UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

ALEXSANDRA PEREIRA RODRIGUES

MONGUBA (*Pachira aquatica* Aubl.) FRUITS: EVALUATION OF COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF THE SEEDS AND OIL OBTAINED BY USING SUPERCRITICAL CO_2 EXTRACTION

FRUTOS DE MONGUBA (*Pachira aquatica* Aubl.): AVALIAÇÃO DA COMPOSIÇÃO E PROPRIEDADES FÍSICO-QUÍMICAS DAS SEMENTES E DO ÓLEO OBTIDO POR EXTRAÇÃO COM CO_2 SUPERCRÍTICO

Campinas
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Thesis presented to the School of Food Engineering of the University of Campinas in partial fulfilment of the requirements for the degree of Doctor in Food Science.

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

Orientadora: Drª Glaucia Maria Pastore

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL
DA TESE DEFENDIDA PELA ALUNA ALEXSANDRA
PEREIRA RODRIGUES E ORIENTADA PELA PROFª
DRª GLAUCIA MARIA PASTORE

Campinas
2021

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

R618m

Rodrigues, Alexsandra Pereira, 1992-
Monguba (*Pachira aquatica* Aubl.) fruits : evaluation of composition and physicochemical properties of the seeds and oil obtained by using supercritical CO_2 extraction / Alexsandra Pereira Rodrigues. – Campinas, SP : [s.n.], 2021.

Orientador: Glaucia Maria Pastore.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Compostos fenólicos. 2. Atividade biológica. 3. Tocoferóis. 4. Triacilgliceróis. 5. Atividade antioxidante. I. Pastore, Glaucia Maria. II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Frutos de monguba (*Pachira aquatica* Aubl.) : avaliação da composição e propriedades físico-químicas das sementes e do óleo obtido por extração com CO_2 supercrítico
Palavras-chave em inglês:
Phenolic compounds
Biological activity
Tocopherols
Triacylglycerols
Antioxidant activity
Área de concentração: Ciência de Alimentos
Titulação: Doutora em Ciência de Alimentos
Banca examinadora:
Glaucia Maria Pastore [Orientador]
Bruno Nicolau Paulino
Mário Roberto Maróstica Junior
Francisco Fabio Cavalcante Barros
Alessandra Sussulini
Data de defesa: 21-07-2021
Programa de Pós-Graduação: Ciência de Alimentos

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0003-1654-9973
- Currículo Lattes do autor: http://lattes.cnpq.br/1952972157215686

Agência(s): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).
Nº do Processo: 88882.329537/2019-01 e 2015/50333-1.

Comissão Examinadora

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Universidade Estadual de Campinas – IQ

Membro titular

A ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

Agradecimentos

Primeiramente, agradeço a Deus pela dádiva da vida, por colocar as pessoas certas no momento certo. Pelos obstáculos vividos, os quais foram o motivo de “buscar uma solução para o problema” e assim, conduziu-me para finalização de mais uma etapa da minha vida.

A toda minha família, agradeço o apoio e incentivo durante os momentos de desabafo e reflexão, em especial aos meus pais e aos meus irmãos. Com carinho, dedico mais essa conquista a vocês.

Aos amigos e companheiros de laboratório do Bioaromas e Compostos Bioativos e aos de outros laboratórios, deixo meu sincero agradecimento pelos momentos de descontração, trocas de experiência e conhecimentos transmitidos. Não citarei nomes porque posso acabar esquecendo de alguém, mas agradeço por todos estes momentos.

Ao senhor Ivo e a dona Luzia pelo acolhimento em sua casa e as meninas da pensão, pelas conversas e risadas. Muito obrigada!

A todos os professores que marcaram e fizeram parte da minha vida, em especial a Gláucia Maria Pastore e Pedro Henrique Ferreira Tomé. A Gláucia por aceitar este projeto desde o primeiro momento que apresentei, pela orientação, dedicação e apoio, e por proporcionar os recursos necessário para realização desta pesquisa. Ao Pedro, que durante a graduação mostrou o “caminho das pedras”. O caminho da ciência, que não é tão reconhecido aos olhos de fora da academia, mas que ao mesmo tempo é gratificante, principalmente quando colhemos os frutos. Sou grata a vocês pelos ensinamentos e incentivos que contribuíram para o meu crescimento pessoal e intelectual.

Agradeço a banca pela disponibilidade e aceite em participar da minha defesa, além das contribuições e sugestões.

A todos os funcionários da Unicamp que direta ou indiretamente ajudaram na realização e conclusão desta tese.

Juntos faremos a diferença!

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de

Financiamento 001 e da Fundação de Amparo à Pesquisa do Estado de São Paulo - Brasil - (FAPESP) - processo nº 2015/50333-1.

RESUMO

As árvores da *Pachira aquatica* Aubl., conhecida vulgarmente por monguba, situadas na floresta tropical são encontradas em terrenos úmidos e alagadiços, expandindo-se desde a parte sul do México até o norte do Brasil. A monguba, assim como os demais frutos exóticos, apresentam domínios que se concentram e ficam retidos pela população local. Apesar de recentemente a monguba ter ganhado a atenção dos pesquisadores, muitas lacunas ainda precisam ser esclarecidas e esta tese vem contribuir para o preenchimento de uma das lacunas. Nessa tese focamos no óleo da monguba, pois com a revisão literária notamos que a monguba é uma espécie promissora devido à grande adaptabilidade geográfica com rápido desenvolvimento e por suas sementes apresentar elevado rendimento lipídico, permitindo a sua aplicação em vários setores industriais. Por esta razão, buscamos determinar os compostos fitoquímicos e açúcares das sementes da monguba, que ainda não haviam sido elucidados na literatura e assim avaliar a atividade antioxidante destes compostos e do óleo da monguba. Comparar o rendimento e qualidade do óleo obtido através do uso de tecnologia verde usando a extração de CO_2 supercrítica com a técnica de extração convencional (Soxhlet). Além disso, aprofundamos nossa pesquisa e determinamos os triacilgliceróis que compõem o óleo e assim entendemos a influência desses compostos no teor de sólidos, ponto de fusão, cristalização, polimorfismo e textura. Por fim, avaliamos os efeitos biológicos do óleo. Os resultados obtidos foram bastante promissores mostrando que tanto as sementes como o óleo da monguba apresentam potencial para aplicação alimentícia e farmacêutica de cosméticos. Entretanto, percebemos que muitas pesquisas ainda precisam ser realizadas no âmbito científico, para contribuir com pesquisas futuras e orientar no desenvolvimento de novos produtos.

ABSTRACT

The *Pachira aquatica* Aubl. trees, commonly known as monguba, located in the rainforest are found in wet, swampy terrain, spreading from southern Mexico to the northern of Brazil. Monguba, like other exotic fruits, have domains concentrated and retained by the local population. Although monguba has recently gained the attention of researchers, many gaps still need to be clarified and this thesis is willing to contribute to fill of them. In this thesis we focused on monguba oil, because in the literature review, we have noticed that monguba is a promising species due its great geographic adaptability with rapid development and because its seeds have high lipid yield, allowing its application in various industrial sectors. For this reason, we aimed to determine the phytochemical compounds and sugars of monguba seeds, which had not been elucidated yet in the literature and thus, evaluate the antioxidant activity of these compounds and monguba oil. Comparing the yield and quality of the oil obtained via green technology using supercritical CO_2 extraction with the conventional extraction technique (Soxhlet). In addition, we deepen our research and determined the triacylglycerols that from the oil and, thus, understood the influence of these compounds on solids content, melting point, crystallization, polymorphism, and texture. Finally, we evaluated the biological effects of the oil. The results obtained were very promising showing that both the seeds and the oil of monguba have potential for food and cosmetic pharmaceutical applications. However, we realize that plenty of research still needs to be done in the scientific field to contribute to future research and to guide the development of new products.

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General introduction

The biodiversity in Brazil is mostly diverse than any country in the world, with almost 19% of its flora. It embraces two ecological hotspots, the Cerrado and the Atlantic rainforest (Mata Atlântica) (Forzza et al., 2012). Globally, the tropical rainforest is the most biodiverse terrestrial biome on earth, it is estimated that approximately 45% of global plant diversity occurs in this biome (Eiserhardt et al., 2017). In this tropical region biome, we found the trees of *Pachira aquatica* Aubl. which covers from southern Mexico to South America (Janick & Paull, 2006; Robyns, 1964). In Brazil, the *Pachira aquatica* tree is found in the Northern and Northeast regions, and in the Southeast region (Rodrigues & Pastore, 2021), also it is popularly known as monguba, munguba or castanheira do Maranhão (de Oliveira et al., 2007; Peixoto & Escudeiro, 2002).

Monguba plant is used in traditional medicine by healers and midwives for the treatment of diabetes, vaginal infections, weakness, anemia, edema, and fatigue (Andrade-Cetto & Heinrich, 2005; Arvigo et al., 1998; Coe, 2008; Hernandez-Galicia et al., 2002). However, the toxicity effects of the monguba plant are still inconclusive and needs further research.

The seeds of monguba fruit tastes like peanuts, and the local inhabitants mix roasted, powdered seeds with milk to make a substitute for coffee (Rodrigues & Pastore, 2021). Monguba seeds are a good source of proteins and amino acids (leucine, valine, lysine, tryptophan, threonine, phenylalanine, and methionine) (Jorge & Luzia, 2012; Oliveira et al., 2000; Silva et al., 2010, 2015), carbohydrates, fibers (Jorge & Luzia, 2012; Rodrigues et al., 2019; Silva et al., 2020), and minerals (Leterme et al., 2006; Rodrigues et al., 2019). However, the predominant component presented in monguba seeds is lipids. According to the literature the seeds of monguba had shown an oil yield of, approximately, 53%. However, this yield value can variate depending on the geographical location (Rodrigues & Pastore, 2021). Monguba oil is composed of saturated and unsaturated fatty acids, prominently, palmitic, stearic, oleic, linoleic acids (Jorge & Luzia, 2012; Lopes et al., 2020; Rodrigues et al., 2019, 2021), and uncommon fatty acid (sterculic and malvalic acids)

(de Bruin et al., 1963; Spitzer, 1991), besides minor compounds such as tocopherols and sterols (Jorge & Luzia, 2012; Lopes et al., 2020; Rodrigues et al., 2019, 2021).

Plant-derived lipids are the second most important source for human nutrition, after carbohydrates, in addition, they are sources of several essential vitamins and nutrients. About 80% of the traded fats and oils are supplied from vegetable lipids, which most are used in the processed food sector (Murphy, 2006). This explains the growing interest in exotic vegetable oils that have monounsaturated fatty acids, carotenoids, and phytosterols in their composition.

Although knowing the physicochemical composition and nutritional properties of the oil is important, study the triacylglycerol composition and its behavior during the crystallization phases are factors that also must be considered because the triacylglycerol composition affects the polymorphic conformation, the microstructure, and solids contents, which, consequently, affect the texture properties.

Based on this information, this thesis comprehends a detailed study of the physicochemical composition of monguba seeds and its oil. For this purpose, we have quantified the phenolic compounds and carbohydrates in the seeds and extracted the oil from the monguba seeds using supercritical fluid extraction with carbon dioxide (CO_2) as solvent under different conditions, and the physical properties of monguba oil were evaluated. Moreover, we investigated the cytotoxic and photoprotective effects of monguba oil obtained by supercritical CO_2 process. Therefore, the present thesis aims to draw attention to potential applications of monguba, as well to warn the importance of Brazilian biodiversity conservation.

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Objectives

General objectives

The aim of this thesis was evaluating the physicochemical composition of seeds and oil; and the obtaining of high-quality oil by using green extraction based on supercritical carbon dioxide (SC-CO_2) of monguba seeds (*Pachira aquatica* Aubl.). The following specific objectives were defined to achieve the overall goal of this thesis:

Specific objectives

❖ Present current knowledge and perspectives about monguba and its potential use as a food and therapeutic plant.

❖ To evaluate the physicochemical and proximate composition of monguba seeds.

❖ To extract and characterize the soluble and insoluble-bound phenolic compounds of monguba seeds.

❖ To extract oil from monguba seeds using SC-CO_2 extraction.

❖ To evaluate the impact of SC-CO_2 process under different conditions (temperature and pressure) on the yield oil and physicochemical composition of monguba seeds.

❖ To evaluate the composition of triacylglycerols and their behavior in crystallization, microstructure, and textural properties of monguba oil obtained by SC-CO_2 process.

❖ To evaluate the cytotoxic activity of monguba oil against melanoma cell line and its photoprotective effect against UV irradiation.

Chapter 1

Critical Review

A review of the nutritional composition and current applications of monguba (*Pachira aquatica* Aubl.) plant

Alexsandra Pereira Rodrigues and Glaucia Maria Pastore

Article published in Journal of Food Composition and Analysis

(Impact Factor 3.721 – Qualis A1)

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(Corrigendum of this research article can be seen in the Annex 3)

The article can be accessed through the following link:
https://doi.org/10.1016/j.jfca.2021.103878

Journal of Food Composition and Analysis 99 (2021) 103878

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Critical Review

A review of the nutritional composition and current applications of monguba *(Pachira aquatica* Aubl.*)* plant

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ARTICLE INFO

Keywords:
Munguba
Chestnut
Phytochemical
Nutritional value
Health benefits
Functional food
Oilseed

ABSTRACT

The monguba (*Pachira aquatica* Aubl.) tree is distributed from southern Mexico to South America. This plant has a variety of applications, including handicraft works, the consumption of its seeds as an alternative to coffee consumption, and the use of monguba bark and seeds in the treatment of vaginal infections in folk medicine. This review aims to draw the attention of researchers to the nutraceutical potential, physicochemical and nutritional composition, and bioactive compounds in the monguba plant for applications in the food, and other industries. After searching for publications containing '*Pachira aquatica*', 'monguba' or 'munguba' in titles and abstracts from 1878 to 2021 in six databases, the resulting publications were analyzed, and 54 full-text articles were included in this review. We observed that monguba has not been much explored and it is not widely known, though it is a novel oilseed with nutritional potential since it is rich in oils, proteins, minerals, fibers and phytochemicals. However, in vivo studies are required to verify the current uses of monguba in folk medicine by analyzing potential effects of the fruit, in addition to the compounds related to such bioactivity.

1. Introduction

The *Pachira aquatica* Aubl. plant is often found on wetlands near lakes and rivers. It is native to the tropical regions, and it is distributed from southern Mexico to South America (Janick and Paull, 2006; Robyns, 1964). *Pachira aquatica* is also known as monguba, castanheira d'água, munguba, castanheira do Maranhão, among other names (de Oliveira et al., 2007; Peixoto and Escudeiro, 2002). *Pachira aquatica* is a fast-growing tree that grows around 30-meter high. Moreover, monguba is used for city urbanization and as an ornamental plant (Infante, 2004; Janick and Paull, 2006; Lorenzi, 2008; Silva et al., 2012). Monguba has been known and used by healers, indigenous, and midwives for the treatment of some diseases, which use has been adopted by the local community. There are also reported antimicrobial activities against some pathogenic microorganisms.

Monguba seeds are consumed by animals such as swine and cattle, and by people, who consume them boiled, raw or baked (Janick and Paull, 2006; Lorenzi, 2008). The main components of monguba seeds are lipids, proteins, carbohydrates, calcium, magnesium, and potassium, in addition to bioactive compounds such as tocopherols, phenolic compounds and significant amounts of the essential amino acids threonine and tryptophan (Jorge and Luzia, 2012; Oliveira et al., 2000; Rodrigues et al., 2019).

Currently, there has been a growing interest in the health potential of bioactive compounds from plant-based foods. Therefore, in this review, we expect to draw the attention of nutrition and bioactive compound researchers to the potential of *Pachira aquatica* for further studies and applications. The nutritional properties and the phytochemical composition of monguba are the focus of this review. In addition, we discuss the botanical and toxicity information of this plant, as well as the antioxidant and biological properties of this plant phytochemicals, and the use of monguba as a food, a cosmetic, and therapeutic.

2. Strategy and criteria for selecting of publications

The data collection for this review started by searching for the keywords '*Pachira aquatica*', 'monguba' and 'munguba' in publications from 1878 to 2021 in six databases: Scopus, Science Direct, Google Scholar, Scielo, Medline (PubMed), and Web of Science. All studies that mentioned at least one of these words in the title and abstract were

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https://doi.org/10.1016/j.jfca.2021.103878
Received 9 September 2020; Received in revised form 2 March 2021; Accepted 3 March 2021
Available online 5 March 2021

selected for full-text review. After removal of all the duplicates, the initial search resulted in 209 references from all databases. Then, the authors screened the full-texts for the inclusion criteria, and only 54 studies were included in this review. The inclusion criteria were (I) growth and habitat, (II) biological properties, (III) folk medicine, (IV) nutritional composition, (V) phytochemical compounds, (VI) industrial field applications.

3. Monguba (*Pachira aquatica* Aubl.) tree: botanical information, taxonomy, growth habitat

3.1. Taxonomy

The classification report about *Pachira aquatica* was retrieved from the Plants Database of the United States Department of Agriculture (USDA) (USDA-NRCS, 2019).

Kingdom: Plantae.
Subkingdom: Tracheobionta.
Superdivision: Spermatophyta.
Division: Magnoliophyta.
Class: Magnoliopsida.
Subclass: Dilleniidae.
Order: Malvales.
Family: Bombacaceae.
Genus: *Pachira* Aubl.
Species: *Pachira aquatica* Aubl.

It is important to highlight that although *Pachira aquatica* was previously classified as belonging to the Bombacaceae family, molecular studies have shown that the species is actually a member of the Malvaceae family (Lorenzi, 2008).

3.2. Botanical information

The genus Pachira has 24 species, among which two species (*Pachira aquatica* Aubl. and *Pachira insignis* Schum) contain edible seeds. *Pachira aquatica aquatica* (popularly known as "*monguba*", "*apombo*", "*cacao de playa*", "*wild cacao*", "*sapotolón*", "*pumpo*", "*mamorana grande*", etc.) is native to the tropical regions of America and it is distributed from southern Mexico (Gulf of Mexico), Belize and Guatemala to Panama and South America (Guyana and Brazil). In Brazil, monguba is found in the Northern and Northeast regions, represented by the states of Amazônia, Pará, Maranhão and Pernambuco, and in the Southeast region in the states of Rio Janeiro and São Paulo. The monguba tree was also introduced into Guangdong, Africa, Gaboon, Congolese Republic, southern Yunnan and Taiwan. Moreover, the monguba tree is becoming an ornamental plant in the United States, Asia and Europe (Cheng et al., 2017; De Bruin et al., 1963; Hamad et al., 2017; Infante, 2004; Janick and Paull, 2006; Li et al., 2009; Lorenzi, 2008; Robyns, 1964). The species grows normally on inundated river banks, flooded plains, and on a range of fertile permeable soil types. The monguba trees can also stand sandy or clayey soils as long as drainage is adequate. Preferentially, this tree is always found in moist ground; however, it could be found in a variety of Brazilian urban regions since it easily adapts to the soil (Janick and Paull, 2006; Lorenzi, 2008; Peixoto and Escudeiro, 2002).

The monguba tree grows under partial shade or full sun and it can reach 4–30 meter high depending on the climatic conditions. This tree has a huge and dense canopy, a rounded shape with a trunk of 25–90 cm diameter, and a thick, smooth and gray to brownish bark (Fig. 1A and B). The leaves are palmate, composed of 5–9 lanceolate leaflets, and clustered towards the branch ends, are shiny green to smooth dark green with papyraceous to leathery texture, and the leaf beam is glabrous (Fig. 1C). The margin of the leaf is undulating, entire and glabrous. The monguba tree has foliage all year round (De Bruin et al., 1963; de Oliveira et al., 2007; Du Bocage and Sales, 2002; Infante, 2004; Janick and Paull, 2006; Jorge and Luzia, 2012; Peixoto and Escudeiro, 2002).

The flowering of *Pachira aquatica* can occur at different times of the year depending on growing region, but this event can be observed specially from September to November or from December to August (Hernández-Montero and Sosa, 2016; Infante, 2004; Lorenzi, 2008). The flower buds of *Pachira aquatica* are large (28 cm long), yellowish, narrowly cylindrical and slightly curved at the apex (Fig. 1D). The flower buds take about 6 weeks to achieve total flower opening. Floral anthesis occurs during the early evening, when the flowers mostly exhale an intense odor (sweet and floral) (Angel-Coca et al., 2014; Hernández-Montero and Sosa, 2016; Pale-Ezquivel et al., 2018; Peixoto and Escudeiro, 2002). The flowers are hermaphroditic, bell-shaped,

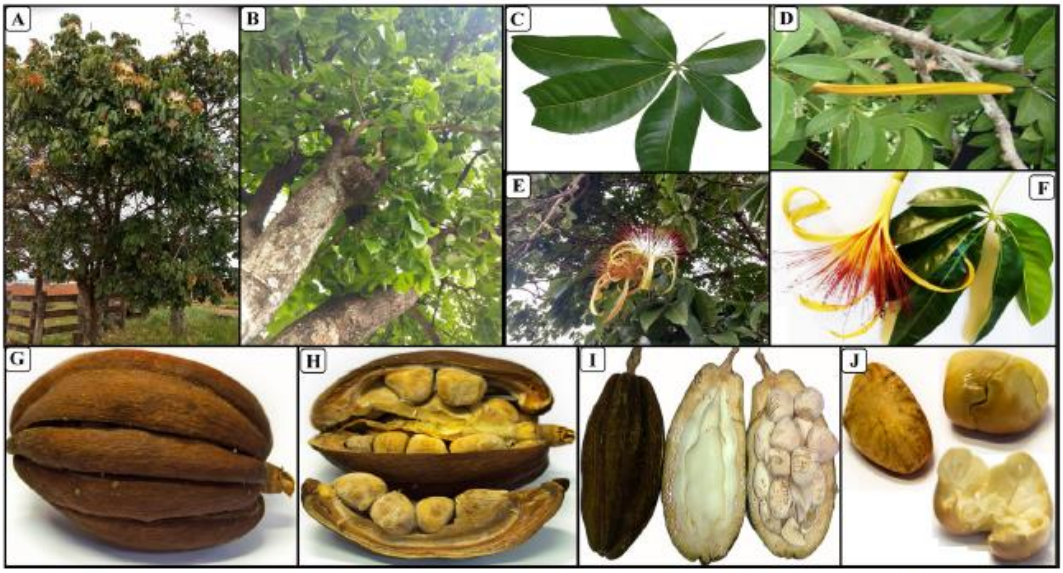


Fig. 1. *Pachira aquatica* Aubl. A, Tree and flowers; B, Trunk and canopy; C, Leaves; D Flower bud; E and F, Leaves and flowers; G, Ripe fruit; H, Open ripe fruit and seeds; I, Immature fruit (left: peel and right: open fruit and seeds undeveloped);J, Seeds (left: seed with endocarp, right side up: seed without endocarp, and right side below: open seed). Authors: Alexsandra Pereira Rodrigues (picture from A, B, C, E to J), and Catalina Angel-Coca (picture D).

solitary (sometimes they are grouped in three or four), and can reach up to 24 cm in diameter and 21.5–35 cm in length (Fig. 1E and F). The petals are narrow, 17 cm long, 2.1 cm wide, greenish, whitish or yellowish, and they have an acute to obtuse shape with a sharp apex. The stamens are heterodyne, and their basal part is white with pinkish-reddish extremities, and they can contain from 200 to 260 filaments per flower, and its anthers are reddish to scarlet. The calyx is cupuliform and tubular, puberulent to tomentulous, its fasciculate is brown-yellowish to greenish, and it has many white silky-plush trichomes on the inner surface (nectary). Each ovaries has a style up to 28 cm long, which is slightly dilated with white basal portion, plush texture, reddish apex, and lobed reddish stigma (Angel-Coca et al., 2014; Du Bocage and Sales, 2002; Hernández-Montero and Sosa, 2016; Infante, 2004; Janick and Paull, 2006; Peixoto and Escudeiro, 2002).

