

# UNIVERSIDADE ESTADUAL DE CAMPINAS FACULDADE DE ENGENHARIA DE ALIMENTOS

## FLORISVALDO GAMA DE SOUZA

Exploratory study of the fruit and oil of the buritirana palm (*Mauritiella armata*) from the Brazilian Cerrado: biometric attributes, physicochemical, chemical, nutritional, functional and antioxidant and antibacterial potentials.

Estudo exploratório do fruto e óleo da palmeira buritirana (*Mauritiella armata*) do Cerrado Brasileiro: atributos biométricos, físico-químicos, químicos, nutricionais, funcionais e potenciais antioxidantes e antibacterianos.

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> Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutor em Ciência de Alimentos.

Orientadora: Profa. Dra. Glaucia Maria Pastore Coorientadora: Dra. Iramaia Angélica Neri-Numa

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Papa Francisco

#### **RESUMO**

Buritirana é uma espécie pouco estudada. Portanto, o objetivo desta tese foi explorar a palmeira buritirana (Mauritiella armata) através da caracterização de seus frutos e óleo obtidos de diferentes frações, temperaturas e métodos de extração a fim de apresentá-la como uma nova frutífera com potencial para a indústria alimentícia e não alimentícia. Os frutos apresentaram forma oblonga. A polpa representou 16,58% do peso do fruto inteiro (10,07 g). Os teores de umidade, cinzas e fibra solúvel foram semelhantes para a fração inteiro sem semente (WS) e polpa. Embora o teor de carboidratos totais tenha sido o mesmo para semente e casca (23,24 g.100 g<sup>-1</sup>), a semente apresentou maiores teores de proteínas e fibras insolúveis. Com exceção da glicose (1256,63 mg.100 g<sup>-1</sup>), a semente apresentou as maiores concentrações de mono, di e oligossacarídeos. O conteúdo mineral variou de 0,43 a 800 mg.100  $g^{-1}$  em todas as frações. A fração casca apresentou o maior teor de vitamina C. Os resultados físico-químicos indicam que a fração polpa e WS apresentam potencial para a produção de produtos alimentícios derivados de frutas. Ácidos protocatecuicos e quínicos e epicatequina/catequina foram encontrados em todas as frações. A capacidade antioxidante DPPH, teor de fenólicos e flavonóides totais foram maiores na polpa; Os valores de TEAC e ORAC<sub>HF</sub> foram menores na semente. Os compostos orgânicos voláteis não foram identificados e as frações não apresentaram atividade antibacteriana. Em relação ao óleo, o maior rendimento de extração foi encontrado na polpa e inteiro sem semente a 60 °C (18,06 e 14,55 g.100 g<sup>-1</sup> da amostra liofilizada (fdw), respectivamente) e na casca a 40 °C ( $8,31 \pm 0,73$ g.100 g<sup>-1</sup> fdw). Durante a cinética de extração, a polpa apresentou os maiores rendimentos de óleo (41,57%) e carotenóides totais (8,34 mg.g<sup>-1</sup>) após 61 minutos (min) a 40 °C. O potencial antioxidante, perfil de ácidos graxos e teor de a-tocoferol foram dependentes tanto da fração quanto da temperatura, sendo o ácido oleico o principal ácido graxo. O óleo da fração inteira sem semente apresentou o maior número (20) de compostos fenólicos identificados. A extração a 60 °C reduziu a intensidade relativa da maioria dos compostos na fração inteiro sem semente e polpa. Além disso, aumentou a intensidade dos compostos na casca. Para os métodos de extração, o CO2 supercrítico apresentou o maior rendimento de extração (13,44%). O maior rendimento e teor de carotenóides ocorreu próximo aos 60 minutos na cinética de extração. A maior capacidade antioxidante pelos ensaios ORAC lipofílico e total (45,793  $\mu$ mol TE.g<sup>-1</sup>) e o maior teor de  $\alpha$ -tocoferol (0,41119 mg.g<sup>-1</sup>) foram encontrados no óleo extraído por prensagem, enquanto a extração com CO<sub>2</sub> supercrítico apresentou o maior potencial antioxidante TEAC (1,385 µmol TE.g<sup>-1</sup>). Os ácidos oleico e palmítico foram os principais ácidos graxos encontrados no óleo de buritirana para ambos os métodos de extração. O CO<sub>2</sub> supercrítico apresentou maior intensidade relativa para compostos fenólicos. All-*E*-Lutein, all-*E*- $\alpha$ -caroteno, all-*E*- $\beta$ -caroteno e 9-*Z*- $\beta$ -caroteno foram a maioria nos métodos avaliados. Apenas o óleo de CO<sub>2</sub> supercrítico revelou compostos voláteis, e nenhum apresentou atividade antibacteriana. Assim, essas técnicas podem ser consideradas boas alternativas para a extração de óleos naturais com potencial nutricional, funcional e tecnológico.

Palavras-chave: Caracterização abrangente; Composição química; Compostos bioativos; CO<sub>2</sub> supercrítico; Potenciais antioxidantes e antibacterianos.

#### ABSTRACT

Buritirana is a poorly studied species. Therefore, the objective of this thesis was to explore the buritirana palm (Mauritiella armata) through the characterization of its fruits and oil obtained from different fractions, temperatures and extraction methods in order to present it as a new fruitful with potential for the food and nonfood industries. The fruits presented an oblong shape. The pulp represented 16.58% of the whole-fruit weight (10.07 g). The moisture, ash and soluble fiber contents were similar for the whole fraction without seed (WS) and pulp. Although the total carbohydrate content was the same for seed and peel (23.24 g.100  $g^{-1}$ ), the seed showed higher protein and insoluble fiber contents. Except for glucose (1256.63 mg.100  $g^{-1}$ ), the seed showed the highest concentrations of mono-, di- and oligosaccharides. The mineral content ranged from 0.43 to 800 mg.100  $g^{-1}$  in all fractions. The peel fraction showed the highest content of vitamin C. The physicochemical results indicate that the pulp and WS fraction have potential for the production of fruit-derived food products. Protocatechuic and quinic acids and epicatechin/catechin were found in all fractions. The antioxidant capacity DPPH, phenolic content and total flavonoids were higher in the pulp; TEAC and ORAC<sub>HF</sub> values were lower in the seed. Volatile organic compounds were not identified, and the fractions did not show antibacterial activity. Regarding oil, the highest extraction vield was found in the pulp and whole without seed at 60 °C (18.06 and 14.55 g.100 g<sup>-1</sup> of the freezedried sample (fdw), respectively) and in the peel at 40 °C (8.31  $\pm$  0.73 g.100 g<sup>-1</sup> fdw). During the extraction kinetics, the pulp had the highest yields of oil (41.57%) and total carotenoids (8.34 mg.g<sup>-1</sup>) after 61 minutes (min) at 40 °C. The antioxidant potential, fatty acid profile, and  $\alpha$ -tocopherol content were dependent on both fraction and temperature, with oleic acid being the main fatty acid. The oil from the whole fraction without seed had the largest number (20) of identified phenolic compounds. The extraction at 60 °C reduced the relative intensity of most compounds in the whole without seed and pulp. Moreover, it increased the intensity of the compounds in the peel. For the extraction methods, supercritical  $CO_2$  presented the highest extraction yield (13.44%). The highest yield and content of carotenoids occurred close to 60 minutes in the extraction kinetics. The highest antioxidant capacity by the lipophilic and total ORAC assays (45.793  $\mu$ mol TE.g<sup>-1</sup>) and the highest  $\alpha$ -tocopherol content (0.41119 mg.g<sup>-1</sup>) <sup>1</sup>) were found in the oil extracted by pressing, while the extraction with supercritical  $CO_2$ showed the highest antioxidant potential TEAC (1.385 µmol TE.g<sup>-1</sup>). Oleic and palmitic acids were the main fatty acids found in buritirana oil for both extraction methods. Supercritical  $CO_2$  showed a higher relative intensity for phenolic compounds. All-E-Lutein, all-E- $\alpha$ - carotene, all-E- $\beta$ -carotene, and 9-Z- $\beta$ -carotene were the majority in the evaluated methods. Only the supercritical CO<sub>2</sub> oil revealed volatile compounds, and none showed antibacterial activity. Thus, these techniques can be considered good alternatives for the extraction of natural oils with nutritional, functional and technological potential.

**Keywords**: Comprehensive characterization; Chemical composition; Bioactive compounds; Supercritical CO<sub>2</sub>; Antioxidant and antibacterial potentials.

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#### **1. GENERAL INTRODUCTION**

For some years now, plants (roots, stems, leaves, flowers and fruits) and plant products (extracts, syrups, etc.) have been used as phytotherapics, and their medicinal characteristics are the focus of scientific investigations around the world because they demonstrate diverse biological properties and economic viability (Singh, Sharma, & Sarkar, 2012). In this context, the consumption of fruits and vegetables is not only linked to the sensorial aspect and the consumer's preference; moreover, it has been associated with the reduction of the risk of several diseases due to the presence of essential nutrients and bioactive substances (phenolic compounds, alkaloids, terpenoids, carotenoids and glycosides) that act positively in the body by maintaining the homeostasis of the redox state, stimulating the immune system and modulating hormonal metabolism, among others (Horst et al., 2010; Almeida et al., 2011; Alissa & Ferns, 2012; Harasym & Oledzki, 2014; Rufino et al., 2010).

Native fruits are widely used for fresh consumption or for the production of jams, jellies, juices and wines, among others. They present different values depending on the flavor and aroma, the added nutritional value, the availability of fruits that do not yet have commercial plantations, the ease or difficulty of harvesting and postharvest conservation. In addition to being used in the formation of domestic orchards, many native fruits are part of the beekeeping flora and can be used on larger scales, depending on the demand presented by the market (Vieira et al., 2006).

Despite unclear mechanisms and few reported studies, hypotheses point out that the high antioxidant capacity of carotenoids, tocopherols, and phenolic compounds may also be effective in reducing oxidative stress in the progression of diabetes mellitus, as well as other chronic noncommunicable diseases (Song et al., 2005; Matsui et al., 2001). Due to their chemical structures, dietary bioactive compounds exert multiple activities interacting with several particularly relevant pathways, for example, for glucose homeostasis (Guash-Ferré et al., 2017). These include reducing intestinal absorption of dietary carbohydrates, modulating enzymes involved in glucose metabolism, improving  $\beta$ -cell function and insulin action, and stimulating insulin secretion and their antioxidant and anti-inflammatory properties (Bahadoran, Mirmiran, & Azizi, 2013).

Thus, when acquiring scientific and technological knowledge of native fruitful species, there is a tendency to a greater motivation for the generation of a new fruitful chain, characterized by new qualities, specific to each fruit, and that, in addition to the sensory

attributes of appearance and flavor, the functional value of food is more sought after by consumers who are increasingly seeking a better quality of life (Santos-Serejo et al., 2009).

Therefore, the species presented in this work is an underused native fruitful with few reports in the literature that presents greater potential for exploitation in the short and medium term, based on its economic, nutritional, functional potential with the prospect of being used as ingredients for various interests with functional and technological appeal. *Mauritiella armata* is also known as buritirana, among other popular names and due to its fruits being similar to the fruits of the buriti (*Mauritia flexuosa*) it is nicknamed buriti mirim, since it has a smaller size. This species is found freely vegetating in humid soils of low elevation areas of the Amazon and Cerrado biomes of Brazil and in the Guianas (Gomes, 2007; Smith, 2015).

In addition to being appreciated for its beautiful ornamental appearance, this palm tree offers stems, leaves and flowers for the production of handicrafts and other products that contribute to the livelihood of local residents. Its fruits are consumed in natura, but they can also be used for the elaboration of derivatives, such as juices, fermented beverages, sweets, jellies, among others. In addition, buritirana has been applied for the treatment of diseases associated with the skin and as tea for rheumatism (Anunciação et al., 2019; Coimbra, 2019; Martins, 2012; Noblick & Barreto, 2018).

Like other species of the same family (Arecaceae), such as oil palm (*Elaeis oleifera* H.B.K.), açaí (*Euterpe oleraceae*), buriti and macaúba (*Acrocomia aculeata*), which have fruits with high nutritional and functional value, buritirana also has a high content of oil rich in bioactive compounds, expressive antioxidant capacity and unsaturated fatty acids with great potential for biodiesel production. In addition, the residue obtained from oil extraction can serve as animal feed, as well as ingredients (colorants, texturizing additives, flavorings, antimicrobials, antioxidants) for the elaboration of by-products of the food industry with high added value (Resende, Franca, & Oliveira, 2019; Souza et al., 2020).

Cold pressing, despite present a low extraction yield or even a possible exposure of bioactive compounds to oxygen, is still a technique used to obtain oil with biological and technological attributes commonly used in the food and non-food industries (Mouahid, Dufour, & Badens, 2017; Roselló-Soto et al., 2019).

Currently, extraction with supercritical  $CO_2$  has been highlighted contributing to the preservation of thermo-labile bioactive compounds extracted from solid or liquid samples. In addition to the high extraction yield, it occurs in a continuous process, uses non-toxic and non-flammable solvents, therefore leaving no traces of solvent in the purchased product (Asl, Niazmand, & Yahyavi, 2020). The speed with which  $CO_2$  penetrates the matrix is because of its fluidity and better diffusion coefficient (Singh, Ahmad, & Ahmad, 2015; Saini & Keum, 2018). However, for better efficiency in the extraction and maintenance of constituents of biological interest, some parameters must be observed, such as temperature, pressure, particle size, among others, so that the phytochemical characteristics, antioxidants and yield of the oil obtained are the best possible (Fachri, Sari, Yuwanti, & Subroto, 2020; Filho et al., 2008; Guedes et al., 2020; Narváez-Cuenca et al., 2020).

Thus, this work shows for the first time a comprehensive study on the fruits and oil of the buritirana palm to provide subsidies for future research aimed at elucidating the physiological and molecular mechanisms in the prevention and treatment of comorbidities, as well as the study of its application as an ingredient in the development of food, pharmaceutical, cosmetic and/or cleaning products.

#### 2. OBJECTIVES

2.1 General objective

The objective of this thesis was to explore the buritirana palm (*Mauritiella armata*) through the characterization of its fruits and oil obtained from different fractions, temperatures and extraction methods, in order to present it as a new fruitful with potential for the food and non-food industries.

2.2. Specific objectives

i. To motivate the exploration and possible integration of these fruits in commercial cultures as sources of added-value ingredients;

ii. To present the physical-chemical and nutritional aspects, the main bioactive compounds, the biological properties and the innovative potential of four Brazilian palm-tree fruits of the Arecaceae family;

iii. To carry out first time comprehensive study to define the biometric and physicochemical characteristics, proximate composition, mineral content, carbohydrate and phenolic profiles, volatile compounds, antioxidant capacity and antibacterial activity of buritirana and its fractions;

iv. Demonstrate the potential and characteristics of buritirana oil as being important not only for the food, cosmetic and pharmaceutical industries, but also for the small producer;

v. To assess the percentage of oil in different fractions of buritirana at 40 and 60 °C through extraction with supercritical  $CO_2$ . In addition, to determine the antioxidant capacity, the  $\alpha$ -tocopherol content, and the phenolic and fatty acid profile. In the fraction with the highest oil content monitor its yield and total carotenoids in the extraction kinetics;

vi. To obtain buritirana oil from two extraction methods and to evaluate the overall yield, extraction kinetics, phytochemical composition, antioxidant capacity, volatile organic compounds, and antibacterial activity.

#### **3. CHAPTER I**

#### **REVIEW ARTICLE**

# Brazilian fruits of Arecaceae family: an overview of some representatives with promising food, therapeutic and industrial applications

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#### **Highlights**

An overview is given about the innovation hotspots of Arecaceae family.
Oil-rich palm trees present great functional and economic potential.
Arecaceae fruits represent a good source of PUFAs, tocopherols and β-carotene.
Arecaceae family intake are linked with several biological effects.
Arecaceae species may represent a new agro-commodities.

#### Abstract:

The Arecaceae family is widely distributed and comprises about 2600 species, in which 48 of them are native to Brazil and occurs in transition biomes between the Amazon, Cerrado and Caatinga. In addition to being used as a source of food and subsistence, they are also rich in lipophilic bioactive compounds, mainly carotenoids, polyunsaturated fatty acids, tocopherols and vitamin A. Moreover, they have considerable content of phenolic compounds, fibers and minerals. Therefore, the objective of this review is to present the physical-chemical and nutritional aspects, the main bioactive compounds, the biological properties and the

innovative potential of four Brazilian palm-tree fruits of the Arecaceae family. Due to the presence of bioactive compounds, these fruits have the potential to promote health and can be used to prevent chronic non-communicable diseases, such as obesity, type 2 diabetes and others. Furthermore, these raw materials and their by-products can be used in the development of new food, chemical, pharmaceutical and cosmetic products. To ensure better use of these crops, promote their commercial value, benefit family farming and contribute to the country's sustainable development, it is necessary to implement new cultivation, post-harvest and processing techniques. Investing in research to publicize their potential is equally important, mainly of the ones still little explored, such as the buritirana.

#### **Graphical abstract**



**Keywords:** Bioactive compounds, biological properties, *Mauritia flexuosa, Mauritiella armata, Euterpe oleracea, Acrocomia aculeata*. Biofuel.

#### Chemical compounds studied in this article:

Lutein (PubChem CID: 5281243)  $\beta$ -carotene (PubChem CID: 5280489) Vitamin A (PubChem CID: 445354) Vitamin E (PubChem CID: 14985) Caffeic acid (PubChem CID: 689043)  $\rho$ -coumaric acid (PubChem CID: 637542) Kaempferol (PubChem CID: 5280863) Orientin (PubChem CID: 5281675) Rutin (PubChem CID: 5280805) Catechin (PubChem CID: 9064)

#### **1. Introduction**

The palm tree family (Arecaceae) comprises around 2600 species in 181 genera which are mostly distributed in tropical and subtropical areas worldwide, although arborescent palm trees can also occur in arid regions of desert climates (Emilio et al., 2019). Depending on the ecosystem in which they occur, the Arecaceae species may differ both morphologically and functionally, including grasses, shrubby species and large woody trees (Balslev et al., 2016; Emilio et al., 2019). Furthermore, a variety of palm trees produces edible fruits with different textures, shapes (eg.: indehiscent nuts, berries or drupes), colours and chemical compositions. Some of them, such as date palm, oil palm, true sago palm and coconuts, are important to agricultural crops in obtaining starches, oils, fibers, thatch and woods (Neri-Numa et al., 2018; Rodgkiss, 2016; Tekula, 2015).

In this context, Brazil's palm flora is very rich and exuberant, containing around 480 native species (the country was originally called "Pindorama", which means the "land of many palms" in the Tupi-Guarani language) of wide occurrence in transition biomes between the Amazon, Cerrado and Caatinga (Barreto, Parise, & Almeida, 2019; Coradin et al., 2011) The Brazilian palm trees represent a socio-ethnobotanical and ecological importance for both indigenous and cabocla peoples from the Amazonian forest, Cerrado and Caatinga regions. Moreover, they are not only used as sources of food and subsistence, but also in the production of fuel, folk medicines, fibres, forage shelter and traditional handcrafts (Campos, Araújo, Gaoue, & Albuquerque, 2019; Neri-Numa et al., 2018; Smith, 2015)

Except for the oil palm (caiaué, *Elaeis oleifera* H.B.K.) and açaí (*Euterpe oleracea* Mart.), the trade of native Brazilian species is unrepresentative. Most of them are exclusively addressed for the local and regional markets or grown specifically for ornamental and landscape purposes (Brandão, Castro, & Futemma, 2018; Coradin et al., 2011; Oliveira et al., 2017). Nevertheless, owing to their high nutritional value, organoleptic characteristics and phytochemical composition, species such as buriti (*Mauritia flexuosa* L.f.)(Nobre et al., 2018) buritirana (*Mauritiella armata* Mart. Burret), açaí (*Euterpe oleracea* Mart.) (Marques et al., 2016) and macaúba (*Acrocomia aculeata*) (Silva et al., 2018; Oliveira et al., 2016; Nesello et al., 2018) have drawn attention (albeit shyly) from researchers and the industrial sector as they can integrate the market niches for the food, pharmaceutical and cosmetical sectors.

Therefore, motivated by the exploration and possible integration of these fruits in commercial cultures as sources of added-value ingredients, the objective of this review is to present the physical-chemical and nutritional aspects, the main bioactive compounds, the biological properties and the innovative potential of four Brazilian palm-tree fruits of the Arecaceae family.

#### 2. Ethnobotanical description, chemical constituents and biological properties

The morphological characteristics and molecular structures of the main bioactive compounds of some Arecaceae representatives described in this review are represented by **Figures 1** and **2**. The geographical distribution, botanical aspects as chemical constituents and beneficial properties are discussed individually in the following topics for each palm-tree fruit.



**Figure 1.** Molecular structures of the main bioactive compounds found in some fruits from Arecaceae family.



**Figure 2.** Representative fruits from Arecaceae family: **A**) Buriti (*Mauritia flexuosa*), **B**) Buritirana (*Mauritiella armata*), **C**) Açaí (*Euterpe oleracea*) and **D**) Macaúba (*Acrocomia aculeata*) Source: Afonso Rabelo/National Amazon Research Institute (INPA) and Ademar Filho.

#### 2.1. Buriti (Mauritia flexuosa L.)

*Mauritia flexuosa* is an edible palm fruit popularly known as buriti, aguaje or moriche, which is widely distributed in South America, mainly in swamp areas across the Amazon and Cerrado Regions, where it grows in homogenous forests known as "buritizais" (Hoek, Solas, & Peñuela, 2019; Nobre, Sousa, Silva, Melo Coutinho, & Costa, 2018; Virapongse, Endress, Gilmore, Horn, & Romulo, 2017). It is a dioecious, high and exuberant palm (also called buritizeiro) tree of the *Mauritia* genus, presenting unique characteristics which allow us to easily distinguish this species from the others, such as the erect, cylindrical, hairless and solitary stem, measuring up to 30 m high and 50-60 cm in diameter, the 20-30 huge fan-shaped leaves measuring 5 m long and up to 3 m wide and the inter-foliar inflorescences (Cattani & Baruque-Ramos, 2016; Vieira et al., 2016).

Depending on both region and edaphoclimatic conditions, each buritizeiro can produce 4-7 bunches, with 400 to 2000 fruit units each, varying from one to the other. The estimated annual production average is up to 300 kg of fruit/palm tree per year, 3.29 to 23.00 Mg.ha<sup>-1</sup> of fresh fruits per hectare per year, which represents  $0.79 \pm 0.23$  Mg.ha<sup>-1</sup> of pulp and 57.50  $\pm$  17.00 Kg of oil.ha<sup>-1</sup> (Santos, 2005; Goodman et al., 2013; Barbosa, Lima, & Mourão Junior, 2010).

Morphometrically, the buriti fruit has an oval-shaped drupe, comprising an epicarp covered with triangular reddish-brown scales, measuring 4-7 cm long, 3-5 cm in diameter and weighing 25-40 g. It also has an edible mesocarp with a thick and soft pulp in yellow-orange vibrant colouring due to the high concentration of carotenoids. A spongy epicarp surrounds 1-3 oblong and hard whitish integumentary seeds (**Figure 2A**) (Ferreira, Costa, Pinheiro, Souza, & Carvalho, 2018; Moura Filho & Penna, 2017). In general, the buriti fruits comprise 20% skin, 10-20% mesocarp, 10-20% epicarp and 40-45% seeds since the mesocarp is the most explored fruit part due to the pleasant taste and nutrient content (Koolen, Silva, Silva, Paz, & Bataglion, 2018).

The buriti palm tree is known as the "tree of life", playing multiple key roles of ecological, socioeconomic and cultural importance (Neri-Numa et al., 2018; Hoek et al., 2019). Apart from serving as feed and shelter for several animal species, the buriti palm is considered a great warehouse of carbon, which is an important ally in reducing global warming. Furthermore, various ethnic groups explore this palm for feeding (fruits and inflorescences), shelter, folk medicine, crafts and handwork (Gilmore et al., 2013; Koolen et al., 2018; Hoek et al., 2019; Virapongse et al., 2017).

Regarding feeding, the buriti pulp is consumed in many ways (e.g.: fresh, juices, candies, ice-cream, wines and fermented beverages), since culinary preparations are also commercialized in local markets, albeit inexpressively (Koolen et al., 2018; Manhães et al., 2015). Conversely, young leaves and the oil extracted from the buriti pulp have been addressed as products with high market value (Virapongse, 2017).

It is important to point out that the buriti is still produced in an extractive way, although this plant is easily propagated and represents a great option for sustainable managing in its naturally occurring areas (Koolen et al., 2018). Sustainable practices are useful to promote regional development, expand business investment opportunities, and reduce the vulnerability of the food system (Ferreira et al., 2018; Manzi & Coomes, 2009). Therefore, the buriti palm represents an important resource to supply raw materials for a variety of approaches, such as meeting the demands in the food, cosmetic and fuel industries due to their sensory, physicochemical and biological characteristics which will be discussed below.

Regarding the physicochemical and nutritional aspects, depending on the cultivar and/or morphotypes, as well as the occurrence region, season and harvest period, the nutrient and non-nutrient contents can vary (Koolen et al., 2018; Milanez et al., 2018). As shown in **Table 1,** the buriti pulp is very rich in minerals, such as Ca, K, Na, Mg, Fe, Mg, Mn, Zn, and Cu, proteins, vitamins A, B, C, E, fibers, and unsaturated oils. It also has high contents of

protein (around 6.50 g.100 g<sup>-1</sup> of crude pulp) and fats (from 14.28 to 45.70 g.100 g<sup>-1</sup>) (Cândido & Silva, 2017; Darnet, Silva, Rodrigues, & Lins, 2011; Sandri et al., 2017; Vásquez-ocmín, Solís, Torres, Alvarado, & Luján, 2009). Likewise, it was observed that the carbohydrates range from 7.28 to 26.20 g<sup>-1</sup> (Carneiro, 2011; Silva & Pighinelli, 2017; Sandri et al., 2017). Moreover, it was observed that the ripe buriti pulp is rich in pectic polysaccharides and unusual linear polysaccharides have also been identified, such as  $(1\rightarrow 5)$ - $\alpha$ -L-arabinan,  $(1\rightarrow 3)$ - $(1\rightarrow 4)$ - $\alpha$ -D-glucan and  $(1\rightarrow 4)$ - $\beta$ -D-xylan (Cantu-Jungles et al., 2015; Cordeiro et al., 2015).

As seen in **Table 2**, the fruits and oils of the buriti present a wide variety of chemical structures from different classes of secondary metabolites, including fatty acids, tocochromanols, phytosterols, carotenoids and phenolic compounds (Koolen et al., 2018). Chemical composition studies have shown that the buriti pulp contains high levels of monounsaturated fatty acids (73.03 to 79.43 g.100 g<sup>-1</sup>), which are very similar to those found in olive oil, with the predominance of oleic acid (72.21 to 78.57 g.100 g<sup>-1</sup>), followed by palmitic, linoleic and  $\gamma$ -linolenic acids (Cândido & Silva, 2017; Cruz et al., 2020; Koolen et al., 2018).

The buriti oil is rich in tocopherols,  $\alpha$ -tocotrienol and  $\delta$ -tocotrienol, in which both  $\beta$ -tocopherol and  $\alpha$ -tocopherol represent around 80% of the total methyl tocols (Serra et al., 2019). The phytosterols comprise from 18.30 to 23.32 g.100 g<sup>-1</sup>, in which the campesterol and  $\beta$ -sitosterol (0.60 g.100 g<sup>-1</sup> and 7.66 g.100 g<sup>-1</sup>, respectively) are the most abundant metabolites (Bataglion et al., 2019).

In addition, due to its chemical composition and physicochemical characteristics, this fruit has great potential for biodiesel production (Beltrão & Oliveira, 2007). The buriti oil has a density of 0.91 g.cm<sup>3</sup>, a saponification index of 190.00 mg KOH.g<sup>-1</sup>, an iodine index of 7.24 mg I<sub>2</sub>.100 g<sup>-1</sup>, a peroxide index of 3.95 mEq.g<sup>-1</sup>, the solidification point at 13 °C and the caloric power of 9256 Kcal.g<sup>-1</sup> (Iamaguti, 2014).

The carotenoids are undoubtedly the most studied class of bioactive compounds from the buriti, since the  $\beta$ -carotene is the most abundant carotenoid in its pulp (9098.00 µg.100 g<sup>-1</sup>) (Sandri et al., 2017). However, considerable amounts of  $\xi$ -carotene,  $\delta$ -carotene,  $\gamma$ carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, and xanthophyll (lutein) (Agostini-Costa, 2018) can also be found. Consequently, it is also a great source of vitamin A, like some food matrices traditionally recognized (e.g.: carrots, guava, pitanga, papaya and passion fruit) (Ferreira et al., 2018).

