$ -tocopherol.

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