Pachira aquatica fruits are produced between January and September, predominantly between April and June, and they take four months to mature. In Mexico, the monguba fruiting period extends from January to September (Hernández-Montero and Sosa, 2016; Infante, 2004; Lorenzi, 2008). The shape of the *Pachira aquatica* fruit is similar to the cocoa fruit. Its fruit is a large, subglobular capsule, ellipsoid to oblong-ellipsoid and slightly longitudinally 5-furrowed, with an obtuse round and emarginate apex. It is dark brown when ripe, it contains a semi-woody peel, and its inside peel contains a fibrous mesocarp (Fig. 1G and H). The large seeds (1.2–2 cm in diameter) are protected by the white pericarp of spongy consistency when the fruit is immature (Fig. 1I). The fresh fruit measures 12.5–30 cm in length, 6–13 cm in diameter, weighs 713.32–1,522 g, and there are from 18 to 39 seeds per fruit on average (Du Bocage and Sales, 2002; Espitia et al., 2018; Infante, 2004; Janick and Paull, 2006; Peixoto and Escudeiro, 2002). Seeds (Fig. 1J) have an average length, width and thickness of 2.5–5, 2.8–3.7 and 2–4 cm, respectively, with a mean weight of 13 g (de Oliveira et al., 2007; Espitia et al., 2018; Infante, 2004; Silva et al., 2012).

The *Pachira aquatica* tree has rapid growth, and it is used for the afforestation of cities because of its beautiful flowers and leaves. Monguba seeds taste similar to peanuts or *Castanea vesca* nuts and are usually consumed raw, boiled, fried, roasted, or ground into flour for bread making (Bailey et al., 1976; Jorge and Luzia, 2012; Silva et al., 2014). Local inhabitants use to toast the seeds and mix the powder in milk to obtain a substitute for coffee, resulting in a drink which aroma and color are very similar to chocolate. In Nigeria, *Pachira aquatica* seed is used for the production of local condiments (Lawal et al., 2015). Moreover, its leaves and flowers can be cooked and consumed such as in salads (Dourado et al., 2015; Lorenzi, 2008; Oliveira et al., 2000; Viana et al., 2011). Monguba wood, which is soft, light, porous and fibrous, is used in the manufacture of pulp for paper, boxes and packaging, in handcrafting, and in carpentry work. The fibers of the wood and fruits can be used for mattress production and pillow stuffing. In addition, monguba bark provides a dark red ink and it is used to dye sails, lines, and fishing nets (Viana et al., 2011).

4. Composition and nutritional value of *Pachira aquatica* Aubl. Fruit

4.1. Proximate and physicochemical composition

Fresh monguba fruits show pH of 6.69, acidity of 1.30 g oxalic acid/100 g and 24.00°Brix (Rodrigues et al., 2019). Similar values of pH were reported by Becker et al. (2018), while the authors obtained acidity via citric acid of 0.34 g/100 g, and vitamin C content of 5.20 mg/100 g. Green ripe monguba seed flour present pH of 6.27 and titratable acidity (ATT) of 4.83 g/100 g (Lopes et al., 2020). In addition, Silva et al. (2020) observed pH of 5.66, acidity of 3.66 g/100 g using citric acid, and 21.33°Brix in raw monguba seeds.

Previous studies of monguba seeds reported that it as a good source of lipids (38.39–53.90 % of dry matter, DM), protein (11.74–16.90 % DM) and carbohydrates (25.83–41.60 % DM) (Jorge and Luzia, 2012; Lopes et al., 2020; Oliveira et al., 2000; Silva et al., 2015, 2020). Becker et al. (2018) reported lower values of lipids (18.67 %), protein (2.44 %), and carbohydrates (7.17 %). This difference in the proximate composition can be influenced by a variety of factors such as weather conditions, geographical localization, and soil type.

Brazil nut shows higher lipids (65 % DM) and protein (22 % DM) content (Moodley et al., 2007) than monguba seeds, while peanut oil had two times more protein and four time more fiber than monguba seeds (Zahran and Tawfeuk, 2019). However, *Moringa oleifera* and Maya nut presented lower content of lipids, protein and crude fiber (del Carmen Quintero-Hilario et al., 2019; Zhao et al., 2019) than monguba seeds.

Rodrigues et al. (2019) recently reported 13.04 % fresh weight (FW) of raw fiber in monguba seeds, while Silva et al. (2020) obtained 17.75 % DM of raw fiber in raw monguba seeds (Table 1). The raw fiber data in monguba seeds was higher than reported for Maya nut (3.98–6.70 % FW) and *Moringa oleifera* (2.94–7.50 %) (del Carmen Quintero-Hilario et al., 2019; Zhao et al., 2019). Therefore, monguba seeds may be a novel source of fibers, because of 100 g of monguba seeds corresponds to an intake of approximately 49.52 % of the recommended daily fiber intake according to the American Heart Association (Van Horn, 1997). In addition, the World Health Organization (WHO) recently published that the daily intake of fibers can promote health benefits (Reynolds et al., 2019).

4.2. Carbohydrates

Rodrigues et al. (2019) recently quantified the individual carbohydrates and the raffinose family of oligosaccharides in monguba seeds. In this study, they observed a total of mono- and disaccharides around 4.24 g/100 g and 0.63 g/100 g of dry weight, respectively (Table 1), and they also detected seven classes in the raffinose family of oligosaccharides, namely: sucrose, maltose, glucose, fructose, raffinose, verbascose, and stachyose.

Even though monguba seeds present significant amounts of carbohydrates, these compounds have not been explored anywhere else besides our previously mentioned study. Analyses such as quantification of starch content, production of functional sugars and quantification of soluble and insoluble fibers would contribute to the current literature about monguba composition.

4.3. Proteins and amino acids

A study that analyzed the amino acid composition of monguba seeds after extraction of their protein reported 28.27 % protein in defatted monguba flour (Silva et al., 2010). The major protein fraction in defatted monguba flour was globulin, followed by albumin, glutelin, and prolamine. The main essential amino acids were leucine, valine and lysine, corresponding to 7.97, 7.16 and 5.27 g per 100 g protein, respectively. Consequently, the highest non-essential amino acids in monguba flour were aspartic acid (12.70 g/100 g) and glutamic acid (17.11 g/100 g). As expected, the amount of sulfated amino acids was considerably lower in monguba oilseed when compared to other vegetable flours. Oliveira et al. (2000) reported that monguba seeds present significant amounts of tryptophan, threonine and phenylalanine + tyrosine, and that methionine + cysteine are their first-limiting amino acids.

Regarding the proteins of monguba seeds, Azevedo et al. (2011) studied their functional properties and characterized them after an acetylation process. The results of modified lysine showed that the lysine amount increases as the concentration of acetic anhydride increases. The authors also observed that acetylated derivatives reduce their solubility as pH increases. Moreover, the absorption capacity of water and oil improved after acetylation in purified native protein from monguba seed. The emulsifying capacity increased at pH 7 and 9. As a follow-up of this study, Silva et al. (2015) characterized two isolated proteins at pH 2 and 10, which resulted in a yield of 82.06 % and 92.18 % protein,

Table 1
The proximate composition and physicochemical proprieties of monguba (*Pachira aquatica* Aubl.) seeds and its oil.

	Parameter	Value	Refs.
Seeds			
	Moisture (%)	3.47-5.55	
	Lipid (%)	38.39-53.90	
	Protein (N x 6.25) (%)	11.74-16.90	
	Ash (%)	3.7-7.49	
	Total Carbohydrate (%)	25.83-41.60	
	Total Raw Fiber (%)	12.38-17.75	
	Starch (%)	9.7	
	Total Energy Value (Kcal/100g)	529.01-560	
	pH	5.66-6.69	
	TSS (° Brix)	21.33-24.00	
	TTA (g/100g)	1.30-4.83	
Vitamins (mg/100g)			(Becker et al., 2018; De Bruin et al., 1963; Janick & Paull, 2006; Jorge & Luzia, 2012; Silva et al., 2015; Silva et al., 2010; Lopes et al., 2020; Silva et al., 2020)
	Ascorbic acid	5.20-25.4	
	Vitamin A (IU)	1300	
	Niacin	4.02	
	Riboflavin	0.06	
	Thiamine	0.03	
Amino acids (g/100g)			
	Leucine	7.97	
	Isoleucine	4.97	
	Threonine	3.71	
	Phenylalanine	4.90	
	Lysine	5.27	
	Valine	7.16	
	Methionine+Cysteine	2.42	
	Histidine	1.51	
	Aspartic acid	12.70	
	Glutamic acid	17.11	
	Serine	5.38	
	Glycine	5.06	
	Arginine	8.56	
	Alanine	5.38	
	Proline	3.73	
Oil			
	Moisture (%)	0.3	
	Free acid (mg KOH/g)	2.06-3.8	
	Peroxide value (meq/Kg)	0-4.88	
	Melting point (°C)	38.6-41.9	
	Saponification value (mg KOH/g)	181.70-208	(De Bruin et al., 1963; Jorge and Luzia, 2012; Raiser et al., 2018, 2020)
	Density (g/cm^3)	0.917-0.929	
	Iodine value (I_2/100g)	27.4-85.6	
	Refraction index	1.45-1.47	

Total carbohydrate content was calculated by difference: 100 − (moisture + ashes + lipids + proteins).
The total energy value was estimated by considering the conversion factors of 4 kcal/g for protein and carbohydrate and 9 kcal/g for lipids.

respectively, and great solubility. At pH 10 absorption and emulsifying capacity were higher than at pH 2. There was higher activity and emulsion stability at pH 2 and 8 in the isolated proteins. The defatted monguba flour showed tannin content of 6.34 mg/g. It is known that the tannins are responsible for decreasing the digestibility of proteins and absorption of nutrients. However, it is necessary to evaluate the anti-nutritional compounds in monguba seeds and whether the amount of these compounds could affect the absorption of nutrients.

As assumed above, the authors suggest that the high emulsifying capacity of monguba seed protein is a potential for its application in the food industry for the production of ice cream, mayonnaise, and meat emulsion.

4.4. Lipids and tocopherols

Monguba oil has low density, *Sui generis* smell, and a similar behavior to coconut oil, it is yellowish, liquid at higher temperatures (above 25 °C; ranging from 38 to 42 °C) and solid at lower temperatures (Raiser et al., 2018; Silva et al., 2015). (Jorge and Luzia, 2012) reported that palmitic acid (44.93 %) is the major saturated fatty acid in monguba oil while oleic and linoleic acids (39.27 % and 11.35 %, respectively) are the major unsaturated fatty acids in the oil (Table 2). However, another study reported that palmitic acid (56 %) was the major oil in monguba seeds while oleic acid (7.5 %) and linoleic acid (5%) were at low concentrations in monguba oil (De Bruin et al., 1963). The same authors also reported sterculic-type acid (26.5 %) in monguba oil. Sterculic-type acid is a naturally occurring cyclopropene fatty acid that may inhibit tumour growth and that is derived from a number of plant families (i.e., Sterculiaceae, Malvaceae, Tiliaceae, and Bombacaceae) (Khoo et al., 1991; Yano et al., 1972). Spitzer (1991) reported a higher amount of palmitic acid (63.3 %) than the other authors, and lower concentration of sterculic acid (8.16 %) in monguba oil. Similar value of palmitic acid was reported by Rodrigues et al. (2019). An even higher value of palmitic acid (75.27 %) was observed in the monguba oil by Raiser et al. (2020) via ultrasound-assisted method using hexane as a solvent. Zhao et al. (2019) characterized *Moringa oleifera* oil and they found lower saturated and higher unsaturated fatty acids content, among which oleic acid was predominant (70.2 %). This concentration of oleic acid was higher than those reported from monguba oil (Jorge and Luzia, 2012; Rodrigues et al., 2019; Spitzer, 1991; Raiser et al., 2020; De Bruin et al., 1963; Sunday, Gillian, & John, 2019).

According to the literature monguba oil has a total of saturated fatty acids, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ranging from 46.67–84.87 %, 6.62–39.30% and 5.24–11.81%, respectively. Similar values of MUFA and PUFA were reported by Standards Malaysia (MS814:2007) from palm oil (Standards Malaysia, 2007). However, peanut oil showed a higher composition of MUFA (55.47–62.93 %) and PUFA (20.81–25.35 %) and lower composition of palmitic acid (12.01–15.00 %) (Zahran and Tawfeuk, 2019). In addition, soybean, sunflower, corn and rapeseed oil also presented higher MUFA and PUFA concentrations than those reported from monguba oil (Li et al., 2011).

The main tocopherol that has been reported in monguba oil is γ-tocopherol followed by α-, β- and δ-tocopherol (Fig. 2) with concentrations of 34.66 mg/kg, 15.23 mg/kg, 1.13 mg/kg and 0.26 mg/kg, respectively (Jorge and Luzia, 2012). High values of γ-tocopherol (51.35 mg/kg) were reported by Rodrigues et al. (2019) using the same organic solvent (petroleum ether) used by Jorge and Luzia (2012), while Lopes et al. (2020) reported 612.90 mg/kg of γ-tocopherol using n-hexane by Soxhlet method (Table 2). These differences in tocopherol content in monguba oil might be due to varietal differences, maturity time and growing conditions which, in agreement with the observation by Rodrigues et al. (2021) affect the chemical composition of monguba seeds and suggested that tocopherol content differences among the fruits might also be related with the time of fruit maturity. Lopes et al. (2020) also evaluated the effects of different extraction conditions and pressurized solvents in *Pachira aquatica* seed oil, and they obtained higher oil yields (32.8–41 %), palmitic acid (78–11-79.92 %) and linolenic acid (6.32–7.35 %), γ-tocopherol (705.00–820.80 mg/kg), stigmasterol (11.01–15.45 mg/100 g), and β-sitosterol (110.14–130.47 mg/100 g)

Table 2
The main compounds from monguba *(Pachira aquatica)* plant.

	Compound	Value	Refs.
Seeds			
Carbohydrates (g/100g DW)			
	Glucose	0.29	
	Fructose	0.23	
	Sucrose	3.72	
	Maltose	n.d.	(Rodrigues et al., 2019)
	Raffinose	0.23	
	Stachyose	0.41	
	Verbascose	n.d.	
Minerals (mg/100g)			
	Calcium	55.89-158.38	
	Iron	0.44-4.0	
	Phosphorus	302.3	
	Potassium	700-1461.84	
	Sodium	1.14-76.1	(Becker et al., 2018; Janick and Paull, 2006; Rodrigues et al., 2019)
	Magnesium	87.53-303.99	
	Manganese	0.20-1.01	
	Zinc	0.99-2.58	
	Cupper	0.75-2.26	
Phenolic compounds (μg/g DW)			
	Gallocatechin	1.08	
	Quercetin	0.34	
	Protocatechuic acid	4.17	
	4-Hydroxybenzoic acid	118.88	
	Gentisic acid	1.05	(Rodrigues et al., 2019)
	Chlorogenic acid	58.82	
	Caffeic acid	445.54	
	p-Coumaric acid	26.33	
	Vanillic acid	2.90	
	Ferulic acid	116.07	
Oil			
Fatty acids (%)			
	Palmitic acid	44.90-76.19	
	Stearic acid	1.77-8.68	
	Malvalic acid	1.63	
	Oleic acid	6.62-39.30	(Dourado et al., 2015; Jorge & Luzia, 2012; Rodrigues et al., 2019; Spitzer, 1991; Sunday et al., 2019; Lopes et al., 2020; Raiser et al., 2020; De Bruin et al., 1963)
	Linoleic acid	5.00-11.35	
	Linolenic acid	0.24-0.46	
	Sterculic acid	8.16-26.5	
	Saturated fatty acids (SFA)	46.67-84.87	
	Monounsaturated fatty acids (MUFA)*	6.62-39.30	
	Polyunsaturated fatty acids (PUFA)	5.24-11.81	
Tocopherols (mg/kg oil)			
	α-tocoferol	15.23	
	β-tocoferol	1.13	(Jorge & Luzia, 2012; Rodrigues et al., 2019; Lopes et al., 2020)
Phytosterols (mg/100g oil)	γ-tocoferol	34.66-820.8	
	δ-tocoferol	0.26	
	Campesterol	10.11-14.19	(Lopes et al., 2020)
	Stigmasterol	11.01-17.00	
	β-sitosterol	89.70-130.47	

n.d., not detected.
DW, Dry weight.
* Malvalic and Sterculic acid values not included.

content by using pressurized n-propane as a solvent. Rodrigues et al. (2021) who studied the oil yield and tocopherol content of monguba oil by supercritical carbon dioxide extraction (in range of 6.63–39.64 % and 48.37–54.75 mg/kg, respectively). The total tocopherol content of *Moringa oleifera* oil and commercial oils such as soybean, sunflower, corn, and canola analyzed by (Grilo et al., 2014; Zhao et al., 2019) was higher that the content observed in the monguba oil extracted by Jorge and Luzia (2012) and Rodrigues et al. (2019). However, the study of Lopes et al. (2020) observed the opposite result. Based on this data, studies comparing the bioactive content of monguba oil based on planting location or genotype (for example, plants differing in fruit or seed color) are necessary since the maturity time and growing conditions of the plant could influence the bioactive composition of this oil.

Regarding the physicochemical properties, monguba oil showed low moisture, melting point and saponification values ranging from 38.6–41.9 °C and 103.86–208.0 mg/KOH g oil, respectively, and density of 0.917 g/cm^3 (Table 1) (Camargo, 2008; De Bruin et al., 1963; Jorge and Luzia, 2012; Raiser et al., 2018). In this way, monguba oil can be a new source of oil for human consumption (Jorge and Luzia, 2012).

4.5. Minerals: macro and microelements

Rodrigues et al. (2019) used a flame atomic absorption spectrometer to determine the concentration of some minerals in monguba seeds (Table 2). Among the macroelements, potassium (1461.84 mg/100 g dry weight, DW) was the main mineral, followed by magnesium, calcium and sodium (304.00, 158.37 and 1.90 mg/100 g DW, respectively). The microelements analyzed were Fe (1.65 mg/100 g DW), Zn (2.58 mg/100 g DW), Cu (2.26 mg/100 g DW), and Mn (1.01 mg/100 g DW). Similar results were reported by Leterme et al. (2006) who observed that monguba contained more P, Mg, Zn, Fe and Cu than other fruits and other starchy foods.

Another study that determined the concentrations of minerals: macro and microelements in fruits of the Amazon Rainforest, including monguba, used Inductively Coupled Plasma-Atomic Optical Spectrometry (ICP AOS) and reported 87.53, 55.89, 1.14, 0.99, 0.75, 0.44 and 0.20 mg/100 g of Mg, Ca, Na, Zn, Cu, Fe and Mn in monguba, respectively. Surprisingly, we found that monguba had much higher amounts of Ca, K, Mg, Cu, and Zn (results are expressed on a wet weight basis) than native Amazon Rainforest fruits, Maya nut, *Moringa oleifera* seed, almond, hazelnut, macadamia nut and pecan nut (Becker et al., 2018; del Carmen Quintero-Hilario et al., 2019; Zhao et al., 2019).

These minerals are essential for human nutrition, and they play an important role in many metabolic processes such as blood pressure maintenance, hemoglobin production, and immune system improvement (WHO, 2018). Thus, the consumption of monguba seeds could contribute to these health benefits.

5. Phytochemicals of *Pachira aquatica* Aubl. Seeds

Polyphenols are defensive secondary metabolites synthesized by plants. They play a fundamental metabolic role in plants, such as defense against predators and microorganisms, protection against ultraviolet radiation and oxidative stress, and as signal compounds to attract pollinators. These chemical compounds are divided into flavonoids and polyphenolic compounds (Vuolo et al., 2018), and have shown promising antioxidant properties. Some authors have evaluated and identified the total phenolic composition of monguba seeds although *in vitro* techniques for the analysis of total phenolic content are non-specific and present disadvantages such as interaction with proteins, sugars and other compounds (Sánchez-Rangel et al., 2013).

5.1. Flavonoids and anthocyanins

Flavonoids compounds are mostly found in fruits and vegetables, they are responsible for the color and aroma of flowers and fruits, and

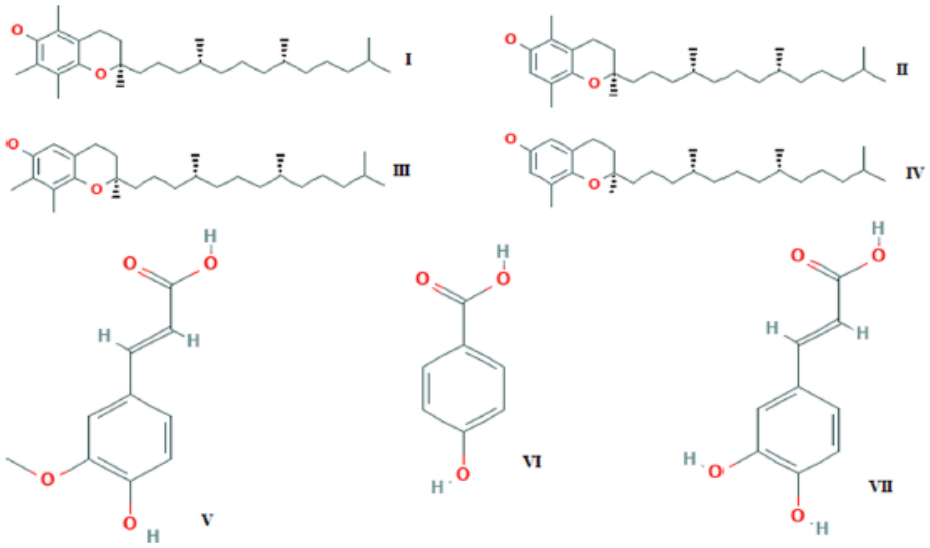


Fig. 2. Structure of tocopherols and principal phenolic acids identified in monguba oil. I) α-tocopherol, II) β-tocopherol, III) γ-tocopherol, IV) δ-tocopherol, V) ferulic acid, VI) 4-hydroxybenzoic acid, and VII) caffeic acid.

they participate in the synthesis of vitamins and enzymes, and reduce lipid peroxidation effects (Vuolo et al., 2018). Recent studies have reported the antiproliferative and anti-inflammatory effects of flavonoids and in the long-term, a flavonoid-rich diet decreases neuroinflammation and oxidative stress as well as the attenuating of Alzheimer's disease symptoms (Bakoyiannis et al., 2019; Bondonno et al., 2020; Park et al., 2019).

Anthocyanins are naturally occurring pigments and members of the group of flavonoids. They are responsible for the attractive color (blue, orange, purple, and red) of many fruits and vegetables. Pre-clinical trials have associated anthocyanins with health benefits, including anticancer, anti-inflammatory, anti-hyperglycemic, anti-obesity, neuronal health and prevention of vascular dysfunction (Burton-Freeman et al., 2016; Saulite et al., 2019; Thilavech et al., 2018).

Silva et al. (2014) suggested that *Pachira aquatica* seeds can be considered a good source of anthocyanin and flavonoids, reporting yellow flavonoids (27.64 mg/100 g) and anthocyanin (7.80 mg/100 g) in these seeds. Recently, Silva et al. (2020) reported total flavonoid and anthocyanin values of 29.40 mg/100 g and 1.42 mg/g, respectively, in raw monguba seeds.

In comparison to the total flavonoid content of blackberry, red raspberry, strawberry, blueberry, and cherry fruits (87.03 mg/100 g, 9.61 mg/100 g, 38.17 mg/100 g, 47.53 mg/100 g, and 59.62 mg/100 g FW, respectively) (de Souza et al., 2014), monguba seeds present lower total flavonoid content. Diverse peanut cultivars showed higher total flavonoid content (62.79–86.27 mg/100 g DW) than monguba seeds (Yang et al., 2020) Regarding total anthocyanin, strawberry varieties showed values (0.38–1.76 mg/g FW) (Dzhanfezova et al., 2020) very similar to the total anthocyanin content reported for monguba seeds. Amaranth and quinoa seeds had lower total anthocyanin content (Paśko et al., 2009) than monguba. On the other hand, the total anthocyanin content of blackberry, red raspberry, strawberry, blueberry, and cherry fruits (58.61 mg/100 g, 14.69 mg/100 g, 16.03 mg/100 g, 29.72 mg/100 g, and 26.27 mg/100 g FW, respectively) (de Souza et al., 2014) was higher than monguba seeds.