Composition (Units per 100 g)	Buriti	References	Açaí	References	Macaúba	References
Moisture	73.45 g <sup>a</sup>	Lescano et al. (2018);	2.75 g <sup>c</sup>	Silva et al. (2019);	40.19 g <sup>b</sup>	Costa, Oliveira, &
Ash	1.41 g <sup>a</sup>	Milanez, Neves,	$4.00 \text{ g}^{a}$	Gordon et al. (2012);	$1.50  \mathrm{g}^{\mathrm{b}}$	Costa (2018); Dario
Total fats	13.75 g <sup>a</sup>	Colombo, Shahab, &	48.00 g <sup>a</sup>	Carvalho, Silveira,	23.62 g <sup>b</sup>	et al. (2018);
Total proteins	$4.30  g^{a}$	Roberto (2018);	12.00 g <sup>a</sup>	Mattietto, Oliveira,	5.31 g <sup>b</sup>	Lescano et al.
-	-	Resende, Franca, &	-	& Godoy (2017);	-	(2015); Prates-
Total sugars	3.08 g <sup>a</sup>	Oliveira (2019);	36.00 g <sup>c</sup>	Schauss et al. (2006);	6.92 g <sup>b</sup>	Valério, Celayeta,
Total fiber	8.32 g <sup>a</sup>	Schiassi, Souza, Lago,	11.00 g <sup>c</sup>	Garzón, Narváez-	13.89 g <sup>b</sup>	& Cren (2019);
pH	5.74 <sup>a</sup>	Campos, & Queiroz	5.23 °	Cuenca, Vincken, &	5.50 <sup>b</sup>	Silva et al. (2018)
Total acidity	0.06 g <sup>a</sup>	(2018); Serra et al.	1.20 g <sup>a</sup>	Gruppen (2017);	1.43 g <sup>b</sup>	
Soluble solids <sup>d</sup>	13.40 <sup>a</sup>	(2019); Hamacek, Della	6.46 <sup>a</sup>	Rufino et al. (2011)	29.70 <sup>b</sup>	
Energy value	93.08 kcal <sup>b</sup>	Lucia, Silva, Moreira, &	533.90 kcal <sup>a</sup>		258.47 kcal <sup>b</sup>	
Macrominerals		Pinheiro-Sant'ana				
Calcium	311.00 mg <sup>a</sup>	(2018); Aguiar & Souza	423.00 mg <sup>a</sup>		113.00 mg <sup>b</sup>	
Magnesium	127.00 mg <sup>a</sup>	(2017); Manhães &	172.00 mg <sup>a</sup>		123.00 mg <sup>b</sup>	
Potassium	183.55 mg <sup>b</sup>	Sabaa-Srur (2011)	930.00 mg <sup>a</sup>		$2.36 \text{ mg}^{b}$	
Sodium	11.25 mg <sup>b</sup>		6.80 mg <sup>a</sup>		$0.13 \text{ mg}^{b}$	
Phosforus	6.90 mg <sup>b</sup>		186.00 mg <sup>a</sup>		6.97 mg <sup>b</sup>	
Microminerals						
Manganese	1.79 mg <sup>b</sup>		13.30 mg <sup>a</sup>		321.00 µg <sup>b</sup>	
Iron	1.77 mg <sup>b</sup>		7.80 mg <sup>a</sup>		4134.00 µg <sup>b</sup>	
Zinc	$0.60 \text{ mg}^{b}$		2.10 mg <sup>a</sup>		4514.00 µg <sup>b</sup>	
Cupper	0.15 mg <sup>b</sup>		-		137.00 µg <sup>b</sup>	
Free fatty acids						
saturated						
Capric (C10:0)	110.00 mg <sup>a</sup>		-		$0.11 \text{ mg}^{b}$	
Lauric (C12:0)	110.00 mg <sup>a</sup>		$30.00 \text{ mg}^{\circ}$		0.59 mg <sup>b</sup>	
Myristic (C14:0)	110.00 mg <sup>a</sup>		$70.00 \text{ mg}^{\circ}$		0.39 mg <sup>b</sup>	
Palmitic (C16:0)	16120.00 mg <sup>a</sup>		21670.00 mg <sup>c</sup>		20.11 mg <sup>b</sup>	
Palmitoleic(C16:1)	540.00 mg <sup>a</sup>		3960.00 mg <sup>c</sup>		2.56 mg <sup>b</sup>	
Margaric (C 17:0)	80.00 mg <sup>a</sup>		150.00 mg <sup>c</sup>		0.08 mg <sup>v</sup>	

**Table 1**. Nutrients, non-nutritive composition and antioxidant capacity of buriti (*Mauritia flexuosa*), açaí (*Euterpe oleracea* Mart.) and macaúba (*Acrocomia aculeata*).

Stearic (C18:0) Monounsaturated	990.00 mg <sup>a</sup>	-	3.09 mg <sup>b</sup>
Oleic (C18:1) Polyunsaturated	78480.00 mg <sup>a</sup>	62180.00 mg <sup>c</sup>	65.71 mg <sup>b</sup>
Linoleic (C18:2)	780.00 mg <sup>a</sup>	11070.00 mg <sup>c</sup>	5.46 mg <sup>b</sup>
Linolenic (C18:3)	120.00 mg <sup>a</sup>	660.00 mg <sup>c</sup>	1.02 mg <sup>b</sup>
Arachidic (C20:0)	280.00 mg <sup>a</sup>	130.00 mg <sup>c</sup>	0.21 mg <sup>b</sup>
Gadoleic (C20:1)	200.00 mg <sup>a</sup>	-	0.16 mg <sup>b</sup>
Behenic (C22:0)	110.00 mg <sup>a</sup>	90.00 mg <sup>c</sup>	0.06 mg <sup>b</sup>
Lignoceric (C24:0)	-	-	0.08 mg <sup>b</sup>
Vitamins			e
Vitamin C	59.93 mg <sup>b</sup>	-	118.19 <sup>b</sup>
Ascorbic acid	7.42 mg <sup>b</sup>	84.00 mg <sup>a</sup>	15.41 mg <sup>b</sup>
Vitamin E	6.45 mg <sup>b</sup>	-	-
Vitamin A	1899.33 RAE <sup>b</sup>	300.60 RAE <sup>a</sup>	599.78 RAE <sup>b</sup>
Antioxidants			
compounds			
Total phenolic	20.65 mg GAE <sup>a</sup>	4786.00 mg GAE <sup>a</sup>	269.00 mg GAE <sup>b</sup>
compounds	-	-	-
Total carotenoids	63.68 mg <sup>a</sup>	1100.00 μg <sup>a</sup>	24.80 mg <sup>b</sup>
β-carotene	21.64 mg <sup>b</sup>	-	56000.00 μg <sup>b</sup>
$\alpha$ -carotene	2.30 mg <sup>b</sup>	-	-
Total carotenes	-	-	46.00 μg <sup>b</sup>
Total xanthophylls	-	-	494.00 µg <sup>b</sup>
Antioxidant			
capacity			
ABTS	1302.00 μmol TE <sup>a</sup>	120566.00 μmol TE <sup>°</sup>	-
DPPH (EC <sub>50</sub> )	186.00 g/DPPH <sup>a</sup>	102.00 g/DPPH <sup>a</sup>	2389.00 μg <sup>b</sup>
DPPH	26 56 umol TE <sup>a</sup>	21049 00 umol TE <sup>a</sup>	_
ORAC	2470 00 µmol TE <sup>a</sup>	$157242.00 \text{ µmol TE}^{\circ}$	4202.00 umol TE <sup>b</sup>
FRAP	8890.00 umol	-	-
	$\operatorname{Fe}_2\operatorname{SO}_4^{a}$		

Saccharides			
Rhamnose	0.95 g <sup>a</sup>	-	-
Fucose	2.77 g <sup>a</sup>	0.09 g <sup>a</sup>	-
Arabinose	41.50 g <sup>a</sup>	0.65 g <sup>a</sup>	-
Xylose	24.43 g <sup>a</sup>	10.59 g <sup>a</sup>	-
Mannose	9.48 g <sup>a</sup>	0.03 g <sup>a</sup>	-
Galactose	7.63 g <sup>a</sup>	0.29 g <sup>a</sup>	-
Glucose	13.24 g <sup>a</sup>	0.25 g <sup>a</sup>	-

<sup>a</sup> Values expressed on the basis dry weight; <sup>b</sup> Values expressed on the basis fresh weight; <sup>c</sup> Freeze-dried sample; <sup>d</sup>  $^{\circ}$ Brix; GAE galic acid equivalent; RAE-Retinol equivalent; TE- Trolox equivalent; ABTS- 2,2 azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) free radical scavenging assay; DPPH- 2,2diphenyl-1picrylhydrazyl free radical scavenging assay; EC<sub>50</sub> – Half maximal effective concentration; ORAC- Oxygen radical absorbance capacity assay; FRAP- Ferric Reducing Antioxidant Power.

Compounds (Units per 100 g)	Concentration	Source	References
	Buriti ( <i>Mauri</i> i	tia flexuosa)	
Phenolics	Durin (intuition	na jienaosa)	
Catechin	35 0 mg <sup>b</sup>	Puln	
Caffeic acid	$372.0 \text{ mg}^{\text{b}}$	Puln	
Rutin	$146.0 \text{ mg}^{\text{b}}$	Puln	
Orientin	$529.0 \text{ mg}^{\text{b}}$	Puln	
Quercetin	$184.0 \text{ mg}^{\text{b}}$	Puln	
Anigenin	$15.0 \text{ mg}^{\text{b}}$	Puln	
Luteolin	$42.0 \text{ mg}^{\text{b}}$	Puln	
Kaempferol	$369.0 \text{ mg}^{\text{b}}$	Puln	Nonato et al. (2018);
Flavonoids	$1247.0 \text{ ug}^{\text{b}}$	Puln	Resende, Franca, &
Proanthocyaniding	$5008.1 \text{ mg}^{a}$	Peel	Oliveira (2019); Serra et
Tocopherols	5000.1 mg	1001	al. (2019)
a-tocopherol	45.1 mg	Oil	
β- tocopherol	76.2 mg	Oil	
y tocopherol	5.7 mg	Oil	
γ- tocopherol	13.0 mg	Oil	
a togotrianol	13.9 mg	Oil	
δ. tocotrianol	9.1 mg	Oil	
0- tocothenoi	1.1 mg	()II	
Dhanalias	Açal (Eulerpe ou	eraceae Mart.)	
Hydroxybenzoic acids and deriva	tives <sup>c</sup>		
Protocatachuic acid havosida	$0.0 \text{ mg}^{a}$	Dulp	Carzón Narvánz Cuanca
Protocatechuic acid	$1.7 \text{ mg}^{a}$	Pulp	Vincken & Gruppen
n Hudrovybanzoia agid	1.7  mg $1.0 \text{ mg}^{a}$	Pulp	(2017): Corvelho
Vanillia agid	$1.0 \text{ mg}^{a}$	r ulp Pulp	(2017), Calvallo, Silvoire Mettiotto
Suringia acid	$11.0 \text{ mg}^{a}$	r ulp Pulp	Oliveira, & Codey (2017)
Hydroxycinnamic acids and co	4.0 mg	ruip	Onvena, & Oodoy (2017)
Hydroxyferulovl quipic acid	$0.7 \text{ mg}^{a}$	Duln	
Sinapovl rhamposa	$1.4 \text{ mg}^{a}$	r ulp Pulp	
5 O coffeeval quipie soid	1.4  mg	r ulp Dulp	
p Coumaria agid havasida	$4.5 \text{ mg}^{a}$	Fulp	
Configure acid	$1.0 \text{ mg}^{a}$	Fulp	
Callele acid	1.9 mg $a$	Pulp	
Feruloyi sinapic acid isomer 1	1.5  mg $1.4 \text{ mg}^{a}$	Pulp	
Caffaavi shikimia aaid isamar 1	1.4 mg $1.7 \text{ mg}^{a}$	Fulp	
Carleoyi sinklinic acid isomer 2	1.7  mg	Pulp	
Feruioyi sinapic acid isomer 2	0.8  mg	Pulp	
Carreovi shikimic acid isomer 2	5.4  mg	Pulp	
Sinapoyi nexose	1.0  mg	Pulp	
Flower side <sup>e</sup>	2.5 mg	Pulp	
Flavonoids	150 <sup>a</sup>	D 1	
Orientin	15.0 mg	Pulp	
Isovitexin	12.0 mg	Pulp	
Homoorientin	9.9 mg "	Pulp	
Vitexin	9.8 mg "	Pulp	
Luteolin	0.9 mg "	Pulp	
Scoparin	0.6 mg "	Pulp	
Chrysoeriol	0.5 mg <sup>a</sup>	Pulp	
Taxifolin deoxyhexose isomer 1	2.8 mg <sup>a</sup>	Pulp	
Taxifolin deoxyhexose isomer 2	1.3 mg <sup>a</sup>	Pulp	

**Table 2.** Bioactive compounds found in Arecaceae family.

Taxifolin	1.2 mg <sup>a</sup>	Pulp				
Rutin	3.4 mg <sup>a</sup>	Pulp				
Isorhamnetin rutinoside	1.7 mg <sup>a</sup>	Pulp				
Dihydrokaempferol isomer 1	0.3 mg <sup>a</sup>	Pulp				
Dihydrokaempferol isomer 2	0.5 mg <sup>a</sup>	Pulp				
Cyanidin 3-glucoside	61300.0 μg <sup>f</sup>	Pulp				
Cyanidin 3-rutinoside	132900.0 µg <sup>f</sup>	Pulp				
3,4-Dihydroxybenzoic acid	1731.0 μg <sup>f</sup>	Pulp				
4-Hydroxybenzoic acid	1078.0 µg <sup>f</sup>	Pulp				
Vanillic acid	3029.0 µg <sup>f</sup>	Pulp				
Caffeic acid	$144.0 \mu g^{f}$	Pulp				
Syringic acid	1175.0 μg <sup>f</sup>	Pulp				
p-Coumaric acid	$308.0 \mu g^{f}$	Pulp				
Isoorientin	166.0 µg <sup>f</sup>	Pulp				
Orientin	111.0 µg <sup>f</sup>	Pulp				
Ferulic acid	760.0 μg <sup>f</sup>	Pulp				
Macaúba (Acrocomia aculeata)						
Carotenoids						
Violaxanthin isomer	14.0 μg <sup>b</sup>	Mesocarp				
(all-E)-Violaxanthin	168.0 μg <sup>b</sup>	Mesocarp				
(all-E)-Neoxanthin	25.0 μg <sup>-b</sup>	Mesocarp				
(all-E)-Luteoxanthin	11.0 μg <sup>b</sup>	Mesocarp				
(all-E)-Antheraxanthin	109.0 μg <sup>b</sup>	Mesocarp				
(13Z)-Lutein	26.0 μg <sup>b</sup>	Mesocarp				
(all-E)-Lutein	62.0 μg <sup>b</sup>	Mesocarp				
(13Z)-Zeaxanthin	10.0 μg <sup>b</sup>	Mesocarp				
(all-E)-Zeaxanthin	57.0 μg <sup>b</sup>	Mesocarp				
(9Z)-Lutein	12.0 μg <sup>b</sup>	Mesocarp	Schex et al. (2018)			
(13Z)-β-Carotene	$4.0 \ \mu g^{b}$	Mesocarp				
(all-E)-β-Carotene	41.0 µg <sup>b</sup>	Mesocarp				
(9Z)-β-Carotene	2.0 μg <sup>-b</sup>	Mesocarp				
Phytofluene 2	60.0 μg <sup>b</sup>	Mesocarp				
Phytofluene 5	23.0 μg <sup>b</sup>	Mesocarp				
Phytoene 1	20.0 µg <sup>b</sup>	Mesocarp				
Phytoene 2	228.0 µg <sup>b</sup>	Mesocarp				
Tocopherol		_				
α-Tocopherol	2724.0 μg <sup>b</sup>	Mesocarp				
<sup>a</sup> Values expressed on the basis	dried weight: <sup>b</sup> Value	a avpraged on the basis	fresh weight: <sup>c</sup> Expressed			

<sup>a</sup> Values expressed on the basis dried weight; <sup>b</sup> Values expressed on the basis fresh weight; <sup>c</sup> Expressed as vanillic acid equivalents; <sup>d</sup> Expressed as 5-*O*-caffeoyl quinic acid equivalents; <sup>e</sup> Expressed as rutin equivalents; <sup>f</sup> Freeze-dried sample.

Finally, the polar content of the buriti has attracted much attention in recent years. Despite its low concentration compared to the carotenoids and tocopherols levels, the phenolic compounds also contribute to the antioxidant properties of this plant matrix (Bataglion et al., 2019). In general, the buriti phenolic profile is composed of phenolic acids, hydroxycinnamic acid derivatives, flavonoids, flavonoid glucosides, and anthocyanins, although the most commonly found are quinic acid, caffeic acid, orientin, apigenin, hesperetina, luteolin, morin, quercetin, kaempferol, and catechins, among others (Bataglion et al., 2019; Nonato et al., 2018; Resende, Franca, & Oliveira, 2019).

Few studies have reported the biological effects of buriti. However, due to its diversity of secondary metabolites, some health benefits have been attributed to this fruit. A study has recently evaluated the effect of the buriti pulp flour on biomarkers of oxidative damage in target organs of diabetic rats. It was observed that the supplementation of the buriti flour reduced the oxidative damage in the liver and heart, without interfering in glycemic and lipidic parameters, aminotransferase activities and histological profile (Lopes et al., 2018). A preliminary study using Wistar rats reported that the buriti oil can be a useful tool in wound treatment as it presented an increase of fibroblast and collagen fibers followed by a complete reephitelialization (Batista et al., 2012). Likewise, a study described that the enzymatically interesterified buriti oil (containing tocopherols, carotenoids and polyphenols) can modulate HepG2 endogenous antioxidant enzyme by *in vitro* models using human liver cells (Falcão et al., 2017). **Table 3** shows additional data reported in the literature concerning the biological effects of different products of buriti.
**Table 3.** Biological effects of some representatives from Arecaceae family.

			Biological effects	
Fruit	Source	Model	Effects	Reference
	Pulp oil	Antimicrobial assay and	The fractions presented antioxidant and antimicrobial activities;	Nonato et al. (2018)
	extract	antibiotic activity in vitro	The synergistic effect of the fraction and cerotaxime $\downarrow$ (MIC) of the antibiotic from 1.024 to 256 µg/mL.	
	Pulp oil extract	In vitro antibacterial assays	The greatest inhibitory activity for <i>Staphylococcus aureus</i> 358 (MIC $256 \mu \text{g/mL}$ ).	Nobre, Sousa, Silva, Coutinho, & Costa (2018)
	Oil	In vitro antimicrobial activity	Highest activity against Salmonela typhimurium.	Santos et al. (2018)
Buriti ( <i>Mauritia</i>	Oil	In vitro insecticidal activity	Death insect (Sitophilus. zeamais) after 24 h.	Santos, Fernandes, Lopes, & Sousa (2015)
flexuosa)	Crude or refined oil	Hypocholesteroloemic effect in rats $(n = 30)$	$\downarrow$ Total cholesterol, $\downarrow$ LDL, $\downarrow$ triglycerides and $\downarrow$ AST.	Aquino et al. (2015)
	Peel oil extract	Antiplatelet/Antithrombotic activities <i>in vitro</i> and <i>in vivo</i>	↓ Secretion and aggregation of platelet and thrombus growth; ↓ ADP-induced platelet aggregation; ↓ platelet release of sP-selectin.	Fuentes et al. (2013)
	Oil extract	(mice) Anti-inflamatory <i>in vivo</i> (n = 36)	$\downarrow$ acute and chronic inflammation, $\uparrow$ fibroblast proliferation and $\downarrow$ macroscopically edema.	Barbosa et al. (2017)
Açaí (Euterpe oleraceae)	Pulp	Antimicrobial and antibiofilm activity in vitro	Effective against planktonic cells and biofilms of <i>S. aureus</i> ; ↓ the proliferation of HepG2 cells.	Dias-Souza et al. (2018)
	Oil	Histological and histochemical in vivo; male Wistar rats ( $n = 24$ )	↑ liver tissue integrity losses with increased the açaí oil doses.	Marques et al. (2019)
	Lyophilized açaí pulp	<i>In vivo</i> (male Wistar rats $n = 20$ ) colitis-associated carcinogenesis and the modifying effect of C3R on the motility of RKO colon adenocarcinoma cells <i>in vitro</i>	$\downarrow$ ACF development and incidence of tumors with high grade dysplasia, $\downarrow$ cell proliferation in colon tumors; $\downarrow$ (C3R) reduce the motility of RKO cells; $\uparrow$ gene expression of negative regulators of cell proliferation (Dlc1 and Akt3) and inflammation (Ppar $\alpha$ ).	Fragoso et al. (2018)
	Pulp	To assess the effects of açaí consumption on plasma lipids, apolipoproteins, the transfer of lipids to HDL, and some biomarkers of redox metabolism in women ( $n = 40$ )	<ul> <li>↑ concentration Apo A-I; ↓ ROS, ↓ ox-LDL and malondialdehyde;</li> <li>↑ activity antioxidative paraoxonase 1;</li> <li>↑ transfer cholesteryl esters to HDL.</li> </ul>	Pala et al. (2018)
	Concentrate açaí juice	Anti-lipidaemic and anti- inflammatory effects on 3T3-L1 mouse adipocytes	<ul> <li>↓ Intracellular lipids by PPARy2;</li> <li>↓ adipogenic transcription factors, mRNA, pro-inflammatory citokines; ↑ adiponectin expression.</li> </ul>	Xie et al. (2012)

		Pulp	Simulated <i>in vitro</i> gastrointestinal digestion; pH-controlled, anaerobic, batch-culture fermentation model	Following <i>in vitro</i> digestion, 49.8% of the total initial polyphenols were available; $\downarrow$ <i>Bacteroides-Prevotella spp.</i> and the <i>Clostridium-histolyticum</i> groups; $\uparrow$ short-chain fatty acids produced.	Alqurashi et al. (2017)
		Clarified açaí juice	Anti-leishmanial action	$\downarrow$ number of promastigotes and caused morphological alterations; $\downarrow$ IL-17 cytokine levels at all tested concentrations and $\downarrow$ number of intracellular amastigotes in macrophages infected with Leishmania.	Silva et al. (2018)
		Pulp	Activity of antioxidant enzymes; Biomarkers of protein oxidation	$\uparrow$ Catalase and total antioxidant activities; $\downarrow$ ROS, serum carbonyl protein.	Barbosa et al. (2016)
		Pulp	Lipidic peroxidation markers in women	↓ oxLDL (62%); ↓ MDA (72%) concentrations; ↑ TAC (14%). ↑ paraoxonase activity (25%) in the high-risk group.	Barbosa et al. (2017)
		Frozen concentrated juice	Anti-lipidaemic and anti- inflammatory <i>in vivo</i> (3T3-L1 mouse)	↓ expression of pro-inflammatory cytokines; ↓ generation of reactive oxygen species; ↓ cellular adhesion molecule; ↓ C/ebp $\alpha$ , C/ebp $\beta$ , Klf5 and Srebp1c.	Martino, Dias, Noratto, Talcott, & Mertens-Talcott (2016)
Macaúba (Acrocomia aculeata)		Pulp oil	<i>In vivo</i> model (rats) of fructose- induced diabetes and <i>In vitro</i> antioxidant and cytotoxic potential	↓ high glucose levels induced by a high fructose-diet in rats; ↓ high plasmatic glucose induced by STZ; ↓ glucose levels; good <i>in vitro</i> antioxidant activity, no sign of cytotoxicity in LLC-PK1 cells.	Silva et al. (2018)
		Dehydrated pulp	Lipid oxidation and quality of eggs of quails $(n = 120)$	The antioxidant potential of diets was improved; $\downarrow$ malondialdehyde content in the egg yolks; $\downarrow$ degree of lipid oxidation.	Nesello et al. (2018)
	NG (1	Pulp oil	Toxicity induced by cyclophosphamide; Adult male Wistar rats $(n = 70)$	$\uparrow$ content of β-carotene; $\uparrow$ gene C-kit expression and normalized the antioxidant enzymes levels which were changed by CP.	Arena et al. (2018)
	Macauba (Acrocomia aculeata)	Kernel Oil	<i>In vivo</i> (Male albino Wistar rats $n = 40$ ) blood glucose level and the fatty acid deposit in the epididymal adipose tissue	Type 2 diabetic rats ↓ blood glucose level; A small fraction of total dietary medium chain fatty acid was accumulated in the epididymal adipose tissue.	Nunes et al. (2018)
	Leaf Extracts	Antimicrobial potential in vitro	There was no significant antimicrobial activity against the tested microorganisms <i>Staphylococcus aureus</i> (ATCC 6538); <i>Enterococcus faecalis</i> (ATCC 4083); <i>Escherichia coli</i> (ATCC 25922); <i>Pseudomonas aeruginosa</i> (ATCC 27853); <i>Candida</i> <i>albicans</i> (4006); <i>Candida parapsilosis</i> (40038).	Oliveira et al. (2016)	
	Almond and pulp oil	<i>In vivo</i> antigenotoxic, antimutagenic, immunomodulation, and apoptotic potentials; Swiss male	Both oils were chemoprotective at all doses; Both AO and PO demonstrated immunomodulatory activity; The oils $\downarrow$ the capacity of cyclophosphamide to trigger apoptosis in the liver, spleen, and kidney cells.	Magosso et al. (2016)	

	mice $(n = 140)$		
Pulp oil	Cytotoxic, genotoxic, mutagenic	Did not ↑frequency or rate of DNA damage; Did not exhibit	Traesel et al. (2015)
-	in vivo. (Male and female	cytotoxic, genotoxic, or mutagenic effects.	
	Wistar rats $n = 70$ )		

AST, aspartate aminotransferase; ACF, aberrant crypt foci; PPARy2, peroxisome proliferator activated receptor gamma; MDA, malondialdehyde; TAC, total antioxidant capacity; STZ, Streptozotocin; MIC, minimum inhibitory concentration; C3R, cyanidin 3-rutinoside; Pparα, Peroxisome proliferator activated receptor alpha; AO, almond oil; PO, pulp oil; CP, cyclophosphamide; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ox-LDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; ADP, Adenosine 5'-diphosphate; ATCC, American Type Culture Collection; HepG2, hepatocellular carcinoma cells; RKO, colon carcinoma cell line; Akt-3, Akt serine/threonine kinase 3; C/ebpα, C/ebpβ, Klf5 and Srebp1c, adipogenic transcription factors; mRNA, messenger ribonucleic acid; LLC-PKI, porcine kidney cells; Dlcl, tumor suppressor gene; Apo A-1, apolipoprotein A-I.

Due to both nutritional and phytochemical properties along with the versatility of uses, the buriti palm tree represents a new profit opportunity for the agriculture and cattle raising sectors, since it can be used fresh, as flour or oil, not to mention its uses in pharmaceutical and cosmetic formulations as emollient and adjuvant in sun protection (Cruz et al., 2020; Neri-Numa et al., 2018; Nobre et al., 2018). In addition, the buriti residue flour can be employed in the feed enrichment of commercial laying hens, without interfering in the price of feed and losses in gross revenue (Rufino, Cruz, Tanaka, Melo, & Feijó, 2017). Likewise, it is possible to supplement rations for prawn with the buriti oil, without commercial and/or zootechnical damages (Guerra et al., 2019). Moreover, crossbred lactating goats that received the buriti oil to replace ground corn showed neither significant differences concerning the feeding behavior nor physiological and hematological variables (Lima et al., 2018).

#### 2.2. Buritirana (Mauritiella armata (Mart.) Burret)

*Mauritiella armata* (Mart.) Burret is often found in areas of low elevations throughout the Amazon Basin, in the Guianas and in northeastern and central Brazil. It is known as caranaí, buritirana, buriti mirim, buriti bravo, palmilla, yumuna aguajillo; bieneñí, moriche negra and caraña, among other denominations (Gomes, 2007; Smith, 2015).

It is a dioecious and short palm of the genus *Mauritiella*, which presents solitary thin trunks, either in armed groups with rigid roots and spines or aculeos along its stem, measuring up to 8 m in height. It contains 12-14 small fan-shaped leaves and inter-leaf inflorescence with hanging branches. In the Amazon region, the buritirana fruits are used for presenting climacteric behavior. As soon as they start falling in the flooded soil, their bunches are cut and hung at home until they ripen completely (Hiura & Rocha, 2018; Noblick, 2019). Morphologically, the buritirana fruits (**Figure 2B**) are globose to oblong, with overlapping orange scales instead of orange or dark red, which are similar to the buriti fruits, but much smaller. They have a fleshy and fibrous mesocarp with a lemon-yellow pulp, 1–2 mm thick, a very thin endocarp, almost imperceptible. It also has a very hard seed,  $23-25 \times 10-15$  mm, a homogeneous endosperm. It is is separated by the base of a spine-covered stem and may also present spines at the margins of the segments, and globose to oblong-ellipsoid fruits are presented only in the former (Hiura & Rocha, 2018; Noblick, 2019; Noblick, 2019; Smith, 2015).

The Buritirana is considered a very beautiful palm tree of ornamental value and with multiple uses. Its stems are used to manufacture bows, its petiole is used in the production of bottle stoppers, the leaves are used in making broom, and the stipe in rural building, besides being used in the manufacture of beds, shelves and others. In folk medicine buritirana is used to treat skin burns and as tea for rheumatism. For processing, the fruits are commonly softened in hot water for about 1 hour, then the peel is removed and the pulp is scraped from the mesocarp. From its attractive and nutritious fruits, it is possible to prepare refreshing drinks, sweets, jams, wine and produce regional handicrafts (Anunciação et al., 2019; Coimbra, 2019; Martins, 2012; Noblick & Barreto, 2018;).

In terms of centesimal composition, the buritirana is composed of water (54.66 %), ash (0.31%), fat (21.01%) proteins (2.15%), carbohydrates (21.62%), calories (284.15 Kcal) and total solids (45.21%) (Ruiz & Villena, 2011). It has also been observed that the leaves, roots and petioles have amounts of flavonoids 7.92 mg. g<sup>-1</sup>; 5.93 mg. g<sup>-1</sup> and 0.93 mg. g<sup>-1</sup>, respectively. Moreover, a bioprospective study of the buriti has reported that such plant parts also have a radical scavenging activity on DPPH assay, ranging from IC<sub>50</sub>= 50.60 EC<sub>50</sub> µg. mL<sup>-1</sup> to IC<sub>50</sub>= 245.20 EC<sub>50</sub> µg. mL<sup>-1</sup> (Royo et al., 2019). With regards to the carotenoids profile, Anunciação et al. (2019) reported that the fruit comprises mainly all-trans-β-carotene (373.00 µg.100 g<sup>-1</sup>), followed by all-trans- $\alpha$ -carotene (230.00 µg.100 g<sup>-1</sup>), all-trans-lutein (198.00 µg.100 g<sup>-1</sup>) and 9-cis- $\beta$ -carotene (11.00 µg.100 g<sup>-1</sup>).

# 2.3. Açaí (Euterpe oleracea Mart.)

Açaí (*Euterpe oleracea*), also known as açaí, açaí-do-Pará, cabbage heart, is a tropical species. It is widely spread in northern South America, lowlands and the flooded forest land of the Amazon River estuary. Although its greatest abundance is in the Amazon region in Brazil, it also occurs in the Brazilian States of Tocantins, Pará, Maranhão and Amapá, as well as in Guyana and Venezuela (Rufino et al., 2011; Marques et al., 2016; Cantu-Jungles, Iacomini, Cipriani, & Cordeiro, 2017; Yamaguchi, Pereira, Lamarão, Lima, & Veiga-Junior, 2015). This species is a multi-stemmed palm, with up to 25 stems per clump, its trunks reach 20 m high with a maximum diameter of 18 cm, presenting a set of 8-14 compounds. The leaves are pinned in a spiral, with 40- 80 pairs of opposites or sub-opposites leaflets. The inflorescence is below the leaf and the flowers are arranged in triads; a female flower flanked by two male flowers. The açaízeiro produces 5-7 viable bunches per plant, which corresponds to 25 kg of fruit/plant/year in average, reaching maximum productivity of

12 t of fruit/hectare/year (Yamaguchi, Pereira, Lamarão, Lima, & Veiga-Junior, 2015; Vieira et al., 2018).