5.2. Phenolic acids

As flavonoids compounds, phenolic acids are also found in fruits and vegetables and they play an important protective role in oxidative stress conditions. Phenolic acids are mainly divided into two groups: hydroxybenzoic acids and hydroxycinnamic acids. Hydroxycinnamic acids are found in fruits and vegetables as esters, glycosides, and/or conjugated with proteins. The most common hydroxycinnamic acids are caffeic, ferulic, ρ-coumaric, ρ-hydroxycinnamic, and sinapic acids (Erukainure et al., 2018). Whereas hydroxybenzoic acids are found in soluble form; conjugated with sugars, organic acids or bound with lignin. The compounds that are most often found in this group are gallic, ρ-hydroxybenzoic, protocatechuic, syringic, and vanillic acids (Kumar and Goel, 2019). Phenolic acids have shown neuroprotective properties, and they act as an antidepressant, antihypertensive, anti-inflammatory, antihyperglycemic and anticarcinogenic (Agunloye et al., 2019; Barauna et al., 2018; Zaitone et al., 2019). A recent study identified ten phenolic compounds in *Pachira aquatica* seeds (Table 2) which were mostly flavonoids (1.42 μg/g DW) and phenolic acids (773.75 μg/g DW) (Rodrigues et al., 2019). Specifically, we observed that the main phenolic acids in monguba seeds were caffeic acid (445.54 μg/g DW), 4-hydroxybenzoic acid (118.88 μg/g DW), and ferulic acid (116.07 μg/g DW) (Fig. 2).

By comparing monguba seeds with other edible nuts, differences in phytochemical composition can be observed. The content of phenolic compounds in raw baru nuts showed high flavonoid content (1,111.0 μg/g) and phenolic acid content (3,008.0 μg/g), among which gallic acid (2,240.0 μg/g) was the main phenolic compound followed by catechin (872.0 μg/g), ferulic (454.0 μg/g), epicatechin (239.0 μg/g), and ρ-coumaric acids (143.0 μg/g) (Lemos et al., 2012). On the other hand, the soluble extracts of kernel cashew nuts presented high content of flavonoids, among which epigallocatechin (1,640,000.0 μg/g) was the most abundant, followed by catechin (702.0 μg/g) and epicatechin (95.0 μg/g), and the predominant phenolic acid was gallic acid (215.0 μg/g) (Chandrasekara and Shahidi, 2011). Pistachio seeds also presented flavonoid compounds as the most abundant compounds, mainly as quercetin-3-*O*-rutinoside (98.08 μg/g), genistein (69.15 μg/g), genistein-7-*O*-glucoside (47.02 μg/g) and daidzein (42.45 μg/g) (Tomaino et al., 2010).

Monguba pulp extract had considerably higher total phenolic compounds (13.28 g GAE/100 g) than other native Amazon fruits (Becker et al., 2018), amaranth and quinoa seeds (Paśko et al., 2009), and some berry fruits (de Souza et al., 2014). Higher total phenolic compound was obtained in common fruits using aqueous extract (Wolfe et al., 2008).

Even though there are few studies about the phenolic composition of *Pachira aquatica*, this fruit has considerable potential for application in food and health. Nevertheless, extreme weather events affect the concentration of bioactive compounds and should be investigated in further research. Furthermore, an extensive investigation on the health effect of monguba bioactive compounds using in vivo trials is critical because in vivo methods would show if these compounds are bioaccessible, bioavailable, and bioactive, which realistically assesses health benefits.

5.3. Alkaloids and carotenoids

Carotenoids play a vital role in photosynthesis due to their eminent photoprotective and antioxidant properties. This chemical substance is a natural pigment responsible for colors yellow-orange to red in fruits and vegetables. Carotenes are pure hydrocarbons, which includes α-carotene, β-carotene and lycopene. Moreover, carotenoids that contain one or more oxygen as a functional group are referred to xanthophylls such as lutein, β-cryptoxanthin and zeaxanthin. In vitro, in vivo and ex vivo studies have demonstrated the potential of these compounds to minimize the risk of chronic diseases such as cancers, reduce the chances of cardiovascular diseases and prevent oxidative damage (Nabi et al., 2020). Monguba seeds showed total carotenoid value of 0.37 mg/100 g (Silva et al., 2020), and concentrations of β-carotene (010 mg/100 g) and lycopene (0.09 mg/100 g) (Silva et al., 2014).

Basically, alkaloids serve as plant defense chemicals against predators and herbivores, and to a lesser degree, they can inhibit or otherwise deregulate vital processes of microorganisms or competing plants. In addition, alkaloids can also be derived from fungi, bacteria and even animals. These compounds are known for their toxic or adverse effects. However, in the medicinal area, alkaloids are used as anesthetics, narcotics, analgesics, respiratory stimulants, to dilate pupils, to relax the skeletal muscles, as well as to treat infections, health disorder and cancers. Caffeine is one of the alkaloids that can modulate receptors of neurotransmitters, that is, it is used as an stimulant in medicinal applications (Wink, 2016), which perhaps explains the use of monguba seeds as a tonic by indigenous healers. Nevertheless, depending on the concentration, this compound can cause toxicological effects due to binding to neurotransmitter receptors and neurotransmitter-degrading enzymes (Wink, 2016).

The presence of alkaloids in monguba seeds has already been reported in the literature, however their quantification and concentrations have not yet been reported. Therefore, we suggest further research to address alkaloids concentration, evaluate its impact on health and toxicological effects.

6. Antioxidant capacity and biological activity of *Pachira aquatica* Aubl. Seed and oil

6.1. Antioxidant activity

Some authors have evaluated the in vitro antioxidant activity of monguba compounds even though this analysis approach does not accurately yield the antioxidant effect of compounds due to interferences such as other compounds in the plant matrix, due to the variability in the mechanism for sample chemistry analysis that each in vitro method apply, in addition to the non-specificity of these methods (Amorati and Valgimigli, 2015). Rodrigues et al. (2019) evaluated the antioxidant activity of compounds (soluble and insoluble phenolic) in monguba seeds and oil through oxygen radical absorption capacity (ORAC) and trolox equivalent antioxidant capacity (TEAC) tests. The insoluble-bound fraction is found in the vegetable cell wall matrix and corresponds to the insoluble form, whereas free, esterified, and glycosylated fractions are the soluble forms of phenolic compounds present in monguba seeds. The phenolic compound extracts in monguba seeds were 1.90 μMol TE/g in the insoluble-bound fraction, 5.07 μMol TE/g in the glycosylated fraction, 5.49 μMol TE/g in the free fraction, and 8.69 μMol TE/g in the esterified fraction via TEAC assay; and 3.91 μMol TE/g in the insoluble-bound fraction, 14.06 μMol TE/g in the glycosylated fraction, 12.92 μMol TE/g in the free fraction, and 20.46 μMol TE/g in the esterified fraction via ORAC assay. The authors observed that the esterified fraction showed the highest antioxidant capacity compared to the other fractions, and presented the highest concentration of phenolic acids such as caffeic acid and ferulic acid. On the other hand, monguba seed oil presented 10.70 μMol TE/g and 3.30 μMol TE/g via ORAC and TEAC assays, respectively (Rodrigues et al., 2019). We highlight that this study is the first to analyze and show the antioxidant capacity of monguba seed and its oil. A higher ORAC value in common fruits using aqueous extract was obtained by Wolfe et al. (2008).

Using the method of the superoxide radical scavenging capacity (RSC) to evaluate the antioxidant capacity of Amazon fruits, Becker et al. (2018) observed that monguba fruit showed good inhibition (75.74 %) of superoxide anion radicals generated than uxi and biribá fruits. In addition, Silva et al. (2020) reported 7.88 g/g of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in raw monguba seeds. A higher DPPH scavenging activity was observed in extracts of Maya nut (790 g/g), walnut (830 g/g), peanut (455 g/g/) and almond (420 g/g) by Ozer (2017). In this way, *Pachira aquatica* has considerable potential to be used to prevent degenerative diseases. However, in vivo and in vitro (using cells) methods are required to evaluate the antioxidant effect, in addition to evaluating which compounds contribute to this effect.

6.2. Anti-inflammatory activity

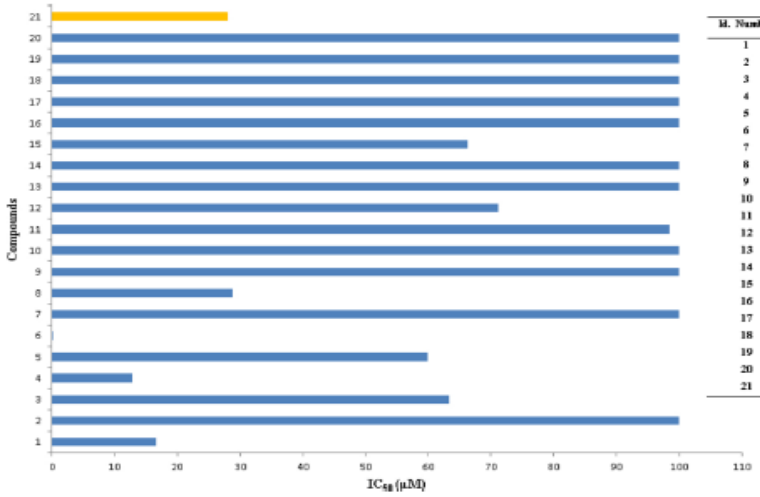
The isolated compounds from *Pachira aquatica* stem have been evaluated by inhibition of formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced superoxide anion generated by human neutrophils (Cheng et al., 2017). The extracts of *Pachira aquatica* stem were separated by chromatography on a silica gel column, and the different fractions were purified. Cheng et al. (2017) identified 20 compounds, in which two of them were identified for the first time in monguba. The anti-inflammatory tests performed by Cheng et al. (2017) showed that 11-hydroxy-2-O-methylhibiscolactone A (1), isohemigossylic acid lactone-7-methyl ether (4), Hibiscone D (5), 3,5,6,7,8,3′,4′-heptamethoxyflavone (11), and 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (12) exhibited more effective inhibition against fMLP-induced superoxide anion generation (Fig. 3) Among the isolates, Gmelofuran (6) exhibited the most effective inhibition of superoxide anion generation in response to fMLP. Therefore, the authors of this study suggested that *Pachira aquatica* stems and its isolated compounds exhibited great inhibition of superoxide anion generation (Cheng et al., 2017). In this way, these compounds could be used as potential candidates for the treatment or prevention of inflammatory diseases.

6.3. Antimicrobial activity

Various authors evaluated the antifungal activity of some parts of *Pachira aquatica*. A compound named isohemigossypolone was identified in the bark of *Pachira aquatica*, and revealed antifungal effects against *Pythium ultimum*, *Cladosporium herbarum* and *Saccharomyces cerevisiae* (Shibatani et al., 1999). In addition, the ethanol extract from monguba seeds showed growth inhibition and fungicide action on Fusarium sp. growth at the concentration of 25 mg/mL via disc diffusion method (Souza et al., 2014).

Recently, a study analyzed the compounds of *Pachira aquatica* leaf oil against two bacteria commonly reported on humans, *Mycobacterium tuberculosis* and *Helicobacter pylori*. In this study, the authors observed that the oil from *Pachira aquatica* leaf was able to inhibit the activity of these bacteria. Therefore, the compounds from *Pachira aquatica* leaf have potential to treat these infections (Gamal El-Din et al., 2018).

The extract from diverse parts of *Moringa oleifera* exhibited antifungal and antibacterial activity against *Trichophyton mentagrophytes*, *Aspergillus flavus*, Penicillium spp., *Aspergillus niger*, *Aspergillus oryzae*,



Id. Number	Compound	IC_{50} (μM)
1	11-Hydroxy-2-O-methylhibiscolactone A	16.65
2	O-Methylhibiscone D	> 100
3	Hibiscolactone A	63.22
4	Isohemigossylic acid lactone-7-methyl ether	12.77
5	Hibiscone D	59.89
6	Omelofuran	0.15
7	2-O-Methylisohemigossylpolone	> 100
8	5-Hydroxyauranetin	28.84
9	Kaempferol 3,7, 4'-trimethyl ether	> 100
10	Santin-7-methyl ether	> 100
11	3,5,6,7,8,3',4'-Heptamethoxyflavone	98.51
12	5,4'-Dihydroxy-3,6,7,8-tetramethoxyflavone	71.12
13	Retusin	> 100
14	5,4'-Dihydroxy-3,7-dimethoxyflavone	> 100
15	Scopoletin	66.24
16	Benzophenone	> 100
17	Lupenone	> 100
18	2α,3β-Dihydroxyfurs-20(29)-ene	> 100
19	(23Z)-Cycloart-23-en-3β,25-diol	> 100
20	(24R)-Cycloart-25-en-3β,24-diol	> 100
21	Ibuprofen (Control)	27.85

Fig. 3. The inhibitory response of the isolated compounds (1-20) present in the stems of *Pachira aquatica* on superoxide anion generation by human neutrophils in formyl-L-methionyl-L- leucyl-L-phenylalanine-induced.

Aspergillus nidulans, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia, and *Pseudomonas aeruginosa* (Dhakad et al., 2019). Peanut seed oil showed antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Listeria ivanovii, Listeria inocua, Enterococcus hirae* and *Bacillus cereus* (Sebei et al., 2013). However, the antimicrobial activity of monguba plant has still not been tested against fungi and bacteria that generally are present in food or in hospital areas.

Considering the antimicrobial property of the monguba tree reported by literature, the compounds of this plant could be used as natural preservatives against microorganisms and may be further applied in food, agriculture, and pharmacy areas. Nevertheless, more studies that prove the antifungal and antibacterial (Gram-negative and Gram-positive bacteria) properties from other parts of monguba plant are necessary.

6.4. Insecticide activity

Our literature review search resulted in only one work analyzing the insecticide activity of monguba, which was specifically about the insecticide activity of ethanolic extracts from *Pachira aquatica* seeds on *Hypothenemus hampei* Ferrari (Souza et al., 2012). The extracts showed great insecticide activity on adult bugs using 25 mg/mL concentration of ethanolic extract from monguba seeds. Moreover, monguba seed extract also showed repellent action against bugs, preventing them from getting close to coffee fruits. Nevertheless, further studies are necessary to prove the efficacy and efficiency against agricultural crop pests, in addition to analyzing any possible toxicity of monguba seed ethanolic extract at various concentrations.

7. Uses in traditional medicine of *Pachira aquatica* Aubl. Plant

Other review articles have investigated monguba plants and their potential for healing diseases. Two studies showed that the infusions of *Pachira aquatica* bark and roots could exhibit hypoglycemic effects in the treatment of diabetes (Andrade-Cetto and Heinrich, 2005; Hernandez--Galicia et al., 2002). According to Coe (2008) and Arvigo et al. (1998), the Mayas healers and Rama midwives use *Pachira aquatica* bark and seeds to treat vaginal infections, weakness, anemia, low blood pressure, kidney conditions, edema, exhaustion or fatigue, besides its use as a tonic, and for strengthening in the elderly. However, further research is necessary to prove all these human health benefits and ensure consumer safety and welfare.

8. Antinutrients of *Pachira aquatica* Aubl. Seeds

Monguba seeds are a great food, but consumption of its raw seeds is not highly recommended. Peixoto and Escudeiro (2002) reported that consumption of its raw seeds causes laxative effects. Therefore, monguba seeds intake must follow suitable heat treatment. Oliveira et al. (2000) showed that raw monguba seed flour has higher trypsin inhibitor content (2.60 g/kg flour) when compared to other seeds, and haemagglutinating activity of 113 $HU.10^{-3}$/kg flour levels of lectin activity. In this same study, the authors also fed rats a raw monguba seed diet, and they observed loss of appetite, and that most rats died within 6–8 days. Survived rats had steady weight loss, developed bald skin, and presented enlarged stomach, liver, pancreas, kidneys, heart and lungs, and atrophy of spleen. Thus, the authors concluded that raw seeds of monguba were toxic to growing rats.

9. Toxicity studies of *Pachira aquatica* Aubl. Plant and seeds

Coe et al. (2012) evaluated whether the plant extracts of medicinal species used in Nicaguara healers were cytotoxic using the brine shrimp lethality assay (BSLA). They observed that less than 10 % of aqueous extracts of the species plant was considered cytotoxic, although over 90 % species had alkaloids. Almost 75 % of medicinal species showed LC_{50} ranging from 1000 to 5000 μg/mL and 87 % of the species were cytotoxic at levels above the 1000 μg/mL threshold, however, the majority of the species tested had been considered "noncytotoxic" by BSLA. Coe et al. (2012) showed that the aqueous extract of *Pachira aquatica* plant (bark and seeds) is not considered cytotoxic. *Pachira aquatica* was lethal to 50 % of the Brine Shrimp at 2684 μg/mL, and tested positive for alkaloids. However, the authors suggested that the aqueous extract of *Pachira aquatica* plant may not be cytotoxic because it contained a low concentration of alkaloids or the type of alkaloid was not toxic to the test, which is explained by the fact that the water was not effective in removing all the compounds from the plant tissue. Plant toxicity can have either a positive or negative impact. It is positive when it aims to kill pathogens such as bacteria and parasites. On the other hand, it can be harmful if it affects the health of someone negatively (Coe et al., 2012).

A recent preclinical trial with *P. aquatica* oil extracted by cold press showed that a dose of 2000 mg/kg, administered for 14 days, did not have toxicity effects and no cause mortality in the Wistar rats treated orally with monguba oil. Changes in vital and reproductive organs were

not observed, and hematological analysis was normal for the animal species. However, the histopathological study observed alterations in the lungs of the animals indicating initial pneumonia. Therefore, Marcelino et al. (2020) suggested further investigation of some toxic effect of monguba oil mainly on the spleen since the seeds are rich in lipids. Thus, these preclinical results demonstrated that *P. aquatica* oil has low toxicity effects in the short-term after acute oral exposure (Marcelino et al., 2020).

This review observed that the adverse effects after intake of monguba seeds are yet inconclusive. Therefore, further pre-clinical and clinical trials are needed focusing on the alkaloids in monguba seeds and their toxic effects, and on the maximum safe intake of monguba oil and the possible toxic effects after its oil consumption.

10. Other studies with *Pachira aquatica* Aubl. Oil

10.1. Lipase

A preliminary study isolated lipase from *Pachira aquatica* seeds and tested it. Lipase from monguba seed reached a maximum activity at 40 °C using p-nitrophenyl palmitate and soybean oil as a substrate. Lipase exhibited maximum stability at 4 °C during incubation for 3 h, and an optimum pH of 8.0. The lipolytic activity of lipase in the presence of organic solvents was high with methanol and glycerol. The enzyme was able to hydrolyze both soluble and insoluble emulsified substrates, and it presented high lipolytic activity in the presence of calcium and magnesium cations (Polizelli et al., 2008a).

The same study group evaluated the activity and stability of the lipase extracted from *Pachira aquatica* seed on the surfactant and commercial detergents effects. They observed that bile salts increased lipase activity in all concentrations tested. Various concentrations of the surfactant sodium dodecyl sulfate (SDS) and sodium octyl sulfate (SOS), as well as nonionic surfactants (Triton X-100 and Tween 80) inhibited lipase activity. On the other hand, glycholate increased lipase activity when the concentration of surfactant was 10 mM. At various concentrations of polyethylene glycol (PEG), the hydrolytic activity of lipase proportionally increased in aqueous solution, especially after 4 h compared to the control (Polizelli et al., 2008b).

Regarding the effect of commercial detergents on *Pachira aquatica* lipase stability and activity, Polizelli et al. (2013) noted that (I) hydrogen peroxide decreases the relative activity of *Pachira aquatica* lipase; (II) lipase retained approximately 100 % of its activity when using commercial detergents at 3.3 mg/mL; (III) incubation of wastewater mixture with lipase resulted in release of 1.7 mM of free fatty acids after 60 min of incubation, and (IV) degradation of fat particles was observed after 72 h incubation with 30 mg/mL of lipase. The maximum lipase hydrolysis was 14.3 % of particle mass reduction.

Bonine et al. (2014) evaluated the thermal stability of the immobilized lipase from *Pachira aquatica* seeds using beads of calcium chloride, sodium alginate (Alg) and poly(vinyl alcohol) (PVA). They observed that immobilized lipase prepared with calcium alginate could be used for up to 6 activity cycles, and that the beads with Alg + PVA polymers could be used for up to 9 cycles. The best result for the thermal stability of lipase was observed with the Alg + PVA when compared with the free enzyme or with the immobilized enzyme on beads of alginate. In this way, the authors concluded that immobilized lipase in alginate and Alg + PVA spheres improved its thermal stability and could allow its utilization in oil hydrolysis. Thus, the authors suggested that *Pachira aquatica* seed lipase could be used in the oil industry and can be useful for vegetable oil degradation, as well as in the treatment of wastewater containing oil and fat.

10.2. Cosmetic formulations

Some studies have reported the stability of *Pachira aquatica* oil in cosmetic emulsions. The emulsions were formulated with a variety of combinations of plant oils and butters, including *Pachira aquatica* seed oil (Dourado et al., 2015; Raiser et al., 2018). The emulsion systems maintained a pH that is compatible with the skin, in addition to their appearance, homogeneity and color at 25°C for 3 months. In addition, the emulsions remained stable after vibrational and centrifugation tests. On the other hand, the cosmetic performance of emulgel type formulations presented high adhesiveness and, therefore, high viscosity, which imparts an undesired sticky sensory impression in pharmacotechnical presentations (Dourado et al., 2018).

Although monguba oil cannot be used in emulgel formulations due to some fatty acids that can cause a sticky sensory impression, these results show that monguba oil could be used in cosmetic emulsion formulations because it has shown a good level of spreadability and a pH similar to the skin. According to Kim et al. (2008), palmitic acid had a good skin permeation when elaborated in the cosmetic emulsions. In addition, monguba oil incorporation into cosmetic emulsions has been suggested as an alternative to synthetic antioxidants due to its antioxidant capacity (Raiser et al., 2018), but further in vitro and in vivo studies are required to evaluate the antioxidant potential of monguba oil as discussed in the topic 6.1.

11. Conclusion and perspectives

This review shows that *Pachira aquatica* fruit is promising for exploration. The literature shows the potential of *P. aquatica* fruit as a new source of fibers and minerals, mainly potassium, calcium, phosphate, magnesium, zinc, iron, and copper. Moreover, it can be used as a new raw material to obtain palmitic acid, which corresponds to around 60 % of monguba's fatty acid content. There is also evidence of the potential of the biocompounds (tocopherols, phenolic composition, and others) that have been identified in different parts of the monguba plant. This plant also has activity against some fungi, bacteria, and pests, in addition to its anti-inflammatory activity, and its uses in folk medicine to prevent and treat diseases. However, there is still little information about the harmful properties of *Pachira aquatica* plant. Thus, this critical point needs further exploration, especially considering that the action of the antinutritional factors and of alkaloids present in *P. aquatica* seeds.

The studies mentioned in this review reveal the variety of potential uses and further exploration of *P. aquatica*. For instance, according to the literature, monguba seed is rich in lipids and proteins, however, there is no research evaluating which kinds of extraction of monguba seed oil would result in optimized bioactive content and whether there are any modifications of physical characteristics and physicochemical stability resulting from the extraction process. Moreover, there are no reports about the application of monguba proteins as a substitute for food ingredients in the development of mayonnaise, meat emulsion, etc. Furthermore, the effect of gastrointestinal digestion in vitro and in vivo on the bioaccessibility of minerals, and phenolic compounds and their antioxidant capacity are of interest for the food industry such as for the enrichment of food formulations. Likewise, exploring the bioavailability of minerals is critical because fibers, vitamins, phenolics and minerals can interfere in mineral bioavailability. In addition, toxicological, preclinical, and clinical trials should be conducted to confirm these effects on human health and ensure consumer safety and welfare. Another topic that needs further research is medicinal application of monguba plant. As demonstrated by Cheng et al. (2017) and for its application already known by indigenous healers, monguba plant exhibits anti-inflammatory actions and is used in the treatment of edemas. Therefore, in vivo research focusing on the action of monguba's bioactive compounds on anti-inflammatory activity is necessary and in the long-term, these compounds may be incorporated into the pharmaceutical industry.

Author statement

Glaucia Maria Pastore encouraged Alexsandra Pereira Rodrigues to

investigate monguba plant and contributed to the implementation of the research. Alexsandra Pereira Rodrigues developed the theoretical framework, the main conceptual ideas, and wrote the article.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

The author Alexsandra Pereira Rodrigues would like to thank Gustavo Araujo Pereira to read and suggestions for the manuscript and Catalina Angel-Coca to give permission to use the image of monguba buds. The authors are thankful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support offered through fellowships (Finance Code 001). The authors are grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (process numbers: 2015/50333-1) and CAPES-PROEX (23038.000795/2018-61) for financial support.

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Chapter 2

Research Article

Chemical Composition and Antioxidant Activity of Monguba (*Pachira aquatica*) Seeds

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Article published in Food Research International

(Impact Factor 4.972 – Qualis A1)

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The article can be accessed through the following link:

https://doi.org/10.1016/j.foodres.2019.01.014

Food Research International 121 (2019) 880–887

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Chemical Composition and Antioxidant Activity of Monguba (*Pachira aquatica*) Seeds

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ARTICLE INFO

Keywords:
Carbohydrates
Fatty Acids
Mineral Composition
Phenolic Compounds
Tocopherols
Bioactive Compounds

Chemical compounds studied in this article:
4-hydroxybenzoic acid (PubChem CID: 135)
Caffeic acid (PubChem CID: 689043)
Ferulic acid (PubChem CID: 445858)
Magnesium (PubChem CID: 5462224)
Potassium (PubChem CID: 5462222)
Zinc (PubChem CID: 23994)
Stachyose (PubChem CID: 439531)
Sucrose (PubChem CID: 5988)
Palmitic acid (PubChem CID: 985)
γ-tocopherol (PubChem CID: 92729)

ABSTRACT

Monguba fruit has a seed with a chestnut-like flavor that can be consumed boiled, fried, and roasted. These nutritious seeds also have been used in popular medicine to treat several diseases. Nevertheless, the nutritional and functional potential of monguba seed is still underexploited. In this sense, we investigated the nutritional and functional components of monguba seeds. These seeds showed high total content of sugars, mainly sucrose, whereas the content of the raffinose family oligosaccharides was low. The mineral assay showed high amount of minerals, namely potassium, calcium, magnesium and zinc, which indicate that monguba seeds can be a new source of these minerals. UHPLC-ESI-MS/MS analysis showed caffeic, ferulic and 4-hydroxybenzoic acids as the main phenolic compounds, mainly in the esterified form, in these seeds. Monguba seed showed high lipid content, in which the main compounds were palmitic acid and γ-tocopherol. The soluble and insoluble phenolic fractions from monguba seeds showed high antioxidant activity measured by the oxygen radical absorption capacity (ORAC) and the trolox equivalent antioxidant capacity (TEAC) assays. Therefore, the monguba seeds have great potential to be explored by food, pharmaceutical and cosmetic industries due to their chemical composition.

1. Introduction

Pachira aquatica Aubl is a tree belonging to the Bombacaceae family, and is found from Southern Mexico to Guyana, and in Northeastern Brazil. This plant was also introduced in Guangdong, Southern Yunnan, and Taiwan as a cultivated plant (Cheng et al., 2017; Jorge & Luzia, 2012; Oliveira et al., 2000; Silva, Azevedo, & Azevedo, 2015). It is known as Malabar chestnut, French peanut, Guiana chestnut, monguba (Brazil), false cocoa, or pumpo (Guatemala), and it is commercially sold under the name 'money tree' (Cheng et al., 2017; Silva et al., 2015). Monguba can be found frequently in wetlands, from which comes its scientific name 'aquatica', however its adaptability to different climates and soil conditions has enabled its cultivation as an ornamental plant in different regions of Brazil (Peixoto & Escudeiro, 2002; Santana, dos Santos, Silva, & das Virgens, 2016).

Monguba fruits are football-shaped and surrounded by a brown wooden peel containing large seeds (see Fig. S1A-B in the Supplementary material). These seeds show a chestnut-like flavor and eventually are consumed boiled, fried or roasted, and can also be ground into flour for baking bread (Bailey, Bailey, & Bailey Hortorium, 1976; Jorge & Luzia, 2012; Silva, Amaral, Braga, Sousa, & Figueiredo, 2014).

Some studies have shown that monguba seeds present high amount of lipids (44%), 12.9% of protein, and that they could contribute to the recommended daily intake of fibers and minerals (de Bruin, Heesterman, & Mills, 1963; Oliveira et al., 2000). Also, Oliveira et al. (2000) observed high amount of essential amino acids such as tryptophan, threonine and phenylalanine/tyrosine. Leterme, Buldgen, Estrada, and Londoño (2006) found the mineral content in monguba seeds ranging from 3.44 to 3.69% and containing more phosphate,

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https://doi.org/10.1016/j.foodres.2019.01.014
Received 2 May 2018; Received in revised form 29 December 2018; Accepted 7 January 2019
Available online 09 January 2019
0963-9969/

magnesium, zinc, iron and copper than some fruits and other starchy foods such as banana, papaya, maize, cashew fruit, potato, jaboticaba, mango, acerola, and zapote.

Monguba parts have pharmacological properties, and it is known that monguba twigs and leaves have been used in urticarial treatments, whereas infusions with monguba root can be used for diabetes mellitus control (Alfaro, 1984; Hernandez-Galicia et al., 2002). Recently, a study reported that monguba stems are active on neutrophil pro-inflammatory responses, based on which the authors suggested that monguba can be further developed as potential candidate for the treatment or prevention of various inflammatory diseases (Cheng et al., 2017). Monguba bark and seed are used as traditional healers in eastern Nicaragua for treatments of diarrhea, diabetes, infections, skin rashes, sores and anemia (Coe, Parikh, Johnson, & Anderson, 2012). Although there are studies on the chemical composition and bioactivity of the different parts of monguba plant, the nutritional and chemical composition, functional properties, and antioxidant capacity of monguba seeds have been poorly assessed. In this approach, the aim of the present study was to evaluate the nutritional and functional components of monguba oil and seeds. The functional sugars and oligosaccharides, minerals, and the soluble and insoluble forms of phenolic compounds in monguba seeds were evaluated. The fatty acids and tocopherols profile of monguba seed oil were also assessed. Furthermore, we evaluated the antioxidant activity of the different phenolic fractions from monguba seed.

2. Materials and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2′-azobis(2-methylamidinopropane)-dihydrochloride (AAPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS), fluorescein, Folin-Ciocalteu reagent, phenolic compounds (gallic acid, gallocatechin, protocatechuic acid, epigallocatechin, catechin, chlorogenic acid, 4-hydroxybenzoic acid, epicatechin, caffeic acid, vanillic acid, gentisic acid, p-coumaric acid, sinapic acid, ferulic acid, rutin, quercetin and naringenin), tocopherols (α-, β-, γ- and δ-tocopherol), sugars (fructose, glucose, sucrose and maltose), raffinose family oligosaccharides (RFOs; stachyose, verbascose and raffinose) and polyols (sorbitol, mannitol and xylitol) standards were purchased from Sigma-Aldrich (St. Louis, USA). All phenolic, tocopherols, sugars, RFOs, and polyols standards show purity ≥ 95%. HPLC grade methanol, formic acid, sodium hydroxide solution 50% from Sigma-Aldrich (St. Louis, USA), whereas sodium acetate were from Merck (Frankfurt, Germany). Ultrahigh-purity water was produced using a Millipore-Milli-Q system (Millipore, Bedford, USA).

2.2. Plant material and sample preparation

Monguba fruits (2 kg) at full physiological maturity (when the fruit showed brown peel with a slight opening) were collected from trees located in the city of Uberlândia (18°54′41″ south latitude, 48°15′44″ west longitude and 843 m altitude), in the state of Minas Gerais, Brazil. The fruits were transported from the collection site to the laboratory up to 24 h after collection. The seeds were manually separated from the peel and approximately 200 g of seeds were freeze-dried. The freeze-dried seeds were ground using a knife mill, which resulted flour was stored in a freezer (−20 °C) for further analysis. The Genetic Heritage Management Board (CGen) under number A50A9D1, following the Law n° 13.123/2015 and its regulations, regulated the activity of access to Genetic Heritage.

2.3. Physicochemical characterizations

The AOAC (Association of Official Analytical Chemists) guide was used to determine total soluble solids content (TSS; method n° 932.12), pH (method n° 970.21) and total titratable acidity (TTA; method n° 942.15) (Horwitz & Latimer, 2005). The TTA was expressed as g oxalic acid (OA) per 100 g sample fresh weight (g OA/100 g FW).

2.4. Proximate composition

The proximate composition of monguba seeds was determined following the AOAC (Horwitz & Latimer, 2005) methods for moisture content (n° 925.26), crude protein (n° 920.87), lipids (n° 933.05), ashes (n° 940.26) and raw fiber (n° 991.43), while the total carbohydrates content was calculated by difference following Eq. 1:

$$\text{Total Carbohydrates} = [100\text{-}(\text{moisture}+\text{ashes} + \text{lipids}+\text{proteins})]. \quad (1)$$

2.4.1. Mineral analysis

The content of Ca, Mg, Fe, Zn, K, Cu, Mn and Na were determined using a flame atomic absorption spectrometer (FAAS) (Perkin Elmer, USA, model AAnalyst-200) and analyzed according to the method described by Silva, Orlando, Rebellato, and Pallone (2017), with slight modifications. For the mineralization, 0.4 g of sample and 4 mL of nitric acid were at room temperature overnight. After, tubes remained for 2 h at 110 °C in a digestion block. Then, 2 mL of nitric acid and 2 mL of hydrogen peroxide 30% were added and the tubes were heated at 130 °C for 2 h. The samples were solubilized in an ultrasound bath for 5 min. The digested was measured to 25 mL with ultrapure water and filtered in ash-free filter paper. The mineral content was expressed as mg/100 g of sample dry weight (DW).

2.4.2. Monguba seed oil composition

Monguba seeds oil was extracted using a Soxhlet extractor following the method described by Jorge and Luzia (2012), with some modifications. The freeze-dried sample (20 g) was washed with petroleum ether at 60 °C for 5 h. The ethereal fraction was evaporated at room temperature (± 25 °C) for overnight. The oil obtained was stored in amber glass bottle at freezer until analysis. Monguba seeds oil was analyzed for its fatty acids and tocopherols composition, antioxidant activity, and total phenolic compounds.

2.4.2.1. Fatty acids composition. The fatty acid methyl esters (FAMES) were separated according to the Hartman and Lago (1973). The fatty acid composition was determined according to AOCS Ce 1f-96 (AOCS, 2009) using the capillary gas chromatography (CGC Agilent 6850 Series GC System, Santa Clara, CA, USA). The chromatography was operated under the following conditions: injection volume of 1.0 µL, detector temperature of 280 °C, and injector temperature of 250 °C. The oven temperature was programmed as follows: initial temperature of 110 °C (5 min), 110–215 °C at a rate of 5 °C/min, 215 °C (24 min). Helium was used as the carrier gas. Qualitative composition was determined by comparing peak retention times with the respective standards for fatty acids. The proportion of each compound was estimated dividing its mean area by the total area of the chromatogram and expressed as percentage.

2.4.2.2. Tocopherols composition. The tocopherol profile in monguba seed oil was determined according to AOCS Ce 8–89 methodology (AOCS, 2009) using the high performance liquid chromatography (Perkin Elmer SERIES 2000 HPLC) coupled to fluorescence detector. The samples were diluted in hexane (1% w/v). The chromatography was operated under the following conditions: isocratic pump; mobile phase of 99:1 hexane/isopropanol; flow of 1.0 mL/min; injected volume of 20.0 µL; fluorescence detector with excitation: 290 nm and emission: 330 nm (Freitas et al., 2018). The qualitative composition of tocopherols was made by comparison of the retention times of peaks with standards tocopherols. Calibration curves were constructed with commercial standards (α-tocopherol 1.37–13.70 µg/mL, β-tocopherol

1.15–11.50 μg/mL, γ-tocopherol 1.68–16.80 μg/mL and δ-tocopherol 1.73–17.30 μg/mL) to quantify the tocopherols in monguba seed oil, and the results were expressed as mg/g of oil.

2.4.3. Sugars and oligosaccharides analyses by HPAEC-PAD

The samples were prepared according to Pereira, Arruda, de Morais, Eberlin, and Pastore (2018), with some modifications. The freeze-dried monguba seeds (1 g) were added into a Falcon tube containing 20 mL ultrapure water and homogenized with the aid of Ultra-Turrax for 60 s. Then, the sample was centrifuged at 4000*g* for 10 min at 5 °C (Hettich Zentrifugen, model Rotanta 460R, Tuttlingen, Germany). The supernatants were collected and filtered using cotton, followed by a 0.22 μm filter, then stored in the freezer until further analysis. The same preparation conditions were followed in commonly consumed seeds, grain and cereals (soybean, beans, corn, rice, chia, quinoa, sesame and linseed) (Table S2 in the Supplementary material) to compare the sugar/oligosaccharides content of monguba seed with commonly consumed seeds, grain, and cereals.

Sugars, polyols and raffinose family oligosaccharides (RFOs) were analyzed by High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAEC-PAD). Each sample was diluted in deionized water, and 25 μL of diluted sample were then injected into the HPAEC-PAD system using an autosampler. The flow rate through the column was of 1.0 mL/min. The column temperature was kept at 30 °C. The chromatographic column used for the mono- and disaccharides, polyols and RFOs analyses was a Carbopac PA1 (250 × 4 mm, 10 μm particle size). The isocratic mobile phase consisted of 120 mM NaOH. Calibration curves were constructed with commercial standards, which concentration ranged from 0.25 to 12.5 μg/mL. Identification of sugars, polyols and RFOs was determined by comparison with the retention time of standard and samples. Calibration curves were constructed with commercial standards (0.25–12.5 μg/mL) to quantify the sugars, polyols and RFOs in the extracts. The results were expressed as g/100 g of sample dry weight.

2.5. Determination of phenolic compounds

2.5.1. Phenolic compounds extraction

The freeze-dried sample (1 g) was firstly washed three times with hexane due to high amount of oil in monguba seeds. After this step, the extraction and fractionation (free, esterified, glycosylated and insoluble-bound forms) of phenolic compounds from defatted monguba seeds (DMS) were determined according to Arruda, Pereira, de Morais, Eberlin, and Pastore (2018). The extraction and fraction steps were realized three times.

2.5.1.1. Extraction of soluble phenolic compounds. The DMS was homogenized with 15 mL of a mixture of methanol-acetone-water (7:7:6, *v/v/v*) and left in ultrasonic bath for 20 min at room temperature (± 25 °C). The mixture was centrifuged (4000 g, 15 min, 5 °C). The supernatant was collected and the solid residue was resubmitted to extraction. This procedure was repeated three times. Then, the supernatants were combined, whereas the solid residue was stored for further extraction of insoluble-bound phenolic compounds. The supernatant was evaporated under vacuum at 35 °C and the remaining aqueous phase was acidified to pH 2 using 6 M HCl, then centrifuged (4000 *g*, 5 min, 5 °C) to remove precipitates. This aqueous phase was used for the fractionation of soluble phenolic compounds.

2.5.1.2. Fractionation of soluble phenolic compounds. The free phenolic compounds (F1) were extracted three times with diethyl ether-ethyl acetate (1:1, v/v) at a solvent to aqueous phase ratio of 1:1 (*v/v*). The remaining aqueous phase after the F1 extraction was hydrolyzed with 4 M NaOH containing 10 mM EDTA and 1% ascorbic acid at 150 rpm for 4 h at room temperature (± 25 °C) to release esterified phenolics. The pH of the hydrolysate was adjusted to 2 using 6 M HCl, and the phenolic compounds (F2, esterified) released from soluble esters were extracted using the procedure as described for F1. The aqueous phase that was left after the F2 separation was hydrolyzed with 5 mL 6 M HCl at 150 rpm for 60 min at 75 °C to release glycosylated phenolics. Phenolic compounds (F3, glycosylated) released from soluble glycosides were extracted as described for F1.

2.5.1.3. Hydrolysis and extraction of insoluble-bound phenolic compounds. The solid residue from the soluble phenolics extraction was hydrolyzed with 4 M NaOH containing 10 mM EDTA and 1% ascorbic acid at a solid to solvent ratio of 1:20 (*w/v*) at 150 rpm for 4 h at room temperature (± 25 °C) to release insoluble-bound phenolics. The pH of the mixture was adjusted to 2 using 6 M HCl and centrifuged (4000 *g*, 5 min, 5 °C). Phenolic compounds (F4, insoluble-bound) released from insoluble-bound phenolics were extracted as described for F1. Each organic phenolic fractions (F1, F2, F3 and F4) that was obtained as described above was dehydrated with anhydrous sodium sulfate, and evaporated to dryness under vacuum at 35 °C. The dry residues were dissolved into 5 mL of methanol, and these solutions were used for total phenolic content analysis, and antioxidant capacity assays.

2.5.2. Identification and quantification of phenolic compounds with UHPLC–ESI-MS/MS

Phenolic compounds analysis in phenolic fractions (free, esterified, glycosylated and insoluble-bound forms) from monguba seeds was performed using an HPLC system coupled to a triple quadrupole mass spectrometer (LCMS 8040, Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source following the method described by Arruda et al. (2018), with some modifications. The chromatographic separation of phenolic compounds was determined using a Shim-pack XR-ODS III column (2.2 μm, 2.0 mm i.d., 150 mm, Shimadzu, Kyoto, Japan), a mobile phase flow of 0.4 mL/min (solvent A was 0.1% formic acid in water and solvent B was methanol). The gradient elution was operated under the following conditions: 0–1 min, 5% B; 1–4 min, 5–60% B; 4–7 min, 60–70% B; 7–10 min, 70–100% B; 10–10.50 min, 100% B; 10.50–11 min, 100–5% B; 11–15 min, 5% B. The autosampler temperature was maintained at 10 °C and the injection volume was 10 μL.

The ESI source was operated in the negative ion mode through the following parameters: capillary voltage, 3.5 kV; heat block temperature of 300 °C; desolvation line temperature of 250 °C; drying gas flow (N_2) of 20 L/min; nebulizing gas flow (N_2) of 3 L/min; collision induced dissociation gas pressure (Ar) of 224 kPa. For each standard, deprotonated molecule $[M–H]^-$ was used as precursor ion and the two most abundant product ions were chosen for quantification and confirmation for the Multiple Reaction Monitoring (MRM). The results were expressed in μg per g of sample dry weight (μg/g DW).

2.6. Determination of antioxidant capacity

2.6.1. Hydrophilic and lipophilic Trolox equivalent antioxidant capacity (TEAC) assay

The ABTS free radical-scavenging activity of monguba seeds phenolic fractions was estimated using the method described by Leite-Legatti et al. (2012), with some modifications. The $ABTS^{\bullet+}$ radical cation was produced by reacting 7 mM $ABTS^{\bullet+}$ with 140 mM potassium persulfate. The mixture was stored at room temperature (± 25 °C) in the dark for 16 h for radical formation. The $ABTS^{\bullet+}$ solution was diluted with deionized water to yield an absorbance of 0.700 ± 0.02 at 734 nm. Then, 1000 μL of $ABTS^{\bullet+}$ solution was added to 200 μL of samples with different concentrations and mixed vigorously. The reactive mixture was stored at room temperature (± 25 °C) for 6 min before the absorbance at 734 nm was measured. A standard curve was obtained by using Trolox standard solution within a concentration range from 5 to 150 μmol/L.

The antioxidant activity of monguba seed oil was determined as follows: the $ABTS^{\bullet+}$ solution was diluted with ethanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. The oil diluted in ethanol (200 µL) was added to 1000 µL of $ABTS^{\bullet+}$ solution and the absorbance was recorded at 734 nm after 6 min of incubation at room temperature (± 25 °C). Standard curve was prepared as in the hydrophilic TEAC assay.

2.6.2. Hydrophilic and lipophilic oxygen radical absorption capacity (ORAC) assay

Hydrophilic-ORAC (H-ORAC) of phenolic fractions was determined according to Leite-Legatti et al. (2012). Briefly, monguba seeds phenolic fractions were diluted with 75 mmol/L phosphate buffer at pH 7.4. Trolox calibration solutions (50 to 600 µmol/L) were prepared to obtain a standard curve. Diluted samples, Trolox calibration solution, and buffer solution (20 µL) were added to the wells of a 96-well plate. Then, 120 µL of fluorescein (0.378 µg/mL), and 60 µL of AAPH (108 mg/mL) were added to each well, followed by incubation at 37 °C. The fluorescence intensity was monitored every 60 s for 80 cycles by a microplate reader (NOVOstar, model. BMG Labtech, Offenburg, Germany) with excitation and emission filters at 485 nm and 520 nm, respectively. The results were calculated as described in Eqs. (2) and (3), and they were expressed as µmol Trolox equivalents per g of sample dry weight (µmol TE/g DW):

$$AUC = 1 + \sum_{i=1}^{i=80} fi/fo. \quad (2)$$

where f0 is the initial fluorescence reading at 0 min and fi is the fluorescence reading at time *i*.

$$NAUC = AUC_{sample} - AUC_{blank}. \quad (3)$$

NAUC was plotted against sample concentration, and the results were compared to the standard curve (NAUC *versus* Trolox concentration).

Lipophilic-ORAC (L-ORAC) assay was used to evaluate the antioxidant activity from monguba seed oil. Briefly, it was added into each microplate well: 20 µL randomly methylated β-cyclodextrin (RMCD) 7% solution (acetone:water, 1:1 *v*/v), Trolox or monguba seed oil previously diluted, 120 µL of fluorescein (0.378 µg/mL, pH 7.4) and 120 µL de AAPH (108 mg /mL). Trolox calibration solutions (50 to 600 µmol/L) were made to obtain a standard curve. Trolox and sample dilution were prepared with RMCD 7% solution, while fluorescein and AAPH solutions were prepared as in the hydrophilic-ORAC assay.

2.7. Statistical analysis

Each analysis was determined in triplicate and the data are reported as the mean values ± standard deviation.

3. Results and discussion

3.1. Proximate composition of monguba seeds

Table 1 shows the proximate composition and physicochemical properties of monguba seeds. Ripe monguba seeds showed pH 6.69, TTA equal to 1.30 g OA/100 g and TSS of 24.00°Brix. The seeds showed low moisture (5.33 g/100 g), which is important for reducing susceptibility to the biochemical reactions and ensuring microbiological safety. The crude protein content was 12.06%, which is similar to those reported by Oliveira et al. (2000) (12.9%), Silva et al. (2015) (13.75%) and Jorge and Luzia (2012) (11.86%), whereas these results were lower than the Brazil nut (18.6%) (Santos et al., 2013).

Monguba seeds showed considerable total carbohydrate content (35.03%), and high total fiber content (12.38%) within the class of the total carbohydrate. Regarding total fiber content monguba seeds showed similar total fiber content when compared to oat (Gutkoski & Trombetta, 1999). Previous studies showed that consumption of fibers reduces the postprandial plasma glucose and insulin levels, the levels of the total cholesterol and constipation, and can prevent some diseases such as cardiovascular diseases (Bernstein, Titgemeier, Kirkpatrick, Golubic, & Roizen, 2013; Hong, Zi-jun, Jian, Ying-jie, & Fang, 2012). Soluble plant fibers can maintain the intestinal barrier function and reducing the digestive transit time. Therefore, potential toxic exposures have less time in contact with the intestinal wall (Andersen et al., 2018). Thus, monguba seeds are a rich source of total fibers, since 100 g of fresh seeds corresponds to 49.52% of the recommended daily fiber intake (25 g) according to the Food and Drug Administration (FDA) (FDA, 2016a).