There are two varieties of açaí. One comprises a blackish-purple epicarp, named dark açaí (**Figure 2C**) while the other has a green epicarp, known as white açaí. In general, the berries are small, rounded and grouped in clusters. Its globose drupe has 1-2 cm of diameter with one yellowish-brown seed, covered by a thin layer of about 2 mm thick (mesocarp) (Costa, Silva, & Vieira, 2018; Dall' Acqua et al., 2015; Yamaguchi et al., 2015).

Commercially, it is a very appreciated fruit, which is preferably consumed as frozen desserts, refreshing drinks, energetic beverages, cream, cereal bars, chewy candies, chocolate, popsicles, powder, although it also composes alcoholic beverages with tequila, wine, and vodka (Arrifano et al., 2018; Costa, Ooki, Vieira, Bedani, & Saad, 2017; Silva, 2017; Faber & Yuyama, 2015; Pinto et al., 2017). Furthermore, the açaí is well distributed in the medicinal sector as supplements and vitamins. It represents a promising ingredient for the cosmetic sector, mainly for personal care products (Silveira et al., 2019; Garbossa & Campos, 2016; Heitor et al., 2017).

From the physicochemical point of view, the açaí is considered a good "add-on" in the human diet due to its high content of unsaturated fatty acid, such as oleic acid (64.19 %), anthocyanins (4.03 mg.g<sup>-1</sup>), carotenoids (41.53  $\mu$ g.g<sup>-1</sup>), total phenolic compounds (37.97 mg GAE.g<sup>-1</sup>), ORAC (841.66  $\mu$ mol Eq.Trolox.g<sup>-1</sup>),  $\alpha$ -tocopherol (342.37  $\mu$ g.g<sup>-1</sup>),  $\gamma$ tocopherol (8.00  $\mu$ g.g<sup>-1</sup>), vitamin E (0.34  $\mu$ g.g<sup>-1</sup>) (Silveira et al., 2019; Lucas, Zambiazi, & Costa, 2018; Silva et al., 2019; Bataglion et al., 2015). The occurrence, purification and partial characterization of xylans from the alkaline açaí extract have been previously studied. Experimental results indicated that the alkali extract provided around 70% of (1  $\rightarrow$  4)- $\beta$ -Dxylan and 4.2% of dry matter açaí pulp. It is currently the biggest source of xylan found in fruit pulps. Moreover, this molecule can be useful in both industrial and biomedical approaches (Cantu-Jungles et al., 2017b).

The açaí berry also received the title of "superfruit" due to the variety of bioactive compounds and their antioxidant behavior. In addition, some comprehensive studies have shown its anti-lipidemic, neuroprotective, hypocholesterolemic, therapeutic, anti-inflammatory and anti-cancer properties, which can be useful not only in the medicinal and pharmaceutical fields, but also as a functional ingredient to be applied in the food industry (Heitor et al., 2017; Yamaguchi, Pereira, Lamarão, Lima, & Veiga-Junior, 2015; Romualdo et al., 2015; Peixoto, Roxo, Krstin, Wang, & Wink, 2016; Torma et al., 2017). **Tables 1** and **2** show further information regarding its nutritional content and bioactive compounds.

Recently, Machado et al., (2019) studied the anti-inflammatory effect of the açaí extract and its mechanism by a RAW 264.7 murine macrophage model using phytohemagglutinin. As a result, it was observed that  $1 \mu g.mL^{-1}$  of the açaí extract was able to modulate redox status by decreasing NLRP3 inflammasome levels and reducing proinflammatory cytokines. It also promotes cell cycle arresting and suppression of cellular growth. In another study, Bem et al. (2018) assessed the effect of the açaí seed extract (ASE) associated with exercise training on hepatic steatosis induced by high-fat (HF) diet plus streptozotocin (STZ) by using eight groups of male Wistar rats subdivided into Sedentary diabetic (D), Training D, ASE Sedentary D and ASE Training D against their respective controls. Experimental results evidenced that the ASE intake associated with exercise training may reduce hepatic steatosis in diabetic rats, decrease lipogenic proteins expression (77.30 %) and increase the antioxidant defense (63.10 %), pAMPK expression (70.20 %), cholesterol transporters (71.10 %) and the pLKB1/LKB1 ratio (57.10 %). Additionally, a reduction in blood glucose levels (70.20 %), total cholesterol (81.20 %), both aspartate aminotransferase (51.70 %) and hepatic triglyceride levels (66.80 %) and steatosis (72.00 %) were also observed in the ASE Training D group in comparison to the Sedentary D group.

Likewise, the ASE supplementation protects C57BL/6 male mice from obesityassociated hepatic steatosis, reducing oxidative stress and modulating the intrahepatic RAS with up-regulation of the ACE2/MASr axis, which is related to the prevention of hepatic remodeling as well as the NF- $\kappa$ B expression and inflammation (Romão et al., 2020). It was also described that the ASE exhibits a great variety of phenolic structures and, consequently, a high antioxidant activity. In human endothelial cells (HUVEC), for instance, it can positively modulate the Nrf2 signaling pathway, modulating the redox status, which emphasizes the possible crucial role of this transcription factor for the anti-inflammatory and antihypertensive effects attributed to the ASE (Soares et al., 2017).

Moreover, Silva et al. (2018) reported that the incorporation of 15% and 30% of the açaí seed flour (ASF) in the mice's diet promoted protective effect against weight gain, prevented insulin resistance and improved their lipid profile, which had lower concentrations of glucose, insulin and leptin. Thus, the ASF reduced lipogenesis, preventing the development of fatty liver diseases and hypertrophic obesity. It is noticed that their use prevents the development of obesity and its comorbidities, especially hepatic steatosis, as it increases cholesterol excretion. Other biological effects of *in vivo* and *in vitro* models from different sources of the açaí fruit are presented in **Table 3**.

#### 2.4. Macaúba (Acrocomia aculeata)

Acrocomia aculeata is a native palm of wide occurrence in tropical and subtropical Americas, also known as ucajá, bocaiúva, coco espinho, mbocayá, coyol "gru-gru", macaw palm, macaya Paraguay palm kernel, etc (Aires & Queiroz, 2016). It is a perennial, heliophilous, and arborescent palm whose single stem reaches 10-15 m in height and 20-30 cm in diameter. The knot region is covered with dark pointed spines about 10 cm long and the roots can be underground or aerial (Aoqui, Favaro, & Moreno, 2012; Bazzo & Pereira, 2018). The green leaves are arranged in different planes, providing a feathery aspect to the crown. They are pinned with a length that varies from 4-5 m, with approximately 130 leaflets on each side and spines in the central region (Amaral, 2007).

The macaúba fruit (**Figure 2D**) is an edible globose drupe whose diameter ranges from 2.5-5.0 cm. Its orange and mucilaginous fibrous mesocarp strongly adheres to the endocarp, which is hard and dense to protect one oleaginous seed coated with a thin brown integument, and makes up the edible portion together with the pulp. Depending on the geographic location, the macaúba produces 3-7 bunches per plant per year (one bunch may weigh more than 25 kg), 200 plants/hectare and has an estimated oil productivity of 5000 kilos/hectare and fruits of 25000 kilos/hectare during the harvest period. (Aires & Queiroz, 2016; Bazzo & Pereira, 2018; Lescano et al. 2015; Mota, Corrêa, Grossi, Castricini, & Ribeiro, 2011). The fruit, which is the greatest product of macaúba palm tree, weighs 22.07 g. It consists of 4.22 g of cellulose skin, 11.00 g of edible pulp and 5.36 g of seeds. Both pulp (mesocarp) and kernel (endosperm) are rich in oil (Lescano et al., 2015; Aires & Queiroz, 2016).

The fruits of the native trees have peculiar characteristics and are known for their therapeutic and nutritional aspects due to their bioactive compounds of industrial interest, such as carotenoids and phenolic constituents, besides a high content of fatty acids (Silva & Fonseca, 2016). The oil obtained from the macaúba pulp fruit is yellow-orange, due to the presence of carotenoids, mainly  $\beta$ -carotene, which corresponds to about 80.00% of the total carotenoids (Aoqui, Favaro, & Moreno, 2012).

The macaúba pulp is rich in nutrients and has antioxidant action. It can be consumed fresh or processed as flour, ice cream, cakes, pastries, biscuits and jellies (Silva et al., 2018; Vianna, Hiane, Jordão, & Pott, 2015). Another characteristic of the macaúba fruit is the oil obtained from both mesocarp and seed, which has shown potential use in food

products, pharmaceuticals and cosmetics (Prates-Valério, Celayeta, & Cren, 2019; Teixeira, Motta, Ribeiro, & Castro, 2017).

Similarly to the buriti, the macaúba also has great potential to be explored in the production of biodiesel. The macaúba oil productivity is estimated at 5.0 Mg oleic-palmitic acid and 1.4 Mg lauric oil in plantations with an average density of 200 plants/ha, which is 70 times greater than the productivity of the buriti and palm oil. In addition, the macaúba stands out because it grows satisfactorily in drier environments, demanding less compared to these two species (Clement et al., 2005). The mesocarp has the highest oil content and is mainly rich in oleic acid (53.40-65.90 %), palmitic acid (13.26-18.70 %) and linoleic acid (8.75-17.70 %). On the other hand, the macaúba almond oil is mainly rich in lauric acid (38.89-58.60 %), oleic acid (12.80-25.50 %), palmitic acid (4.70-17.35 %) and myristic acid (8.00-11.00 %) (Mota et al., 2011). The purified macaúba oil has an acidity index of 0.41 mg KOH.g<sup>-1</sup>, a saponification index of 200.21 mg KOH.g<sup>-1</sup>, an iodine index of 56.70 g I<sub>2</sub>. 100 g<sup>-1</sup>, a peroxide index of 16.83 mEq.Kg<sup>-1</sup>, a density of 0.92 g.cm<sup>-3</sup> and a viscosity of 54.78 mm<sup>2</sup>. s<sup>-1</sup> (Moura et al., 2019). Compared to the soybean biodiesel (the main biodiesel production base in Brazil), the macaúba biodiesel production may be more advantageous due to the lower amount of linolenic acid (less oil degradation by oxidation) (Mota et al., 2011).

Rosa et al. (2019) discovered fatty acids, campesterol 13.92 mg.100 g<sup>-1</sup> and  $\beta$ sitosterol 52.77 mg.100 g<sup>-1</sup>. Prates-Valério et al. (2019) found a total of 85.50 mg.kg<sup>-1</sup> of Tocols, being 11.20 mg.Kg<sup>-1</sup> of  $\gamma$ -tocotrienol, 29.70 mg.Kg<sup>-1</sup> of  $\alpha$ -tocotrienol and 44.60 mg.Kg<sup>-1</sup> of  $\alpha$ -tocopherol, and also discovered relatively high amounts of total carotenoids 248.00 mg.kg<sup>-1</sup>. The carotenoids, tocopherols, tocotrienols present may not only contribute to increasing the oil stability due to their antioxidant action and preservation properties, but also promote health effects concerning the edible oil. **Table 1** shows its nutrients, non-nutritive composition and antioxidant capacity; and **Table 2**, more bioactive compounds, their sources and concentrations, respectively.

Regarding the metabolic effects of the macaúba fruits, **Table 3** shows different models that demonstrate its antioxidant, antilipidemic, anti-inflammatory, antimicrobial, antigenotoxic, antimutagenic effects, among others. The diet rich in long-chain fatty acids (LCFA) increased body mass and decreased glucose tolerance in WT and GPR84 KO animals, while the diet rich in medium-chain fatty acids had no effect on these parameters. However, the liver mass was increased following LCFA feeding in WT, but not in GPR84 KO mice (Du Toit et al., 2018), as the macaúba oil has a high content of fatty acids, polyphenols, oligo-elements and  $\beta$ -carotene. Dario et al., (2018) developed a formulation by

using nanocarriers as sunscreen agents, photoaging prevention and skin cancer. This feature has presented potential application as a natural effective strategy against ultraviolet rays.

### 3. Innovation potential of some representatives of Arecaceae family

Regarding patent applications granted for the fruits of the Arecaceae family, when searching in the Questel Intellectual Property Portal on the Internet: <URL: http // www.orbit.com> by using the following search terms: *Mauritia flexuosa, Euterpe oleracea* and *Acrocomia aculeata*, it was possible to observe a total of 529, with 109 from 2006-2010, 200 from 2011-2015 and 198 from 2016-2020. However, no request was found for the *Mauritiella armata. Euterpe oleracea* is the fruit of this family that has the largest number of patent applications (358). Among the top 10 markets, the United States has the highest number (91), followed by Spain, China, Japan, Brazil, Canada, Korea, India, France and Germany. The ranking of the top 10 players who lead the international patent market involves companies such as Mary Kay, Colgate Palmolive, Evonik operations, Nissin foods, Remedev, Symrise, Capsum, Henkel and Nanning zhijan zhifan technol.

Some of the patent applications for these fruits include processes for the synthesis of nanoparticles using natural fruit oils, preparation of dermatological kits, medicines to treat kidney problems, obtaining natural oils to be used in industry, among others (**Table 4**). A Brazilian invention provided knowledge about the preparation and pharmaceutical use of the *Euterpe oleracea* extract compositions and their antihypertensive properties (Moura, 2006). In addition, the soluble extract of the *Euterpe oleracea* seed was also used in the preparation and production of a cappuccino by using whey (Maifrede, 2020).

Meanwhile, the lyophilized shell of the *Mauritia flexuosa* can be used as an antioxidant agent for applications in formulations of medicines, cosmetics, food ingredients and nutritional products (Pereira et al., 2018). The *Mauritia flexuosa* leaf extract was used to obtain a pharmaceutical formulation with microbicidal action against various bacteria and fungi of clinical interest for the treatment of fungal and bacterial infections (Figueirêdo et al., 2018). An invention developed by the European Patent Office used active ingredients extracted from *Acrocomia aculeata* in the preparation of a nutritional supplement, which can be used to prevent or treat inflammatory processes and oxidative stress related to the aging process and many other pathologies, such as prostatic hyperplasia benign, prostatitis and osteoarthritis (Canavaciolo et al., 2015).

T	T:41 a	Publication	Publication		Technology	Applicant/
rruit	The	number	date	Claim	domain	Assignee
	Skin care composition	WO2016164735	2016-10-13	Cosmetic preparation for topical use to	Organic fine	CGTN ( parent
	having skin damage			reduce DNA fragmentation.	chemistry	company C
	prevention and					TUNA)
	damage repairing					
	properties					
	Synthesis of metallic	BR102016009410	2016-05-10	Synthesis process of metallic	Materials,	Federal
Duriti	nanoparticulas using			nanoparticles, using vegetal and animal	metallurgy	University of
Mauritia	natural reducing			oils.	Micro-structure	Minas Gerais -
(Maurina	agents, metallic				and nano-	UFMG
jiexuosa)	nanoparticulas and				technology	
	uses					
	Dermatological kit	EP3203977	2016-04-13	Cosmetic preparation for topical use in	Organic fine	Medical Brands
	comprising			the treatment of brown spots.	chemistry	Research
	compositions based on					
	hibiscus flower and					
	buriti oil					
Açaí	Preparation method	CN108785476	2018-11-13	Preparation of a solid composition and	Pharmaceuticals	Bai Dongyue
(Euterpe	and application of			application to preparation of medicines		
oleraceae	solid composition			for tonifying kidney and strengthening		

Mart.)	containing acai and	yang, resisting oxidation, resisting			
	herba cistanches	fatigue, resisting caducity, resisting	fatigue, resisting caducity, resisting		
		tumors, regulating immunity and			
		protecting liver.			
	Acai berry extracts for KR20140140338 2014-12	2-09 Preparation of dried extract to improve Pharmaceuticals	Bioport Korea		
	improving blood	blood circulation and relieve			
	circulation and	hypertension.			
	hypertension and				
	process for preparation				
	thereof				
	Acai berry health CN104783164 2015-0	7-22 Preparation of supplement for health Food chemistry	Guizhou		
	care product for Yin-	care.	Guangjitang		
	deficiency		Pharmaceutica		
	constitution		1		
	Oil of macaúba as BR1020140249 2015-12	2-01 Use of macaúba oil as anionic Chemical	Federal		
	aniônic collector in 72	collector in flotation of minerals. engineering	University of		
Macaúba (Acrocomia aculeata)	the flotation of		Goias		
	minerals				
	Process of BR1020140327 2015-0	7-21 Solid state fermentation of macaúba Food chemistry	Petrobras		
	production of 86	residue for protein enrichment,			
	voluminous for	reduction of lipids and antinutritional			
	animal ration	compounds for use it as voluminous			

					in animal ration.		
	Process for the	ne oil	BR1020140214	2014-10-14	Esterification reaction catalyzed by a	Basic materia	als Petrobras
	treatment acids by 63		63		solid fermented enzyme.	chemistry	
	means of reaction of					Biotechnology	
	esterification						
	catalyzed	for					
	fermented	solid					
	enzymes						
Source: <u>https://www.orbit.com</u> .							

These results demonstrate the potential of the fruits of the Arecaceae family, which have technological and functional importance. The use of these fruits is essential for emerging approaches related to their use in several technological domains, as well as for carrying out future research that seeks to add value to their products and also elucidate their real biological effects on human health.

# 4. Concluding remarks

In this review, it was demonstrated that the fruits of the *Arecaceae* family are good sources of bioactive compounds and antioxidants such as carotenoids, polyunsaturated fatty acids, tocopherols, vitamin A, and phenolic compounds. Due to the presence of these compounds, these fruits have the potential to promote health and, therefore, need to be studied, so that the possible mechanisms involved in the prevention of chronic non-communicable diseases, such as obesity and type 2 diabetes, are elucidated.

Given the possible applications in the development and elaboration of new food, chemical and pharmaceutical products obtained from the fruits and by-products of these studied species, it is necessary to implement new cultivation, post-harvest and processing techniques, as well as investing in research to publicize their potential, especially for those that are still little explored, such as the buritirana. Thus, it is possible to promote its commercial value, benefit family farming and contribute to the country's sustainable development.

#### **Credit authorship contribution statement:**

Florisvaldo Gama de Souza and Iramaia Angélica Neri-Numa: Contributed equally on Conceptualization, writing - review and editing. Fábio Fernandes de Araújo, David de Paulo Farias and Aline Wasem Zanotto: contributed equally to Writing- original draft; Glaucia Maria Pastore: Conceptualization, Supervision and Funding acquisition.

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# **4. CHAPTER II**

#### **RESEARCH ARTICLE**

# Characterization of buritirana (*Mauritiella armata*) fruits from the Brazilian cerrado: biometric and physicochemical attributes, chemical composition and antioxidant and antibacterial potential

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#### Abstract:

The buritirana is a little-explored species of the Arecaceae family. The biometric and physicochemical characteristics, nutritional and chemical composition and antioxidant and

antibacterial potential of the buritirana fruit fractions were evaluated here for the first time. The fruits presented an oblong shape. The pulp represented 16.58% of the whole-fruit weight (10.07 g). The moisture, ash and soluble fiber contents were similar for the whole fraction without seed (WS) and pulp. Although the total carbohydrate content was the same for seed and peel (23.24 g.100 g<sup>-1</sup>), the seed showed higher protein and insoluble fiber contents. Except for glucose (1256.63 mg.100 g<sup>-1</sup>), the seed showed the highest concentrations of mono-, di- and oligosaccharides. Mineral content ranged from 0.43 to 800 mg.100 g<sup>-1</sup> in all fractions. The peel fraction showed the highest content of vitamin C. The physicochemical results indicate the pulp and WS fraction have potential for the production of fruit-derived food products. Protocatechuic and quinic acids and epicatechin/catechin were found in all fractions. The assay antioxidant capacity DPPH, phenolic content and total flavonoids were higher in the pulp; TEAC and ORAC<sub>HF</sub> values were lower in the seed. Volatile organic compounds were not identified, and the fractions did not show antibacterial activity.

**Keywords:** antimicrobial activity; bioactive compounds; carbohydrate profile; food fibers; functional potential; nutritional composition; phenolic compounds; proximate composition; volatile organic compounds

# **1. Introduction**

For many years, Brazil has attracted the attention of researchers around the world due to its native plant biodiversity. Many of these plants have bioactive substances that have been associated with the prevention and treatment of diseases [1,2].

The Brazilian flora is rich in native palm species with socioeconomic, nutritional and functional importance, and they can be found in areas of transition between the Cerrado, Amazon and Caatinga biomes. Some of these palm trees, such as the oil palm (*Elaeis oleifera*) and açaí (*Euterpe oleraceae*), have been commercially exploited, while buritirana (*Mauritiella armata* Mart.) is still underutilized and needs further studies to promote its cultivation and commercialization [3,4].

*Mauritiella armata* is a species of the Arecaceae family popularly known as buritirana, buriti bravo, yumuna aguajillo and buriti mirim [5]. The fruits of this species are morphologically similar to those of the buriti (*Mauritia flexuosa*), but smaller in size. Its climacteric fruits are drupes round to oval in shape, arranged in clusters, and are covered with small peels that look like overlapping fish scales of a light-orange color. They have a fibrous mesocarp with soft pulp, which varies from yellow to light green and covers a very thin endocarp containing a very hard seed [4,6]. Its fruits are generally marketed and consumed *in natura*, or their pulp is used to produce sweets, jams, beverages, creams, wines and other food products [7].

In local communities, buritirana is used for the treatment of skin burns and rheumatism [8], probably due to the presence of phenolic compounds, which have a high antioxidant capacity and can be effective in combating various diseases [2,9]. Thus, studies on the buritirana are necessary to locate the phenolic compounds, assess their antioxidant capacity and understand the potential benefits of this species.

Few studies have been carried out on the buritirana fruits. Anunciação et al. [10] found some carotenoids in them, such as all-trans- $\beta$ -carotene (373.00 µg.100 g<sup>-1</sup>), all-trans- $\alpha$ -carotene (230.00 µg.100 g<sup>-1</sup>), all-trans-lutein (198.00 µg.100 g<sup>-1</sup>) and 9-cis- $\beta$ -carotene (11.00 µg.100 g<sup>-1</sup>); and Ruiz and Villena [11] reported the significant presence of lipids (21.01%), proteins (2.15%), carbohydrates (21.62%) and calories (284.15 Kcal). Souza et al. [12] evaluated the oil extracted from the fruits by supercritical CO<sub>2</sub> and found high yield, total carotenoids, saturated and unsaturated fatty acids,  $\alpha$ -tocopherol and antioxidant capacity. However, no work has assessed the chemical composition and nutritional, biological or technological potential of the buritirana fruit and its fractions. Therefore, this is the first study carried out to determine the biometric and physicochemical characteristics, proximate composition, mineral content, carbohydrate and phenolic compounds profiles, volatile organic compounds, antioxidant capacity and antibacterial activity of buritirana and its fractions.

#### 2. Materials and Methods

#### 2.1. Chemicals and Reagents

The following chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA): Trolox (6-hydroxy-2578-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazil (DPPH), 2,2'-azobis (2-methylamidinopropane)-dihydrochloride (AAPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS), fluorescein, Folin–Ciocalteu reagent, methanol and formic acid grade HPLC, quinic acid (PubChem CID: 6508) and all phenolic compound standards (protocatechuic acid (PubChem CID: 1794427), epicatechin (PubChem CID: 72276), p-coumaric acid (PubChem CID: 637542), sinapic acid

(PubChem CID: 637775), ferulic acid (PubChem CID: 445858), rutin (PubChem CID: 5280805), quercetrin (PubChem CID: 5280459) with a purity of  $\geq$ 96%. The other solvents and reagents used in this study were of analytical grade. All solutions were prepared with ultra-pure water (18 M $\Omega$  cm<sup>-1</sup>) obtained from a Milli-Q water purification system (Millipore, Bedford, UK).

# 2.2. Samples

Buritirana fruits in the physiological maturation stage were harvested from 20 plants in November 2018 at Fazenda Moreira ( $10^{\circ}33'11''$  S;  $48^{\circ}43'50''$  W), located in Porto Nacional, TO, Brazil. The fruits were harvested in the afternoon. An exsicate (access number 203433- **Anexx 1**) was identified and deposited in the Herbarium UEC of the Institute of Biology of the State University of Campinas (UNICAMP), Brazil. The fruits collected were washed and dried in open air at room temperature and then packed in polyethylene packaging for 3 days to ripen completely. Then, the fractions were obtained by the manual pulping of the fruits using stainless steel knives; they were named as follows: WS (whole without seed), PU (pulp), PE (peel) and SE (seed). The fractions were frozen at -20 °C, freeze-dried for 72 h (LIOTOP, model L101, São Carlos, Brazil) and ground using a knife grinder (Marconi, model MA340, Piracicaba, Brazil). They were stored in dark polyethylene bags at -40 °C until the moment of preparing the extracts and carrying out the analyses.

#### 2.3. Biometric Characteristics

Twenty fruits obtained from different trees were individually weighed on a semianalytical balance to obtain the average of the total mass of the fruit. With the aid of a digital caliper, the length (l) and the basal (b), central (c) and apical (a) widths of each fruit were measured as illustrated in **Figure 1**. After manual pulping of each fruit, the pulp, peel and seed fractions were weighed in a semi-analytical balance. The percentage of their fractions was calculated as the ratio between the pulp, peel and seed masses, respectively, and the total mass of the fruit multiplied by 100.



Figure 1. Buritirana fruit (Mauritiella armata). (l) Length; (b) basal, (c) central and (a) apical width.

2.4. Proximate Composition, Physicochemical Characterization and Vitamin C Content

The freeze-dried fruit fractions were subjected to analyses of protein, dietary fiber, ash, moisture and pH according to standard methods [13]. The carbohydrate content and total titratable acidity (TTA) were determined according to the 040/IV and the 312/IV methods [14], and the total fat content was determined according to [15]. The vitamin C content was assessed according to [16]. The total energy value was estimated using the conversion factors of 4 kcal.g<sup>-1</sup> for protein or carbohydrate content, 9 kcal.g<sup>-1</sup> for the lipid content and 2 kcal.g<sup>-1</sup> for dietary fiber content: Energy (kcal) =  $[4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g lipid}) + 2 \times (\text{g fiber})]$  [17]. The total soluble solids (TSS) and the ratio of TSS/TTA [13] were assessed only for WS and PU fractions. Thus, for PE and SE fractions, the results are expressed as "not measured—n.m". All analyzes were performed in triplicate.

2.5. Carbohydrate Profile (Mono-, Di- and Oligosaccharides)

The freeze-dried fractions were prepared, and the carbohydrate profile was determined according to [18]. An amount of 1 g of each fraction was diluted in 10 mL of ultra-pure water, homogenized in UltraTurrax (UltraTurrax IKA, T25, Werke, Germany) at 11,000 rpm  $30.s^{-1}$  at room temperature and then centrifuged ( $4000 \times g$ , 5 min, 5 °C; Hettich Zentrifugen, model Rotanta 460R, Tuttlingen, Germany). The supernatant was filtered with a 0.22 µm regenerated cellulose membrane filter.

Carbohydrates were identified and quantified by high-performance anionexchange chromatography coupled with a pulsed amperometric detection system (HPAEC-PAD) model DIONEX ICS-5000 (Thermo Fisher Scientific, Waltham, MA, USA). The flow rate was 1.0 mL.min<sup>-1</sup>, the column temperature was maintained at 30 °C, and the injection volume was 25  $\mu$ L.

A chromatographic column (Carbopac PA1  $250 \times 4$  mm, 10 µm particle size) was used to determine the monosaccharides, disaccharides and polyols (glucose, fructose, sucrose, maltose, cellobiose, raffinose, arabinose, verbascose, stachyose, xylitol and sorbitol). The isocratic mobile phase with 60% A (0.2 M NaOH) and 40% C (ultra-pure water) was used for 25 min. Afterward, 100% A was used for 5 min for column cleaning. Then, for a new run, column stabilization was performed using 60% A and 40% C.

A column (CarboPac PA100,  $250 \times 4$  mm, 8.5 µm particle size) was used to analyze fructo- and malto-oligosaccharides (1-kestose (GF2), nystose (GF3) and 1fructofuranosylnystose (GF4), maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6) and maltoheptaose (G7). The gradient was performed as follows: 0–2 min, 48.5% A, 1.5% B (1 M sodium acetate containing 0.2 M NaOH); 2–18 min, 48.5–30% A and 1.5–20% B; 18–25 min, 100% B; and 25–30 min, 48.5% A and 1.5% B.

Carbohydrates were quantified using a linear calibration curve for carbohydrate standards (glucose, fructose, sucrose, G3, G4, G5, G6 and G7 from Sigma-Aldrich, St. Louis, MO, USA) and GF2, GF3 and GF4 (Wako Pure Chemical Industries, Osaka, Japan). All standards presented purity grade  $\geq 98\%$ . The carbohydrate contents of the samples were expressed in mg.g<sup>-1</sup>.

# 2.6. Minerals

The contents of iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), potassium (K), manganese (Mn) and copper (Cu) were evaluated according to [19]. After mineralization with nitric acid (7%, analytical grade; Sigma-Aldrich, St. Loius, MO, USA) and hydrogen peroxide (2 mL, 30%; Synth, Diadema, Brazil), the samples were digested, and flame atomic absorption spectrophotometry (FAAS, Analyst 200, PerkinElmer, Waltham, MA, USA) was used to determine the mineral content. FAAS was used in absorption mode with a deuterium lamp to correct background radiation and with hollow cathode lamps to determine Fe (248.3 nm), Ca (422.67 nm), Mg (285.21 nm), Mn (279.48 nm), Cu (324.75 nm) and Zn (213.86 nm) contents. The K content was determined with FAAS in emission mode at 766.5 nm. Standard
solutions of Fe, Ca, Mg, K, Mn, Cu and Zn (Sigma-Aldrich) at a concentration of 1000  $\text{mg.g}^{-1}$  were used to construct the analytical curves.