Table 1
Proximate composition and physicochemical parameters of monguba (*Pachira aquatica*) seeds.

Parameter	Monguba Seed
Moisture (%)	5.33 ± 0.07
Lipid (%)	43.42 ± 1.57
Protein (N x 6.25) (%)	12.06 ± 1.08
Ash (%)	4.16 ± 0.11
Total Carbohydrate (%)	35.03 ± 1.55
Total Fiber (%)	12.38 ± 2.15
Total Energy Value (Kcal/100 g)	529.01 ± 11.52
pH	6.69 ± 0.04
TSS (°Brix)	24.00 ± 0.04
TTA (g/100 g)	1.30 ± 0.03

Data presented as means ± standard deviation of triplicate determination (n = 3).
Total carbohydrate content was calculated by difference: 100 − (moisture + ashes + lipids + proteins).
The total energy value was estimated by considering the conversion factors of 4 kcal/g for protein and carbohydrate and 9 kcal/g for lipids.

The individual quantification of sugars and RFOs showed considerable concentration of sucrose (3.72 g/100 g DW) and low total amount of RFOs (0.63 g/100 g DW) in monguba seeds (Table 2). The sucrose of monguba seeds was very close to that found for soybean (3.90 g/100 g DW) (Table S2 in the Supplementary material). Ho and Bhat (2015) showed that durian fruit has most of its sucrose content (5.57 g/100 g DW) similar to monguba seeds. Soybean and chia showed higher amount of total mono and disaccharides (5.18 g/100 g DW and 5.52 g/100 g DW, respectively) than monguba seeds (4.24 g/100 g DW).

The RFOs are made up of water-soluble and non-reducing sugars. This group of carbohydrates are fermented in the large intestine by the bacterial microbiota, causing gastrointestinal discomfort and diarrhea (Atta-Ur-Rahman, 2017). However, when RFOs are consumed in adequate amount, they may also play important biological roles, such as stimulation of bifidobacterial growth and suppression of bacterial

Table 2
Individual quantification of mono- and disaccharides, and raffinose family oligosaccharides (RFOs) of monguba (*Pachira aquatica*) seeds.

Compound	Monguba seed (g/100 g DW)
Glucose	0.29 ± 0.02
Fructose	0.23 ± 0.03
Sucrose	3.72 ± 1.00
Maltose	n.d.
Total Mono- and Disaccharides	4.24 ± 0.15
Raffinose	0.23 ± 0.01
Stachyose	0.41 ± 0.02
Verbascose	n.d.
Total RFOs	0.63 ± 0.03

Data presented as mean ± standard deviation for the triplicate determination (*n* = 3).
n.d., not detected.

pathogen. In this way, monguba seeds could be indicated to people with inflammatory bowel disease (IBD) due to their lower RFO concentrations when compared with soybean, black bean, golden linseed (Table S2, Supplement material), chickpea, cowpea, pea and peanut (Atta-Ur-Rahman, 2017).

Regarding the oil fraction, monguba seed showed 43.42% of oil, which is approximate to values previously reported in the literature (Jorge & Luzia, 2012; Oliveira et al., 2000). However, oilseeds such as macadamia, pecans, pine nuts, Brazil nuts, hazelnuts, walnuts, almonds, peanuts, pistachios, cashews, and coconuts exhibited higher total lipid content than monguba seeds (Yang, 2009). Different cocoa cultivars also showed high total fat levels, which ranged from 46.08 to 56.37% (Liendo, Padilla, & Quintana, 1997). Similarly, raw rapeseed also showed high total lipid content than monguba seeds (Yoshie-Stark, Wada, & Wäsche, 2008). On the other hand, monguba seeds showed higher total lipid content than those reported for soybean seed (Qin et al., 2012), sunflower, olive, cotton, corn, sunflower, olive, cotton, corn (Erol, Özcan, & Er, 2011), flaxseed (Bozan & Temelli, 2008) and Indian almond (Akpakpan & Akpabio, 2012).

The fatty acid qualitative analysis showed that the palmitic acid was the main fatty acid among all fatty acids in monguba seed oil. High chromatogram proportion of palmitic acid (60.92%) was observed in monguba seed oil, followed by oleic acid (7.67%), and linoleic acid (6.56%) (Table 3). Similar profile of fatty acids was reported by de Bruin et al. (1963) and Pereira et al. (2013), whereas Jorge and Luzia (2012) observed different results for palmitic acid (44.93%), oleic acid (39.27%) and linoleic acid (11.35%). Dourado et al. (2015) analyzed the fatty acids composition in *P. aquatica* and *P. retusa* species and reported 44.9% and 60% of palmitic acid, and 39.3% and 19.2% of oleic acid, respectively. Variation in the chemical composition could also be due to different geographical origins, climatic conditions and processing methods. Other fatty acid composition in oils has been analyzed in olive, sunflower, corn, peanut and soybean, which showed concentrations of 72.77%, 27.52%, 28.02%, 39.97% and 23.17% of oleic acid, respectively, and 9.47%, 60.11%, 57.98%, 37.75% and 54.87% of linoleic acid, respectively (Monfreda, Gobbi, & Grippa, 2012; Ribeiro, Grimaldi, Gioielli, & Gonçalves, 2009).

Monguba seed oil can be applied in pharmaceuticals and cosmetic emulsions. According to Kim et al. (2008), palmitic acid showed the highest skin permeation effect among the saturated fatty acids tested, while Dourado et al. (2015) showed that the *Pachira aquatica* oil presents the closest saturation band relationship to that of the epidermis. Furthermore, the high content of fatty acids in monguba seed oil such as oleic and palmitic acids provide an appropriated texture and spreadability for cosmetic use.

The total tocopherols content for monguba seed oil was 513.5 mg/kg. The γ-tocopherol was the only tocopherols identified. Some commonly eaten oils such as soybean, sesame and corn showed γ-tocopherol value higher than monguba seed oil (Schimidt & Pokorny, 2005). A lower value was found in another study (51.27 mg/kg), which predominant tocopherols were γ-tocopherol (34.66 mg/kg) and α-tocopherol (15.23 mg/kg) (Jorge & Luzia, 2012). However, the isomers γ-tocopherol and δ-tocopherol presented higher antioxidant activity in food lipids (Schimidt & Pokorny, 2005). Crude oil from rapeseed, sunflower and soybean showed the following ranges of total tocopherol, respectively: 464–1458 mg/kg, 725–1892 mg/kg and 1094–2484 mg/kg Monguba seed oil showed total tocopherols content higher than some nuts, *e.g.* almond, hazelnut, pistachio, Brazil nut, peanut and macadamia, ranging from 471.2 to 59.8 mg/kg of oil (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2006; Yang, 2009). The intake of seeds rich in tocopherols can prevent the emergence of degenerative diseases, for example Alzheimer, as tocopherols have been appointed as protective of lipids oxidative stress and free radicals generation (Kontush & Schekatolina, 2004; Yoshihara, Fujiwara, & Suzuki, 2010).

Regarding the lipophilic compounds of monguba seed oil, the phenolic compounds extracted from monguba seed oil showed high antioxidant activity by TEAC and ORAC assays (3.30 μMol TE/g and 10.70 μMol TE/g, respectively), when compared with other well-known vegetable oils such as soybean, extra virgin olive, corn, sunflower and peanut (Pellegrini et al., 2003).

Monguba seeds also showed high amount of ash (4.16%). Regarding minerals, potassium (1461.84 mg/100 g DW) was the main mineral, followed by magnesium and calcium (304.00 and 158.37 mg/100 g DW, respectively) (Fig. 1). Leterme et al. (2006) showed a mineral composition of monguba seeds (1081–1782 mg/100 g DW of potassium, 412–503 mg/100 g DW of magnesium and 117–194 mg/100 g DW of calcium) similar to the present study. Furthermore, the ripe durian fruit, which also belongs to the Bombacaceae family showed a lower amount of minerals (1381.1 mg/100 g DW of potassium, 70.1 mg/100 g DW of magnesium and 17.2 mg/100 g DW of calcium) than monguba seed (Haruenkit et al., 2010). In a recent study, Grela, Samolińska, Kiczorowska, Klebaniuk, and Kiczorowski (2017) analyzed the mineral content of some leguminous species and found higher amounts of potassium (847–1180 mg/100 g DW), magnesium (86–222 mg/100 g DW) and calcium (49–698 mg/100 g DW) than monguba seeds.

Table 3
Fatty acids and tocopherol composition of monguba (*Pachira aquatica*) seed oil.

Fatty acid (%)[a]	Monguba seed oil
Palmitic acid	60.92 ± 0.11
Stearic acid	1.77 ± 0.01
Oleic acid	7.67 ± 0.01
Linoleic acid	6.56 ± 0.01
Unknow	6.81 ± 0.01
Unknow	11.39 ± 0.08
Others[b]	4.91 ± 0.04
Σ Saturated fatty acids	62.68 ± 0.11
Σ Unsaturated fatty acids	14.21 ± 0.01
Tocopherol (mg/kg)	
α-tocoferol	n.d.
β-tocoferol	n.d.
γ-tocoferol	513.5 ± 9.00
δ-tocoferol	n.d.

n.d., not detected.
Data presented as means ± standard deviation of triplicate analyses (n = 3).
[a] Qualitative analysis, in which the proportion of each compound was estimated dividing its mean area by the total area of the chromatogram and expressed as percentage.
[b] Sum of fatty acids with values below 1.0%.

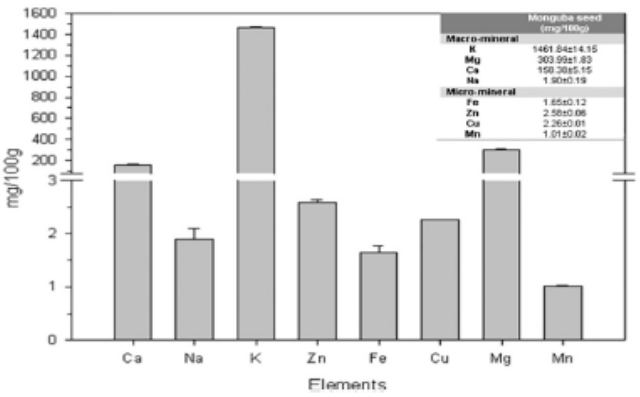

Fig. 1. Macro- and micro-mineral content of monguba (*Pachira aquatica*) seeds (mg/100 g DW). Ca: calcium; Na: sodium; K: potassium; Zn: zinc; Fe: iron; Cu: copper; Mg: magnesium; Mn: manganese.

Monguba seeds showed a higher potassium content than banana (TACO, 2017), which is known as a good source of this mineral. Therefore, monguba seeds could be a new source of potassium, since 100 g of dry seeds correspond to 41.65% of the recommended daily potassium intake according to the World Health Organization (WHO) recommendations (WHO, 2012), which suggests a potassium intake of at least 3510 mg/day for adults. Monguba seeds also showed high amount of zinc and copper, which were higher than Brazil nut (1.40 and 3.50 mg/100 g DW, respectively). Regarding the zinc content, it was similar to those for peanut, chickpea and nut (TACO, 2017). The intake of 100 g of monguba seeds can provide approximately 17.20% of the recommended daily intake for zinc (15 mg) (FDA, 2016b). In this way, monguba seeds could also be a new source of zinc. However, it is necessary more studies to assess the mineral bioavailability of monguba seeds, because the seeds have antinutritional factors that adversely impact protein digestion (trypsin and chymotrypsin inhibitors), mineral absorption (lectins, phytates and oxalates), and starch digestion (amylase inhibitors and saponins) (Shi, Mu, Arntfield, & Nickerson, 2017; Singh, Singh, Singh, & Kaur, 2017).

Phytic acid is the principal storage form of phosphorus in seeds, and it has significant roles in metabolism and pathogen resistance in plants. Nevertheless, phytic acid has the ability to chelate metal ions of minerals such as calcium, copper, magnesium, iron and zinc, thereby adversely affecting their absorption in the gastrointestinal tract (Tiwari & Singh, 2012). Another form of antinutrients is the tannins, which belongs to the group of polyphenolic compounds. The tannins cause a decrease in the digestibility of protein and carbohydrates due to formation of insoluble enzyme-resistant complexes (Reddy, Pierson, Sathe, & Salunkhe, 1985). Besides it, the polyphenols could react with proteins and enzymes, and be also prone to act as trypsin and amylase inhibitors (Deshpande, Cheryan, Salunkhe, & Luh, 1986). Therefore, it is necessary more studies about the mineral bioavailability of monguba seeds.

3.2. Phenolic compounds profile

In this study 10 phenolic compounds were identified, among which flavonoids and phenolic acids were the main classes (Table 4). Among the fractions that were studied (free, esterified, glycosylated and insoluble-bond), the esterified fraction showed the highest phenolic compound content (74.58%), followed by glycosylated (13.02%), free (8.22%) and insoluble-bound (4.18%) fractions. Caffeic acid was the most abundant compound in monguba seeds, corresponding to 57.5% of the total phenolic composition. Low concentrations of flavonoids were found in glycosylated fractions such as gallocatechin and quercetin. The glycosylated fractions showed the major diversity of phenolic compounds, whereas the esterified fractions showed the highest phenolic compound contents. In addition, soluble phenolics contributed significantly to the total phenolics of monguba seeds, since they accounted for > 95.8% of the phenolic composition of monguba seeds.

Phenolic compounds are common in several oilseeds, and monguba seeds showed high amount of phenolics, in a total of 775.17 μg/g DW. Caffeic, ferulic and 4-hydroxybenzoic acids were the main phenolic compounds, as shown in Table 4. Pająk, Socha, Gałkowska, Rożnowski, and Fortuna (2014) reported an amount of 127.4 μg/g DW in sunflower seeds, which free phenolic acids occurred in the largest amount, namely protocatechuic (50.8 μg/g DW), caffeic (25.5 μg/g DW), ferrulic (16.9 μg/g DW) and gallic (11.2 μg/g DW) acids. Alu'datt, Rababah, Ereifej, and Alli (2013) observed that the predominant individual phenolic compounds in full-fat and defatted flaxseed were ferulic, sinapic and p-coumaric acids.

3.3. Antioxidant activity

The health-beneficial effects derived from phenolic compounds in fruits and vegetables have been attributed to their antioxidant activity. The antioxidants play an important role in removing excessive amounts of reactive oxygen and nitrogen species under oxidative stress conditions, thereby preventing the occurrence of some diseases such as coronary heart disease, cancer and Alzheimer's disease (Gülçin, 2012). In this approach, the antioxidant activity of the different phenolic fractions of defatted monguba seed was investigated.

The esterified fraction showed the highest antioxidant activity measured by TEAC and ORAC assays, followed by free and glycosylated fractions (Table 5). In this way, soluble phenolics (free, glycosylated, and esterified fractions) contribute significantly to the antioxidant activity of monguba seeds. The antioxidant activity assessed by ORAC assay was similar from durian fruit fresh (Isabelle et al., 2010), whereas durian fruit and avocado methanolic extracts showed higher antioxidant activity levels by TEAC assay than this study (Poovarodom et al., 2010). Cocoa extract showed higher values in antioxidant activity assays than monguba seeds (Cádiz-Gurrea et al., 2014).

The antioxidant activity of the phenolic compound standards (Table 5) was in the decreasing sequence of ferulic acid > caffeic acid > chlorogenic acid through the TEAC assay, whereas the ORAC

Table 4
Concentration of identified phenolic compounds in four phenolic fractions (free, esterified, glycosylated and insoluble-bound) of monguba (*Pachira aquatica*) seeds.

Polyphenol Sub-class	Compound	Concentration (μg/g DW)				
		Free	Esterified	Glycosylated	Insoluble-bound	Σ (Free to Insoluble-bound)
Flavanols	Gallocatechin	n.d	n.d.	1.08 ± 0.27	n.d.	1.08 ± 0.27
	Σ Flavanols	–	–	1.08 ± 0.27	–	1.08 ± 0.27
Flavonols	Quercetin	n.d.	n.d.	0.34 ± 0.04	n.d.	0.34 ± 0.04
	Σ Flavonols	–	–	0.34 ± 0.04	–	0.34 ± 0.04
Σ Flavonoids		–	–	1.42 ± 0.31	–	1.42 ± 0.31
Hydroxybenzoic acids	Protocatechuic acid	n.d.	2.44 ± 0.30	0.59 ± 0.02	1.15 ± 0.12	4.17 ± 0.37
	4-Hydroxybenzoic acid	n.d.	44.33 ± 6.72	73.87 ± 5.11	0.68 ± 0.03	118.88 ± 11.69
	Gentisic acid	n.d.	n.d.	1.05 ± 0.06	n.d.	1.05 ± 0.06
	Σ Hydroxybenzoic acids	–	46.77 ± 7.00	75.50 ± 5.13	1.83 ± 0.09	124.10 ± 12.08
Hydroxycinnamic acids	Chlorogenic acid	58.82 ± 0.85	n.d.	n.d.	n.d.	58.82 ± 0.85
	Caffeic acid	1.35 ± 0.15	412.21 ± 52.17	7.88 ± 1.27	24.10 ± 0.41	445.54 ± 53.96
	p-Coumaric acid	0.30 ± 0.02	24.37 ± 0.30	0.52 ± 0.03	1.15 ± 0.19	26.33 ± 2.42
	Vanillic acid	1.91 ± 0.26	n.d.	n.d.	0.99 ± 0.07	2.90 ± 0.33
	Ferulic acid	1.31 ± 0.66	94.75 ± 20.06	15.64 ± 1.03	4.37 ± 1.36	116.07 ± 22.09
	Σ Hydracycinnamic acids	63.69 ± 1.29	531.32 ± 71.37	24.04 ± 2.33	30.60 ± 1.71	649.66 ± 75.12
Σ Phenolic acids		63.69 ± 1.29	578.09 ± 78.36	99.54 ± 7.39	32.43 ± 1.13	773.75 ± 81.19
Σ (flavonoids + phenolic acids)		63.69 ± 1.29	578.09 ± 78.36	100.96 ± 7.59	32.43 ± 1.13	775.17 ± 87.40

n.d., not detected.
Data presented as mean ± standard deviation for the triplicate determination (n = 3).

Table 5
Antioxidant activity of different phenolic fractions from monguba (*Pachira aquatica*) seed, and antioxidant activity of the main phenolic compounds found in these fractions.

Seed phenolic fraction	TEAC (μMol TE/g)	ORAC (μMol TE/g)
Insoluble-bound	1.90 ± 0.03	3.91 ± 0.37
Glycosylated	5.07 ± 0.02	14.06 ± 1.82
Free	5.49 ± 0.09	12.92 ± 0.64
Esterified	8.69 ± 0.16	20.46 ± 0.99
Phenolic compound standard[a]		
Chlorogenic acid	7.52 ± 0.20$_c$	5.84 ± 0.52$_b$
Caffeic acid	13.29 ± 0.28$_b$	18.84 ± 1.89$_a$
Ferulic acid	60.82 ± 1.76$_a$	7.98 ± 0.97$_b$

Data represent mean values for each sample ± standard deviations (n = 3). Means followed by the same lowercase letters in a column are not significantly different ($p > .05$).

[a] Antioxidant activity of phenolic compounds standards was expressed as μMol TE/g of standard.

assay provided the following caffeic acid > ferulic acid > chlorogenic acid. Comparing these data with the antioxidant activity of seed phenolic fractions and their individual phenolic composition, it is possible to observe that the esterified fraction (highest antioxidant fraction by TEAC and ORAC assay) showed the highest content of caffeic and ferulic acids (Table 4). In this way, we can partially attribute the antioxidant activity of monguba seed to the presence of acid phenolic compounds, mainly caffeic and ferulic acids.

4. Conclusion

Monguba seeds showed high amount of mono- and disaccharides, whereas the amount of raffinose family oligosaccharides was low. High amount of potassium and zinc was found in monguba seeds, which can be a novel source of these minerals, since 100 g of dry seeds correspond to 41.65% and 17.20% of the recommended daily intake for potassium and zinc, respectively. However, further studies are needed to assess the mineral bioavailability of monguba seeds because seeds generally have antinutritional factors. Monguba seeds also showed high content of oil (43.42%), in which the palmitic, oleic and linoleic acids and γ-tocopherol were the main compounds in the lipid fraction of monguba seeds. Monguba seed oil could be used as raw material for the development of new skin care products due to its high amount of palmitic acid. Regarding the phenolic compounds, UHPLC-ESI-MS/MS showed caffeic, ferulic and 4-hydroxybenzoic acids as the main phenolic compounds. These phenolic acids were mainly observed in the esterified fraction, which showed the highest *in vitro* antioxidant activity. These findings clearly show that monguba seed may contribute to various biological and nutritional applications.

Conflict of interest

The authors report no conflict of interest.

Acknowledgements

The authors Alexsandra Pereira Rodrigues, Gustavo Araujo Pereira and Henrique Silvano Arruda are thankful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support offered through fellowships (Finance Code 001). We would like thankful to Pedro Henrique F. Tomé by performing the proximate composition analysis at the Federal Institute of Triângulo Mineiro, and the Fatty and Oil Laboratory of the University of Campinas by performing the fatty acids and tocopherol analyses, and Thomson Mass Spectrometry Laboratory of the University of Campinas for their infrastructural support. This work was supported by the São Paulo Research Foundation (FAPESP) (process numbers: 2015/50333-1) and CAPES-PROEX (23038.000795/2018-61).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.01.014.

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Chapter 3

Research Article

Obtaining high-quality oil from monguba (*Pachira aquatica* Aubl.) seeds by using supercritical CO_2 process

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Article published in The Journal of Supercritical Fluids

(Impact Factor 3.744 – Qualis A1)

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The article can be accessed through the following link:

https://doi.org/10.1016/j.supflu.2021.105192

The Journal of Supercritical Fluids 171 (2021) 105192

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Obtaining high-quality oil from monguba (*Pachira aquatica* Aubl.) seeds by using supercritical CO_2 process

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HIGHLIGHTS

- The highest global yield was obtained at 35 MPa and 60 °C.
- Palmitic acid was the main fatty acid in all process settings studied.
- Pressure and temperature did not affect the γ-tocopherol content and the antioxidant activity.
- High-quality monguba oil was extracted by using supercritical CO_2.

GRAPHICAL ABSTRACT

ARTICLE INFO

Keywords:
Emerging technology
Fatty acids
Kinetic
Antioxidant activity
Tocopherol

ABSTRACT

Monguba seeds show high content of extractable oil, approximately 54% (w/w). Literature data have reported the recovery of monguba seed oil using conventional methods. However, the use of emerging and environment-friendly methods should be applied, especially the supercritical fluid process. Therefore, we evaluated herein the effect of supercritical CO_2 process (15–35 MPa and 40–60 °C) on yield, fatty acid profile, tocopherols content, and antioxidant activity of monguba oil. The highest global yield (39.64 g/100 g) was obtained by using 35 MPa and 60 °C. Palmitic acid was the main fatty acid, which partially justifies the highest global yield observed at 60 °C. Supercritical process operated at 60 °C, 35 MPa for 160 min showed global yield equal to the Soxhlet method. The use of supercritical CO_2 allows the recovery of high content of high-quality monguba oil without affecting the antioxidant activity and the γ-tocopherol content.

1. Introduction

The *Pachira aquatica* Aubl. tree is found from Southern Mexico to South America [1,2]. It belongs to the Malvaceae family due to its genetic similarity to the species of this family [3]. The *Pachira aquatica* plant is popularly known as monguba, munguba, and Guiana chestnut [4,5]. Monguba seeds show high content of oil (approximately 54%) in which palmitic acid and total unsaturated fatty acids (*e.g.* oleic and linoleic acids) stand out in values of 44.90–76.19% and 13.42–51.11%, respectively. Other important properties of the monguba oil include high content of bioactive lipid compounds, such as vitamin E and phytosterols. Monguba seeds have also been reported as a source of proteins, amino acids (essential and non-essential), minerals, and fiber [1,4,6–8].

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https://doi.org/10.1016/j.supflu.2021.105192
Received 4 September 2020; Received in revised form 2 February 2021; Accepted 4 February 2021
Available online 10 February 2021

The consumer demand and awareness of bioactive compounds and their health benefits have driven the food industry to use functional foods. Technology advances allow food scientists to extract high content of bioactive compounds from food without impacting the environment. Much effort has been made to develop extraction processes involving eco-friendly solvents, such as supercritical CO_2 [9].

Supercritical fluid extraction (SFE) is more advantageous than conventional techniques due to the use of low temperature and non-toxic solvents, high selectivity, and short process time [10–13].

Carbon dioxide (CO_2) is commonly used in SFE due to its low critical points (31 °C, and 7.3 MPa). It has also been explored to extract heat-labile substances from food, especially bioactive lipid compounds [13,14]. CO_2 acquires intermediate physicochemical properties of liquids and gases at critical point (*e.g.* gas viscosity and liquid density), which enhances its penetration into the matrix and, as a consequence, improves the extraction yield [15,16].

The oil from monguba seeds has been recovered by using conventional methods. Nonetheless, the quality of the oil can be affected since these methods commonly employ toxic solvents and high temperatures. As previously presented, the monguba seed has a high content of oil with an interesting profile [6]. It is important to use extraction methods that do not affect its quality. Thus, supercritical CO_2 is an alternative to obtain high-quality oil since it is non-toxic, eco-friendly, and residue-free [4,6]. In this context, the effects of temperature and pressure on total yield, tocopherol content, fatty acid profile, and antioxidant activity of the monguba seed oil were evaluated. The extraction kinetics was evaluated and the monguba oil obtained by the supercritical carbon dioxide (SC-CO_2) and Soxhlet method were compared.

2. Materials and methods

2.1. Plant material and sample preparation

Monguba fruits at full physiological maturity (Fig. 1A) were collected in Uberlândia (18°54′41″ south latitude, 48°15′44″ west longitude, and 843 m altitude), MG, Brazil. The fruits were transported from the collection site to the laboratory within 24 h after collection. The seeds were manually separated from the peel. Approximately 250 g of seeds was freeze-dried (−50 °C and 86 μHg) and grounded by using a handheld food processor. The flour (Fig. 1C and D) particles were distributed, with sizes ranging from 2.36 mm/μm and 500 mm/μm, according to sieve mesh size. The monguba seed flour was stored in a freezer (−18 °C) until analysis.

The Genetic Heritage Management Board (CGen) under number A214301, following the Law n° 13.123/2015 and its regulations, regulated the activity of access to Genetic Heritage.

2.2. Extraction of monguba oil

2.2.1. Conventional extraction

The oil was extracted from monguba seeds with a Soxhlet extractor. The freeze-dried sample (10 g) was wrapped in filter paper and inserted into the Soxhlet apparatus connected to a solvent flask containing 300 mL of petroleum ether. The system was heated to boiling and reflux was maintained at 60 °C for 5 h. The ethereal fraction was evaporated by using a rotary evaporator at 45 °C [6]. The oil obtained was collected in an amber glass bottle and stored in a freezer (−18 °C) until analysis. This process was performed three times.

2.2.2. Supercritical fluid extraction

The SFE process was performed using a commercial Spe-ed SFE unit (Applied Separations, 7071, Allentown, USA) equipped with a cooling bath (Marconi, model MA184, São Paulo, Brazil), a pneumatic pump, an electric oven, a compressor (Shulz S/A, model MS 3, Santa Catarina, Brazil), and a flow totalizer (LAO G0, São Paulo, Brazil). An extraction vessel of 5 mL of volume, 2 cm of diameter, and 1.6 cm of height was used (Thar Designs, Pittsburgh, PA). In each experiment, the extractor was filled with approximately 5 g of monguba seed flour (density 1.4987 g/cm^3). The experiments were performed at pressures of 15, 25, and 35 MPa and temperatures of 40 and 60 °C as presented in Table 1. The supercritical CO_2 solvent flow rate was set at 5 g/min and the solvent-to-feed ratio (S/F) was kept constant at 25. The static period of the system was maintained for 5 min. The oil extract was collected in an amber glass bottle and stored in a freezer (−18 °C) until analysis. SFE was performed twice for each process setting.

2.2.3. Extraction kinetic

The extraction kinetic was performed using the pressure and temperature that promoted the recovery of the highest amount of oil (global oil yield). Kinetic experiments were performed using a commercial Spe-ed SFE unit previously described in Section 2.2.2. Approximately 20 g of monguba

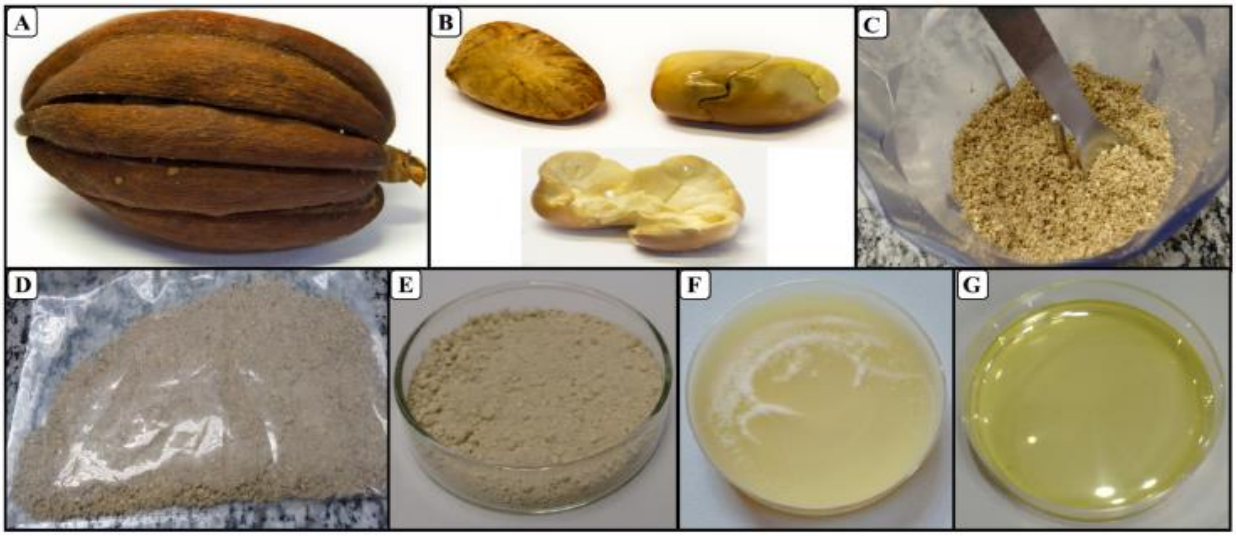

Fig. 1. *Pachira aquatica* Aubl. A, maturity stage of monguba fruit; B, fresh seeds; C and D, freeze-dried monguba seed flour (this material was employed in supercritical extraction, raw material); E, monguba seed flour after supercritical extraction; F and G, oil extracted using SC-CO_2 (solid and liquid phases, respectively).

Table 1
Global yield, γ-tocopherol purity and recovery and antioxidant activity from monguba seed oil by supercritical CO_2 extraction and conventional method.

Conditions	Solvent	Pressure (MPa)	Temperature (°C)	CO_2 density (kg/m³)[b]	Yield (g/100 g RM[d])	ORAC (μmol/g oil)	γ-Tocopherol purity (mg/kg oil)	γ-Tocopherol recovery (mg/kg RM)
1	Supercritical CO_2	15	40	780.23	7.42 ± 0.77	470.17 ± 99.24	51.32 ± 6.22	3.69 ± 0.66
2	Supercritical CO_2	15	60	604.09	6.63 ± 0.29	1086.90 ± 419.52	48.37 ± 0.50	8.73 ± 0.74
3	Supercritical CO_2	25	40	879.49	16.97 ± 0.20	809.51 ± 131.49	51.45 ± 4.34	14.06 ± 3.21
4	Supercritical CO_2	25	60	782.55	21.93 ± 1.57	992.51 ± 181.48	54.75 ± 10.28	3.20 ± 0.03
5	Supercritical CO_2	35	40	934.81	26.93 ± 1.71	712.37 ± 423.52	52.22 ± 11.92	12.01 ± 2.25
6	Supercritical CO_2	35	60	862.94	39.64 ± 2.09	739.04 ± 349.25	49.36 ± 1.85	19.57 ± 0.73
Soxhlet	Petroleum Ether	101.7[a]	60	640[c]	54.29 ± 2.86		25.14 ± 0.73	–

Values expressed as mean ± standard deviation.
[a] Atmospheric pressure value in Campinas, Brazil.
[b] NIST Chemistry WebBook database.
[c] Density value at 20 °C.
[d] RM: Raw material (results expressed on dry basis).

flour (raw material, RM) was placed in a 25 mL stainless-steel extraction vessel (2.0 cm of diameter and 8.0 cm of height). Extraction conditions were kept at 60 °C and 35 MPa, and supercritical CO_2 as a solvent. The solvent flow rate was held constant at 5 g/min for 250 min. The oil extracts were collected at 1, 3, 6, 10, 14, 18, 24, 30, 40, 50, 60, 80, 100, 130, 160, 200, and 250 min. The extraction kinetics was performed twice to remove operational bias.

2.2.4. Global oil yield

The global oil yield was calculated as the ratio between the extracted mass and the amount of monguba flour (raw material, RM) used on a dry basis (Eq. (1)).

$$Y(g/100g\ RM) = \frac{m_e}{m_f} \times 100 \quad (1)$$

where Y (g/100 g RM) is the global oil yield expressed in g/100 g RM (results expressed on a dry basis), m_e is the extracted mass (g) and m_f is the initial mass of monguba flour (g).

2.3. Fatty acid profile

Fatty acid methyl esters (FAMEs) were separated according to Hartman and Lago [17] and an adaptation based on the AOCS Ce 1b-89 method. The fatty acid composition was performed on a gas chromatograph (Shimadzu, Series 2010 Plus) equipped with a flame ionization detector (FID) using a Restek-Wax column (30 m x 0,32 mm ×;0,25 μm). The chromatograph was operated under the following conditions: injection volume of 1.0 μL on slip mode (1/10) and detector and injector temperatures at 250 °C. The oven temperature was programmed as follows: initial temperature at 60 °C, increasing up to 130 °C at a rate of 20 °C/min for 7 min. Subsequently, the temperature increased to 240 °C at a rate of 30 °C/min for 18 min. Hydrogen gas was used as a carrier gas. The qualitative composition was determined by comparing the retention times of analytes with the authentic standards (F.A.M.E C8-C22, Sigma-Aldrich). The analysis was performed in duplicate and results were obtained by summing the peak area and expressed as percentages.

2.4. Tocopherols content

The tocopherol content in monguba seed oil was determined according to Hashim et al. [18] by using high-performance liquid chromatography (Shimadzu, LC-20 CE) coupled with a fluorescence detector using a silica column (LiChrospher Si-60 250 × 4,6 mm × 5 μm, Merck). Samples were diluted in hexane and filtered through a 0.20 μm filter. The chromatograph was operated under the following conditions: fluorescence detector with emission at 330 nm and excitation at 290 nm; injected volume of 20.0 μL; mobile phase of hexane/isopropanol (99:1 v/v) at the flow rate of 1.0 mL/min. Tocopherol isomers (α-, γ-, and δ-tocopherol) were investigated. The authentic analytical standards (Sigma-Aldrich) were used to plot the standard curve at concentrations ranging from 0.32 to 25.75 mg/mL (R^2 = 0.999). This analysis was performed in triplicate and results were expressed as mg per kg of oil.

2.5. Lipophilic oxygen radical absorption capacity (ORAC) assay

Lipophilic-ORAC (L-ORAC) assay was used to evaluate the activity of lipophilic antioxidants from the monguba seed oil. Briefly, 20 μL randomly methylated β-cyclodextrin (RMDC) 7% solution (acetone:ethanol, 1:1 v/v), Trolox or diluted sample, 120 μL of fluorescein (0.378 μg/mL, pH 7.4), and 120 μL 2,2´-azobis (2-amidinopropane) dihydrochloride (AAPH) (108 mg/mL) were added to each well of the microplate. Trolox and sample dilution were performed with RMCD 7% solution. The standard curve was calibrated with a Trolox solution at concentrations from 16.25 to 650 μmol/L. The fluorescence intensity was monitored by a micro-plate reader (NOVOstar, model. BMG Labtech, Offenburg, Germany) every 1 min for 80 cycles with excitation (485 nm) and emission (520 nm) filters. The area under the curve (AUC) and the net area under the curve (NAUC) of the standards and the samples were determined using Eqs. (2) and (3) and expressed as μmol Trolox equivalents per g of the extracted oil [19]. This analysis was performed in triplicate.

$$AUC = 1 + \sum_{i=1}^{i=80} fi/fo \quad (2)$$

where f0 is the initial fluorescence reading at 0 min and fi is the fluorescence reading at time i.

$$NAUC = AUC_{sample} - AUC_{blank} \quad (3)$$

NAUC was plotted against sample concentration and the results were compared to the standard curve (NAUC *versus* Trolox concentration).

2.6. Statistical analysis

Pressure (15, 25, and 35 MPa) and temperature (40 and 60 °C) of the supercritical fluid process were evaluated with a randomized full factorial design (3 × 2) with two blocks, totalizing 12 experimental runs. The effects of pressure, temperature, and the interactive effects (pressure*temperature) on total yield, tocopherols, fatty acid profile, and antioxidant activity were analyzed by ANOVA ($p < 0.05$) using the Minitab 18® software (Minitab Inc., State College, PA, USA). Data were reported as the mean values ± standard deviation.

3. Results and discussion

3.1. Obtaining monguba oil by using SC-CO_2

The monguba seed oil was recovered by using supercritical CO_2. The effects of pressure (15–35 MPa) and temperature (40–60 °C) on the global yield, tocopherol content, antioxidant activity, and fatty acid profile were evaluated.

3.1.1. Global yield

The temperature (40–60 °C), pressure (15–35 MPa), and the interactive effect between them statistically affected the monguba oil global yield (Table 1 and Supplementary Table 1; Table S1). The highest global yield was observed at 35 MPa and 60 °C, while the lowest value was obtained at 15 MPa and 40 °C (Table 1). The CO_2 density increases with the increase in pressure at a constant temperature, which improves the capacity of the supercritical phase [20]. Although the increase in temperature at a constant pressure corresponds to a decrease in CO_2 density, the vapor pressure of the solutes (to be extracted) increases. The increase in oil recovery is thus due to an increase in the solute solubilities in supercritical CO_2 when the temperature increases. Palmitic acid is the main fatty acid of the monguba oil, and its melting point is higher than 60 °C (approximately 63 °C). Nevertheless, the interactive effect between the pressure and the temperature was statistically significant (Table S1), which indicates that both previously described events affected the monguba oil recovery [21,22]. The effect of temperature and pressure on the global yield can be observed in Fig. 2.

The global yield obtained by Soxhlet with petroleum ether as a solvent (54.29 g/100 g) was higher than SC-CO_2 (39.64 g/100 g). The solvent recycling, high solvent/solute ratio, and the solvent used in the Soxhlet method can partially explain this high global yield. The petroleum ether solvent can easily access the active sites inside the solid plant material and solubilize the target molecules [21]. It is also interesting to note that the global yield of 39.64 g/100 g was obtained after 30 min using SC-CO_2 (35 MPa and 60 °C), while the process time of the Soxhlet extraction was 300 min. In addition, petroleum ether, a flammable and harmful solvent, was employed in the Soxhlet method and thermolabile compounds can be destroyed at high temperatures. Although the Soxhlet extraction showed a high global yield, it can affect the chemical and sensory properties of the monguba oil [23,24].

Lopes et al. [25] showed a global yield of 8.74 g/100 g of monguba seeds using SC-CO_2 at flowing conditions of 22 MPa and 40 °C. The process presented herein recovered a large amount (16.97 g/100 g) of oil from monguba seeds using SC-CO_2 at process conditions (25 MPa and 40 °C) close to those applied by Lopes et al. [25] (Table 1). This difference in the oil yield may be due to the maturity stages of the fruits, geographic variations, and climatic conditions, as well as the process and solvents employed.

Ultrasound-assisted extraction and the use of n-hexane as a solvent has been recently applied to recover the monguba oil. A yield of 47.70 g/100 g was observed after 2 h of extraction at 35 °C [26]. This value was higher than the global yield obtained herein using the best supercritical setting (35 MPa and 60 °C). Although Raiser et al. [26] proposed the ultrasound-assisted technique as a substitute for the Soxhlet method to obtain the monguba oil with shorter extraction time, the use of n-hexane (a toxic solvent) with the ultrasound-assisted extraction is not recommended since it is an emergent technology commonly employed due to its sustainable aspect. Generally recognized as safe (GRAS) solvents are an alternative to be used with this technology.

The global yield of the monguba oil obtained by SC-CO_2 was higher than the palm kernel oil (0.47–7.82 g/100 g), soybean oil (13.5–19.3 g/100 g), and pecan nut cake oil (0.58–43.65 g/100 g) extracted by SC-CO_2 [27–29], whereas the global yield of cocoa butter (31.32 g/100 g) was similar to the monguba oil (39.64 g/100 g) at 35 MPa and 60 °C [30].

3.1.2. Fatty acid profile

Table 1 shows the fatty acid profile of the monguba oil recovered at different process settings. By analyzing the statistical data, it was observed that the temperature affected the fatty acid profile ($p < 0.05$) (Table S1). For saturated fatty acids (palmitic and stearic acids and their sum), the increase in temperature (40 °C from 60 °C) positively affected the extraction, while oleic and linoleic acids were negatively affected by the increase in temperature. However, the pressure and temperature did not affect the sum of unsaturated fatty acids ($p > 0.05$). As shown in Table 2, the lowest temperature (40 °C) led to an increase in unsaturated fatty acids. The best conditions showed values of 9.27% of oleic acid and 9.91% of linoleic acid at 40 °C and 25 MPa. On the other hand, when the maximum temperature (60 °C) was applied, it was possible to obtain higher values of saturated fatty acids. Although these process parameters require further studies to prove such effects, under the conditions of this study, the temperature may be used as a selection parameter of fatty acids during the extraction.

When comparing the data with the literature, we observed that the fatty acid profile of the monguba oil recovered by supercritical CO_2 was very close to the reports using conventional and emerging technologies [4,6,25]. It indicates that the process maintained the composition and stability of fatty acids from the monguba seeds. The main fatty acid was

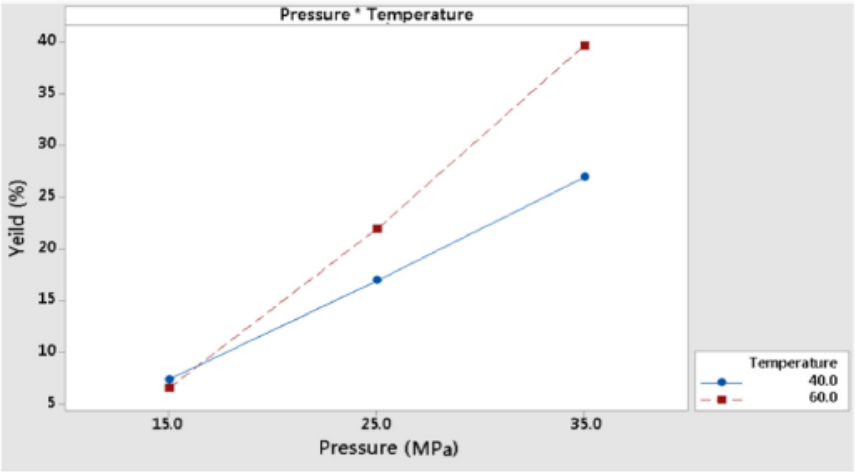

Fig. 2. Effect of the interaction between temperature and pressure on the global yield of monguba oil obtained by SC-CO_2 process.

Table 2
Fatty acids profile (% total fatty acids) from monguba seed oils by different method extraction.

Conditions	Solvent	Variables[a]	C 16:0	C 18:0	Σ Saturated Fatty acids	C 18:1	C 18:2	Σ Unsaturated Fatty acids
1	Supercritical CO_2	15/40	80.11 ± 1.23	2.72 ± 0.03	82.83 ± 1.32	8.32 ± 0.61	8.84 ± 0.65	17.16 ± 1.32
2	Supercritical CO_2	15/60	82.07 ± 2.41	2.81 ± 0.04	84.88 ± 3.75	7.30 ± 1.20	7.82 ± 1.26	15.12 ± 3.75
3	Supercritical CO_2	25/40	78.20 ± 0.18	2.62 ± 0.04	80.82 ± 0.06	9.27 ± 0.08	9.91 ± 0.14	19.18 ± 0.06
4	Supercritical CO_2	25/60	81.19 ± 0.18	2.84 ± 0.00	84.03 ± 0.62	7.77 ± 0.13	8.20 ± 0.05	15.97 ± 0.62
5	Supercritical CO_2	35/40	81.09 ± 1.73	2.87 ± 0.06	83.96 ± 3.90	7.84 ± 0.81	8.20 ± 0.98	16.04 ± 1.61
6	Supercritical CO_2	35/60	80.97 ± 0.26	2.94 ± 0.02	83.91 ± 0.24	7.86 ± 0.12	8.23 ± 0.16	16.09 ± 0.24
Soxhlet	Petroleum Ether	atm[b]/60	79.34 ± 1.05	2.99 ± 0.07	82.33 ± 1.12	7.65 ± 0.48	8.93 ± 0.56	16.58 ± 1.04

Values expressed as mean ± standard deviation.
[a] Pressure (MPa)/Temperature (°C).
[b] atm = Atmospheric pressure (101.7 MPa).

palmitic acid (78.20–81.19%) followed by linoleic and oleic acids, which showed values of 7.82–9.91% and 7.30–9.27%, respectively (Table 2).

The main fatty acid in the monguba seed oil corroborates with previous reports [4,6,25,26]. Jorge and Luzia [4] reported 44.93% of palmitic acid, 39.27% of oleic acid, and 11.35% of linoleic, whereas Rodrigues et al. [6] obtained 60.92% of palmitic, 7.67% of oleic, and 6.56% of linoleic acids, respectively, using the Soxhlet method and petroleum ether as a solvent. Lopes et al. [25] extracted the oil from monguba seeds using SC-CO_2 and isopropanol, reporting 75.25–79.21% of palmitic, 5.45–6.59% of oleic, and 9.61–10.64% of linoleic acids. They also extracted the oil from monguba using the Soxhlet method and n-hexane as a solvent, reporting values of 76.19% of palmitic, 6.62% of oleic, and 9.38% of linoleic acids. Raiser et al. [26] obtained 75.27% of palmitic acid, 8.19% of oleic acid, and 6.91% of linoleic acid using the ultrasound-assisted extraction and n-hexane as a solvent. Despite the use of different technologies, the fatty acid profile was similar in all reports.

3.1.3. *Antioxidant activity and γ-tocopherol content*

Unlike the fatty acid profile, the statistical analysis (Table S1) showed that the pressure and the temperature did not affect the antioxidant activity and the γ-tocopherol content of the monguba oil. Furthermore, the antioxidant and γ-tocopherol data were not correlated according to the Pearson coefficient (r = −0058 and −0046, ORAC/γ-tocopherol purity and ORAC/γ-tocopherol recovery, respectively). Therefore, unidentified lipophilic compounds can contribute to the antioxidant activity of the monguba oil. It is important to point out that the antioxidant activity of the monguba oil recovered by SC-CO_2 was higher than the Soxhlet method (petroleum ether as a solvent) (Table 1).

The γ-tocopherol content from Soxhlet with petroleum ether (25.14 mg/kg) was lower than SC-CO_2 (54.75 mg/kg) (Table 1). On the other hand, Lopes et al. [25] reported a γ-tocopherol content of 705.0–820.8 mg/kg. These authors employed pressurized propane to obtain the monguba oil, which can partially explain the difference between the data.