# 2.7. Bioactive Compounds and Antioxidant Capacity

## 2.7.1. Extract Preparation

The extract of all freeze-dried and ground fractions was obtained according to [20], with some modifications. An amount of 1.0 g of the samples was homogenized in UltraTurrax (UltraTurrax IKA, T25, Werke, Germany) with 15 mL of ethanol–acetone–water solution (7:7:6, v/v/v). This solution was mixed in an ultrasonic bath (UNIQUE, model UCS-2850, 25 kHz, 120 W, Brazil) for 30 min at room temperature and then centrifuged at 4000 g for 5 min at 5 °C (Hettich Zentrifugen, model Rotanta 460R, Tuttlingen, Germany). The supernatants were collected and reserved. This procedure was repeated twice more. Subsequently, the supernatants were collected and concentrated under vacuum at 35 °C (Rotavapor model RII, Büchi Labortechnik, Flawil, Switzerland) to remove the organic solvents and suspended in 10 mL of water.

#### 2.7.2. Phenolic Compounds Profile

The analysis of phenolic compounds profile was performed by ultra-high performance liquid chromatography (UHPLC) using an Acquity chromatograph coupled with a TQD Acquity mass spectrometer (Micromass-Waters, Manchester, UK) with electrospray ionization (ESI) and using a C18 BEH Waters Acquity column (2.1 mm × 50 mm × 1.7 µm particle size). A specific chromatographic method for the samples was developed according to [21], with modifications. Phase A was composed of acidic water (0.1% formic acid) and phase B of acetonitrile (HPLC grade). The gradient started at 5% A and 90% B; it increased to 50% B in 7.5 min, then to 100% B in 8.0 min, keeping these concentrations for 1.0 min and returning to the initial conditions in 9.1 min until 10 min. The conditions of the ESI analysis in negative mode were: capillary voltage of -3.00 kV, cone of -30.00 V, source temperature of 150 °C and desolvation temperature of 350 °C. In the tandem mass spectrometry analysis (MS-MS), used to examine the fragmentation of ions, a Collision-Induced Dissociation (CID) was performed at 20 V of collision energy. Initially, a full scan (*m*/*z* 100 to 1000) was carried out to check whether phenolics were present compared to a panel of standards. Finally, the

analysis of the samples and the calibration curve of the standards in selected ion mode (SIM) were performed at m/z 191 (quinic acid), m/z 153 (protocatechuic acid), m/z 353 (chlorogenic acid), m/z 289 (catechin/epicatechin), m/z 163 ( $\rho$ -coumaric acid), m/z 609 (rutin), m/z 223 (synaptic acid), m/z 193 (ferulic acid) and m/z 447 (quercetrin).

# 2.7.3. Total Phenolics Compounds (TPC)

This assay was performed according to [22], with some modifications. The sample solubilized in ethanol was added to 25 mL of the aqueous solution of the reagent Folin–Ciocauteal at 10% and to 2.0 mL of sodium carbonate at 7.5% and then incubated in water-bath for 6.0 min at 45 °C for development of the color. The absorbance was measured in a spectrophotometer (Beckman, model DU600, Fullerton, CA, USA) at 760 nm compared to a blank. To quantify the total phenolic compounds, a curve of gallic acid standard was built, and the results are expressed in mg of gallic acid equivalent.g<sup>-1</sup> of freeze-dried sample (mg GAE.g<sup>-1</sup> fdw).

## 2.7.4. Total Flavonoid Content (TFC)

The total flavonoid content was determined using the method proposed by [23], through sample reactions with NaNO<sub>2</sub>, AlCl<sub>3</sub> and NaOH, followed by absorbance reading on a spectrophotometer (Beckman, model DU600, Fullerton, CA, USA) at 510 nm. The quantification of total flavonoids of the samples was performed using a standard curve prepared with catechin and expressed as catechin equivalents. The final result is expressed in mg of catechin equivalents.g<sup>-1</sup> of freeze-dried sample (mg CE.g<sup>-1</sup> fdw).

## 2.7.5. DPPH Scavenging Assay

The assay was performed using 200  $\mu$ L of the sample with 1000  $\mu$ L of the DPPH • solution (0.004% *w/v*) according to [24], with some modifications. Both the sample and the standard curve were incubated for 30 min and protected from light at room temperature. The absorbance of the remaining DPPH was measured at 517 nm against a blank on a spectrophotometer (Beckman, model DU600, Fullerton, CA, USA). The results are expressed as micromoles of Trolox equivalents.g<sup>-1</sup> of freeze-dried sample ( $\mu$ mol TE.g<sup>-1</sup> fdw).

#### 2.7.6. Trolox Equivalent Antioxidant Capacity (TEAC) Assay

The total antioxidant capacity was determined through the test with ABTS<sup>++</sup>, obtained by reacting 5.0 mL of ABTS 7 mM with 88  $\mu$ L of 140 mM potassium persulfate (final concentration of 2.45 mM), according to the method described by [25]. The system allowed resting at room temperature for 12 to 16 h in the absence of light. Once ABTS<sup>++</sup> was formed, it was diluted with distilled water until an absorbance value of 0.700 ± 0.02 at 734 nm was obtained. The absorbance reading was obtained from the reaction of 50  $\mu$ L of sample and 250  $\mu$ L of ABTS<sup>++</sup> solution against the 734 nm blank in a microplate reader. The results are expressed as micromoles of Trolox equivalents.g<sup>-1</sup> of freeze-dried sample ( $\mu$ mol TE.g<sup>-1</sup> fdw).

# 2.7.7. Oxygen Radical Absorbance Capacity-Hydrophilic Fraction (ORAC<sub>HF</sub>) Assay

The assays were performed according to the method described by Prior et al. [26]. A dark microplate of polystyrene with 96 wells was used. The volume of 20  $\mu$ L of sample blank or Trolox standard plus 120  $\mu$ L of fluorescein and 60  $\mu$ L of AAPH [2,2'- azobis (2'- metilpropionamidine) dihydrochloride] was added to each well. The temperature was maintained at 37 °C for 80 min. Fluorescence was determined and recorded every minute on a NovoStar Microplate reader (New Brunswick Scientific Classic Series, model C76, Offerburg, Germany) with fluorescence filters (excitation and emission wavelengths of 485 and 520 nm, respectively). Trolox was used to prepare the standard curve, and the results are expressed as micromoles of Trolox equivalents.g<sup>-1</sup> of freeze-dried sample ( $\mu$ mol TE.g<sup>-1</sup> fdw).

# 2.8. Analysis of Volatile Organic Compounds (VOCs) in GC-MS

## 2.8.1. Sample Conditions and SPME Extraction

Buritirana samples remained frozen at -40 °C for 36 months. After this period, they were unfrozen and pulped manually. The mass of 500 mg of the edible fraction (PU) was ground and homogenized with ultra-pure water (1:4, *w*:*v*) in 20 mL flasks with a screw cap containing a Teflon-coated septum for VOCs headspace microextraction [27]. SPME extractions were performed from the headspace of the samples according to the following

conditions: DVB/CAR/PDMS fiber; equilibrium time of 10 min; extraction time of 15 min; and extraction temperature of 50 °C.

#### 2.8.2. GC-MS Analysis Procedure

The compounds were separated using an Agilent 7890A gas chromatography system (Agilent Technologies, Santa Clara, USA) equipped with a GC DB-WAX column (30 m  $\times$  0.25 mm  $\times$  0.15 µm) and Agilent 5975C inert MSD with Triple-Axis Detector, using He as a carrier gas. The VOCs were desorbed for 5 min by inserting the SPME fiber into a GC injector (270 °C). The GC oven was programmed to maintain a temperature of 70 °C for 1 min, then to increase to 140 °C at a rate of 3 °C.minute<sup>-1</sup>, and finally to 210 °C at a rate of 5 °C.minutes<sup>-1</sup>. The column flow rate was 1.0 mL.minute<sup>-1</sup>. The MS was scanned in the range of 45–650 amu at 70 eV. The total analysis time was 39.33 min. Compounds were identified using NIST 14.0 database, and Linear Retention Index (LRI) was calculated with a series of n-alkanes (C7–C40).

# 2.9. Antibacterial Activity

# 2.9.1. Micro-organism and Culture Condition

The bacterial strains used in this study were *Escherichia coli* ATCC 10231, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 5061, *Bacillus cereus* ATCC 10876, *Salmonella choleraesuis* ATCC 10708 and *Peseudomonas aeruginosa* ATCC 13388. All strains were periodically harvested in Mueller–Hinton medium (Kasvi) (bacteria, 37 °C for 1 day) and stored under refrigeration conditions.

## 2.9.2. Determination of Minimum inhibitory Concentration (MIC)

Extracts of PU, PE, WS and SE fractions were used to assess antimicrobial susceptibility using the broth microdilution method according to CLSI [28]. These methods assessed the ability to inhibit bacterial growth by evaluating different known concentrations in a 96-well microplate. Initially, the compounds were transferred into the first well, and serial dilutions were made in the range of  $4.0-0.001 \text{ mg.mL}^{-1}$ . Streptomycin sulfate (Sigma-Aldrich<sup>®</sup>) was used in the range of  $0.5-0.0039 \text{ mg.mL}^{-1}$  as the reference antibiotic control.

The antibacterial activity was carried out using Mueller–Hinton broth (Kasvi). The bacteria strains were standardized in Mueller–Hinton broth to  $10^6$  CFU.mL<sup>-1</sup>. The inoculum was added to all wells, and the plates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of the sample that inhibited visible growth. As indicated by 2,3,5-triphenyltetrazolium chloride staining, dead cells did not stain.

# 2.10. Experimental Design and Statistical Analysis

A completely randomized design (DIC) was used for the experiment, the data were analyzed by ANOVA and, when differences were detected, the Tukey test was used for multiple means comparison using a significance level of 5%. The Principal Component Analysis (PCA) was used to understand possible variable–variable and variable–samples relations. The PCA was performed on standardized data to avoid the effect of different magnitude levels of response variables. Statistical analyses were carried out using the R 4.0.2 (2020) and the package FactorMineR 1.32 for exploratory multivariate data analysis. All analyses were performed in triplicate, and the results are expressed as mean  $\pm$  standard deviation.

# 3. Results and Discussion

#### 3.1. Biometric Characteristics

The buritirana fruits evaluated were oval drupes with not very hard peels similar to small overlapping fish scales light orange in color. The seed is covered by a fibrous layer and a lemon-yellow pulp with a characteristic odor.

The whole fruit had a mass of 10.07 g (**Table 1**). The buritirana fruit showed a lower mass in comparison with buriti (*Mauritia vinífera*) [29], which presented a WS fraction of 60.43 g, PU of 13.55 g, PE of 15.68 g and SE of 31.19 g. Moreover, the buritirana seed (**Table 1**) presented the highest mass, followed by the peel and pulp (6.46 g > 1.94 g > 1.67 g), representing 64.15%, 19.27% and 16.58% of the fruit, respectively. These values were higher than those of buriti pulp (*Mauritia flexuosa*) grown in Goiás and Pará (16.43% and 8.53%, respectively) [30].

<b>Biometric Characteristics</b> $(n = 20)$							
Mass of whole fruit $10.07 \text{ g} \pm 1.02$							
Mass of peel	$1.94 \text{ g} \pm 0.13$	* 19.27%					
Mass of pulp	$1.67 \text{ g} \pm 0.60$	* 16.58%					
Mass of seed	$6.46\ g\pm 0.58$	* 64.15%					
Longitudinal length	$29.20\ mm \pm 0.89$						
Apical diameter	$19.30\ mm \pm 1.13$						
Central diameter	$24.75\ mm \pm 1.52$						
Basal diameter	$21.25\ mm \pm 1.33$						

Table 1. Biometric characteristics of the buritirana fruit and its fractions.

\* Percentage (w/w) of each fraction related the whole fruit.

The length (29.20 mm) and the apical (19.30 mm), central (24.75 mm) and basal (21.25 mm) widths indicate that the buritirana fruit had an oblong shape. Ruiz and Villena [11] evaluated the buritirana fruit grown in Iquito, a region of Loreto, Peru, and found similar results for length (25–35 mm) and width (20–30 mm).

3.2. Proximate Composition, Physicochemical Characteristics and Vitamin C of Buritirana Fractions

The seed presented the highest moisture (**Table 2**) in comparison with the other fractions (p < 0.05). The amount of total carbohydrates was the highest (p > 0.05) in SE and PE fractions (23.24% for both), followed by the WS (17%) and PU (8.06%) fractions. The PU and WS fractions differ statistically and showed the highest amounts of lipids (20.20% and 16.57%, respectively), followed by the PE (13.43%) and SE (0.27%) fractions. These results are similar to those found by Souza et al. [12]

For proteins, the PU and SE fractions showed similar values (5.96% for both, p < 0.05), followed by WS and PE fractions (5.53% and 5.34%, respectively). The ash content varied from 1.58 g ± 0.03 (PU) to 3.77 g ± 0.02 (SE) (p < 0.05). The WS, PE and PU fractions showed the highest total energy, (381.74 Kcal.100 g<sup>-1</sup>, 368.78 Kcal.100 g<sup>-1</sup>, 381.88 Kcal.100 g<sup>-1</sup>, respectively; p < 0.05) followed by the SE fraction (280.72 Kcal.100 g<sup>-1</sup>.

There was no difference between PU and PE fractions (p > 0.05). The highest energy value of the WS and PE fractions can be explained by the fact they also have the highest lipid amount, since lipids have 9 Kcal.g<sup>-1</sup> and proteins and carbohydrates only have 4

Kcal.g<sup>-1</sup>. The total energy of the PU fraction corresponds to ~7 portions (50 g of dehydrated buritirana pulp), 18.43% of the recommended daily intake, 2000 Kcal for adults [31].

The PU fraction showed the highest soluble fiber content with  $(10.53 \pm 1.97 \text{ g}.100 \text{ g}^{-1})$ , which was similar (p > 0.05) to those of the WS ( $10.33 \pm 0.68 \text{ g}.100 \text{ g}^{-1}$ ) and PE ( $8.04 \pm 2.26 \text{ g}.100 \text{ g}^{-1}$ ) fractions. The lowest soluble fiber content was found in the SE fraction ( $5.56 \pm 0.14 \text{ g}.100 \text{ g}^{-1}$ , p < 0.05). Fiber consumption is related to fecal volume, viscosity and fermentation, which may contribute to the reduction in hyperglycemia, hypertension and oxidative stress in people with type 2 diabetes [32,33]. This indicates that the buritirana fruit is rich in dietary fiber and can be used to treat chronic diseases associated with the intestine.

The concentrations of vitamin C, in decreasing order, were: PE (242.45 mg.100  $g^{-1}$ ) > WS (223.53 mg.100  $g^{-1}$ ) > PU (205.00 mg.100  $g^{-1}$ ) > SE (106.33 mg.100  $g^{-1}$ ). However, there were no statistical differences between the WS and PE fractions. Buritirana fractions were revealed to be a great source of vitamin C compared to several Brazilian tropical fruits [34]. Additionally, the vitamin C in the peel of buritirana represents about 12% of the vitamin C content of acerola (*Malpighia emarginata*), which after camu-camu (*Myrciaria dubia*), is considered one of the fruits with the highest concentration of vitamin C among Brazilian tropical fruits [35,36].

Regarding pH, the seed was the only one (p < 0.05) that presented low acidity (pH 5.88), while the other fractions can be considered very acidic (pH < 4.0). Acidic fruits are widely used for the preparation of sweets and jellies, as they do not require the addition of organic acids to reach the optimum gelation point. The TSS reflects the organic acids, vitamins, minerals and total soluble sugars in a fruit, and is usually represented by the total soluble carbohydrates. The TSS value in the WS fraction was 10.48 g and 8.18 g in the PU fraction. The TTA was  $1.28 \pm 0.00$  and  $1.71 \pm 0.04$  for WS and PU and resulted in a TSS/TTA ratio of 8.18  $\pm$  0.08 and 4.83  $\pm$  0.06, respectively, with an emphasis on the WS fraction. The ratio is related to the flavor attribute and indicates the balance between sugars and organic acids; it is considered an important parameter to determine the ripeness and palatability of the fruits [33].

Table 2.	Proximate	composition,	saccharides,	minerals	and	vitamin	Сс	contents,	and	physicochemical	characteristics	of	the	buritirana	fruit
fractions.															

Characteristics		Fractions						
Chara		WS	PU	PE	SE			
	Moisture g	$1.73 \pm 0.13$ c	$1.58 \pm 0.03 \text{ c}$	$2.02\pm0.11~b$	$3.77 \pm 0.02$ a			
	Total carbohydrates g	$17.00\pm0.75~b$	$8.06 \pm 0.15 \text{ c}$	$23.24 \pm 0.90$ a	$23.24 \pm 0.88$ a			
	Total fat g	$16.57\pm0.10~b$	$20.20 \pm 1.98$ a	$13.43 \pm 1.01 \text{ c}$	$0.27 \pm 0.03 \text{ d}$			
Drovimate composition	Protein g	$5.53\pm0.14~b$	$5.96 \pm 0.09$ a	$5.34\pm0.05\ b$	$5.96 \pm 0.27$ a			
upits per 100 g	Ash g	$1.73 \pm 0.13 \text{ c}$	$1.58\pm0.03~c$	$2.02\pm0.11~b$	$3.77 \pm 0.02$ a			
units per 100 g	Total fiber g	$71.22\pm0.98~b$	$65.46 \pm 2.41 \text{ c}$	$73.35\pm1.12~b$	$80.74 \pm 0.14$ a			
	Soluble fiber <sup>1</sup> g	$10.33 \pm 0.68$ a	$10.53 \pm 1.97$ a	$8.04 \pm 2.26 \text{ ab}$	$5.56\pm0.14\ b$			
	Insoluble fiber g	$60.89 \pm 0.30 \text{ c}$	$53.32 \pm 1.58 \text{ d}$	$65.32 \pm 1.15 \text{ b}$	$75.18 \pm 0.00 \text{ a}$			
	Total energy value Kcal	381.74 ± 4.16 a	368.78 ± 19.20 a	381.88 ± 3.70 a	$280.72 \pm 3.93 \text{ b}$			
Monosaccharides	Glucose mg	$1228.00 \pm 34.00 \text{ b}$	$1387.87 \pm 13.50$ a	$1034.23 \pm 23.60$ c	$1256.00 \pm 4.20 \text{ b}$			
units per 100 g	Fructose mg	$453.00 \pm 46.00 \text{ c}$	$547.50\pm5.30~b$	$390.63 \pm 10.28 \text{ c}$	$801.67 \pm 4.04$ a			
Disaccharides	Sucrose mg	n.d	n.d	n.d	$3038.37 \pm 28.76$ a			
units per 100 g	Maltose mg	$479.00 \pm 11.00$ a	$466.80 \pm 21.52$ a	$509.97 \pm 27.82$ a	$523.32 \pm 23.87$ a			
Oligosaccharides	1- Kestose (GF2) mg	157.91 ±10.21 b	n.d	n.d	2646.49 ±137.65 a			
units per 100 g	Nystose (GF3) mg	$106.45 \pm 4.57 \text{ b}$	n.d	n.d	$209.15 \pm 12.08$ a			
	Fe mg	3.29 ± 0.26 a,b	$2.88\pm0.15~b$	$3.58 \pm 0.33$ a	$2.70\pm0.13~b$			
	Zn mg	$2.23 \pm 0.07 \text{ b}$	$2.15\pm0.02~b$	$2.38 \pm 0.04$ a	$1.94 \pm 0.03 \text{ c}$			
Minerals	Ca mg	$51.46 \pm 2.82$ c	$65.189 \pm 0.71$ a	$34.46 \pm 1.20 \text{ d}$	$60.05 \pm 0.60 \text{ b}$			
units per $100 \text{ g}$	Mg mg	$46.17 \pm 0.66 \text{ c}$	$49.12 \pm 1.47$ b	43.96 ± 1.03 c	$112.96 \pm 0.87$ a			
units per 100 g	K mg	608.67 ± 9.04 c	672.64 ± 39.80 b	528.12 ± 23.36 d	$800.01 \pm 8.91$ a			
	Mn mg	$3.37 \pm 0.10$ a	$3.54 \pm 0.08$ a	$3.08 \pm 0.11 \text{ b}$	$2.15 \pm 0.07 \text{ c}$			
	Cu mg	$0.44 \pm 0.02 \text{ b}$	$0.43 \pm 0.04$ b	$0.45\pm0.03~b$	$0.79 \pm 0.00 \text{ a}$			
Vitamin units per 100 g	Vitamin C mg	223.53 ± 11.164 a,b	$205.00\pm11.16\ b$	$242.45 \pm 10.08$ a	$106.33 \pm 10.71 \text{ c}$			
Physicochemical	Total titratable acidity (g citric acid)	$1.28\pm0.00\ b$	$1.71 \pm 0.04$ a	$1.05\pm0.07\ c$	$0.25\pm0.02\;d$			
characteristics	pH	$3.36\pm0.00\ b$	$3.37\pm0.00\ b$	$3.37\pm0.00~b$	$5.88 \pm 0.02$ a			
units per 100 g	Total soluble solids g	$10.48 \pm 0.08 \text{ a}$	$8.18\pm0.08\ b$	n.m	n.m			
	Ratio TSS/TTA	$8.18\pm0.08~a$	$4.83\pm0.06\ b$	n.m	n.m			

<sup>1</sup>: Soluble fibers were calculated as the difference between total fiber and insoluble fibers. TSS: total soluble solids; TTA: total titratable acidity; WS: whole without seed; PU: pulp; PE: peel; SE: seed. Averages with different letters in the line indicate statistical differences according to Turkey test (p < 0.05). n.d: not detected; n.m: not measured. n = 3. Season: November.

#### 3.3. Carbohydrate Profile: Mono-, Di- and Oligosaccharides

Total carbohydrates were investigated and classified and quantified as mono-, diand oligosaccharides as shown in Table 1. Among monosaccharides, glucose was the main simple sugar found in all fractions, and the PU fraction presented the highest value at 1687.87  $\pm 13.50 \text{ mg}.100 \text{g}^{-1}$  (p < 0.05). The SE fraction showed the highest fructose content (801.67  $\pm$ 4.04 mg.100 g<sup>-1</sup>), about 1.8, 1.5 e 2.0 times greater than that of the WS, PU and PE fractions, respectively. The values of glucose and fructose were lower than those found in buriti (*Mauritia flexuosa*) (13240 mg.100  $g^{-1}$  for glucose and 2770 mg.100  $g^{-1}$  for fructose) but higher than those found in açaí (*Euterpe oleraceae*) (250 mg.100  $g^{-1}$  for glucose and 90 mg.100  $g^{-1}$  for fructose) [4]. Regarding the disaccharides, sucrose was found only in the seed  $(3038.37 \pm 28.76 \text{ mg}.100 \text{ g}^{-1})$ . The sucrose concentration found in this study was higher than that found in the uvaia seed  $(1352 \pm 0.10 \text{ mg}.100 \text{ g}^{-1})$  [37]. The maltose was found in all fractions and ranged from 466.80  $\pm$  21.52 mg.100 g<sup>-1</sup> in the PU to 523.32  $\pm$  23.87 mg.100 g<sup>-1</sup> in the SE; therefore, there was no statistical difference among the fractions (p > 0.05). The maltose content of the WS, PU, PE and SE fractions was higher than that of the edible fractions and seeds of uvaia (Eugenia pyriformis) and aracá-boi (Eugenia stipitata) [27,37]. The buritirana has a high concentration of maltose, which represents about 50% of the maltose content in adulterated apple juices [38]. Maltose is a very important fermentable sugar, with potential to stabilize an emulsion in cooling and freezing processes, and when hydrolyzed it turns into glucose, which is widely used to reduce sweetness and increase the brightness of base products of fruits, such as jellies, among others. [39,40].

Malto-oligosaccharides were not found in any of the fractions evaluated. Fructooligosaccharides were found in the seed, generally GF2 and GF3, and was approximately 16.7 and 2.0 times higher than in the WS fraction, respectively; GF2 and GF3 concentrations in WS and SE fractions were  $157.91 \pm 10.21 \text{ mg.g}^{-1}$  and  $2646.49 \pm 137.65 \text{ mg.g}^{-1}$ , respectively (**Table 2**). This is the first study that includes an analysis of the carbohydrate profile of the buritirana fruit. Other studies on native fruits from different regions of Brazil have shown the presence of the following compounds: GF2 (63.80 mg.100 g<sup>-1</sup>) and GF3 (374.91 mg.100 g<sup>-1</sup>) in the juá-açu pulp (*Solanum oocarpum* Sendtn.); GF2 (26.81 mg.100 g<sup>-1</sup>) and GF3 (66.69 mg.100 g<sup>-1</sup>) in the fruta-do-lobo pulp (*Solanum lycocarpum* St. Hill) [33]; GF2 (27  $\pm$  0.03 mg.100 g<sup>-1</sup>) in the edible fraction of *Eugenia stipitata* [27]; and GF2 (21  $\pm$  0.04 mg.100 g<sup>-1</sup>) in the uvaia seed (*Eugenia pyriformis*) [37]. Among the reported fruits, the juá-açu pulp showed the highest levels of GF2 and GF3. However, in comparison to the juá-açu seed, the value of GF2 (2646.49 mg.100  $g^{-1}$ ) of the buritirana seed was 41.5 times higher, and the value of GF3 was only 1.8 times lower. Oligosaccharides are specific groups of carbohydrates that have prebiotic activity and are applied as food ingredients, additives for cosmetics, pharmaceutical products and preservatives for fruits and vegetables. They are associated with several functional properties, such as improvement of intestinal function, mineral absorption, regulation of lipid and glycemic metabolism and reduction in the risk of developing colon cancer [27,41]. This reinforces the importance of investigating these compounds and shows that buritirana has great potential for the production of food products with functional attributes.

#### 3.4. Mineral Content

All fractions of buritirana have nutritional potential in micro- and macrominerals (**Table 2**), but there were differences among the fractions (p < 0.05). The highest mineral contents in mg.100 g<sup>-1</sup> were: Fe (3.58 ± 0.33, PE), Zn (2.23 ± 0.07, WS), Ca and Mn (65.189 ± 0.71 and 3.54 ± 0.08, PU) and Mg, K and Cu (112.96 ± 0.87, 800.01 ± 8.91 and 0.79 ± 0.00, SE). On the other hand, the lowest contents were: Fe, Zn and Mn (2.70 ± 0.13, 1.94 ± 0.03 and 2.15 ± 0.07, SE), Ca, Mg and K (34.46 ± 1.20, 43.96 ± 1.03 and 528.12 ± 23.36, PE) and Cu (0.43 ± 0.04, PU).

Fruit from the same family, buriti (*Mauritiaflexuosa*), açaí (*Euterpeoleraceae*) and macaúba (*Acrocomia aculeata*), were also studied regarding mineral content [4] by convention; the highest mineral content (mg.100 g<sup>-1</sup>) found here will be compared with those of these fruits. The content of Fe in buritirana was higher than that of buriti, but lower than that of macaúba and açaí; the Zn content was lower than that of macaúba and higher than that of buriti and açaí; the Mg content was lower than that of the three other fruits; the K content was lower than that of açaí but higher than that of buriti and macaúba; the Cu content was higher than that of buriti and macaúba (açaí, not reported); the Ca content was lower than that of buriti and macaúba. Minerals are essential elements for good maintenance of the body, and according to [42], the consumption of 100 g of edible buritirana fractions (WS and PU) provides the recommended daily intake (RDA) of Ca for all lifestyle groups; Cu represents 50% of the RDA for children 0–8 years; Mg provides more than 50% of the RDA for children aged 7 months–3 years); Mn also supplies the RDA

of all groups; and finally, Zn provides about 74% of the RDA for children aged 1–3 years) [42]. All fractions of the buritirana studied are sources rich of minerals for people of different lifestyles, sex and age, as they present more than the minimum of 15% of the recommended dietary intake per 100 g of sample [43]. PE and SE fractions, which are not edible, can be used as sources of minerals to enrich food.

**Figure 2** shows a clear and concise interpretation of the possible relationships among the responses of proximate composition, carbohydrate profile, physicochemical characteristics, bioactive compounds, including antioxidant properties and vitamin C content, and the fractions. The first two main components explain 71.5% and 26.7% of the data variability, totaling 98.2%. This value was higher than that recommended by Abdi and Williams [44], who reported that the first selected main components must correspond to a minimum of 75% of the variability among the samples.



**Figure 2.** Graphical representation of the Principal Component Analysis (PCA) of the buritirana fruit fractions: (**a**) Fractions: WS: whole without seed; PU: pulp; PE: peel; and SE: seed. (**b**) Responses of proximate composition, carbohydrate profile, physicochemical characteristics, vitamin C content, bioactive compounds and antioxidant capacity; Mo: moisture; Ptn: protein; Car: total carbohydrates; Fat: total fat; VtC: vitamin C; TF: total fiber; IF: insoluble fiber; SF: soluble fiber; EV: Energy value; TTA: total titratable acidity; Glu: glucose; Fru: fructose; Suc: sucrose; Mal: maltose; GF2: 1- kestose; GF3: nystose; Ca: calcium; Mg: magnesium; Fe: iron; Zn: zinc; K: potassium; Cu: copper; Mn: manganese; pH: hidrogenionic potential.; TPC: total phenolic compounds; TFC: total flavonoids; TEAC: Trolox equivalent absorbances capacity; DPPH: DPPH scavenging; ORAC<sub>HF</sub>: oxygen radical absorbance capacity; QA: quinic acid; FA: ferulic acid; PA: protocatechuic acid; ChA: chlorogenic acid; E/C: epicatechin/catechin; p.CA:  $\rho$ -Coumaric acid; RU: rutin; QE: quercetrin.

The SE fraction (**Figure 2a**) had greater values for Mo, Ash, TF, IF, Fru, Suc, Mal, K, Mg, Cu, GF2, GF3, pH, ChA and PA (**Figure 2b**) as they are in the same geometric space as the SE. Among the nine phenolic compounds investigated, six were present in the seed, and among these, two were below the LOQ (**Table 3**). Thus, since the phenolic compounds are directly related to the antioxidant capacity, it can be observed (**Table 4**) that

in all the assays, SE presented lower antioxidant potential compared to the other fractions. On the other hand, oligosaccharides such as GF2 and GF3 were identified in a greater proportion in the buritirana seed and can contribute to the formulation of products with functional claim. Since these compounds have prebiotic action, they can regulate the gut microbiota and help to prevent and fight against diseases [45,46]. Therefore, the characteristics of this fraction can add value to a part of the buritirana fruit that is usually discarded, as occurs with most fruits that are consumed fresh or utilized in processing units to obtain their derivatives.

Similarly, the PU fraction (**Figure 2a**) was more associated with the highest values of the variables Ptn, Fat, Ca, Mn, SF, Glu, TTA, ORAC<sub>HF</sub>, TPC, TFC, FA, and RU (**Figure 2b** and **Table 2**). Thus, the pulp presented a high energy value and great antioxidant potential. On the other hand, the PE fraction (**Figure 2a**) was better represented by Car, Fe, Zn, VtC, EV, QA, TEAC, p.CA and E/C (**Figure 2b** and **Table 2**). The buritirana peel has potential to be used as a source of dietary fiber and natural antioxidants in food. Finally, the WS fraction, which is in light blue in **Figure 2a**, showed intermediate values when compared to the PU and PE fractions.

## 3.5. Bioactive Compounds and Antioxidant Potential of the Buritirana Fruit Fractions

## 3.5.1. Phenolic Compounds Profile

The phenolic compound profile of the buritirana fruit fractions was determined using UHPLC-ESI-MS. This was the first time that the profile of phenolic compounds of the buritirana fruit fractions was evaluated, and they are shown in **Table 3**. Investigating phenolic compounds is paramount since they are non-nutritive ingredients synthesized by the secondary metabolism of plants and play an important role in human health, in addition to having several bioactive properties [4,47,48]. Nine compounds were identified and quantified: quercetrin, rutin, epicatechin/catechin, and ferulic, sinapic, p-coumaric, chlorogenic, protecatechuic and quinic acids. However, quercetrin and sinapic acids were below the limit of quantification (<LOQ). The quinic and protocatechuic acids and epicatechin/catechin were quantified in all fractions. They showed the highest contents among the acids; their content ranged from  $0.03 \pm 0.07 \,\mu \text{g.mL}^{-1}$  for SE (p < 0.05) to  $6.77 \pm 1.43 \,\mu \text{g.mL}^{-1}$  in the WS fraction. The PU fraction showed the highest content of rutin ( $1.14 \pm 0.01 \,\mu \text{g.mL}^{-1}$ ) and ferrulic acid ( $0.05 \pm 0.00 \,\mu \text{g.mL}^{-1}$ ) (p < 0.05).

			Fractions					
Dhanalia Comnounda	Mass	RT	WS	PU	PE	SE		
Phenone Compounds	[M-H]	(Min.)						
Quercetrin	447	3.90	n.m	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>		
Ferulic acid	193	3.60	$0.04 \pm 0 b$	$0.05 \pm 0$ a	$0.03\pm0.01~c$	n.m		
Sinapic acid	223	3.50	<loq< td=""><td>n.m</td><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	n.m	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>		
Rutin	609	3.40	$0.61 \pm 0 b$	$1.14 \pm 0.01$ a	$0.37\pm0.01~c$	n.m		
<i>p</i> -Coumaric acid	163	3.25	$0.01 \pm 0 a$	n.m	$0.01 \pm 0$ a	n.m		
Epicathecin/Cathecin	289	2.70	$0.03 \pm 0 b$	$0.01 \pm 0 c$	$0.10 \pm 0$ a	$0.01 \pm 0 c$		
Chlorogenic acid	353	2.25	$0.06 \pm 0 b$	n.m	$0.10\pm 0 \; b$	$2.51 \pm 0.1 \text{ a}$		
Protecatechuic acid	153	1.75	$0.06 \pm 0 c$	$0.08 \pm 0 \ b$	$0.04 \pm 0 \ d$	$0.14 \pm 0.01$ a		
Quinic acid	191	0.70	$6.77 \pm 1.43 \text{ b}$	$5.20 \pm 0.11 \text{ c}$	$8.25 \pm 0.24$ a	$1.91 \pm 0.07 \ d$		

Table 3. Phenolic compounds found by UHPLC-ESI-MS in the buritirana fruit fractions.

RT: retention time; LOQ: limits of quantification; WS: whole without seed; PU: pulp; PE: peel; SE: seed; Average with different letters in the line indicate statistical differences according to Turkey (p < 0.05). n.m: not measured; quercetrin LOQ: 0.88 µg.mL<sup>-1</sup>; sinapic acid LOQ: 0.43 µg.mL<sup>-1</sup>.

*p*-Coumaric acid content was similar (p > 0.05) between fractions WS and PE (0.01 µg.mL<sup>-1</sup>) and was not determined in the PU and SE fractions. Phenolic compounds were also investigated in the oil of the buritirana fruit fractions (*Mauritiella armata*), and the authors reported that the fractions WS, PU and PE also presented phenolic compounds similar to those found in this study [12].

## 3.5.2. Total Phenolic Compounds, Total Flavonoids and Antioxidant Capacity

The contents of total flavonoids and total phenolic compounds, as well as the antioxidant capacity of the buritirana fruit fractions, were evaluated by the DPPH, TEAC and  $ORAC_{HF}$  assays. The results are shown in **Table 4**.

Doromotors	Fractions							
1 al alletel s	WS	PU	PE	SE				
TPC <sup>1</sup>	$8.51\pm0.14\ b$	$10.60 \pm 0.08$ a	$4.70\pm0.10\ c$	$1.54\pm0.03~d$				
TFC <sup>2</sup>	$0.53\pm5.97~b$	$0.75\pm0.38~a$	$0.29\pm0.43\;c$	$0.02\pm0.07~d$				
DPPH <sup>3</sup>	$140.75\pm0.30\ b$	$234.25 \pm 4.42$ a	$136.95\pm0.26~\text{b}$	$24.35\pm0.50\ c$				
TEAC <sup>3</sup>	$743.02\pm9.94~b$	$448.40 \pm 9.83 \ c$	$781.09 \pm 4.32 \text{ a}$	$38.44 \pm 1.70 \text{ d}$				
ORAC <sub>HF</sub> <sup>3</sup>	$1.82\pm0.01~b$	$2.12 \pm 0.13$ a	$1.55 \pm 0.17 \text{ b}$	$0.50\pm0.03\ c$				

**Table 4.** Content of total flavonoids, content of total phenolic compounds and antioxidant capacity of the buritirana fruit fractions.

WS: whole without seed; PU: pulp; PE: peel; SE: seed; 1: result expressed as mg GAE.g<sup>-1</sup> fdw (freeze-dried sample); 2: result expressed as mg CE.g<sup>-1</sup> fdw; 3: result expressed as  $\mu$ mol TE.g<sup>-1</sup> fdw; Averages with different letters in the line indicate statistical differences according to Turkey (p < 0.05).

The PU fraction showed the highest content of TPC and TFC and antioxidant capacity according to DPPH (10.60  $\pm$  0.08 mg GAE.g<sup>-1</sup>, 0.74  $\pm$  0.38 mg CE.g<sup>-1</sup> and 234.25  $\pm$ 4.42  $\mu$ mol TE.g<sup>-1</sup>, respectively), and the values differed statistically from those of WS, PE and SE fractions (p < 0.05). An antioxidant capacity of  $140.75 \pm 0.30$  and  $136.95 \pm 0.26$  µmol TE.g<sup>-1</sup> according to DPPH was similar (p > 0.05) between the WS and PE fractions, respectively. For the TEAC assay, the highest (p < 0.05) antioxidant capacity was observed in the PE fraction (781.09  $\pm$  4.32 µmol TE.g<sup>-1</sup>). On the other hand, the PU fraction showed the highest ORAC<sub>HE</sub> values  $(2.12 \pm 0.13 \,\mu\text{mol TE.g}^{-1})$ , while the SE showed the lowest ORAC<sub>HE</sub> values ( $0.50 \pm 0.03 \text{ }\mu\text{mol TE.g}^{-1}$ ). The values for TPC and TFC of the PU fraction were above those found by Araújo et al. [27], who reported values of 9.06  $\pm$  0.42 mg GAE.g<sup>-1</sup> and 1.25  $\pm$ 0.12 mg  $CE.g^{-1}$ , respectively, for the edible fraction of *Eugenia stipitata*, a Brazilian fruit native to the Northeast region. According to the authors, this may be due to environmental variables, since the buritirana comes from the North region of Brazil. In another study on the Eugenia pyriformis (another Brazilian native fruit), the authors found a TEAC value (83.39  $\pm$  $0.79 \text{ }\mu\text{mol TE.g}^{-1}$ ) about 5.4 times lower and 11.1 times higher than those found here for the PU and SE fractions, respectively [37]. Finally, the results found for the antioxidant capacity showed that the ORAC<sub>HF</sub> values for the peel ( $2.66 \pm 0.34 \text{ }\mu\text{mol} \text{ TE.g}^{-1}$ ), pulp ( $4.14 \pm 0.18$  $\mu$ mol TE.g<sup>-1</sup>) and seed (3.16  $\pm$  0.03  $\mu$ mol TE.g<sup>-1</sup>) of the fruta-do-lobo (*Solanum lycocarpum*) St. Hill) were approximately 1.7, 1.9 and 6.3 times higher, respectively, than those we found for the same fractions evaluated [33].

# 3.6. Volatile Organic Compounds and Antibacterial Activity

According to the conditions previously described, no VOCs were identified in the buritirana pulp, and the fractions tested did not show activity against the micro-organisms at any concentration evaluated. There are no reports in the literature that define the aroma and flavor of buritirana, not even among fruits of the same family, to compare their sensory characteristics. The buritirana in nature is not a fruit that has a pronounced aroma like other Brazilian fruits, but when prepared for different products, these sensory characteristics are remarkable. Therefore, according to [49], it can be said that buritirana has its own aroma and flavor. The storage time and temperature most likely have negatively influenced the chemical composition of the fractions, affecting the properties of bioactive compounds that are related to antioxidant and antibacterial activity, among others. Although freezing is widely used to

prolong shelf life, this conservation technique can affect the chemical composition and quality of the fractions [9,50,51].

## 4. Conclusions

The WS and PU fractions showed physicochemical characteristics that indicate that the buritirana has great potential in fruit technology for new products with high added value.

The fractions of the buritirana fruit showed nutritional value since they contained proteins, carbohydrates, vitamin C, fibers (soluble and insoluble) and minerals (Fe, Zn, Ca, Mg, K, Mn and Cu). Therefore, buritirana could be an important source of nutrients, which could contribute to the economy of the local population, as well as to the sustainability of Brazilian fruits since they are widely utilized in the agroindustry. Although the buritirana seed is not an edible fraction, it contains interesting oligosaccharides (1-kestose and nystose) and a high sucrose content, which could be used as valuable ingredients in the food, cosmetic and pharmaceutical industries.

Higher levels of epicatechin/catechin and quinic and protocatechuic acids were found in all fractions of the ten identified phenolic compounds. The results of antioxidant assays associated with the highest concentrations of quinic acid and rutin showed that the WS and PU fractions had the best antioxidant potential, respectively. Thus, these results suggest that these fractions can be further studied in biological assays to demonstrate their functional potential.

Under the conditions described above, no VOCs were identified in the buritirana pulp, and none of the fractions showed inhibition potential against the investigated bacteria. Our study demonstrates the nutritional and functional potential of buritirana and can contribute to future investigations, exploration, propagation and commercialization of this species.

# **Author Contributions**

Conceptualization, F.G.d.S. and F.F.d.A.; Data curation, F.G.d.S., F.F.d.A., E.A.O., F.M.R. and D.W.H.C.; Formal analysis, F.G.d.S., F.F.d.A., E.A.O., F.M.R. and D.W.H.C.; Funding acquisition, J.A.L.P., A.C.H.F.S. and G.M.P.; Investigation, F.G.d.S., F.F.d.A., E.A.O. and A.C.H.F.S.; Methodology, F.G.d.S., F.F.d.A., E.A.O., F.M.R. and

D.W.H.C.; Project administration, G.M.P.; Resources, J.A.L.P., I.A.N.-N., A.C.H.F.S. and G.M.P.; Software, F.M.R. and D.W.H.C.; Supervision, I.A.N.-N. and G.M.P.; Visualization, F.G.d.S., F.F.d.A., I.A.N.-N. and G.M.P.; Writing—original draft, F.G.d.S., F.F.d.A. and I.A.N.-N.; Writing—review and editing, F.G.d.S., F.F.d.A., J.A.L.P., I.A.N.-N., A.C.H.F.S. and G.M.P. All authors have read and agreed to the published version of the manuscript.

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# **5. CHAPTER III**

#### **RESEARCH ARTICLE**

# Evaluation of antioxidant capacity, fatty acid profile, and bioactive compounds from buritirana (*Mauritiella armata* Mart.) oil: A little-explored native Brazilian fruit

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# Highlights

The buritirana fractions showed oil extraction yields from 7.49 to 18.06% Oleic acid was found in greater concentration in the pulp at 40 and 60 °C Peel 40 and 60 °C showed the highest antioxidant potential and  $\alpha$ -tocopherol content 8 flavonoids and 7 phenolic acids were found in the whole without seed fraction Whole without seed at 40 °C showed greater relative intensity of phenolic compounds

# Abstract:

Buritirana (Mauritiella armata Mart.) is a fruit species native to the Amazon and Cerrado region, belonging to the Arecaceae family. It has high nutritional and functional potential, yet little explored. In this study, we evaluated for the first time the overall yield, behavior of total carotenoids in the extraction kinetics, fatty acid profile, bioactive compounds, and the antioxidant capacity of the oil from buritirana fractions obtained by supercritical CO<sub>2</sub>. The highest extraction yield was found in the pulp and whole without seed at 60 °C (18.06  $\pm$  0.40 and  $14.55 \pm 1.10$  g 100 g<sup>-1</sup> of the freeze-dried sample (fdw), respectively), and in the peel at 40 °C (8.31  $\pm$  0.73 g 100 g<sup>-1</sup> fdw). During the extraction kinetics, the pulp had the highest yields of oil (41.57%) and total carotenoids (8.34 mg g<sup>-1</sup>) after 61 minutes at 40 °C. The antioxidant potential, fatty acid profile, and  $\alpha$ -tocopherol content were dependent on both fraction and temperature, with oleic acid being the main fatty acid. The oil from the whole fraction without seed had the largest number (20) of identified phenolic compounds. The extraction at 60 °C reduced the relative intensity of most compounds in the whole without seed and pulp. Moreover, it increased the intensity of the compounds in the peel. These results suggest that buritirana is a good oil source with great bioactive potential to produce new products with functional claims.



# Graphycal abstract

**Keywords:** Brazilian fruit; Supercritical fluid extraction; Oil yield; Chemical composition; Phenolic compounds; Antioxidant potential

# 1. Introduction

Brazilian fruits have been of great interest to the scientific community as they are considered good sources of bioactive compounds and can contribute to health maintenance (Araújo et al., 2019; Souza et al., 2020). Buritirana (*Mauritiella armata* Mart.) is a fruit species, also known as *buriti mirim*, *buriti bravo*, and *yumuna aguajillo*. It belongs to the Arecaceae family and is widely distributed throughout the Cerrado and Amazon region of Brazil. However, it is not commercially explored (Anunciação et al., 2019). This species has fruits ranging from globose to oblong. They have a fleshy and fibrous pulp, with a very thin endocarp that involves a hard seed. The peel is light orange and has overlapping scales. Buritirana is attractive and nutritious. It is consumed *in natura* or used in beverages, sweets, wines, and others. (Anunciação et al., 2019; Souza et al., 2020). Research suggests that the species is rich in lipids and bioactive compounds, such as carotenoids and phenolic compounds. Therefore, it has a great antioxidant capacity and can play an important role in the prevention of diseases, such as obesity, diabetes, cancer, among others (Anunciação et al., 2019; Farias, Neri-Numa, Araújo, & Pastore, 2020; Souza et al., 2020).

Components with biological interest and essential oils are extracted from fruits like buritirana using hydrodistillation, steam distillation, organic solvents, and others. The extracted substances exhibit various biological and technological properties. They can be widely used in food, pharmaceutical, and cosmetic products. (Mouahid, Dufour, & Badens, 2017; Roselló-Soto et al., 2019). However, some disadvantages of these techniques are low extraction yields, high energy costs, and loss or degradation of heat-sensitive compounds (Mouahid et al., 2017).

As an alternative to conventional extraction methods, the supercritical fluid extraction is a continuous, emergent, environmentally safe technique. It uses non-toxic, non-flammable solvents, such as  $CO_2$ . Therefore, the final product does contain solvent traces (Asl, Niazmand, & Yahyavi, 2020). This technique is widely applied in the extraction of thermolabile compounds from complex solid or liquid matrices. Due to its low viscosity and higher diffusion coefficient, the supercritical  $CO_2$  technique rapidly penetrates the matrix, allowing greater extraction and maintenance of bioactive compounds (Singh et al., 2015; Saini & Keum, 2018). However, factors such as temperature, pressure, size and density of particles, extraction time, auxiliary solvents, and matrix complexity can influence chemical characteristics, yield, and antioxidant potential of the oils extracted by supercritical  $CO_2$ 

(Fachri, Sari, Yuwanti, & Subroto, 2020; Filho et al., 2008; Guedes et al., 2020; Narváez-Cuenca et al., 2020).

Few studies have investigated the buritirana composition (Anunciação et al., 2019; Ruiz & Villena, 2011). Moreover, there are no reports in the literature describing a method to extract and characterize the buritirana oil. Thus, studying the potential and characteristics of the oil from this species will be important not only for science and technology, but also for the food, cosmetic, and pharmaceutical industries. The objective of this research is to determine the oil yield in different buritirana fractions and verify the behavior of total carotenoids in the extraction kinetics in order to evaluate the antioxidant capacity and the profile of bioactive compounds using the supercritical  $CO_2$  extraction.

## 2. Materials and methods

#### 2.1. Plant material and sample preparation

The buritirana fruit was harvested in November 2018 in the Moreira farm (302 m 10.3311 S; 48.4350 W 278 m), located in the city of Porto Nacional-TO, Brazil. A voucher specimen (UEC 203433- **Anexx 1**) has been deposited in the Herbarium of the Institute of Biology at the University of Campinas, Brazil (Herbarium UEC). The fruits were cleaned, manually separated into pulp (PU), peel (PE), whole without seed (WS), and seed (SE), and immediately frozen. The fractions were lyophilized (LIOTOP, model L101, São Carlos, Brazil), grounded with a knife mill (Marconi, model MA340, Piracicaba, Brazil), and classified according to size using a vibratory sieve system (model 1868, Bertel, Caieiras SP, Brazil) with 16 to 80 mesh sieves (Tyler, Tyler, Mentor, OH, USA series). The ground material was added to dark vials and stored at -20 °C until further analysis. The ASAE S319.3 method was used to calculate the average diameter ( $d_{mg}$ ) according to Equation 1.

Equation 1: 
$$d_{mg} = \log^{-1} \left[ \frac{\sum_{i=1}^{n} w_{i \log \overline{d_i}}}{\sum_{i=1}^{n} w_i} \right]$$

Where  $d_i = (d_i d_{i+1})^{0.5}$  is the geometric mean between the diameter of sieve i and i + 1, and  $w_i$  is the mass of solid retained by sieve i. The mean particle diameter for each fraction was PU 2.107 ± 0.00 mm, PE 1.513 ± 0.01 mm, WS 1.960 ± 0.03 mm, and SE 0.901 ± 0.06 mm. The particle density ( $d_P$ ) measured by the helium gas pycnometer (Model 1305

Multivolume, Micromeritics Instrument Corporation, Norcross, GA, USA) was PU 1.13  $\pm$  0.01 g cm<sup>-3</sup>; PE 1.02  $\pm$  0.01 g cm<sup>-3</sup>; WS 1.12  $\pm$  0.01 g cm<sup>-3</sup>, and SE 1.30  $\pm$  0.01 g cm<sup>-3</sup>. The bulk density (d<sub>b</sub>) of the particles was calculated using the extractor volume and the mass of PU, PE, WS, and SE. The results were 1.060 g cm<sup>-3</sup>, 1.036 g cm<sup>-3</sup>, 1.254 g cm<sup>-3</sup>, 1.055 g cm<sup>-3</sup>, respectively. The porosity of the particle bed was calculated using the bulk density and the particle density, as  $\epsilon = 1 - (d_b/d_P)$ , where  $\epsilon$  is the bed porosity and d<sub>P</sub> is the true density of the sample. The results were 0.062 (PU), 0.016 (PE), 0.120 (WS), and 0.062 (SE), respectively.

## 2.2. Supercritical CO<sub>2</sub> extraction

The extraction process was performed in a commercial laboratory-scale supercritical fluid extraction unit (Speed 7071, Applied Separations, Allentown, USA). For each assay,  $5.4 \pm 0.5$  g of raw material was placed in a 5.0 mL stainless-steel extraction column (height: 66.8 mm; internal diameter: 20.0 mm, and external diameter: 31.7 mm). The system was then heated and pressurized under the desired conditions and maintained at a static period for 5 min. The solvent flow rate was set to 5.0 g min<sup>-1</sup>. The solvent (S) to feed (F) ratio was kept constant at S/F = 15. The effects of the buritirana fractions (PU, PE, WS, and SE) and temperature (40 and 60 °C) at a constant pressure of 30 MPa were studied. The total extraction yields were calculated as the ratio between the extracted mass and the amount of raw material used in the freeze-dried sample. The recovered extract was stored at -20 °C in the absence of light for further analyses.

#### 2.3. Extraction kinetics

The kinetic experiments were performed under the optimal extraction conditions to maximize the oil extraction yield. The best results for the extraction yield were obtained in the PU at 40 °C. This fraction was used in the kinetic test. 21.0 g of the raw material was placed in a 300 mL stainless-steel extraction vessel, and the  $CO_2$  flow rate was held constant at 17.0 g.min<sup>-1</sup>. The extraction process was performed in the same commercial SFE instrument described in Section 2.2. A 210-minute extraction was used to reach the diffusion-controlled period. The kinetic experiments were repeated twice.

## 2.4. Antioxidant capacity

#### 2.4.1. Trolox equivalent antioxidant capacity (TEAC) assay

The antioxidant capacity obtained by the TEAC assay was performed according to the methodology described by Re et al. (1999). After the ABTS radical formation, the samples were diluted in distilled water until obtaining an absorbance value of  $0.700 \pm 0.02$  at 734 nm. The absorbances were read in a microplate reader (SpectrostarNano, BMG Labtech, Germany). The results were expressed as µmol Trolox equivalent (TE) g<sup>-1</sup> of the freeze-dried sample (fdw).

#### 2.4.2. Oxygen radical absorbance capacity- ORAC assays

The antioxidant capacity of hydrophilic ( $ORAC_{HF}$ ) and lipophilic ( $ORAC_{LF}$ ) ORAC assays were performed according to the methods described by Prior et al. (2003). The reactions were carried out in polystyrene microplates with 96 specific wells for fluorescence and evaluated using a NovoStar microplate reader (New Brunswick Scientific Classic Series, model C76, NJ, USA) with fluorescence filters (excitation wavelengths and emission of 485 and 520 nm, respectively).

For the hydrophilic assay, the samples, standards, and reagents were prepared in a 75 mM potassium phosphate buffer at pH 7.4. To each well of the microplate, 20  $\mu$ L of the sample, standard blank or Trolox, 120  $\mu$ L of fluorescein, and 60  $\mu$ L AAPH [2,2'-azobis (2'-methylpropionamidine) dihydrochloride] were added. The fluorescence behavior was monitored at 37 °C for 80 min immediately after adding AAPH.

For the lipophilic assay, the samples were prepared in a solution of acetone:water (1:1), containing 7.0% methylated cyclodextrin (RMCD). Then, they were stirred and ultrasonified for 30 minutes. AAPH and fluorescein were prepared in a 75 mM potassium phosphate buffer at pH 7.4. The reaction system consisted of adding 20  $\mu$ L of the sample or Trolox standard, 120  $\mu$ L of fluorescein, and 60  $\mu$ L of AAPH solution, resulting in a final volume of 200  $\mu$ L. The fluorescence decay was measured every 1 min for 80 min at 37 °C. The total ORAC (ORAC<sub>T</sub>) was obtained by summing the results of the (ORAC<sub>HF</sub>) and (ORAC<sub>LF</sub>). The results were expressed as  $\mu$ mol TE.g<sup>-1</sup> fdw.

#### 2.5. Chemical composition of the oil

#### 2.5.1. Total carotenoid content

The carotenoid content was determined according to the method described by França et al. (1999), with some modifications. 10 mg of the samples were diluted in 1 mL of the acetone-hexane solution (4:6, v/v) and homogenized. Then, a 250  $\mu$ L aliquot was transferred to a microplate well. The absorbance was read using a microplate reader at 450 nm. The results were expressed as mg of  $\beta$ -carotene.g<sup>-1</sup> fdw.

#### 2.5.2. Determination of the fatty acid profile by gas chromatography (GC-FID)

The oil from the buritirana fractions was methylated through saponification and esterification of the samples according to the method described by Hartman & Lago (1973), with adaptations based on the Ce 1b-89 method (AOCS, 2003). 2.0 mL of methanolic sodium hydroxide 0.5 N was added to 0.5 g of the sample. The mixture was heated at 100 °C for 5 minutes. After cooling, 2.5 mL of a solution of ammonium chloride and sulfuric acid in methanol (2:3:60 w/v/v, prepared under reflux) was added to the reaction and incubated at 100 °C for 5 minutes. Then, 5.0 mL of hexane and 5.0 mL of a saturated saline solution were added and waited 10 minutes until phase separation. The methyl esters were collected and 5.0 mL of Mili-Q water and 0.05 g of anhydrous sodium sulfate were added. This procedure was repeated.

The fatty acid profile was determined in a gas chromatograph (Shimadzu, Series 2010 Plus), equipped with a Restek-Wax column (30 m x 0.32 mm x 0.25  $\mu$ m), coupled to a flame ionization detector (FID). The temperature programming started at 60 °C and reached 130 °C at a rate of 20 °C min<sup>-1</sup>, remaining at that temperature for 7 minutes. Then, the programming reached 240 °C at a rate of 30 °C min<sup>-1</sup> and remained under this condition for 18 minutes. The temperatures of the injector and the detector were 250 °C. Hydrogen was used as carrier gas with a linear velocity of 21.0 cm s<sup>-1</sup>. The injection volume was 1.0  $\mu$ L in a split mode of 1/10. The identification was made by comparing the mix of fatty acid methyl ester standards (F.A.M.E C8-C22/Sigma-Aldrich).

#### 2.5.3. Determination of tocopherols

The analysis of tocopherols in oil samples was performed according to the method described by Hashim, Koehler, Eitenmiller & Kvien (1993) in high-performance liquid chromatography (Shimadzu, LC-20AD) coupled to a fluorescence detector (RF-20A). The mobile phase was composed of hexane/isopropanol (99/1; v/v) with a flow rate of 1.0 mL min<sup>-1</sup>. The hexane-diluted samples were filtered in 0.20  $\mu$ m PTFE filter units and injected (20  $\mu$ L) onto a normal phase silica column (LiChrospher Si-60 250 x 4.6 mm x 5  $\mu$ m, Merck). In the fluorescence detector, the emission and excitation wavelengths were 330 and 290 nm, respectively. The  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol isomers were investigated in the samples based on pure analytical standards (Sigma-Aldrich).

#### 2.6.4. Compound profile by ESI-LTQ-XL-MS/MS

The samples for injection were prepared by diluting 10  $\mu$ L of the extract in 990  $\mu$ L of methanol. After homogenization, a 20  $\mu$ L aliquot of the previous solution was diluted in 480  $\mu$ L of methanol. Positive ionization was achieved by adding formic acid 2%. The samples were injected directly into an ESI-LTQ-XL-MS/MS mass spectrometer (Thermo Scientific, Bremen, Germany) for fingerprint analysis in a mass range of 115-1000 m/z. The selection of molecules was carried out using online databases, such as METLIN (Scripps Center for Metabolomics, La Jolla, CA) and Lipid MAPS (University of California, San Diego, CA - www.lipidmaps.org). To confirm the proposed structures, tandem mass spectrometry (MS/MS) experiments and fragmentation modeling of the Mass Frontier software (v. 6.0, Thermo Scientific, San Jose, CA) were used.

To compare the relative abundance of selected ions in the oils subjected to process variations, a heatmap analysis was performed with the identified molecules using the MetaboanalystR software version 4.0 (Chong, Wishart, & Xia, 2019).

## 2.7. Statistical Analysis

All analyses were performed in triplicate and the results were expressed as average  $\pm$  standard deviation. The data were analyzed by ANOVA. To verify the difference between the means, the Tukey test was used with a significance level of 5%. Multivariate analysis techniques were used, such as the Principal Component Analysis (PCA) to analyze

which treatments are related by interrelated response variables so that the variables are grouped according to their similarity. PCA was performed on standardized data to avoid the effect of different magnitude levels of response variables. Data standardization of each response variable was carried out by subtracting each value by its mean divided by its standard error. All statistical analyses were performed using the R 3.2.4 software and the FactorMineR 1.32 exploratory multivariate data analysis package (TEAM, 2016).

## 3. Results and Discussion

## 3.1. Total extraction yields

The total oil yields obtained from the buritirana fractions using supercritical CO<sub>2</sub> extraction are in **Figure 1**. The results obtained at 40 and 60 °C ranged from 7.49  $\pm$  0.36 to 18.06  $\pm$  0.40 g 100 g<sup>-1</sup> fdw. As observed, there was a statistical difference (p <0.05) between all fractions evaluated. The highest extraction yield was obtained by the PU at both temperatures. However, there was no difference between the temperatures at 40 and 60 °C for this fraction. The WS had the second-highest global yield (14.55  $\pm$  0.78 g 100 g<sup>-1</sup> fdw at 60 °C), followed by the PE (8.31  $\pm$  0.52 g 100 g<sup>-1</sup> fdw at 40 °C). However, no statistical differences were observed between the extraction temperatures for the same fraction. For the SE, a small amount of oil was obtained at both temperatures (222.75 and 78.52 folds lower than the PU yield at 40 and 60 °C, respectively).