Indeed, the fruit ripening, weather, and process settings influenced the result. Lopes et al. [25] used seeds from green ripening fruits, whereas the present study used seeds from ripe monguba fruits (full physiological maturity). It indicates that the fruit maturity stage may affect the γ-tocopherol content [25]. The monguba seed oil showed a higher content of pure γ-tocopherol as compared to the manketti nut oil (12.49 mg/kg), which was extracted by SC-CO_2 (35.46 MPa and 60 °C) [31]. In contrast, the oils from walnut and palm extracted by SC-CO_2 showed higher γ-tocopherol contents than the monguba oil [32,33].

By analyzing the data, it is possible to note that the pressure and temperature did not affect the antioxidant activity and γ-tocopherol contents, but they directly affected the global yield (Tables 1, S1 and 2).

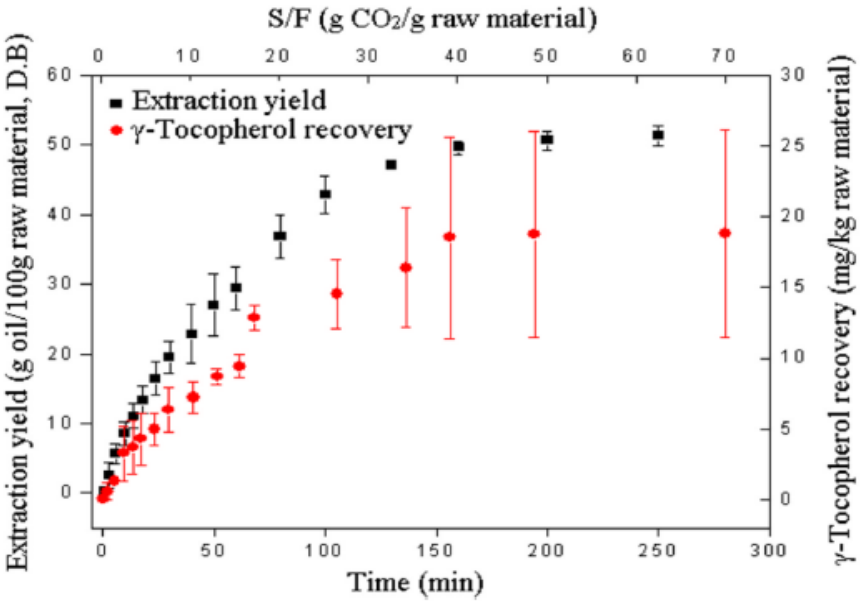

Fig. 3. The overall extraction curve of yield and γ-tocopherol recovery from monguba seed oil. D.B; dry basis.

Table 3
Fatty acids profile (% total of fatty acids) from monguba oil obtained in the extraction kinetic using supercritical CO_2.

Time (min)	C16:0	C18:0	C18:1	C18:2	Others[a]
1	77.39 ± 1.82	2.36 ± 0.10	8.61 ± 0.80	10.61 ± 1.05	1.03 ± 0.23
3	80.28 ± 2.88	2.67 ± 0.32	7.24 ± 1.29	8.80 ± 1.81	1.02 ± 0.24
6	81.32 ± 3.45	2.73 ± 0.39	6.91 ± 1.56	8.12 ± 2.15	0.92 ± 0.21
10	81.49 ± 2.86	2.75 ± 0.35	6.82 ± 1.29	8.01 ± 1.79	0.92 ± 0.20
14	81.71 ± 1.75	2.70 ± 0.32	6.54 ± 0.72	7.86 ± 1.17	1.19 ± 0.44
18	81.67 ± 1.64	2.75 ± 0.26	6.73 ± 0.65	8.11 ± 1.07	0.75 ± 0.17
24	81.09 ± 1.52	2.71 ± 0.17	6.95 ± 0.56	8.17 ± 0.99	1.08 ± 0.35
30	81.34 ± 1.32	2.76 ± 0.17	6.84 ± 0.48	8.15 ± 0.88	0.91 ± 0.24
40	81.65 ± 0.09	2.76 ± 0.04	6.77 ± 0.03	7.96 ± 0.01	0.86 ± 0.19
50	81.49 ± 0.26	2.88 ± 0.05	6.76 ± 0.11	7.92 ± 0.07	0.94 ± 0.18
60	81.27 ± 0.32	2.97 ± 0.06	6.77 ± 0.13	7.93 ± 0.13	1.06 ± 0.22
80	81.40 ± 0.33	3.06 ± 0.06	6.80 ± 0.13	7.78 ± 0.12	0.95 ± 0.19
100	80.08 ± 0.34	3.13 ± 0.07	7.36 ± 0.13	8.37 ± 0.11	1.05 ± 0.16
130	78.53 ± 0.30	3.19 ± 0.05	8.06 ± 0.33	8.95 ± 0.37	1.28 ± 0.17
160	77.33 ± 0.70	3.28 ± 0.05	8.55 ± 0.38	9.40 ± 0.42	1.44 ± 0.19
200	77.27 ± 0.68	3.16 ± 0.06	8.64 ± 0.34	9.60 ± 0.51	1.33 ± 0.16
250	77.48 ± 0.67	3.11 ± 0.03	8.50 ± 0.31	9.61 ± 0.58	1.30 ± 0.15
Max	**81.71**	**3.28**	**8.64**	**10.61**	**1.44**
Min	**77.27**	**2.36**	**6.54**	**7.78**	**0.75**
Mean	**80.16**	**2.88**	**7.34**	**8.55**	**1.06**
SD	**1.78**	**0.24**	**0.78**	**0.81**	**0.19**
CV	**2.22**	**8.50**	**10.64**	**9.51**	**17.79**

Values expressed as mean ± standard deviation.
SD: standard deviation; CV: coefficient of variation.
[a] Sum of fatty acids with values ≤1.0%.

These are important data for the food industry. They show that the supercritical process developed herein is effective since the temperature and pressure did not affect the quality of the oil. Thus, the highest pressure and temperature tested herein to extract the monguba oil (35 MPa and 60 °C) can be used. The extraction kinetics of the fatty acid profile and γ-tocopherol were evaluated at 35 MPa and 60 °C for 250 min

3.2. Extraction kinetic by using SC-CO_2

Fig. 3 shows the overall extraction curve of yield and γ-tocopherol recovery from the monguba seed oil. The typical behavior of the supercritical fluid extraction kinetics begins at a constant extraction rate (CER) period (30 min; 19.55 g/100 g and S/F = 7). The extraction rate is controlled by two mechanisms: convection and diffusional mass transfer. After this period, a falling extraction rate (FER) (130 min; 47.19 g/100 g and S/F = 34) was observed, which means that a transition period begins. Therefore, the kinetic curves present the shape of the diffusion-controlled (DC) curve since the extraction rate is reduced [21,22]. This period was reached at 160 min with an oil yield of 49.64 g/100 g (S/F = 39) (Fig. 3).

Observing the overall extraction curve of the γ-tocopherol recovery, the initial recovery rate of γ-tocopherol was 9.43 mg/kg RM (S/F = 15), while the recovery rate reduces to 16.37 mg/kg RM (S/F = 34) when achieving the FER period (130 min). When the DC period (160 min) was reached, the recovery rate of γ-tocopherol was 18.54 mg/kg RM. In the diffusion-controlled period, the γ-tocopherol extracted was completely removed from the monguba seeds. Therefore, the extraction process is controlled by the diffusion of the solute-solvent and the solute mixture to the surface of the solid matrix [34]. Analyzing the data, both extraction curves could be interrupted at 160 min (S/F = 39) since the recovery rate is almost constant and flattens off towards the end of the extraction (diffusion period). Consequently, the operational time and process costs can be reduced.

Nevertheless, it is interesting to highlight that the extraction using SC-CO_2 obtained 51.27 g/100 g of yield after 250 min, very similar to the total yield (54.29 g/100g) obtained by the Soxhlet method using petroleum ether as a solvent after 300 min of extraction.

Regarding the fatty acid profile, the total mean of palmitic, oleic, and linoleic acids after 250 min were 80.16%, 6.54%, and 7.78%, respectively (Table 3). The qualitative analysis of the fatty acid profile showed that it did not change along the process time, which means that the monguba oil maintained the same fatty acid (saturated and unsaturated) profile until the end of the extraction. Table 3 shows that there is a tendency to extract palmitic acid initially, whereas the intensity of the recovery of oleic and linoleic acids increased after 100 min. Since palmitic acid has the lowest molecular weight and, as a consequence, is more soluble than oleic and linoleic acids, it can partially explain the event mentioned above [35,36].

Although the sensorial properties of the monguba oil recovered by using a supercritical process were not evaluated, it was observed that this oil is odorless and yellowish (Fig. 1G), which indicates that it can be employed in the cosmetic and food industries. Toxicological tests of the monguba oil have not yet been published, which may limit its use. However, local people commonly consume monguba seeds.

4. Conclusion and perspectives

The highest global yield was obtained at 35 MPa and 60 °C and palmitic acid was the main fatty acid in all process settings studied. Furthermore, the γ-tocopherol content and the antioxidant activity were not affected by pressure and temperature. The supercritical CO_2 process can be conducted at 35 MPa and 60 °C for 160 min to obtain an overall yield similar to the Soxhlet method (petroleum ether as a solvent).

The monguba seed can be explored to obtain a saturated fatty acid-rich oil and a remarkable content of unsaturated fatty acids, such as oleic and linoleic. Therefore, the monguba oil can be employed in the food and cosmetic industries. The recovery of the monguba oil by using the supercritical CO_2 process is an eco-friendly alternative as compared to conventional methods. Finally, data showed that high-quality monguba oil can be recovered by using supercritical CO_2.

Declaration of Competing Interest

The authors report no conflict of interest.

Acknowledgements

The authors Alexsandra Pereira Rodrigues and Grazielle Náthia-Neves are thankful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support offered through fellowships (Finance Code 001). This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (process numbers: 2015/50333-1) and CAPES-PROEX (23038.000795/2018-61).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.supflu.2021.105192.

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Chapter 4

Research Article

Composition, crystallization properties and biological effects of monguba (Pachira aquatica Aubl.) oil obtained by using supercritical CO_2 process

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Manuscript submitted to Food Research International
(Impact Factor 4.972 – Qualis A1)

Composition, crystallization properties and biological effects of monguba (*Pachira aquatica* Aubl.) oil obtained by using supercritical CO_2 process

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Highlights

The oil of monguba is composed predominantly of disaturated and trisaturated TAGs.
Monguba oil presents β´ polymorphic form and irregular needle-shaped crystals.
The consistency of monguba oil has been classified with a very hard and brittle.
Monguba oil increases fibroblast cell viability and photoprotective activity.

Abstract:

Vegetable oils and fats are mainly composed of triacylglycerols (TAG), and other minor components such as free fatty acids, mono and diacylglycerols, tocopherols and phytosterols. The TAG composition of non-edible oils and fats is pivotal for food and cosmetics industries, once it is related to crystallization properties and defines the application of the bulk material in end products. In addition, especially for cosmetics purposes, little is known about the skin properties of exotic oils and fats, reinforcing the importance of fundamental understanding in this regard. Thus, this study aimed to characterize monguba oil (MO), a Brazilian unexplored oil with high potential for industrial applications. MO was evaluated regarding its composition, and physical properties, such as solid fat content (SFC), thermal behavior by differential scanning calorimetry (DSC), microstructure, polymorphism and consistency. Furthermore, MO's cytotoxic and photoprotective activities were examined. MO presented mainly palmitic acid and disaturated TAG species, with emphasis in POP (palmitic-oleic-palmitic, dipalmito-olein). The SFC values showed that the monguba oil decreased more slowly, which can be observed by the small difference (2.2x) in solids content between the temperatures of 10 and 30 °C. The concentration of 1µg/mL of monguba oil showed photoprotective activity and was also able to increase the viability of fibroblast cells. Besides presenting cytotoxic activity against melanoma, the skin cancer. Therefore, we suggest that monguba oil can be incorporated into cocoa butter or palm oil as a physicochemical modulator and/or used for the elaboration of confectionery fillings, as well as in the elaboration of ointments/creams using monguba oil as a base for these products.

Keywords: Munguba; Cytotoxic assay; Differential Scanning Calorimetry; Microstructure; Polymorphism; Solid Fat Content; Cosmetics.

1. Introduction

One of the huge biomes that have the most ecologically diverse is the tropical rainforest, localized between the Tropic of Capricorn and the Tropic of Cancer. Such forest is found in Asia, Australia, Africa, South America, Central America, Mexico, and many of the Pacific Islands. South America holds the largest stretch of rainforest being that about one-third of this biome is found in Brazil with the greatest expanses found in Amazonia (Butler, 2019). The search for alternatives to promote renewable exploitation and the preservation of biodiversity is a global interest undertaking, and the commercial exploitation of these oilseed species is one of the alternatives (Saraiva et al., 2009).

In the tropical forest, we can find *Pachira aquatica* Aubl. trees are distributed from southern Mexico to South America on the banks of rivers and lakes (Rodrigues & Pastore, 2021). The fruits of *Pachira aquatica* tree, also known as monguba or munguba, are similar to the shape of the cocoa fruits. A monguba fruit has an average of 39 seeds per fruit, and the seeds are a rich source of oil (53%) whose yield of extraction. The lipid fraction of monguba oil (MO) is composed mainly of palmitic, oleic and linoleic acids, γ-tocopherol and sterols. The oil contains 46.7-84.7% of saturated, 6.6-39.3% of monounsaturated, and 5.24-11.81% of polyunsaturated fatty acids (Rodrigues & Pastore, 2021), and melting point ranging from 38.6 to 41.9 °C, being solid at room temperature (De Bruin et al., 1963; Jorge & Luzia, 2012).

Oils are important components for several industries, with a broad application in cosmetics, pharmaceuticals, and food products. Fats and oils confer desirable characteristics to foods, such as softness, producing a moist mouthfeel and affecting food texture (Rios et al., 2014). Knowing the chemical properties of vegetable oils, such as triacylglycerols (TAGs) and minor unsaponifiable constituents, is very important because these compounds affect stability and nutritional properties. The fatty acid content varies according to the geographic origin of the raw material (Ribeiro et al., 2012).

Despite literature reported diverse studies about MO, such as the optimization process to obtain bioactive compounds and its oil, the physicochemical and chemical composition of oil, there is still no report on TAG composition of MO. Thus, deep knowledge of crystallization processes is therefore essential to develop

new commercial products based on vegetable oils and fats. Since TAG molecules play an important role in the sensory properties (texture, rheology, appearance, melting behavior) of food products lipid-based, in which the macroscopic mechanical, rheological, and physical properties are influenced by the microstructure of the tridimensional network formed, such as crystal aggregation, crystalline size and shape, solidification behavior, and thermal stability, crystal morphology, and intermolecular interactions (De Graef et al., 2012; Macridachis-González et al., 2020).

In recent years, the constant search for the development of products with a greater number of components of plant origin and broad biological relevance, rationally exploiting the Brazilian biodiversity has become more evident (Bajerski et al., 2016). Therefore, knowing the cytotoxic effects of MO against cancer cell lines could be another way to incorporate MO because despite various chemical drugs being an effective anticancer agent, it causes several crucial side effects such as cardiotoxicity, hepatotoxicity, nephrotoxicity, anaemia, nausea, and diarrhea (Ajaykumar, 2020). In addition, investigations based on sun protection studies have captivated interest among specialists due to the adverse effects of products containing high amounts of synthetic UV filters (Lacatusu et al., 2014).

Based on this information and our ongoing studies, we analyzed the composition and crystallization behavior of MO obtained by using supercritical CO_2 extraction. In addition, we evaluated the cytotoxic and photoprotective activity of the oil. In this research, we aimed to provide information for possible uses and applications of MO by knowing its fatty acids and triacylglycerols composition, and crystallization behavior.

2. Materials and Methods

2.1. Plant material and sample preparation

Ripe monguba fruits (2 kg) were collected in Uberlândia (18°54′41″ south latitude, 48°15′44″ west longitude and 843 m altitude), MG, Brazil. The fruits were transported from the collection site to the laboratory up to 24 h after haverst. The seeds were manually separated from peel, then freeze-dried. These freeze-dried seeds were grounded by using a handheld food processor. The particle size of the

flour was classified using a sieve (8-mesh; 2.36 mm/µm). Finally, the flour frozen (-18°C) until analysis.

2.2. Extraction of monguba oil

Monguba oil was extracted via supercritical CO_2 process by a commercial Spe-ed SFE unit (Applied Separations, 7071, Allentown, USA). The extraction process was according to Rodrigues et al. (2021) (temperature of 60°C and pressure of 35 MPa). The oil extract was collected in glass vials and frozen at -18°C to prevent any possible degradation.

2.3. Fatty acids composition

The fatty acid methyl esters (FAMEs) were separated according to (Hartman & Lago, 1973), and for separation, the Ce 1b-89 method was used (AOCS, 2009). The fatty acid (FA) composition was performed in a flame ionization detector (FID) coupled to a gas chromatograph (Shimadzu, Series 2010 Plus), followed the conditions of the analysis according to Rodrigues et al. (2019).

2.4. Triacylglycerol (TAG) composition

The TAGs composition was performed by a gas chromatograph (Agilent 6850 Series GC System), with a capillary column DB-17 HT Agilent (50%-phenyl-methylpolysiloxane, 15 m in length × 0.25 mm of internal diameter and containing 0.15 µm of film). The sample concentration was 100 mg/5 mL of tetrahydrofuran. The amount injected was 1.0 µL on split mode (1/100). The operational temperature followed the conditions: injector temperature at 360°C; detector temperature at 375°C; column temperature at 250°C, increasing up to 350°C at the rate of 5°C/min. Helium gas was used as a carrier gas. The identification of TAGs was performed by comparison of the retention times, according to the procedures of Filho et al. (1995) and PrOleos, a software which establishes the random distribution of fatty acids in the TAGs. The analysis was performed in triplicate.

2.5. Solid fat content (SFC)

SFC was determined by an Nuclear Magnetic Resonance (NMR) spectrometer (Bruker pc120 Minispec, Silberstreifen, Rheinstetten, Germany) and dry baths (0–70 °C) (Duratech, Carmel, USA), according to the directed method described in AOCS official method Cd 16b-93 (AOCS, 2009). Monguba oil was melted at 90°C to completely erase the crystal memory, then the oil was maintained in a dry thermostatic bath at temperatures of 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55°C. The analysis was performed in triplicate. The melting point was calculated for the temperature corresponding to 4% solids content, obtained from the SFC curve given by NMR (Karabulut et al., 2004).

2.6. Thermal behavior by differential scanning calorimetry (DSC)

Thermal analysis of monguba oil were measured with a DSC equipped with TA Thermal Analyzer, model Q2000 V4.7A, and coupled to a RCS90 Refrigerated Cooling System (TA Instruments, Waters LLC, New Castle, USA). The analysis was determined according to AOCS official method Cj 1-94 (AOCS, 2009). Approximately 9 mg of the melted monguba oil was weighed into aluminum pans and sealed. The reference was an empty aluminum pan. Thermal analysis conditions were held at 80 °C for 10 min followed by cooling down to -60°C, at a rate of 10°C/min and held for 30 min at -60°C then programmed to 80°C at 5°C/min. The parameters onset temperature (T_{onset}), final temperature (T_{offset}), peak temperature (T_{peak}), crystallization and melting enthalpy (ΔH) were determined. The TA Universal Analysis V4.7A software was used to obtain the curves. The exothermic or endothermic peak temperatures were obtained from DSC curves and used for the determination of crystal forms. The analysis was performed in triplicate.

2.7. Microstructure images

Pictures were obtained by polarized light microscope (Olympus, model BX 51) coupled to a digital video camera (Media Cybernetics). Monguba oil were melted at 90°C and pipetted onto a pre-heated slide, and then covered with a coverslip. Subsequently, the slides were kept for 24 h at 20°C and 30°C to stabilize the crystalline structure. The slide was kept at 20°C and 30°C for picture acquisition. The crystals formed were carefully examined under the microscope at 20 and 40×

magnification, respectively. Photographs were captured by the software Image Pro-Plus 7.0 (Media Cybernetics), under polarized light.

2.8. X-ray diffraction

The polymorphic form of the fat crystals in monguba oil was determined by using X-ray diffraction, according to Cj 2-95 AOCS official method (AOCS, 2009). The analysis was performed on a Philips PW 1710 diffractometer (PANalytical, Almelo, the Netherlands), using Bragg-Brentano geometry (θ:2θ) with Cu-Kα radiation (λ = 1.54178 Å, voltage 40 kV and current of 30 mA). The measurements were obtained with steps of 0.02° in 2θ and acquisition time of 2 s, with scans from 5 to 40° (range 2θ). Monguba oil was melted at 90°C and stored at 20°C for 24 hours, and evaluated after 1, 7, 14, 21, and 28 days. The identification of the polymorphic forms was determined from the characteristic short spacings of each crystal (Martini et al., 2006).

2.9. Consistency analysis

The consistency was determined with the TA-XT Plus Texture Analyzer equipment (Stable Micro Systems, Surrey, UK). The samples were heated in microwave for 2 min to completely melt the crystals (90 °C), then placed in beakers of 50 mL. Conditioning was performed in an incubator for 24 h at 5°C to induce monguba oil crystallization and after for 24 h at the temperature readings: 10, 20 and 30 °C. An acrylic cone with an apex angle of 60° and no truncation was used in the experiments. Tests were operated in the following conditions: displacement in the sample equal to 10 mm; probe velocity of 2 mm/s; penetration time of 5 s. From these conditions, compressive force in g_f was obtained. Penetration data were converted into yield value, according to (Haighton, 1959):

Equation 1: $$YV = \left(\frac{K \times W}{p^{1.6}}\right)$$

Where: YV = yield value (g_f/cm^2); K = factor of the cone angle (equal to 2815 for a cone of 60°); W = compression force (g_f); p = penetration depth (mm/10).

Samples were analyzed in duplicate, and the results correspond to the calculated average.

2.10. In vitro bioassays

2.10.1. Conditions of the Cell Culture

The 3T3-Swiss albino mouse fibroblast and SK-Mel-103-human metastatic melanoma cells were cultured with RPMI 1640 medium supplemented with 5% of fetal bovine serum (FBS) for SK-Mel-103 cell line and 10% for 3T3 cell line with 0.001% of penicillin/streptomycin, and incubated at 37 °C, under a 5% CO_2 atmosphere.

2.10.2. Cytotoxic activity

The cytotoxic effect of monguba oil was tested against SK-Mel-103 (melanoma) human tumor cell line, and 3T3 (fibroblast) no cancer cell line. Cell viability was evaluated using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl2-H-tetrazolium bromide (MTT) method (Mosmann, 1983). Briefly, 1 mg of monguba oil was dissolved in 10 µL of dimethyl sulfoxide (DMSO) and added 990 µL of Dulbecco's Modified Eagle's Medium (DMEM)/10% fetal bovine serum (FBS). The cells were distributed in 96-well plates (density of $5x10^4$ cell/mL) and exposed to different concentrations of monguba oil (1, 10, 100, and 1000 µg/mL) in DMSO (0.1%) at 37 ºC, with 5% of CO_2, for 48 h. DMSO final concentration was < 0.25% (v/v), which does not interfere with the cell growth of the tested stains. Doxorubicin (0.006, 0.06, 0.62, and 6.20 µg/mL) was used as positive control. After incubation, the culture media with the samples were removed, the plates were washed and 100 µL of MTT (0.5 mg/mL phosphate buffered saline) was added to each well and left to incubate at 35°C for 4 hours with 5% of CO_2. After, the culture media was removed again, and the formazan crystals was dissolved in 100 µL of DMSO for 15 min at 35 ºC with 5% of CO_2. The formazan crystals were quantified by microplate reader (Synergy™ HTX Multi-Mode Microplate Reader, Biotek) at 570 nm (Bahuguna et al., 2017). Inhibition activity was expressed as 50% of cell viability (IC_{50}) values. The experiments were performed in triplicate.