Few studies have reported the oil yield in different buritirana fractions. However, Ribeiro et al. (2018) showed a 26.0% oil yield obtained from the buritirana pulp using the Soxhlet extraction. When studying fruits belonging to the same family as buritirana, these authors reported a yield of 15.0%, 43.9%, 23.2%, and 33.1% for the açaí pulp (*Euterpe oleracea*), bacaba (*Oenocarpus bacaba*), buriti (*Mauritia flexuosa*), and tucumanzinho (*Astrocaryum acaule*), respectively (Ribeiro et al., 2018). For the bacaba-de-leque (*Oenocarpus distichus* Mart.), the lowest yields were obtained (17.5%) at 150 °C and 150 bar using the supercritical CO<sub>2</sub> extraction, while the highest yields (45.9%) were obtained at 60 °C and 270 bar (Cunha et al., 2019). A 45.4% oil yield was found in the pulp of freeze-dried açaí extracted by supercritical CO<sub>2</sub> at 70 °C and 490 bar (Batista et al., 2016).



**Figure 1.** Total yields of the oils obtained from buritirana fractions by supercritical  $CO_2$  extraction. SE: seed; PE: peel; WS: whole without seed; PU: pulp. Different lowercase letters indicate a significant difference by Tukey test (p <0.05).

According to Filho et al. (2008), the increase in the extraction yield may be due to high pressures and temperatures. However, as the temperature increases under constant pressure conditions, the solubility of the solute decreases, and the extraction yield is reduced. This behavior can be related to changes caused by the effect of temperature, pressure, and density of the solvent on vapor pressures of the solute.

#### 3.2. Extraction kinetic

Based on the previous results, the extraction kinetics and the behavior of the total carotenoids were evaluated only in the PU fraction at 40 °C (**Figure 2**) due to the highest extraction yield obtained (see **Figure 1**). After 6 min of extraction, it was observed that the concentration of carotenoids increased over time. The maximum extraction rate was 41.57 g 100 g<sup>-1</sup> after 61 minutes and had total carotenoid content of 8.34 mg g<sup>-1</sup> fdw. After this period, it was observed that the oil extraction rate remained constant and the carotenoids stabilized after 80 minutes, with a concentration of 8.86 mg g<sup>-1</sup> fdw. Similarly, Huang et al. (2020) observed that the *Physalis alkekengi* yield using the supercritical CO<sub>2</sub> extraction increased considerably after 60 minutes of extraction (10.54  $\pm$  0.36%), where the highest carotenoid yield (15.96 mg g<sup>-1</sup>) was recovered after 200 minutes at 50 °C and 40 Mpa. In another study using the oil obtained from carrot peels, 97% of the total carotenoid content was

recovered after only 30 minutes of extraction at 59 °C, 349 bar, and 15.5% of ethanol (Lima, Charalampopoulos, & Chatzifragkou, 2018).

According to França et al. (1999), the carotenoid concentration increases as the  $CO_2$  increases during the extraction process. Initially, the lipids present on the solid surface or in the vicinity of the matrix are rapidly dissolved in  $CO_2$ . When the draining starts, less dissolved compounds in the oil are easily extracted, resulting in a product with a higher concentration of this compound.



**Figure 2.** Overall extraction curve and behavior of total carotenoids in buritirana pulp oil by supercritical  $CO_2$  extraction.  $\blacklozenge$  Extraction kinetic;  $\blacksquare$  Total carotenoids.

# 3.3. Antioxidant capacity

**Table 1** shows the antioxidant capacity obtained by the TEAC and ORAC assays in oils from buritirana fractions using the supercritical CO<sub>2</sub> extraction at 40 and 60 °C. The SE did not present enough amount of oil to be evaluated in these assays. As shown in **Table 1**, the oil with the highest antioxidant capacity for the TEAC, ORAC<sub>LF</sub>, ORAC<sub>HF</sub>, and ORAC<sub>T</sub> assays was extracted from the PE at both temperatures, except for the TEAC of this fraction at 60 °C, which presented  $1.415 \pm 0.09 \,\mu$ mol of TE g<sup>-1</sup> fdw, differing statistically (p <0.05) from the PE at 40 °C. The WS showed no statistical difference at 40 and 60 °C in all assays evaluated. The lowest antioxidant capacity was verified for the TEAC in the PU at 40  $^{\circ}$ C (which was 1.76 folds lower than the PE at 40  $^{\circ}$ C). This fraction did not present antioxidant capacity for the ORAC<sub>HF</sub>.

The antioxidant capacity in oils extracted by supercritical CO<sub>2</sub> has been reported in several other species. According to Luan et al. (2020), the increase in the antioxidant capacity using this method can be attributed to the greater extraction of chemical constituents, such as sterols, tocopherols, and phenolic compounds. In a study by Cunha et al. (2012), it was found that the buriti oil extracted by CO<sub>2</sub> at 60 °C and 20 MPa resulted in a 2.0-fold increase in the antioxidant potential by the ORAC test, while a 6.5-fold increase was obtained in the oil extracted at 60 °C and 25 MPa. The highest antioxidant potential of the bacaba-deleque oil was obtained at 60 °C and 420 bar (238.11  $\mu$ M.TE g<sup>-1</sup>), while the lowest potential was found in the oil extracted at 50 °C and 150 bar (37.56  $\mu$ M.TE g<sup>-1</sup>) (Cunha et al., 2019).

## 3.4. Chemical composition of the oil

The content of fatty acids and  $\alpha$ -tocopherol present in the oil from the buritirana fractions are in **Table 1**. This oil proved to be a good source of saturated (palmitic and stearic) and unsaturated (oleic, linoleic, and linolenic) fatty acids. Regarding the saturated fatty acids, it was observed that there was a statistical difference between the extraction temperatures and the fractions evaluated, with the highest content of palmitic acid found in the PE at 60 °C (22.70  $\pm$  0.234 % fdw) and stearic acid in the PE at 40 °C (2.30  $\pm$  0.037 % fdw). Among the unsaturated fatty acids, oleic was found in the highest concentration in the PU at 40 and 60 °C (69.90  $\pm$  0.266 and 70.00  $\pm$  0.180 % fdw, respectively), followed by the WS for both temperatures, while the lowest concentration was found in the PE at 60 °C (65.10  $\pm$  0.283 % fdw). Linoleic acid showed a statistical difference for all fractions and extraction temperatures, except for the PE at 40 and 60 °C, which had the highest levels and did not differ statistically from each other. Similarly, linolenic acid also had the highest concentration in the oil from this fraction at both temperatures and the lowest content in the PU at 40 and 60 °C (2.30 % fdw).

**Table 1**. Antioxidant capacity, fatty acids profile and  $\alpha$ -tocopherol content in the oils obtained from buritirana fractions by supercritical CO<sub>2</sub> extraction.

		Temperature 40 °C		Temperature 60 °C				
	PU	PE	WS	PU	PE	WS		
Antioxidant capacity								
TEAC ( $\mu$ mol TE g <sup>-1</sup> )	$1.029 \pm 0.10904 d$	$1.813 \pm 0.07611$ a	$1.289 \pm 0.08654$ bc	$1.118 \pm 0.07110 \text{ cd}$	$1.415 \pm 0.08738 \text{ b}$	$1.426 \pm 0.01752 \text{ b}$		
$ORAC_{LF} (\mu mol TE g^{-1})$	$16.245 \pm 1.00301 \text{ b}$	22.069 ± 1.35971 a	$17.527 \pm 1.50153$ b	$12.231 \pm 0.35742$ c	$24.296 \pm 0.84702$ a	$17.439 \pm 1.39966 \text{ b}$		
$ORAC_{HF}$ (µmol TE g <sup>-</sup>	n.m.	$5.055 \pm 0.00893$ a	$1.207 \pm 0.74468 \text{ b}$	n.m.	$5.143 \pm 0.26224$ a	$1.808 \pm 0.74223 \; b$		
) ORAC <sub>T</sub> ( $\mu$ mol TE g <sup>-1</sup> )	$16.245 \pm 1.00301 \text{ b}$	$27.124 \pm 1.35078$ a	$18.734 \pm 0.75684 \ b$	$12.231 \pm 0.35742$ c	$29.440 \pm 0.58478$ a	$19.247 \pm 2.14188 \text{ b}$		
Fatty acids (%)								
Palmitic acid	$21.40 \pm 0.240 \text{ c}$	$22.10\pm0.141\ b$	$22.00\pm0.040\ b$	$21.40 \pm 0.171$ c	$22.70 \pm 0.234$ a	$22.10\pm0.080\text{ b}$		
Estearic acid	$2.10\pm0.010~d$	2.30± 0.037 a	$2.10\pm0.003~\text{d}$	$2.10\pm0.018~d$	$2.20\pm0.034~b$	$2.10 \pm 0.028$ c		
Oleic acid	$69.90 \pm 0.266$ a	$65.60 \pm 0.179$ c	$68.10 \pm 0.114 \ b$	$70.00 \pm 0.180$ a	$65.10 \pm 0.283 \text{ d}$	$68.10\pm0.031~\text{b}$		
Linoleic acid	$4.20 \pm 0.039 \text{ e}$	$6.80 \pm 0.039$ a	$5.10\pm0.054\ c$	$4.20 \pm 0.007 \; f$	$6.80\pm0.027~b$	$5.10\pm0.019\;d$		
Linolenic acid	$2.30\pm0.008~d$	$3.10 \pm 0.00037$ a	$2.70\pm0.024~b$	$2.30\pm0.022~d$	$3.20 \pm 0.011$ a	$2.60\pm0.004\;c$		
Tocopherol								
$\alpha$ -tocopherol (mg g <sup>-1</sup> )	$0.043 \pm 0.00218 \text{ c}$	$0.203 \pm 0.01589$ ab	$0.126 \pm 0.11359 \text{ bc}$	$0.072 \pm 0.00590 \text{ c}$	$0.250 \pm 0.01013$ a	$0.093 \pm 0.04555 \ c$		

n.m.- not mensured; The same letter in line not differ statistically. Results expressed in fdw (freeze-dried sample). PU- pulp; PE- Peel; WS- whole without seed

According to Gustinelli et al. (2018), oils are complex mixtures of fatty acids and glycerol, with triglycerides being one of its main constituents. Although the chemical composition of fatty acids present in the oil from the buritirana fractions has not been reported, several other fruits belonging to the Arecaceae family have been studied. When studying the bacaba-de-leque oil, Cunha et al. (2019) observed that there was no difference in the composition of fatty acids in the oil extracted by supercritical  $CO_2$  at temperatures of 50 and 60 °C and pressures of 150 to 420 bar. The authors also observed that oleic acid was the main fatty acid present in the oil, followed by palmitic and linoleic acids. In another study, the total fatty acid content in açaí extracts obtained by supercritical  $CO_2$  ranged from 0.02 to 65.81%. Moreover, the main saturated fatty acid in all extraction conditions (at 50, 60, and 70 °C and pressures ranging from 150 to 490 bar) was palmitic acid, followed by stearic acid.

For polyunsaturated fatty acids, oleic acid had the highest concentration (65.81%), followed by linoleic (15.54%) and palmitoleic acids (7.08%) (Batista et al., 2016). In the buriti oil, the main fatty acids found in the oil extracted at 60 °C and 15 to 25 Mpa were oleic acid (77.06%) and palmitic acid (15.99%). However, this oil presented concentrations of linoleic acid (1.59%), stearic acid (1.39%), and linolenic acid (1.12%) (Cunha et al., 2012).

The  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol isomers were investigated in the oils from buritirana fractions, where only  $\alpha$ -tocopherol was identified (**Table 1**). It was observed that the PE showed the highest content of  $\alpha$ -tocopherol (0.250 ± 0.01 and 0.203 ± 0.01 mg g<sup>-1</sup> at 60 and 40 °C, respectively). However, there was no statistical difference between them. On the other hand, the PU at 40 °C was 5.8 folds lower than the PE at 60 °C.

Gustinelli et al. (2018) observed that the tocopherol content in bilberry (*Vaccinium myrtillus* L.) was significantly influenced by both temperature and pressure used in the extraction process. The oil extracted at 60 °C and 20 MPa presented the highest content of this compound (129.2  $\pm$  5.0 mg 100 g<sup>-1</sup>), while the oil extracted at 40 °C and 50 MPa showed the lowest concentration (58.0  $\pm$  1.4 mg 100 g<sup>-1</sup>). The oil extracted from several other fruits of the buritirana family is also a good source of  $\alpha$ -tocopherol. According to Ribeiro et al. (2018), its content in the buritirana pulp oil was 22.7 and 1.06 folds higher than in the bacaba and buriti oil, respectively, and 6.6 folds lower than the açaí oil.

Among its isomers,  $\alpha$ -tocopherol is considered an excellent natural antioxidant in the control of oxidative degradations of polyunsaturated fatty acids (C18:2, C18:3), also being associated with the oxidation control of biomolecules and, consequently, combating nontransmissible chronic diseases (Das & Roychoudhury, 2014; Sahari, Moghimi, Hadian, Barzegar, & Mohammadi, 2017).
Regarding the identification of polar compounds present in the oil from different buritirana fractions (**Table 2**) using ESI-LTQ-XL-MS/MS, it was possible to verify that the highest number of compounds (20) was identified in the WS, being 5 organic acids, 7 phenolic acids, and 8 flavonoids. In addition, 5 organic acids, 6 phenolic acids, and 6 flavonoids were also found in the PU, while the PE had the lowest number of identified compounds (14), being 2 organic acids, 4 phenolic acids, and 8 flavonoids. In general, flavonoids were the most representative compounds in the oil from all fractions of this fruit. Molecules such as maleamic acid, cinnamic acid, vanillic acid, caffeic acid, caffeoylquinic acid, quercetin, 5-deoxyleucopelargonidin, catechin hexoside, kaempferol coumaroyl deoxyhexoside, catechin dihexoside, and myricetin were identified in the three samples.

To verify the possible effects of extraction temperatures on the compounds identified in the oils from the different buritirana fractions, a Heatmap analysis was performed, as shown in **Figure 3**. According to Araújo et al. (2021), the Heatmap analysis is carried out by grouping the compounds identified in the oil from the buritirana fractions, represented as a heatmap, and illustrated as a color-coded thermometer that indicates the relative concentration of the metabolites in each fraction. This analysis represents a graph consisted of colors, where the intensity of the color indicates the relative intensity of the color indicates the relative intensity of the color indicates the acquisition of a mass spectrum. In this study, 15 repetitions/acquisitions were made per sample class (each square in the columns represents the relative intensity of the ion in each spectrum).

PU	PE	WS	Mass	MS/MS fragments	Adduct	Molecule	Molecular formula
				Organic acids			
Х	Х	Х	116	70 - 88 - 98	$[M+H]^+$	Maleamic acid	$C_4H_5NO_3$
Х	-	Х	119	101 - 91 - 73	$[M+H]^+$	Succinic acid	$C_4H_6O_4$
Х	-	Х	123	81 - 95 - 67 - 57 - 105	$[M+H]^+$	Benzoic acid	$C_7H_6O_2$
Х	-	Х	139	121 - 111 - 122 - 95 - 94	$[M+Na]^+$	Fumaric acid	$C_4H_4O_4$
Х	Х	Х	149	117 - 121 - 131 - 107 [M+H] <sup>+</sup> Cinnamic acid C		$C_9H_8O_2$	
				Phenolic acids			
Х	Х	Х	169	133 - 123 - 89 - 109 - 107 - 119 - 81 - 95	$[M+H]^+$	Vanillic acid	$C_8H_8O_4$
Х	-	Х	171	89 - 153 - 139 - 143 - 135 - 125 – 111	$[M+H]^+$	Gallic acid	$C_7H_6O_5$
Х	Х	Х	181	163 - 149 - 135 - 153	$[M+H]^+$	Caffeic acid	$C_9H_8O_4$
Х	-	Х	193	175 - 161 - 165 - 147 - 137 - 133 - 120	$[M+H]^+$	Quinic acid	$C_7H_{12}O_6$
-	Х	Х	295	277 - 249 - 263 - 235 - 281	$[M+H-H_2O]^+$	Caffeoyl tartaric acid	$C_{13}H_{12}O_9$
Х	Х	Х	355	267 - 285 - 274 - 337 - 163	$[M+H]^+$	Caffeoylquinic acid	$C_{16}H_{18}O_9$
Х	-	Х	361	317 - 259 - 343 - 301 - 315 - 271 - 280	$[M+H]^+$	Glucosyringic acid	$C_{15}H_{20}O_{10}$
				Flavonoids			
Х	Х	Х	303	257 - 229 - 285 - 165 - 247	$[M+H]^+$	Quercetin	$C_{15}H_{10}O_7$
Х	Х	Х	313	257 - 109 - 95 - 123 - 239 - 137	$[M+K]^+$	5Deoxyleucopelargonidin	$C_{15}H_{14}O_5$
-	Х	Х	415	119 - 295 - 397 - 133 - 277	$[M+H-H_2O]^+$	Apigenin hexoside	$C_{21}H_{20}O_{10}$
Х	Х	Х	453	435 - 407 - 365 - 417 -347 - 305	$[M+Na]^+$	Catechin hexoside	$C_{21}H_{24}O_{11}$
Х	Х	Х	579	265 - 239 - 247 - 503	$[M+H]^+$	Kaempferol coumaroyl deoxyhexoside	$C_{30}H_{26}O_{12}$
-	Х	Х	595	577 - 339 - 313 - 539 - 535 - 357	$[M+H]^+$	Apigenin caffeoyl hexoside	$C_{30}H_{26}O_{13}$
Х	Х	Х	637	581 [525 – 469]*	$[M+Na]^+$	Catechin dihexoside	$C_{27}H_{34}O_{16}$
Х	Х	Х	957	507 - 911 - 419 - 611	$[M+K]^{+}$	Myricetin coumarylrobinobioside deoxyhexoside	$C_{42}H_{46}O_{23}$

Table 2. Compounds identified in the oils from the buritirana fractions obtained by supercritical CO<sub>2</sub> extraction.

\*MS; X: Identified; -: Not identified; PU- pulp; PE- Peel; WS- whole without seed.

It was found that the extraction using supercritical CO<sub>2</sub> at 40 °C reduced the relative intensity of all compounds in the PE, except for the kaempferol coumaroyl deoxyhexoside (class 579) (**Figure 3B**). The temperature at 60 °C reduced the relative intensity of all compounds in the WS (**Figure 3C**), while only 1 organic acid (cinnamic acid, class 149) and 4 phenolic acids (gallic acid, caffeic acid, quinic acid, and glucosyringic acid) showed an increase in the PU relative intensity (**Figure 3A**). When comparing the fractions with the highest number of compounds identified at 40 °C (**Figure 3D**), an increase in the relative intensity of succinic acid, kaempferol coumaroyl deoxyhexoside, and maleamic acid in the PU was observed, while the intensity reduced in all the other compounds. The WS fraction, in turn, had the greatest intensity in all compounds, except for those that were more intense in the PU. The increase in the relative intensity in some compounds can be attributed to a greater extraction or lower degradation during the extraction process using supercritical CO<sub>2</sub> at different temperatures. The degradation of the bioactive compounds extracted by this method is lower than other extraction techniques (Roselló-Soto et al., 2019).

According to Narváez-Cuenca et al. (2020), some thermodynamic parameters and supercritical conditions, such as critical temperature, critical pressure, and molar volume factor, are involved in the extraction process and can influence the solubility of phenolic compounds in oils extracted by supercritical  $CO_2$ .

Currently, this is the first study to identify phenolic compounds in buritirana fractions extracted by supercritical CO<sub>2</sub>. However, other authors have reported the presence of these compounds in the oil from other species. Roselló-Soto et al. (2019) determined the phenolic profile in oils from by-products of "horchata" (beverage extracted from junça tubercle - *Cyperus rotundus*) obtained by supercritical CO<sub>2</sub> at 40 °C and 30 MPa. They found 8 compounds, namely caffeic acid, p-coumaric acid, p-hydroxybenzoic acid, sinensetin, isohydroxymatairesinol, scopoletin, and homovanillyl alcohol. However, the number of compounds identified in the by-product was lower than in buritirana fractions under the same extraction conditions. The guava seed oil (*Psidium guava*) obtained by supercritical CO<sub>2</sub> at 52 °C and 35.7 MPa presented vanillic acid, 4-hydroxybenzaldehyde, vanillin, syringaldehyde, coniferylaldehyde, sinapaldehyde, and cinnamic acid (Narváez-Cuenca et al., 2020). In another study, Silva et al. (2019) evaluated the profile of phenolic compounds in the pomegranate seed oil and identified 5 phenolic acids (2,4-dihydroxyphenylacetic acid, ferrulic acid, p-hydroxybenzoic acid, trans-cinnamic acid, and vanillic acid), and 1 flavonoid (naringenin).



**Figure 3.** Heatmap of compounds obtained from oils of fractions of buritirana by supercritical CO<sub>2</sub> extraction. **A**: Pulp oil extracted at 40 °C vs pulp oil extracted at 60 °C; **B**: Peel oil extracted at 40 °C vs peel oil extracted at 60 °C; **C**: Whole fraction oil without seed at 40 °C vs whole fraction oil without seed at 60 °C; **D**: Pulp oil extracted at 40 °C vs whole fraction oil without seed at 40 °C.

Phenolic compounds are non-nutritive ingredients synthesized by the secondary metabolism of plants. They play an important role in human health and have several bioactive properties such as antimicrobial, anti-inflammatory, anti-tumor, anti-obesity, and antioxidant activities (Araújo et al., 2019; Farias et al., 2020). Thus, the presence of these compounds in the oils from buritirana fractions demonstrates the potential of this fruit in the food, pharmaceutical, and cosmetic industry.

The PCA (Figure 4) provided a simplified interpretation of the relationships between the response variables and the samples, facilitating their correlation and allowing the identification of which treatments are characterized by certain variables. In the PCA graphic representation, the variability of the samples is presented in orthogonal axes, named components. The first component, or main component (CP1), explains most of the variability between the samples, followed by the other components (Almeida et al., 1999). In this experiment, the sum of the first two components (CP1 and CP2) explained 97.1% of the variability, being 91.1% explained by the first component and 6.0% by the second component. The sum of the main components I and II ( $\geq$ 75%) accurately presented the variability between the samples (Abdi & Williams, 2010).



**Figure 4.** PCA of oil from the buritirana fractions obtained by supercritical CO<sub>2</sub> extraction. **a**) representation of the variables of antioxidant capacity and bioactive compounds; and **b**) representation of the buritirana fractions, where PU40 (Pulp oil at 40 °C); PU60 (Pulp oil at 60 °C); WS40 (Oil of the fraction whole without seed at 40 °C); WS60 (Oil of the fraction whole without seed at 60 °C); PE40 (Peel oil at 40 °C);  $\alpha$ - tocol ( $\alpha$ -tocopherol).

According to Figure 4 (b), the PU60 and PU40 treatments located in the upper left and lower left part of the graph, respectively, presented similar characteristics and are related to the highest amounts of oleic acid (C18:1), corroborating the data of Table 1. Oleic acid has also been reported as the main fatty acid present in other species belonging to the same family as buritirana (Souza et al., 2020). The WS60 and WS40 treatments (also located in the upper left and lower left part of the graph, respectively) presented intermediate values of C18:1 as compared to the other treatments. The PE40 and PE60 treatments, located in the upper right and lower right part, respectively, presented similar characteristics for some response variables evaluated and are related to the highest amounts of TEAC, ORAC<sub>LF</sub>, ORAC<sub>HF</sub>, ORAC<sub>T</sub>, C16, C18, C18:2, C18:3, and  $\alpha$ -tocopherol, as shown in Figure 4 (a) and Table 1. The results also indicate that the extraction temperatures differently influenced the response variables in each buritirana fraction.

#### 4. Conclusions

According to the conditions presented in this study, the PU fraction showed the highest extraction yield. Moreover, the maximum point to obtain the oil (41.57%) and carotenoids (8.34 mg g<sup>-1</sup>) was reached after 61 minutes at 40 °C. The antioxidant potential, fatty acid profile, and  $\alpha$ -tocopherol content were dependent on the fruit fraction and extraction temperature, with oleic acid being the main representative of unsaturated fatty acids in all buritirana fractions. The highest number of bioactive compounds (20) was found in the WS, including organic acids, phenolic acids, and flavonoids. The heatmap analysis demonstrated that the extraction at 60 °C reduced the relative intensity of most compounds in the PU and WS, while the relative intensity of the compounds in the PE increased at the same temperature. In turn, the WS showed the highest relative intensity of the compounds at 40 °C.

These results demonstrate that the oil from the buritirana fruit, which is yet little explored, has great bioactive potential and can be used in the production of new products with functional claims. In addition, this research can also encourage the cultivation and greater commercial use of this species in food, cosmetic, and pharmaceutical industries since the buritirana fruit is extractively exploited.

#### **Credit Author Statement**

**Florisvaldo Gama de Souza:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft, Writing review & editing. **Grazielle Náthia-Neves:** Investigation, Data curation, Formal analysis, Methodology, Software. Fábio Fernandes de Araújo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft, Writing - review & editing. Flávia Luísa Dias-Audibert: Data curation, Formal analysis, Methodology, Software. Jeany Delafiori: Data curation, Formal analysis, Methodology, Software. Iramaia Angélica Neri-Numa: Supervision, Visualization, Writing - original draft, Writing - review & editing. Rodrigo Ramos Catharino: Funding acquisition, Resources, Writing - review & editing. Severino Matias de Alencar: Investigation, Funding acquisition, Resources, Writing - review & editing. Maria Angela de Almeida Meireles: Funding acquisition, Resources, Writing - review & editing. Glaucia Maria Pastore: Project administration, Supervision, Funding acquisition, Resources, Writing - review & editing.

#### **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# 6. CHAPTER IV

#### **RESEARCH ARTICLE**

# Influence of extraction methods on the phytochemical, antioxidant and antibacterial properties of buritirana oil

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# Highlights

- $\checkmark$  The best rate of extraction of oil and total carotenoids occurred close to 60 min
- ✓ Higher antioxidant capacity and  $\alpha$ -tocopherol content were found for cold pressing
- $\checkmark$  Fatty acids concentration was similar for two extraction methods
- $\checkmark$  Phenolic compounds showed higher intensity in the oil obtained by supercritical CO<sub>2</sub>
- ✓ All-*E*-Lutein, all-*E*- $\alpha$ -carotene, all-*E*- $\beta$ -carotene were the majority in both methods

# Abstract:

In this study, we evaluated the influence of supercritical CO<sub>2</sub> extraction and cold pressing on the yield, bioactivity and phytochemical composition of buritirana oil. Supercritical CO<sub>2</sub> presented the highest extraction yield (13.44%). The highest yield and content of carotenoids occurred close to 60 minutes in the extraction kinetics. The highest antioxidant capacity by lipophilic and total ORAC assays (45.793 µmol TE.g<sup>-1</sup>) and highest  $\alpha$ -tocopherol content (0.41119 mg.g<sup>-1</sup>) were found in the oil extracted by pressing, while the extraction with supercritical CO<sub>2</sub> showed the highest antioxidant potential TEAC (1.385 µmol TE.g<sup>-1</sup>). Oleic and palmitic acids were the main fatty acids found in buritirana oil for both extraction methods. Supercritical CO<sub>2</sub> showed a higher relative intensity for phenolic compounds. All-*E*-lutein, all-*E*- $\alpha$ -carotene, and all-*E*- $\beta$ -carotene were the major carotenoids in the evaluated samples. Only the supercritical CO<sub>2</sub> oil revealed volatile compounds, and none showed antibacterial activity. These results could add value to buritirana oil.

**Keywords**: *Mauritiella armata*; Extraction methods; Carotenoids; Bioactive compounds; Antioxidant capacity; Antibacterial activity.

## **1. Introduction**

Buritirana or buriti mirim is a native Brazilian species found in humid environments of the Amazon basin and in the Northeast and Midwest regions of Brazil and other South American countries. Its fruit is oblong-rounded with an inedible light orange color peel and has a soft pulp that surrounds a very hard seed. This fruit is widely consumed and marketed *in natura*, and its pulp is used to prepare beverages, jellies, sweets, and wines, among others (Souza et al., 2020). The buritirana fruit is rich in fibers, minerals, vitamin C, fructooligosaccharides, bioactive compounds such as carotenoids,  $\alpha$ -tocopherol, and phenolics, in addition to having an expressive antioxidant capacity (Souza et al., 2022).

In addition, it has a high oil content rich in bioactive compounds and unsaturated fatty acids with great pharmacological and technological potential (for example, for the production of biodiesel) (Souza et al., 2020, 2021). The importance of bioactive compounds is related to their great antioxidant potential, which exerts an effective action in the prevention of several diseases, such as obesity, diabetes, cancer, burns, rheumatism, and xerophthalmia, among others (Farias, Neri-Numa, Araújo, & Pastore, 2020; Souza et al., 2020).

In recent years, there has been a growing concern about obtaining natural products in a safe, practical and efficient way, so that there are no changes in the chemical and biological properties of their constituents (Silva et al., 2019). In this sense, many edible vegetable oils are still extracted by conventional methods, such as mechanical pressing and solvent extraction. However, these techniques may have some disadvantages, such as the degradation of biological compounds due to high exposure to oxygen and light, in addition to having low extraction yields and being more aggressive to the environment (Gu et al., 2019; Molina, González-Fuentes, Loske, Fernández, & Estevez, 2020).

Supercritical extraction has been used as a possible alternative to conventional extraction methods due to its wide availability, nontoxicity, better yield, lack of solvent, and environmental safety (Rai, Mohanty, & Bhargava, 2015; Santos, Bitencourt, Santos, Vieira e Rosa, & Martínez, 2019). However, it can also show some disadvantages, such as not being suitable for matrices with high moisture content and polar compounds, having low processing capacity and high investment costs (Rai et al., 2015; Santos et al., 2019).

To date, a single work has been developed using supercritical  $CO_2$  to obtain buritirana oil and evaluate its chemical composition and antioxidant capacity (Souza et al., 2021). However, here, more comprehensive research was carried out where the oil was obtained by supercritical  $CO_2$  and cold pressing. Cold pressing emerged as an alternative for the extraction of buritirana oil to provide an additional option for segments that still cannot adhere to a more robust technology. In addition, it is intended to optimize the exploitation and commercialization of this species, making it available not only to the various industrial areas but also to family farming.

Therefore, the objective of this study was to obtain buritirana oil by two extraction methods and to evaluate the overall yield, extraction kinetics, phytochemical composition, antioxidant capacity, volatile organic compounds, and antibacterial activity.

# 2. Materials and methods

## Chemicals

Analytical grade reagents were purchased from Labsynth (Diadema, Brazil). HPLC grade methanol was obtained from J. T. Baker (Phillipsburg, NJ, USA), and methyl tert-butyl ether (MTBE) was obtained from TEDIA (Fairfield, OH, USA). Standards of all-*E*lutein (89% purity), all-*E*- $\beta$ -carotene (87% purity), and all-*E*- $\alpha$ -carotene (98% purity) were obtained from freeze-dried marigold flowers (*Tagetes erecta*), pumpkin, and carrot and purified by open column chromatography (MgO (Merck, Germany): Hyflosupercel (1:1), 20 cm). The samples and solvents were filtered through Millipore 0.22 and 0.45 µm membranes, respectively.

# 2.1. Obtaining and preparing the vegetable sample for oil extraction

The buritirana fruits were collected in November 2018 at Moreira farm (10°33'11" S; 48°43'50" W), located near the city of Pugmil-TO, Brazil. A voucher specimen (UEC 203433) was deposited in the Herbarium of the Institute of Biology of the State University of Campinas, Brazil. The buritirana seeds were removed manually with the aid of a stainless steel knife, and the fraction pulp + peel (PP) was obtained. Then, it was frozen at -20 °C, lyophilized (fdw) (LIOTOP, model L101, São Carlos, Brazil) and ground in a knife mill (Marconi, model MA340, Piracicaba, Brazil). The ASAE S319.3 method was used to calculate the average particle diameter (1.960 ± 0.03 mm). The particle true density (d<sub>P</sub>) measured by the helium gas pycnometer (Model 1305 Multivolume, Micromeritics Instrument Corporation, Norcross, GA, USA) was  $1.120 \pm 0.01$  g.cm<sup>-3</sup>. The bulk density (d<sub>b</sub>) of the particles was calculated using the extractor volume and the mass of PP, and the result was 0.627 g.cm<sup>-3</sup>. The porosity of the particle bed ( $\varepsilon$ ) was 0.440, calculated using the bulk density and the particle true density, according to  $\varepsilon = 1-$  (d<sub>b</sub>/d<sub>P</sub>). The samples remained frozen until the time of the extraction process.

# 2.2. Extraction procedures with supercritical CO<sub>2</sub>

The thawed sample was submitted to the extraction process in a supercritical fluid extraction unit (Spe-ed 7071, Applied Separations, Allentown, USA) according to Souza et al. (2021) with slight modifications. Approximately 6.3 g of lyophilized raw material (fdw) was placed in a 5.0 mL stainless-steel extraction column. The system was then heated and pressurized under the desired conditions and maintained at a static period for 5 min. The solvent flow rate was set at 5 g.min<sup>-1</sup>, and the solvent (S) to feed (F) ratio was kept constant at S/F = 15. The extraction temperature was 40 °C at a constant pressure of 30 MPa. The extract obtained was kept protected from light in a freezer at -20 °C for further analyses. The global extraction yields were calculated according to equation 1.

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where y is the global extraction yield, em is the extracted weight and fm is the fresh weight.

2.3. Extraction kinetics with supercritical CO<sub>2</sub> and content of total carotenoids

The extraction kinetics was measured to verify the oil yield over time. Briefly, 21 g (fdw) of sample was placed in a 300 mL stainless steel column to obtain the overall extraction curve with the  $CO_2$  flow rate held constant at 17 g.min<sup>-1</sup> for 210 min.

The total carotenoid content in the extraction kinetics was determined according to França et al. (1999) with some modifications. Ten milligrams of the samples were diluted and homogenized in 1 mL of acetone-hexane solution (4:6, v/v). The absorbance of the mixture was measured at 450 nm in a microplate reader (SpectrostarNano, BMG Labtech, Offerburg, Germany), and the results were expressed as mg  $\beta$ -carotene.g<sup>-1</sup> fdw. The kinetic assays were repeated two times.

# 2.4. Cold pressing extraction

To obtain the oil, 100 g of the sample was added to the stainless steel basket of the manual hydraulic press, which operates with downwards movement of the piston. The extraction was interrupted, and the extract was collected when the piston reached a pressure of 60 tons. The global extraction yields were calculated according to equation 1.

# 3. Analysis of buritirana oil

### 3.1. Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was performed according to Re et al. (1999). Five milliliters of the ABTS solution (7 mM) with 88  $\mu$ L of potassium persulfate (140 mM) was used for the formation of the radical. The mixture was kept at rest, and then an aliquot of this solution was diluted with water, and the absorbance was adjusted to 0.70 ± 0.02 at 734 nm. The absorbance was measured after the mixture of 50  $\mu$ L of sample (diluted in methylated cyclodextrin 7%) and 250  $\mu$ L of ABTS<sup>\*+</sup> solution on a microplate reader at 734 nm. The results were expressed as  $\mu$ M TE.g<sup>-1</sup> fdw.

#### 3.2. Oxygen radical absorbance capacity (ORAC) assays

The oxygen radical absorbance capacity was determined according to Prior et al. (2003). The reactions of the assays were carried out in polystyrene microplate-specific wells for fluorescence and evaluated in a NovoStar microplate reader (New Brunswick Scientific Classic Series, model C76, Offerburg, Germany).

For hydrophilic ORAC (ORAC<sub>H</sub>), the samples were prepared in 75 mM potassium phosphate buffer, pH 7.4. In each well of the microplate, 20  $\mu$ L of sample, blank or Trolox, 120  $\mu$ L of fluorescein and 60  $\mu$ L AAPH [2,2'-azobis (2'-methylpropionamidine) dihydrochloride] were added. Immediately after adding AAPH, the fluorescence was monitored at 37 °C for 80 min.

In the lipophilic ORAC (ORAC<sub>L</sub>), the samples were prepared and homogenized in a solution of acetone/water (1:1) containing 7% methylated cyclodextrin, while the AAPH and fluorescein were prepared in 75 mM potassium phosphate buffer, pH 7.4. The reaction system consisted of 20  $\mu$ L of sample, blank or Trolox, 120  $\mu$ L of fluorescein and 60  $\mu$ L of AAPH. The fluorescence decay was measured for 80 min at 37 °C. Total ORAC (ORAC<sub>T</sub>) was obtained by summing the results of the lipophilic and hydrophilic ORAC. The results were expressed as  $\mu$ mol Trolox equivalent.g<sup>-1</sup> fdw.

#### 3.3. $\alpha$ -Tocopherol content

The  $\alpha$ -tocopherol content was determined according to (Hashim, Koehler, Eitenmiller, & Kvien, 1993). This analysis was carried out in a high-performance liquid chromatograph (Shimadzu, LC-20AD) coupled to a fluorescence detector. Hexane/isopropanol (99:1; v/v) with a flow rate of 1 mL.min<sup>-1</sup> was used as the mobile phase. Twenty microliters of the samples were diluted in hexane and injected into a normal phase silica column (LiChrospher Si-60 250 x 4.6 mm x 5 µm, Merck). In the fluorescence detector, the emission and excitation wavelengths were 330 nm and 290 nm, respectively. 3.4. Saturated and unsaturated fatty acid profiles

The sample was methylated through saponification and esterification according to (Hartman & Lago, 1973), with adaptations based on the (AOCS, 2003) Ce 1b-89 method. Briefly, 2.0 mL of 0.5 N methanolic sodium hydroxide was added to 0.5 g of the sample. The mixture was heated at 100 °C for 5 min. After cooling, 2.5 mL of a solution of ammonium

chloride and sulfuric acid in methanol (2:3:60 w/v/v, prepared under reflux) was added to the reaction and incubated at 100 °C for 5 min. Then, 5.0 mL of hexane and 5.0 mL of a saturated saline solution were added and allowed to stand for 10 min until phase separation. The methyl esters were collected, and 5.0 mL of Milli-Q water and 0.05 g of anhydrous sodium sulfate were added. The extraction procedure was repeated once.

A gas chromatograph (Shimadzu, Series 2010 Plus) equipped with a Restek-Wax column (30 m x 0.32 mm x 0.25  $\mu$ m) coupled to a flame ionization detector (FID) was used. The temperature programming started at 60 °C and reached 130 °C at a rate of 20 °C.min<sup>-1</sup>, remaining at that temperature for 7 min; then, at a rate of 30 °C.min<sup>-1</sup>, the programming reached 240 °C, remaining in this condition for 18 min. The injector and detector temperature were 250 °C, and hydrogen with a linear velocity of 21 cm.s<sup>-1</sup> was used as the carrier gas. The injection volume was 1  $\mu$ L in split mode. The identification was realized by comparison of the mix of fatty acid standards (F.A.M.E C8-C22/Sigma–Aldrich).

## 3.5. Characterization of phenolic compound profiles by ESI-LTQ-XL-MS/MS

The organic compounds were identified by mass spectrometry analysis according to Farias et al. (2020). Ten microliter of oil obtained by supercritical CO<sub>2</sub> was diluted in 990  $\mu$ L of methanol. Prior to direct infusion in an ESI-LTQ-XL-MS/MS mass spectrometer (Thermo Scientific, Bremen, Germany), the sample was positively ionized by the addition of formic acid (2.0%). For structural elucidation, predominant *m*/*z* ions underwent tandem mass spectrometry analysis using helium (He) as the collision gas and collision-induced dissociation energy ranging from 15-50 eV. Molecules identities were achieved by consulting online databases such as METLIN (Scripps Center for Metabolomics, La Jolla, CA metlin.scripps.edu) and Lipid MAPS (University of California, San Diego, CA www.lipidmaps.org), and theoretical structures fragmentation modelling using Mass Frontiers software (v. 6.0, Thermo Scientific, San Jose, CA).

To evaluate the presence and relative intensities of the phenolic compounds characterized in the oils, a heatmap analysis using Ward hierarchical clustering with Euclidean distance was performed by inputting MS data into MetaboAnalyst software, version 4.0 (Chong, Wishart, & Xia, 2019).

#### 3.6. Volatile organic compounds (VOCs)

#### 3.6.1. Sample preparation for SPME extraction and GC-MS analysis

The oil obtained from the two extraction methods remained frozen at -40 °C in an ultrafreezer, and after a period of three years, it was thawed and subjected to analysis of volatile organic compounds. The determination of VOCs was performed according to (Araújo et al., 2021) with some modifications. A 500  $\mu$ L aliquot was suspended and homogenized in 0.5 mL PA ethanol in 20 mL flasks with a screw cap containing a Teflon-coated septum for VOCs headspace microextraction. SPME extractions were performed from the headspace of the samples according to the following conditions: DVB/CAR/PDMS fiber, equilibrium time of 10 min; extraction time of 15 min and extraction temperature of 50 °C.

The compounds were separated using an Agilent 7890A gas chromatography system (Agilent Technologies, Santa Clara, USA) equipped with a GC DB-WAX column (30 m  $\times$  0.25 mm  $\times$  0.15 µm) and an Agilent 5975C inert MSD with a triple-axis detector, using He as the carrier gas. The VOCs were desorbed for 5 min by inserting the SPME fiber into a GC injector (270 °C). The GC oven temperature was programmed to hold at 70 °C for 1 min, then to increase to 140 °C at 3 °C.min<sup>-1</sup> and finally 210 °C for 5 °C.min<sup>-1</sup>. The column flow rate was 1 mL.min<sup>-1</sup>. The MS was scanned in the range of 45–650 amu at 70 eV. The total analysis time was 39.33 min. Compounds were identified using the NIST 14.0 database and the linear retention index (LRI) calculated with a series of n-alkanes (C7-C40).

#### 3.7. Carotenoid profile

Buritirana oil obtained by supercritical fluid extraction (0.5 g  $\pm$  0.1) and hydraulic press (0.3 g  $\pm$  0.1) was solubilized in 10 mL petroleum ether and saponified overnight (~16 h) at room temperature with 30% methanolic KOH, and after that, it was washed until it was alkali free. The extract was frozen (-20 °C) for 16 h for the physical removal of the fatty acids (Rosso & Mercadante, 2007a with adaptations). Then, the carotenoid extracts were separated from the solid portion containing the fatty acids under low temperature and concentrated until dryness.

An aliquot of the extract was redissolved in a MeOH:MTBE mixture [90:10 (v/v)] and analyzed by HPLC-DAD for the determination of the carotenoid composition. Carotenoids were separated on a C30 YMC column (5  $\mu$ m, 250 mm × 4.6 mm) (Waters,

Wilmington, DE) using 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. Chromatograms were processed at 450 nm. The identification of the carotenoids was performed considering the combination of the following parameters: elution order on the C30 column, UV–vis spectral features [maximal absorption wavelength ( $\lambda$ max), spectral fine structure (%III/II), and cis peak intensity (%AB/AII)], comparison to standards analyzed under the same conditions, and data available in the literature (Chisté, Costa, Monteiro, & Mercadante, 2021; Rosso & Mercadante, 2007b). The carotenoids were quantified by HPLC-DAD using external sevenpoint analytical curves (built in triplicate) for all-*E*-lutein (1.9–21.3 µg.mL<sup>-1</sup>), all-*E*- $\alpha$ -carotene (7.8–19.6 µg.mL<sup>-1</sup>), and all-*E*- $\beta$ -carotene (3.0–13.9 µg.mL<sup>-1</sup>). The limits of detection (LOD) and quantification (LOQ) were 0.02 and 0.07 µg.mL<sup>-1</sup> for all-*E*- $\beta$ -carotene, 0.013 and 0.044 µg.mL<sup>-1</sup> for all-*E*- $\alpha$ -carotene, and 0.017 and 0.056 µg.mL<sup>-1</sup> for all-*E*-lutein, respectively. Areas under peaks were compared with the calibration curves. The results were expressed in µg of carotenoids per g sample.

#### 3.8. Antibacterial activity

# 3.8.1. Microorganism and culture conditions

The conditions of the oil samples for the determination of antibacterial activity are mentioned in section 3.6.1. All the bacterial strains (*Escherichia coli* ATCC 10231, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 5061, *Bacillus cereus* ATCC 10876, *Salmonella choleraesuis* ATCC 10708 and *Pseudomonas aeruginosa* ATCC 13388) were periodically harvested in Mueller-Hinton medium (Kasvi) (bacteria, 37 °C for 1 day) and stored under refrigeration.

# 3.8.2. Determination of minimum inhibitory concentration (MIC)

Antimicrobial susceptibility was evaluated by using the broth microdilution method according to (CLSI, 2005). These methods consist of evaluating the ability to inhibit bacterial growth by evaluating different known concentrations in a 96-well microplate. Initially, the compounds were transferred into the first well, and serial dilutions were made in the range of 4.0-0.001 mg.mL<sup>-1</sup>. Streptomycin sulfate (Sigma–Aldrich®) was used as the reference antibiotic control in the range of 0.5-0.0039 mg.mL<sup>-1</sup>.

The antibacterial activity was carried out using Mueller-Hinton broth (Kasvi). The bacterial strains were standardized in Mueller-Hinton broth to  $10^6$  CFU.mL<sup>-1</sup>. The inoculum was added to all wells, and the plates were incubated at 37 °C for 24 hours. The MIC was defined as the lowest concentration of the sample that inhibited visible growth. As indicated by 2,3,5-triphenyltetrazolium chloride staining, dead cells were not stained.

## 3.9. Statistical Analysis

All analyses were performed in triplicate. The experiment was carried out in a completely randomized design, and the data obtained were expressed as the mean  $\pm$  standard deviation. The results were analyzed by ANOVA, and to check the difference between the means, a t-test was used, with a significance level of 5%, using Statistica software version 7.0

#### 4. Results and discussion

#### 4.1. Global yields, extraction kinetics and behavior of total carotenoids

The global extraction yields obtained for buritirana oil by extraction with supercritical CO<sub>2</sub> and cold pressing are shown in **Figure 1**. The statistical analysis showed that there was a difference between the extraction methods used (p < 0.05) and that supercritical CO<sub>2</sub> showed a 1.5-fold greater yield (13.44 ± 0.032%) than that obtained by cold pressing (8.94 ± 0.035%). These results are lower than those found by Souza (2021) when investigating the oil yield (14.55%) of the whole seedless buritirana fraction obtained by supercritical CO<sub>2</sub> at 60 °C and 30 MPa and Ribeiro et al. (2018), who discovered a 26% oil yield from the buritirana pulp extracted by the Soxhlet method. According to Efthymiopoulos et al. (2018), the higher yield promoted by supercritical CO<sub>2</sub> may be related to the employment of higher temperatures, as these temperatures facilitate the solubilization of lipids due to the interruption of cohesive and adhesive interactions between the oil molecules and the matrix, allowing an increase in the diffusion and extraction of lipids.

To date, no study has shown the oil yield of buritirana by comparing extraction by supercritical  $CO_2$  and cold pressing. However, some research was done with fruits of the same family, including açaí (*Euterpe oleracea*), buriti (*Mauritia flexuosa*), tucumanzinho (*Astrocaryum acaule*), and bacaba (*Oenocarpus bacaba*) using different fractions, temperatures, pressures and extraction methods (Batista et al., 2016; Ribeiro et al., 2018).



**Figure 1.** Total oil yields obtained from buritirana by supercritical  $CO_2$  extraction and cold pressing (CP). Different lower case letters indicate a significant difference by the Test t (p < 0.05).

Similarly, several studies have reported the extraction efficiency using supercritical CO<sub>2</sub> compared to other methods. In red pepper seed oil (*Capsicum annuum* L.) yields of 14.60%, 18.39%, 25.27% and 32.87% were found for cold pressing, Soxhlet, supercritical CO<sub>2</sub> and microwave, respectively (Chouaibi, Rezig, Hamdi, & Ferrari, 2019). Likewise, Zheng et al. (2020) reported that the yields obtained from seed oil of *Actinostemma lobatum* Maxim. by Soxhlet extraction  $(32 \pm 1 \text{ g.}100 \text{ g}^{-1})$  and cold pressing  $(29 \pm 1 \text{ g.}100 \text{ g}^{-1})$  were also lower than by supercritical CO<sub>2</sub>  $(36 \pm 0 \text{ g.}100 \text{ g}^{-1})$ .

Regarding the general extraction curve, it was observed that in the total extraction time of 210 min, 28.42% of buritirana oil and 16.67 mg of  $\beta$ -carotene.g<sup>-1</sup> fdw were extracted (**Figure 2**). At 60 min, the oil extraction speed was higher and reached a yield of 25.94%. After this time, there was a small variation (approximately 1%) in the yield until the end of the extraction. The total carotenoid extraction rate also showed an increasing behavior up to 60 min, reaching a yield of 14.61 mg of  $\beta$ -carotene.g<sup>-1</sup>. There was a tendency towards stabilization in the concentration of carotenoids after 140 min (16.00 mg of  $\beta$ -carotene.g<sup>-1</sup>). Thus, it can be evidenced that for both oil extraction and total carotenoid yield, it would not be necessary to spend energy and time until 210 min, since the speed and rate of extraction tends to stabilize after 60 min. As reported by França et al. (1999), the lipids arranged in the matrix that first come into contact with CO<sub>2</sub> are extracted more quickly, resulting in oil with



fewer dissolved compounds and thus a higher carotenoid content in the final product after starting the drainage process.

**Figure 2.** Overall extraction curve and behavior of total carotenoids in buritirana oil by supercritical  $CO_2$  extraction.  $\blacklozenge$  Extraction kinetic of oil;  $\blacksquare$  Extraction kinetic of total carotenoids.

A similar study was carried out by Souza et al. (2021) with buritirana pulp under the same process conditions (40 °C and 30 MPa), and at approximately 60 minutes of extraction, the oil yield was higher (41.57 g.100 g<sup>-1</sup>), while the total carotenoid content was approximately 1.6-fold lower (8.34 mg.g<sup>-1</sup>). Santos et al. (2020b) found a yield of 26% for favela seed oil (*Cnidoscolus quercifolius*) at 60 min of extraction by supercritical CO<sub>2</sub> at 60 °C and 80 °C and 30 MPa. In babassu (*Orbignya phalerata*), a species belonging to the same family as buritirana, higher extraction yields were found by supercritical CO<sub>2</sub> (89.86%) at 80 °C and 25 MPa for 120 min (Oliveira et al., 2019). In another study, Lima and collaborators demonstrated that 97% of the total carotenoid content of the carrot peel was recovered after just 30 min of extraction (Lima, Charalampopoulos, & Chatzifragkou, 2018).

## 4.2. Antioxidant capacity, $\alpha$ -tocopherol content and fatty acid profile

The results of the assays of antioxidant capacity,  $\alpha$ -tocopherol contents and fatty acid profile of the oil obtained by the two extraction methods are shown in **Table 1**. The antioxidant capacity determined by the TEAC assay showed no significant difference (p < 0.05) between the extraction methods evaluated, while all other results for the ORAC assays

differed statistically.  $ORAC_L$  showed the highest antioxidant capacity for both extraction methods evaluated, and in cold pressing, the result was 1.64-fold greater than that in extraction by supercritical CO<sub>2</sub>. On the other hand, no antioxidant capacity was observed in ORAC<sub>H</sub> for cold pressing. Regarding ORAC<sub>T</sub>, the antioxidant capacity for the oil obtained by supercritical CO<sub>2</sub> was 1.60-fold less than that obtained by cold pressing. Souza et al. (2021) evaluated the antioxidant capacity by the TEAC, ORAC<sub>L</sub>, ORAC<sub>H</sub> and ORAC<sub>T</sub> assays of the pulp+peel fraction oil of buritirana obtained by supercritical CO<sub>2</sub> at 40 and 60 °C at 30 MPa and found that the results did not differ statistically between the analyzed temperatures; in turn, they were similar to this study (p > 0.05).

**Table 1**. Antioxidant capacity,  $\alpha$ -tocopherol content and fatty acids profile in the oils obtained of buritirana by supercritical CO<sub>2</sub> extraction and cold pressing.

Denometers	Extraction methods				
Farameters	Supercritical CO <sub>2</sub>	Cold pressing			
Antioxidant capacity					
TEAC ( $\mu$ mol TE.g <sup>-1</sup> )	$1.385 \pm 0.28$ a	$1.027 \pm 0.06$ a			
ORAC <sub>L</sub> (µmol TE.g <sup>-1</sup> )	$27.841 \pm 5.67 \text{ b}$	$45.793 \pm 9.21$ a			
ORAC <sub>H</sub> (µmol TE.g <sup>-1</sup> )	$0.820 \pm 0.29$ a	$0.000\pm0.00~b$			
ORAC <sub>T</sub> (µmol TE.g <sup>-1</sup> )	$28.660 \pm 5.52 \text{ b}$	$45.793 \pm 9.21$ a			
Tocol					
$\alpha$ -tocopherol (mg.g <sup>-1</sup> )	$0.05473 \pm 0.00134 \; b$	$0.41119 \pm 0.00962$ a			
Fatty acids					
Palmitic acid $(g.g^{-1})$	$0.21170 \pm 0.00035$ a	$0.20674 \pm 0.00018 \text{ b}$			
Estearic acid $(g.g^{-1})$	$0.02264 \pm 0.00003 \text{ b}$	$0.02303 \pm 0.00001$ a			
Oleic acid $(g.g^{-1})$	$0.68850 \pm 0.00037 \; b$	$0.69354 \pm 0.00014$ a			
Linoleic acid $(g.g^{-1})$	$0.05198 \pm 0.00044 \ b$	$0.05232 \pm 0.00032$ a			
Linolenic acid (g.g <sup>-1</sup> )	$0.02518 \pm 0.00031$ a	$0.02436 \pm 0.00001 \text{ b}$			

Average with different letters in the line indicate statistical differences according to t-test (p < 0.05). Results expressed in fdw. TE: Trolox equivalent; L: Lipophilic: H: Hydrophilic; T: Total.

Similarly, tea seed oils extracted by cold pressing also showed greater antioxidant potential by DPPH and TEAC assays when compared to supercritical  $CO_2$  (Shao, Liu, Fang, & Sun, 2015). According to the authors, the lower antioxidant capacity verified for supercritical  $CO_2$  can be justified due to its low efficiency in the extraction of more polar compounds. In addition, in cold pressing extraction, the cell wall breaks, increasing the release and extraction of bioactive compounds, which in turn increases the antioxidant capacity of the oil (Shao et al., 2015). On the other hand, the results different from those found here in buritirana oil were reported by Shi et al. (2018) in toasted sesame oil, where supercritical  $CO_2$  showed greater antioxidant potential than cold pressing, being 29.6-fold higher for ORAC and 20.50-fold higher for TEAC. Likewise, the antioxidant capacity by the TEAC and DPPH assays was also 2.89- and 3.80-fold greater, respectively, in red pepper seed oil extracted by supercritical  $CO_2$  when compared to cold pressing (Chouaibi et al., 2019).

Tocopherols are excellent natural antioxidants that can act as hydrogen donors, thus preventing lipid oxidation and ensuring the maintenance of the quality of food products in addition to contributing to the bioactive potential of food (Gu et al., 2019). **Table 1** shows the results of  $\alpha$ -tocopherol in buritirana oil obtained by the aforementioned extraction methods. The  $\alpha$ -tocopherol content differed statistically (p < 0.05) between the studied processes, and the extraction with supercritical CO<sub>2</sub> showed a result approximately 13.4% lower in relation to the cold pressing.

Similarly, Souza et al. (2021) evaluated the oil extracted from whole without seed fraction the buritirana (*Mauritiella armata* Mart.) using supercritical CO<sub>2</sub> at 40 °C/30 MPa and found 0.126 mg.g<sup>-1</sup>  $\alpha$ -tocopherol, which was 2.3-fold higher than the tocopherol content obtained by CO<sub>2</sub> supercritical extraction and approximately 3.3-fold lower than the content found by cold pressing extraction.

The higher  $\alpha$ -tocopherol content obtained in cold pressing extraction may be related to less degradation of these phytochemicals, since the use of high temperatures used in supercritical CO<sub>2</sub> can result in greater degradation/oxidation of compounds (Mustapa et al., 2015). In iron walnut oil (*Juglans sigillata*), cold pressing showed a content of  $\alpha$ -tocopherol 1.50-fold greater than that extracted by supercritical CO<sub>2</sub> (Shao et al., 2015). Different results were found by Zheng et al. (2020), who observed that cold pressing extraction presented the lowest  $\alpha$ -tocopherol concentration when compared to extraction with supercritical CO<sub>2</sub> and Soxhlet; however, there was no significant difference in the total tocopherol content (p > 0.05).

In relation to fatty acids, the results showed that the oil extracted from buritirana by both methods presented a considerable amount of saturated and polyunsaturated acids. The content of these acids was influenced by the extraction method evaluated and differed statistically from each other (p < 0.05). In general, oleic acid was the main constituent, followed by palmitic, linoleic, linolenic and stearic acids. Oleic acid represented 68.85% and 69.35% of the composition of oils obtained by supercritical CO<sub>2</sub> and cold pressing, respectively (**Table 1**). According to Pavlović et al. (2018), the variation in fatty acid content may be related to oxidation reactions due to contact with air during the extraction process.

In the study performed by Chouaibi et al. (2019) to verify the influence of extraction methods on the chemical composition of red pepper seed oil, it was reported that linoleic acid was the main constituent, followed by palmitic and oleic acids. According to the

authors, the levels of linoleic acid were 73.65%, 73.89%, 76.26% and 76.54% for cold pressing, Soxhlet, supercritical CO<sub>2</sub> and microwave, respectively (Chouaibi et al., 2019). Santos et al. (2019) studied passion fruit seed oil (*Passiflora edulis* Sims) obtained by cold extraction and supercritical CO<sub>2</sub> at 313 K and 34 MPa under conditions very close to those used in this research and reported that linoleic (66.84 g.g<sup>-1</sup>) and linolenic acids (0.83 g.g<sup>-1</sup>) were higher for supercritical CO<sub>2</sub>, while palmitic (11.68 g.g<sup>-1</sup>), stearic (2.99 g.g<sup>-1</sup>) and oleic acids (18.35 g.g<sup>-1</sup>) showed higher values for cold pressing. In another study, Pavlović et al. (2018) found that the content of linoleic and palmitic acids extracted from apricot kernel by supercritical CO<sub>2</sub> and cold pressing did not differ statistically, while for oleic acid, the results were 62.73% and 57.33% for cold pressing and supercritical CO<sub>2</sub>, respectively.

4.3. Influence of extraction methods on phenolic compounds in buritirana oil

Different compounds were identified by ESI-LTQ-XL-MS/MS in buritirana oil and are shown in **Table 2**. Of the 20 compounds found, 40% are flavonoids, 35% are phenolic acids, and the remaining 25% are organic acids. Souza et al. (2021) also found results very close to this study.

**Table 2.** Compounds identified by ESI-LTQ-XL-MS/MS in the buritirana oil extracted by supercritical  $CO_2$  and cold pressing.

Compound	Mass	MEME from on to	Adduct	Molecular
Compound		MIS/MIS Tragments	Adduct	formula
Maleamic acid	116	70 - 88 - 98	$[M+H]^+$	$C_4H_5NO_3$
Succinic acid	119	101 - 91 - 73	$[M+H]^+$	$C_4H_6O_4$
Benzoic acid	123	81 - 95 - 67 - 57 - 105	$[M+H]^+$	$C_7H_6O_2$
Fumaric acid	139	121 - 111 - 122 - 95 - 94	$[M+Na]^+$	$C_4H_4O_4$
Cinnamic acid	149	117 - 121 - 131 - 107	$[M+H]^+$	$C_9H_8O_2$
Vanillic acid	169	133 - 123 - 89 - 109 - 107 - 119 - 81 - 95	$[M+H]^+$	$C_8H_8O_4$
Gallic acid	171	89 - 153 - 139 - 143 - 135 - 125 - 111	$[M+H]^+$	$C_7H_6O_5$
Caffeic acid	181	163 - 149 - 135 - 153	$[M+H]^+$	$C_9H_8O_4$
Quinic acid	193	175 - 161 - 165 - 147 - 137 - 133 - 120	$[M+H]^+$	$C_{7}H_{12}O_{6}$
Caffeoyl tartaric acid	295	277 - 249 - 263 - 235 - 281	$[M+H-H_2O]^+$	$C_{13}H_{12}O_9$
Quercetin	303	257 - 229 - 285 - 165 - 247	$[M+H]^+$	$C_{15}H_{10}O_7$
5Deoxyleucopelargonidin	313	257 - 109 - 95 - 123 - 239 - 137	$[M+K]^+$	$C_{15}H_{14}O_5$
Apigenin hexoside	415	119 - 295 - 397 - 133 - 277	$[M+H-H_2O]^+$	$C_{21}H_{20}O_{10}$
Catechin hexoside	453	435 - 407 - 365 - 417 -347 - 305	$[M+Na]^+$	$C_{21}H_{24}O_{11}$
Kaempferol coumaroyl	579	265 - 239 - 247 - 503	$[M+H]^+$	$C_{30}H_{26}O_{12}$
deoxyhexoside				
Apigenin caffeoyl hexoside	595	577 - 339 - 313 - 539 - 535 - 357	$[M+H]^+$	$C_{30}H_{26}O_{13}$
Catechin dihexoside	637	581 [525 – 469]*	$[M+Na]^+$	$C_{27}H_{34}O_{16}$
Myricetin	957	507 - 911 - 419 - 611	$[M+K]^+$	$C_{42}H_{46}O_{23}$
coumarylrobinobioside				
deoxyhexoside				
*MS				

However, instead of 18, 20 compounds were discovered, which differed only by presenting 2 more phenolic acids (caffeoylquinic and glucosyringic acids).

Polyphenols such as phenolic acids and flavonoids are compounds produced by the secondary metabolism of plants that can act in the inhibition and neutralization of reactive species, in addition to having antidiabetic, anticancer, and antiobesity properties, among others (Farias et al., 2020). Thus, the presence of these compounds in buritirana oil may indicate its potential in the development of new products with functional claims.

As shown in **Figure 3**, a heatmap analysis was performed to check the influence of extraction methods on the relative intensity of the bioactive compounds identified in the buritirana oil. Briefly, the heatmap is a graph represented by a thermometer with color codes, where the color intensity indicates the analyzed variable, in this case, the relative concentration of the characterized compounds.



Figure 3. Heatmap analysis of compounds identified in buritirana oil by supercritical  $CO_2$  extraction and cold pressing (CP).

Thus, it was possible to observe that the extraction with supercritical  $CO_2$  showed the highest relative intensity for all compounds identified in the buritirana oil, while in the extraction by cold pressing, the relative intensity of these compounds was reduced, except for kaempferol coumaroyl deoxyhexoside (class 579). Despite the higher relative intensity of the compounds extracted by supercritical  $CO_2$ , the acids maleamic (class 116), caffeic (class 181), gallic (class 171), caffeoyl tartaric (class 295), cinnamic (class 149), quinic (class 193), and vanillic (class 169) seem to be more affected by this method, as they presented lower relative intensity in comparison to the other compounds. These results could be related to the higher sensitivity of these compounds to the temperature used during the extraction or even to a lower solubility in the supercritical  $CO_2$ .

The heatmap analysis used by Souza et al. (2021) to verify the relative intensity of the phenolic compounds in the oil of the WS (whole without seed) fraction of buritirana (*Mauritiella armata* Mart.) obtained by supercritical  $CO_2$  was also greater compared to the other fractions at 40 °C; in addition, the authors confirmed a greater number of flavonoids in the analyzed fraction.

According to Souza et al. (2019), the extraction method and the operating conditions used influence the extraction of bioactive compounds present in the matrix. These authors found that in *Arctium lappa*, extraction by PLE showed higher concentrations of chlorogenic acid (1.84%) and rutin (1.46%) when compared to the extract obtained by supercritical CO<sub>2</sub>. Koubaa et al. (2016) demonstrated that canola seed oil obtained by extraction of supercritical CO<sub>2</sub> was approximately twice as enriched in phenolic compounds as that obtained by cold pressing.

## 4.4. VOCs and antibacterial activity

This is the first time that VOCs have been reported in buritirana oil. **Table 3** shows the VOCs identified only in the buritirana oil obtained by supercritical CO<sub>2</sub>, since no representative volatile compounds were found in the oil extracted by hydraulic press. In this extraction method, compounds of different functional groups were found, such as acids, alcohols, ketones, ethers, esters and terpenes. Esters were dominant, representing approximately 36% of the discovered compounds. Hexanoic acid and ethyl ester showed the highest peak area (29.203%), followed by octanoic acid and ethyl ester (8.088%). These two components together correspond to 79.54% of the volatile constituents of buritirana oil. Hexanoic acid and ethyl ester are responsible for floral/fruity aromas, and octanoic acid and ethyl ester have a waxy flavor; in addition, they are the most commonly found aromatic compounds in passion fruit (Pereira et al., 2019). Despite the low concentrations of the other contributing to the flavor of fruits, VOCs can be used by the food, cosmetic, perfume and beverage industries, and due to their therapeutic properties, they have great potential for human health (Araújo et al., 2021; Pereira et al., 2019).

Compounds	MW	Formule	Cas#	RT (min)	Area (%)	LRI
Pentanoic acid	102	$C_5H_{10}O_2$	109-52-4	3.710	2.035	1126.47
Hexanoic acid	116	$C_6H_{12}O_2$	142-62-1	4.563	0.649	1182.93
Limonene	136	$C_{10}H_{16}$	5989-27-5	4.723	2.712	1193.51
1-butanol-3-methyl	88	$C_5H_{12}O$	123-51-3	5.078	2.458	1212.04
Hexanoic acid, ethyl ester	144	$C_8H_{16}O_2$	123-66-0	5.473	29.203	1230.55
3-hexenoic acid, ethyl ester (E)	142	$C_8H_{14}O_2$	26553-46-8	6.806	0.043	1293.02
Ethyl 5-methylhexanoate	158	$C_9H_{18}O_2$	10236-10-9	7.023	0.644	1302.41
Octanoic acid, ethyl ester	172	172 $C_{10}H_{20}O$ 106-32-1 10.920		8.088	1435.20	
		2				
Cis-linaloloxide	170	$C_{10}H_{18}O$	$121974^{*}$	11.292	0.066	1446.71
		2				
1,6-octadien-3-ol, 3,7-dimethyl	154	$C_{10}H_{18}O$	78-70-6	14.880	0.419	1557.69
Decanoic acid, ethyl ester	200	$C_{10}H_{24}O$	110-38-3	17.941	0.136	1640.64
		2				
Acetophenone	120	$C_8H_8O$	98-86-2	18.130	0.218	1645.97
L-α-terpineol	154	$C_{10}H_{18}O$	10482-56-1	20.092	0.180	1701.10
Diphenyl ether	170	$C_{12}H_{10}O$	101-84-8	29.574	0.031	1900.77

**Table 3-** VOCs found in the buritirana oil extracted by supercritical  $CO_2$ 

\* NIST; MW: molecular weight; RT: retention linear; min: minutes; LRI: linear retention index.

Regarding antibacterial activity, both the oil obtained by supercritical  $CO_2$  and the oil extracted by mechanical pressing showed no action against any of the microorganisms (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella choleraesuis* and *Pseudomonas aeruginosa*) evaluated in the concentration range of 4.0-0.001 mg.mL<sup>-1</sup>. According to Souza et al. (2022), the period and storage conditions may have negatively influenced the chemical composition of the evaluated oils so that the bioactive compounds present lost their antibacterial properties.

# 4.5 Carotenoid profile

The combined information obtained from chromatographic elution on a C30 column and the characteristics of UV–vis (**Table 4**) were considered for the identification and tentative identification of the carotenoids from saponified buritirana oil separated by HPLC (**Figure 4**). All-*E*-Lutein (peak 1), all-*E*- $\alpha$ -carotene (peak 4), and all-*E*- $\beta$ -carotene (peak 6) were positively identified using purified standards. These compounds were previously detected in buritirana pulp (Anunciação et al., 2019). Among the four carotenoids tentatively identified, only 9-*Z*- $\beta$ -carotene was previously detected. All-*E*- $\beta$ -cryptoxanthin, 13-*Z*- $\beta$ -carotene, and 9-*Z*- $\alpha$ -carotene were reported in other species from the Arecaceae family, such as buriti pulp (Rosso & Mercadante, 2007a), orange and yellow peach palm fruits (*Bactris gasipaes*) (Chisté et al., 2021), commercial palm oil and tucumã pulp (Rosso & Mercadante, 2007a).

**Table 4**: Chromatographic, UV-Vis characteristics and carotenoid content of buritirana oil saponified extracted by supercritical  $CO_2$  and hydraulic press, obtained by HPLC-DAD.

Decla	<b>4</b> ( <b>!</b> )	Constancid	$\lambda_{\max}$ (nm)	%111/11	%A <sub>B</sub> /A <sub>II</sub>	Carotenoid content (µg.g <sup>-1</sup> of buritirana oil)		
Реак	$t_{R}$ (min)	Carotenoid				Supercritical CO <sub>2</sub>	Cold pressing	
1	13.41 - 13.49	all-E-lutein <sup>1,e</sup>	401, <b>428</b> , 452	43	0	$0.32\pm0.010^{\text{B}}$	$0.51\pm0.020^{\rm A}$	
2	23.90 -23.96	all-E-β-cryptoxanthin <sup>2</sup>	421, <b>451</b> , 476	30	0	$0.17\pm0.008^{B}$	$0.27\pm0.007^{\rm A}$	
3	27.31 - 27.43	$13-Z-\beta$ -carotene <sup>2</sup>	340, <b>443</b> , 471	n.c.	34	$0.16\pm0.002^{\rm B}$	$1.81\pm0.030^{\rm A}$	
4	29.76 - 29.87	all- $E$ - $\alpha$ -carotene <sup>3,e</sup>	420, <b>445</b> , 475	66	0	$0.52\pm0.030^B$	$2.16{\pm}~0.060^{\rm A}$	
5	30.76 - 30.89	9-Z-α-carotene	330, 419, <b>442</b> , 470	62	20	$0.18\pm0.003^{\rm B}$	$1.82\pm0.040^{\rm A}$	
6	33.73 - 33.89	all- $E$ - $\beta$ -carotene <sup>2e</sup>	422, <b>451</b> , 477	33	0	$0.42\pm0.020^{B}$	$2.16\pm0.004^{A}$	
7	35.66 - 35.82	9-Z- $\beta$ -carotene <sup>2</sup>	340, <b>445</b> , 476	25	13	$0.22\pm0.004^{\text{B}}$	$1.87 \pm 0.030^{\text{A}}$	
an tt-		Total carotenoids (µg.g <sup>-1</sup> of buritirana oil)	τ. Γίωτα 4 <sup>b</sup> υ			$\frac{2.00 \pm 0.06^{\text{B}}}{5}$	$10.60 \pm 0.2^{\text{A}}$	

<sup>a</sup>Numbered according to the chromatogram shown in Figure 4; <sup>b</sup>Retention time on the C<sub>30</sub> column; <sup>c</sup>Solvent, linear gradient of Methanol:MTBE; <sup>d</sup> The peaks were quantified (n = 3) as being equivalent to all-*E*-lutein<sup>1</sup>, all-*E*- $\beta$ -carotene<sup>2</sup>, and all-*E*- $\alpha$ -carotene<sup>3</sup>; <sup>e</sup>Identified (standard available); n.c.: not calculated; Significant (p ≤ 0.05) differences of means within a line are indicated by different capital letters.



**Figure 4**: Chromatogram obtained by HPLC-DAD of carotenoids from saponified supercritical extracted (A) and pressed (B) oil of *Mauritiella armata*. Chromatographic conditions:  $C_{30}$  YMC column (5 µm, 250 mm × 4.6 mm); Mobile phase: linear gradient of a methanol/MTBE mixture; Mobile phase flow of 0.9 mL min<sup>-1</sup>. Peak characterization is given in Table 4.

Hydraulic press presented higher carotenoid extraction efficiency when compared to supercritical fluid extraction (10.6 and 2.0 mg.Kg<sup>-1</sup>, respectively). The low carotenoid content in the buritirana oil extracted by supercritical  $CO_2$  can be related to the cosolvent absence. The total carotenoid contents determined by HPLC in both buritirana oils were lower than those observed for buriti oil (608.39 - 686.89 mg.kg<sup>-1</sup>) (Ferreira et al., 2016; Freitas et al., 2017).

Additionally, the extraction method affected the carotenoid profile. The Z isomer contents were remarkably representative in the sample obtained by hydraulic pressing. While the contents of Z- $\beta$ -carotene and Z- $\alpha$ -carotene isomers represented, respectively, 19 and 9% of the total carotenoids in the oil obtained by supercritical fluid extraction, the pressed oil presented 34 and 17% of Z-isomers of  $\beta$ - and  $\alpha$ -carotene, respectively. This may be associated with the exposure of organic acids from the pulp and the heat generated due to mechanical energy dissipation during pressing, which may promote the isomerization of carotenoids (Mercadante, 2007). Moreover, all-*E*- $\alpha$ -carotene (26%) was shown to be the major compound in the supercritical fluid extracted oil, while pressed oil presented the same amount of all-*E*- $\beta$ -carotene and all-*E*- $\alpha$ -carotene (20%).

## **5.** Conclusions

Buritirana can be considered a good source of natural oil rich in bioactive compounds. Supercritical CO<sub>2</sub> was the most efficient technique for extracting oil from this fruit (13.44%), with the maximum rate and speed of recovery of oil and total carotenoids occurring in approximately 60 min. However, the highest antioxidant capacity,  $\alpha$ -tocopherol content (0.41119 mg.g<sup>-1</sup>) and carotenoids were found in the oil obtained by cold extraction. Regarding the fatty acid content, there was similarity between the two extraction methods used. On the other hand, supercritical CO<sub>2</sub> was responsible for less degradation or even greater extraction of bioactive compounds, mainly phenolic acids, flavonoids, and VOCs.

Thus, we can conclude that extraction with supercritical  $CO_2$  can be considered a potential technique to extract natural oils rich in bioactive compounds. However, despite the lower extraction yield, cold pressing can be an alternative for small producers to obtain buritirana oil in a more accessible way, with quality and good antioxidant capacity. In addition, further studies are needed that seek to evaluate the use of other extraction techniques to encourage greater exploitation of this natural oil and thus make the buritirana a species more exploited commercially.

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## **Conflict of interest**

The authors declare that they have no conflicts of interest.

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### 7. GENERAL DISCUSSION

Widely found in the Brazilian Cerrado, the buritirana has contributed to the subsistence and local economy of the peoples of that region, mainly because it is a very attractive palm tree with ornamental value and multiple uses. The palm tree as a whole is used for the construction of corrals, fences, beds, shelves, roofs, arches and corks, among others. The fruits, in addition to being used to make jellies, sweets, wine, beverages in general, can be made into handicrafts, such as necklaces, curtains, and other products. However, the key point of this research occurred when we discovered that buritirana was also used in folk medicine for the treatment of burns and skin wounds and as tea for rheumatism. However, little was known about this native palm to elucidate such mechanisms, which led us to carry out a literature review (Chapter I) with the objective of exploring and integrating this fruitful into commercial crops as a source of value-added ingredients and pointing it as a new species that can be investigated by the food, pharmaceutical and/or cosmetic industries and promote its commercial value, benefit family farming and contribute to the sustainable development of the country. In this sense, the studies found reported (although very timid) some ethnobotanical and morphological characteristics, few centesimal composition variables and a limited content of bioactive compounds. According to studies in relation to bioactivity, the presence of flavonoids in leaves, roots and petioles and some carotenoids in fruits was found.

In the face of the bibliographic survey carried out, even though it worked as a "fine comb", it still faced a scarcity of data that could indicate the potential of the buritirana. Thus, **Chapter II** consisted of a comprehensive characterization of the fruits and fractions of buritirana, in which the biometric and physicochemical, nutritional and functional attributes, bioactive compounds and antioxidant and antibacterial potential were evaluated. The results confirmed that buritirana is morphologically an oval drupe composed of peel, pulp and seed, representing 19.27%, 16.58% and 64.15%, respectively, in relation to the whole fruit. The association of very acidic pH, low acidity and high total soluble solids content was an important revelation, as these parameters demonstrated that the pulp and pulp+peel fractions can be used in fruit technology for the elaboration of many products. Among the characteristics of proximate composition, all fractions showed significant values of total carbohydrates, proteins, total lipids (except seeds), and fibers (soluble and insoluble), which added a great caloric value to buritirana. Nutritionally, all fractions of buritirana fruit showed significant amounts of minerals, vitamin C, glucose, fructose, sucrose (this only found in the seed) and maltose. On the other hand, fructooligosaccharides (1-kestose and nystose) that

have prebiotic activity were found only in the pulp+peel and seed fractions, and these can compose the formulation of products with functional attributes. Regarding bioactive compounds and antioxidant potential, phenolic compounds such as quercetrin, ferulic acid, sinapic acid, rutin, p-coumaric acid, epicathecin/cathecin, chlorogenic acid, protecatechuic acid, quinic acid and vitamin C contributed to better antioxidant capacity (DPPH, TEAC and ORAC<sub>HF</sub>) of pulp, peel and pulp+peel fractions. Referring to VOCs and antibacterial activity, the pulp did not reveal VOCs of interest, and none of the fractions that were used for the antibacterial assay showed potential against the bacteria tested (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella choleraesuis and Peseudomonas aeruginosa*).

Some data curious, but no less important found in Chapter II, such as the content of total lipids and phenolic compounds, which also corroborated with those mentioned in the literature review (Chapter I), provided the elaboration of Chapter III of this thesis. In this chapter, the extraction of oil from buritirana fractions (pulp, peel, seed and pulp+peel) was proposed for the first time using supercritical CO<sub>2</sub> at two temperatures, 40 °C and 60 °C. Then, the global yield and the behavior of total carotenoids in the extraction kinetics were evaluated, as well as the profiles of fatty acids, tocopherols, phenolic compounds and antioxidant capacity. With the exception of the seed that showed only traces of oil, the other fractions showed different yields (p < 0.05); however, there were no significant differences between the two temperatures in the same fraction (p > 0.05). Based on the fraction that presented the highest yield (pulp = 18.06%) and using a temperature of 40 °C to propose a better maintenance of the bioactive compounds, the oil yield and total carotenoids were monitored over time in the kinetics of extraction. The kinetic curves showed that the highest yield and the highest concentration of carotenoids (41.57 g.100 g<sup>-1</sup> and 8.34 mg of  $\beta$ carotene.g<sup>-1</sup>, respectively) were detected close to 60 minutes of extraction and that after 80 minutes remained constant. Thus, obtaining the oil must be interrupted to reduce the costs involved in the extraction process. The fatty acid composition, represented by saturated (palmitic and stearic) and unsaturated (oleic, linoleic and linolenic) acids, the alphatocopherol content and the antioxidant capacity determined by the TEAC and ORAC<sub>T</sub> assays (ORAC<sub>HF</sub> + ORAC<sub>LF</sub>) were submitted to analysis of principal components to facilitate the interpretation of the results between the variables-variables (analyses) and the treatments (samples), which in turn showed that the highest content of oleic acid was found in the pulp but was similar between the two temperatures of 40 °C and 60 °C (p > 0.05). In addition, the peel was marked with the highest values of palmitic stearic, linoleic, linolenic acids, TEAC, ORAC<sub>T</sub>, ORAC<sub>HF</sub>, ORAC<sub>LF</sub> and  $\alpha$ -tocopherol among the other fractions, but with similarities (p > 0.05) among linolenic acid, ORAC<sub>T</sub>, ORAC<sub>HF</sub>, ORAC<sub>LF</sub> and  $\alpha$ -tocopherol at the investigated temperatures. Regarding the profile of phenolics, the oil from the whole without seed fraction extracted at 40 °C showed the highest number and concentration of phenolic found by ESI-LTQ-XL-MS/MS and heatmap analysis, which were distributed into three categories: a) organic acids (maleamic, succinic, benzoic, fumaric, and cinnamic acids; b) phenolic acids (vanillic, gallic, caffeic, quinic, caffeoyl tartaric, caffeoylquinic, and glucosyringic acids) and c) flavonoids (quercetin, 5-deoxyleucopelargonidin, apigenin hexoside, catechin hexoside, kaempferol coumaroyl deoxyhexoside, apigenin caffeoyl hexoside, catechin dihexoside, and myricetin coumarylrobinobioside deoxyhexoside acid). Thus, buritirana oil also presented functional compounds with expressive antioxidant capacity that can be used in the elaboration of food and nonfood products, being another source of added value for this species.

In view of the interesting results obtained from the buritirana pulp+peel fraction already mentioned previously (Chapter II, and especially Chapter III, which refers to its oil) and not only considering the nutritional, functional, or even technological aspects but also the biological aspects, the fourth and final chapter of this study was created to complement some analyses that could not be performed in the previous chapter, such as VOCs, carotenoid profile and antibacterial activity. In addition, we present another method of oil extraction, mechanical cold pressing, and verify the influence of these extraction methods on yield and phytochemical, antioxidant and antibacterial characteristics. Not only to compare the mentioned variables but also as a means of showing another extraction alternative that allows the small producer to have access to the products and byproducts of the buritirana as a form of exploitation and commercialization. Oil extraction using supercritical CO<sub>2</sub> showed better yield (13.44%) compared to mechanical pressing (8.94%), and over a period of approximately 60 minutes, it reached approximately 26% oil yield and 15 mg of  $\beta\text{-carotene.g}^{\text{-1}}$  of total carotenoids. The methods used to extract the oil directly influenced the phytochemical composition (content of  $\alpha$ -tocopherol, fatty acids, phenolic compounds and VOCs) and the antioxidant capacity (TEAC and ORAC assays), since all the variables analyzed differed statistically from each other (p < 0.05). The phenolic compounds revealed by both methods were similar to those found previously (see results in the previous discussion, Chapter III), but with emphasis on supercritical CO<sub>2</sub>, which showed the highest relative intensity. The profile of volatile compounds was discovered only in the oil obtained by supercritical CO<sub>2</sub> (pentanoic and hexanoic acids; limonene, 1-butanol-3-methyl; hexanoic acid, ethyl ester; 3hexenoic acid, ethyl ester (e); ethyl 5-methylhexanoate; octanoic acid, ethyl ester; cislinaloloxide; 1,6-octadien-3-ol; 3,7-dimethyl, decanoic acid, ethyl ester; acetophenone; 1-αterpineol; and diphenyl ether), and among the mentioned compounds, hexanoic acid, ethyl ester and octanoic acid, ethyl ester were the majority in quantity (29.203% and 8.088%, respectively). Regarding the antibacterial potential, the oils obtained by both extraction methods did not show antibacterial activity against any of the microorganisms tested (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella choleraesuis and Pseudomonas aeruginosa*). Finally, the oils obtained revealed carotenoid profiles similar to those mentioned in other studies carried out in species of the same family. Carotenoids such as all-*E*-lutein, all-*E*-β-cryptoxanthin, 13-*Z*-β-carotene, all-*E*-α-carotene, 9-*Z*-α-carotene, all-*E*-β-carotene (2.16 μg.g<sup>-1</sup> of oil), all-*E*-β-carotene (2.16 μg.g<sup>-1</sup> of oil) and 9-*Z*-β-carotene (1.87 μg.g<sup>-1</sup> of oil) were the predominant carotenoids in the oil obtained by hydraulic press (p < 0.05), as well as total carotenoids (10.6 μg.g<sup>-1</sup> of oil).

## **8. GENERAL CONCLUSION**

Buritirana has characteristics as important as other species of the same family already recognized, such as palm oil, açaí and buriti. The study carried out on buritirana fruits and oil allowed us to reveal physicochemical and chemical composition, nutritional, functional and antioxidant potential results, which indicate that this fruit can be used in the elaboration of food and nonfood products with high added value.

Buritirana fruit is a good source of macro (proteins, carbohydrates, lipids, fibers) and micronutrients (Ca, Fe, Mg, Mn, Cu, K, Zn). It is rich in bioactive compounds, such as vitamin C, carotenoids, phenolics and fructooligosaccharides, as well as having outstanding physicochemical characteristics that show its delicious taste. Buritirana pulp and bark offer oil rich in vitamin E, fatty acids, carotenoids, phenolic and volatile compounds. Both fruit and oil have expressive antioxidant capacity due to the presence of bioactive compounds. In addition to all these aspects, its oil can be used for the production of biodiesel, since it presented a high concentration of oleic acid.

Obtaining buritirana oil using an emerging technology such as supercritical  $CO_2$  was a good choice, as supercritical  $CO_2$ , in addition to showing a high oil yield, ensured better maintenance of the bioactive compounds. On the other hand, mechanical pressing can be used to acquire good quality oil but with less efficiency in yield. Other methods of extraction can be investigated in future works to discover the best technique that allows the maintenance and preservation of the constituents of buritirana oil and that can be used as nutrients and functional compounds with biological appeal for the treatment of diseases.

It is worth mentioning the importance of studying the residue generated from oil extraction, as this byproduct may have the potential to be applied as an ingredient to enrich different formulations of food and nonfood products.

We believe we have established a "bridge", shortening the path for future research, where on one side was a palm fruitful native to the Cerrado, unexplored, and on the other a universe of expectations translated by the desire to promote, through science, the search for answers that could leverage what was apparently static - the Buritirana. However, behind it, there was a source of implicit variables with great potential for science and technology, arousing even more interest in the food and nonfood industries for this species that could be increasingly promising.

Finally, through this thesis, we have given a "start" to new investigations that may contribute not only to encouraging the planned cultivation of buritirana but also to preserving its species, and this species becomes useful for the different industrial segments of food, cosmetics, and pharmaceuticals.

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# ANNEXS

# ANNEX 1- Exsiccate no. (UEC 203433) Herbarium / Institute of Biology / UNICAMP



## ANNEX 2- Genetic patrimony access activity



#### Ministério do Meio Ambiente CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A0D53B3

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	A0D53B3
Usuário:	UNICAMP
CPF/CNPJ:	46.068.425/0001-33
Objeto do Acesso:	Patrimônio Genético
Finalidade do Acesso:	Pesquisa
Espécie	
Mauritiella armata	
Título da Atividade:	Perfil de carotenoides e avaliação do potencial funcional, Bioacessibilidade in vitro e atividade prebiótica da buritirana (mauritiella armata).
Equipe	
Florisvaldo Gama de Souza	UNICAMP
Iramaia Angélica Neri-Numa	Unicamp
Glaucia Maria Pastore	Unicamp

Data do Cadastro:

Situação do Cadastro:

26/02/2019 22:17:30

Concluído

Conselho de Gestão do Patrimônio Genético Situação cadastral conforme consulta ao SisGen em 16:58 de 02/03/2020.



SISTEMA NACIONAL DE GESTÃO DO PATRIMÓNID DENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SIS GEN

## ANNEX 3- Food Research International article presentation cover

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	Contents lists available at ScienceDirect	
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Brazilian fruits of Arecaceae family: An overview of some representatives with promising food, therapeutic and industrial applications

Florisvaldo Gama de Souza<sup>®</sup>, Fábio Fernandes de Araújo<sup>®</sup>, David de Paulo Farias, Aline Wasem Zanotto, Iramaia Angélica Neri-Numa, Glaucia Maria Pastore

Department of Food Science, Faculty of Food Engineering, University of Campinas, Brazil

## ANNEX 4- Publisher's authorization to include the article in the thesis

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	Publication: Food Research International					
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#### ANNEX 5- Foods article presentation cover





- Campinas 13083-862, SP, Brazil; alexandra.sawaya@fcf.unicamp.br
- Correspondence: florisvaldo.gs@gmail.com

## ANNEX 6- Publisher's authorization to include the article in the thesis

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## Characterization of Buritirana (*Mauritiella armata*) Fruits from the Brazilian Cerrado: Biometric and Physicochemical Attributes, Chemical Composition and Antioxidant and Antibacterial Potential

by 😮 Florisvaldo Gama de Souza <sup>1,\*</sup> 🖾 🗐 🔇 Fábio Fernandes de Araújo <sup>1</sup> 🖾 🔇 Eduardo Adilson Orlando <sup>1</sup> 🗁 😮 Fernando Morais Rodrigues <sup>2</sup> 🖾 🔇 Davy William Hidalgo Chávez <sup>3</sup> 🖾 🗐 🔇 Juliana Azevedo Lima Pallone <sup>1</sup> 🖾 🗐 😮 Iramaia Angélica Neri-Numa <sup>1</sup> 🖾 🔇 <u>Alexandra Christine Helena Frankland Sawaya</u> <sup>4</sup> 🖾 and 😩 Glaucia Maria Pastore <sup>1</sup> 🖂

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- <sup>3</sup> Department of Food Science and Technology, Federal Rural University of Rio de Janeiro, Seropédica 23890-000, RJ, Brazil
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- \* Author to whom correspondence should be addressed.

Academic Editor: Gian Carlo Tenore

Foods 2022, 11(6), 786; https://doi.org/10.3390/foods11060786

## ANNEX 7- Food Research International article presentation cover



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Evaluation of antioxidant capacity, fatty acid profile, and bioactive compounds from buritirana (Mauritiella armata Mart.) oil: A little-explored native Brazilian fruit

Florisvaldo Gama de Souza<sup>a,\*</sup>, Grazielle Náthia-Neves<sup>b</sup>, Fábio Fernandes de Araújo<sup>a,\*</sup>, Flavia Luísa Dias Audibert<sup>e</sup>, Jeany Delafiori<sup>e</sup>, Iramaia Angélica Neri-Numa<sup>a</sup>, Rodrigo Ramos Catharino<sup>e</sup>, Severino Matias de Alencar<sup>d</sup>, Maria Angela de Almeida Meireles<sup>b</sup>, Glaucia Maria Pastore<sup>a</sup>

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ANNEX 8- Publisher's authorization to include the article in the thesis

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TOOD RESEARCH INTERNATIONAL INTERNATIONAL	Evaluation of antioxidant capacity, fatty acid p (Mauritiella armata Mart.) oil: A little-explored Author: Florisvaldo Gama de Souza,Grazielle Náthia-Neves,Fábio Fern Neri-Numa,Rodrigo Ramos Catharino,Severino Matias de Aler Publication: Food Research International Publisher: Elsevier Date: April 2021 © 2021 Elsevier Ltd. All rights reserved.	profile, and bioact native Brazilian 1 andes de Araújo,Flavia ncar,Maria Angela de Al	<b>tive comp</b> fruit Luísa Dias A Imeida Meire	ounds from b udibert.Jeany Dela eles et al.	afiori,Irama	a ia Angélica
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# ANNEX 9- Proof of submission in Food Chemistry

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