2.10.3. Photoprotective Activity

The cell viability of the photoprotection bioassay was determined by MTT assay (Mosmann, 1983) with and without the effect of UV radiation on 3T3 cell line. Briefly, 100µL per well of 3T3 cells ($5x10^4$ cells/mL) were inoculated for 24 h (37 °C with 5% of CO_2) in 96-well plates, and subsequently, one plate was exposed to UV radiation (15W, 43 cm, wavelength from 270 to 230 nm) for 5 min and the other plate was kept in dark for the same time. Afterwards, the cells were incubated at 37 °C with 5% of CO_2 for 48 hours. After the samples were removed, 100µL/well of growth medium with MTT (0.5 mg/mL) was added and incubated for 4 hours. Sequentially, growth medium was removed and 100µL/well of DMSO was added. The cell viability was determined after 15 min using a microplate reader at 570 nm.

2.11. Statistical analyses

Databases and statistical analyses were performed by GrafPad Prism® software and submitted to one-way ANOVA followed by Boferroni's test in the case of *in vitro* bioassays. Each analysis was performed in triplicate and the data are reported as mean values ± standard deviation.

3. Results and discussion

3.1. Fatty acid (FA) profile and triacylglycerols (TAGs) composition

The FA profile and TAGs composition of monguba oil are shown in Tables 1 and 2. The predominant known FAs in MO were palmitic, stearic, oleic and linoleic acids. The obtained amount are within those found in the literature (Lopes et al., 2020; Rodrigues et al., 2019; Teixeira et al., 2021). Palm oil showed a similar fatty acid profile to MO (Chikhoune et al., 2020).

Table 1. Fatty acid (FA) profile of monguba oil obtained at 60°C and 35 MPa by supercritical CO_2 process.

FA	**CN:DU[1]**	**Content (% relative)[2]**
Palmitic acid	16:0	59.77 ± 0.05
Stearic acid	18:0	2.88 ± 0.00
Elaidic acid	18:1 trans	1.73 ± 0.01
Oleic acid	18:1	7.95 ± 0.01
Linoleic acid	18:2	7.28 ± 0.00

Unknown	-	7.53 ± 0.00
Unknown	-	9.83 ± 0.01
Others[3]	-	3.15 ± 0.00
SFA		62.65
MUFA		9.68
PUFA		7.28

[1]CN:DU, calculated carbon number: degree of unsaturation.

[2]The values show the average of three independent determinations ± standard deviation.

[3]Sum of fatty acids with values below 1.0%.

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Furthermore, unknown FAs were observed in MO and we suppose that FAs corresponded to cyclopropenoid fatty acid derivatives present in MO (Malvaceae family), such as malvalic (C18:1), sterculic (C19:1) and dihydrosterculic (C19:1) acids. These cyclopropenoid fatty acids were identified by other authors in MO (Bohannon & Kleiman, 1978; De Bruin et al., 1963; Spitzer, 1991).

MO presented TAGs composed of long-chain fatty acids, with carbon number ranging between 48 and 52. MO showed a predominance of TAGs species with 50 carbon number, which corresponded to 70.46% of the triacylglycerols (Table 2). The predominant TAGs of MO are disaturated; saturated-unsaturated-saturated (SUS), followed by trisaturated; saturated-saturated-saturated (SSS) and monosaturated; saturated-unsaturated-unsaturated (SUU). These data correlate to (Sunday et al., 2019), who showed that monguba oil has at the proportion in the TAG 76.50%, 20.12%, and 3.37% of saturated, oleic and linoleic fatty acids, respectively. By using ^{1}H nuclear magnetic resonance spectroscopy, they observed signals that confirm the presence of saturated 1,3 TAG and 2- positions of olelyl, linolelyl esters and indicate the presence of oleic esters together with linoleic esters, however, it was detected no peaks corresponding to linolenic acid (Sunday et al., 2019).

Table 2. Triacylglycerol (TAG) composition of monguba oil obtained at 60°C and 35 MPa by supercritical CO_2 process.

Carbon number	TAG species	Content (%)
48	PPP	18.71 ± 0.62
	PPS	
50	POP	70.46 ± 0.74
	PLP	
	POO	
52	POL	10.83 ± 0.29
	PLL	

Note: The positional distribution of fatty acids between sn-1, sn-2, and sn-3 positions was neglected.

Cocoa butter contained triacylglycerols with carbon numbers between 50 and 56 (long-chain fatty acids) and the presence of disaturated ranged from 81.83% to 96.24%. The main triacylglycerols species were the one with 52 carbon numbers (44.62-52.14%), followed by 54 (22.25-32.55%) and 50 (15.74-24.55%) carbon numbers (Ghazani & Marangoni, 2019; Ribeiro et al., 2013). Palm oil consists mainly of 50 (13.87-39.93%), 52 (42.38-43.13%), and 54 (9.46-26.07%) carbon numbers of triacylglycerols species, with a predominance of monosaturated and carbon number between 44 and 54 (Chen et al., 2007; Chikhoune et al., 2020). Gilabert-Escrivá et al. (2002) and Saraiva et al. (2009) showed that cupuaçu butter contained the triacylglycerols species consisting of 54 (51.3-59.4%) and 56 (20.6-25.9%) carbon numbers, with the triacylglycerol predominantly monosaturated and disaturated. According to the data (Table 2), MO showed TAGs species with 50 carbon numbers higher than palm oil, cocoa and cupuaçu butters. In this group, TAG could contain at least one palmitic acid in each moiety.

The literature suggests that MO presents cyclopropenoid fatty acids and these significantly contributed to the TAGs composition, since they presented noticeable amounts, as obtained in the FA determination (unknown FAs). Determining the TAG composition of the lipid system is important because it affects the organization of the crystal network and therefore helps to understand the crystal morphology, microstructure, and textural properties. However, due to the specificity

of non-common FA present in MO, difficulties were imposed in elucidating single TAGs concentrations.

3.2. Solid fat content and Melting point

MO had a melting point of 41.4°C, a similar values as described elsewhere De Bruin et al. (1963) and Jorge & Luzia (2012). According to Ribeiro et al. (2013) and Chikhoune et al. (2020), the melting point of cocoa butter and palm oil were 34°C and 34.2°C, respectively. These values were lower than that obtained for MO. This effect could be directly related to the higher amount of high melting TAGs in MO than in the mentioned fats, such as PPP and PPS, which have melting point ranging from 44.7°C to 65.9°C, and 46.6°C to 62.6°C, respectively (Berry, 2009).

Regarding solid fat content (SFC), MO showed it in an initial of 66.43% at 10°C; 39.18% at 25°C, and complete melting at 50°C. It was observed that the SFC had a pronounced decreased as the temperature was increased (Figure 1A). Teixeira et al. (2021) reported this same behavior for MO obtained by supercritical CO_2 process under different conditions (40 to 60°C and 20 to 30 MPa). However, the SFC at 30°C (SFC = 3-19%) reported by them was lower than that found in our study (SFC = 29.94%), as well as the temperature of complete melting (34°C). Hypothesis for this difference in complete melting and SFC might be due to the FAs position in glycerol molecule and triacylglycerol heterogeneity (Rasor & Duncan, 2014), and the presence of non-esterified forms of sterols in which the solids content increased because the compounds can organize themselves in a simpler way than the TAGs in a lipid mixture, hampering the organization of the crystalline network (Buscato et al., 2018).

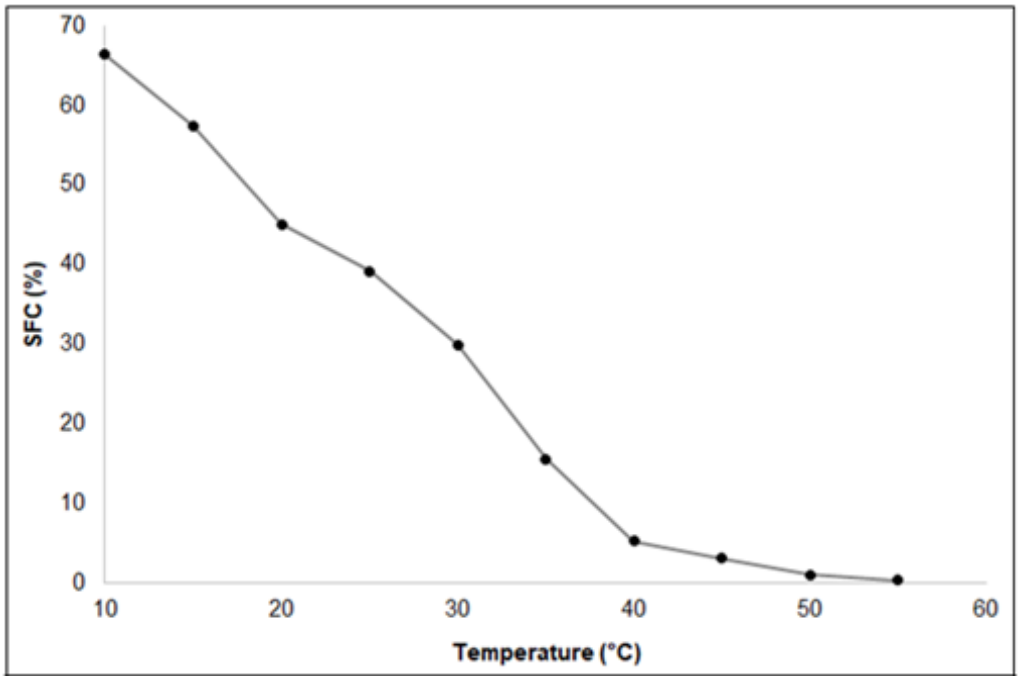


Figure 1. The solid fat content (SFC) of monguba oil obtained by supercritical CO_2 process (60°C and 350 MPa).

MO displayed a higher solid fat content than palm oil at the temperature range from 10 to 25°C, with palm oil showing SFC of 47.97% and 14.65% at 10°C and 25°C, respectively, and complete melting at 40°C (Mello et al., 2020).

In this study, MO showed SFC of 45.02% solid at 20 °C and 39.18% at 25 °C, which are comparatively close to the values reported by Timms (2012), who suggested that confectionery fat should exhibit approximately a solid fat content of 63% at 20 °C, 40% SFC at 25 °C and 0% at 37 °C. In another study Talbot (2009) suggested that fat with SFC lower than 50% at 20 °C is suitable as confectionery fillings.

The SFC of 66.43% at 10°C and 5.23% at 40°C of MO suggests an extremely hard and brittle, with a possible application as a heat-resistant spread (Madalena Maciel Guedes et al., 2017; Teixeira et al., 2021). Therefore, MO could be used in confectionery fillings and in production of margarine or shortening and cosmetic formulations.

3.3. Differential Scanning Calorimetry (DSC)

Figure 2A shows the thermal behavior via DSC of MO. The melting event started at -23.37 °C and finished at 53.84°C, consuming 52.77 J/g of energy to melting, while the crystallization event started at 36.78°C and finished at -25.29°C, releasing 52.06 J/g of energy to crystallize.

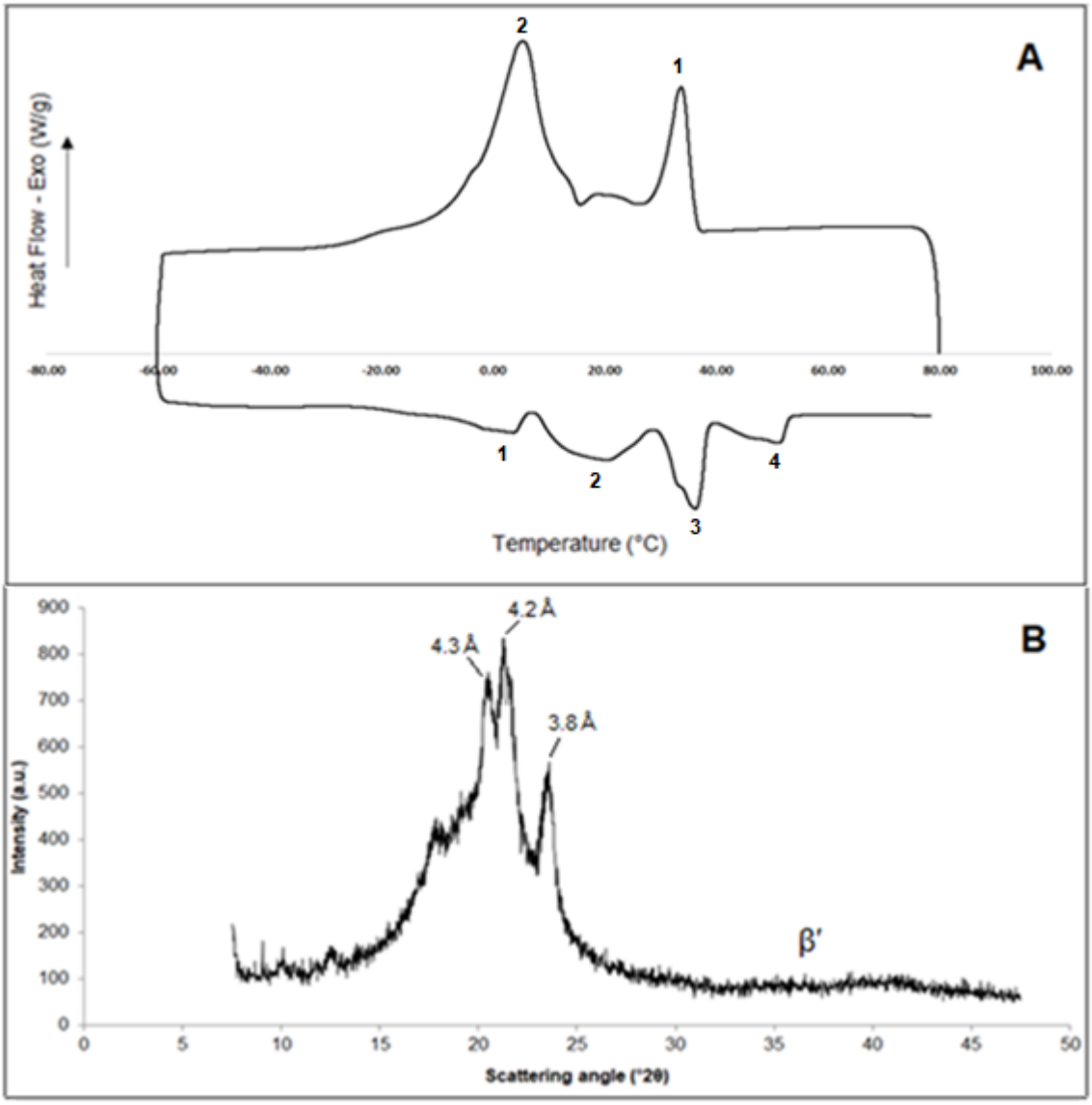


Figure 2. Monguba oil obtained at 60°C and 35 MPa by supercritical CO_2 process. A, Differential scanning calorimeter thermograms; B, X-ray patterns at 20 °C for 28 days after melting.

The melting profile of MO had four endothermic peaks (Table 3); this phenomenon may be due to the slow melting because the temperature rate used.

The first endothermic curve showed T_{peak} at 3.65 °C and enthalpy value of 9.92 J/g, which corresponds to the low melting fraction. While the second and third peaks had T_{peak} at 18.88 °C and 36.39°C with enthalpy values of 19.30 J/g and 16.24 J/g, respectively. Finally, the fourth peak showed T_{peak} at 51.40 °C and enthalpy value of 7.31 J/g, which corresponds to high melting fraction. The complete melting of MO agrees with the findings obtained for SFC (50°C; SFC = 0.96%). It is important to highlight that enthalpy values corroborate with TAG composition found in MO since the major TAG species with 50 carbon numbers shown high enthalpy values.

Table 3. Melting and crystallization onset, peak, offset and enthalpies temperatures of monguba oil.

	Temperature (°C)			ΔH (J/g)
	Onset	**Peak**	**Offset**	
Crystallization				
Peak 1	36.78 ± 0,22	33.70 ± 0.11	-	15.01 ± 0.58
Peak 2	-	5.30 ± 0.31	-25.29 ± 1.06	37.59 ± 1.29
Melting				
Peak 1	-23.37 ± 1.01	3.65 ± 0.23	-	9.92 ± 0.69
Peak 2	-	18.88 ± 1.00	-	19.30 ± 0.90
Peak 3	-	36.39 ± 0.18	-	16.24 ± 0.71
Peak 4	-	51.40 ± 0.26	53.84 ± 0.09	7.31 ± 0.59

Data presented as means ± standard deviation of triplicate determination.

Observing the behavior of MO in DSC, probably the first peak was mostly composed of TAG species consisting of unsaturated fatty acids. Followed by disaturated TAGs in second and third peaks, our hypothesis is that these peaks are composed of TAGs molecules with predominantly of POP, PLP, POO, and unknow fatty acids. And the fourth peak corresponding to trisaturated TAG, consisting predominantly of PPP. Therefore, this composition was responsible to high enthalpy. MO showed a major melting profile into middle (-10°C to 20°C) and high-melting (around 40°C) regions. The peaks at position 2 and 3 corresponds to middle melting region and the peak at position 4 corresponds to high-melting region.

Regarding measurements performed after melting, exothermic peaks were also observed in MO. It was observed that during cooling MO slowly crystallized and two peaks were formed. For first exothermic peak, it was showed T_{peak} at 33.70°C and enthalpy values of 15.01 J/g in second peak had T_{peak} at 5.30°C with enthalpy value of 37.59 J/g. These crystallization peaks corresponds to high melting (hard) fraction and a mix of medium and low melting (smooth) fractions (Fredrick et al., 2008).

The first peak (high melting fraction) corresponded to the crystallization of the saturated FAs, whereas the second peak (low melting fraction) constituted by TAGs with unsaturated FAs. A shoulder is observed after the first peak at 25.30°C, which can be assumed as the solidification of the medium melting fraction. Indicating a rapid crystallization because the peaks could not be completely separated, which in turn can be attributed to an overlap of two crystallization steps. This phenomenon could be due the formation of different polymorphic forms of this process in different fractions, or the combination of both (Fredrick et al., 2008; Jahurul et al., 2019), and the temperature rate used in the methodology may have influenced.

A recent study using supercritical CO_2 extraction under different conditions to obtain MO showed a different DSC data than our study. They identified that the crystallized oil achieved a liquid state approximately at 35 °C (Teixeira et al., 2021). In our study the main peak (peak 3) had 36.39 °C, however the MO only achieved a total melt at 53.84 °C, in which corresponded to the fourth melting peak.

Nevertheless, it is possible to observe that the extraction conditions by supercritical CO_2 process cause an impact on the thermal behavior of oil because it can contribute to extract others fatty acids which with subsequent incorporation of the TAGs molecules, affecting the crystallization. This hypothesis refers to the amount of unknown fatty acids found by Teixeira et al. (2021). They reported lower amount (up to 1.83%) than the one found in our oil (17.36%), which we assume is cyclopropenoid fatty acid derivatives (as described in Section 3.1).

3.4. Polymorphism

The diffractogram and polymorphic structure of MO at 20°C are depicted in Figure 2B. MO showed multiple short spacings at 3.8, 4.2 and 4.3 Å, the pattern observed during all storage time (at 20°C after 1, 7, 14, 21 and 28 days of

stabilization), indicates the β′ polymorphic form. The peaks of β′ form become more defined over time, resulting in slow crystallization and the increasing content of β′ crystals. According to Nissim & Sato (2001) and Sato (2001) the TAGs influence the crystalline phases and high levels of POP tends to have β′ crystals. The β′ form results in ideal rheological and textural properties to produce margarine and shortening (Sato & Ueno, 2011). Therefore, MO could be incorporated in formulations of ice cream, whipped cream, and cakes because this form exhibits a smooth and aerated texture with excellent creaminess properties, being ideal for incorporation of air bubbles and suspension of particles (Gamboa & Gioielli, 2006; Shahidi, 2005).

3.5. Microstructure and textural properties

Micrographs obtained after crystallization at 20 and 30 °C for 24 hours are shown in Figure 3. At 20°C very subtle crystals were formed (Figure 3A), with the presence of small needle crystals. At 30°C (Figure 3B), the microstructure cold be better visualized, represented by a large crystal composed of irregular needle-shaped crystals emanating from a central nucleus, with 184.60 μm in diameter. A similar crystal form was observed in cupuaçu fat, however, the crystal diameter was higher (461.13 μm) (Lannes et al., 2004) than that observed in MO. The crystal morphology from MO agrees with the findings obtained from polymorphism (Figure 2B), which corresponds to β′ crystals formed mainly by disaturated long-chain TAG.

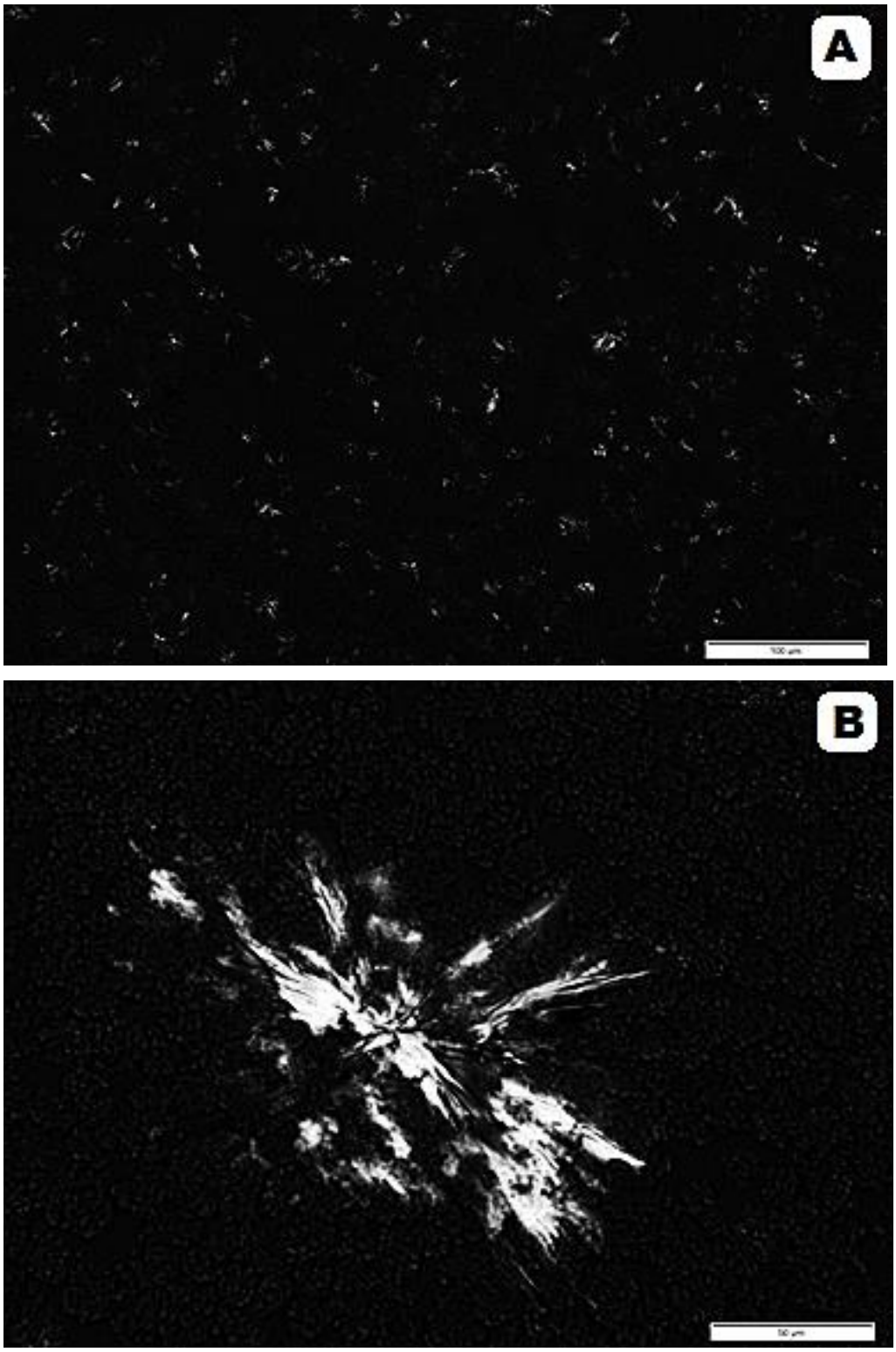
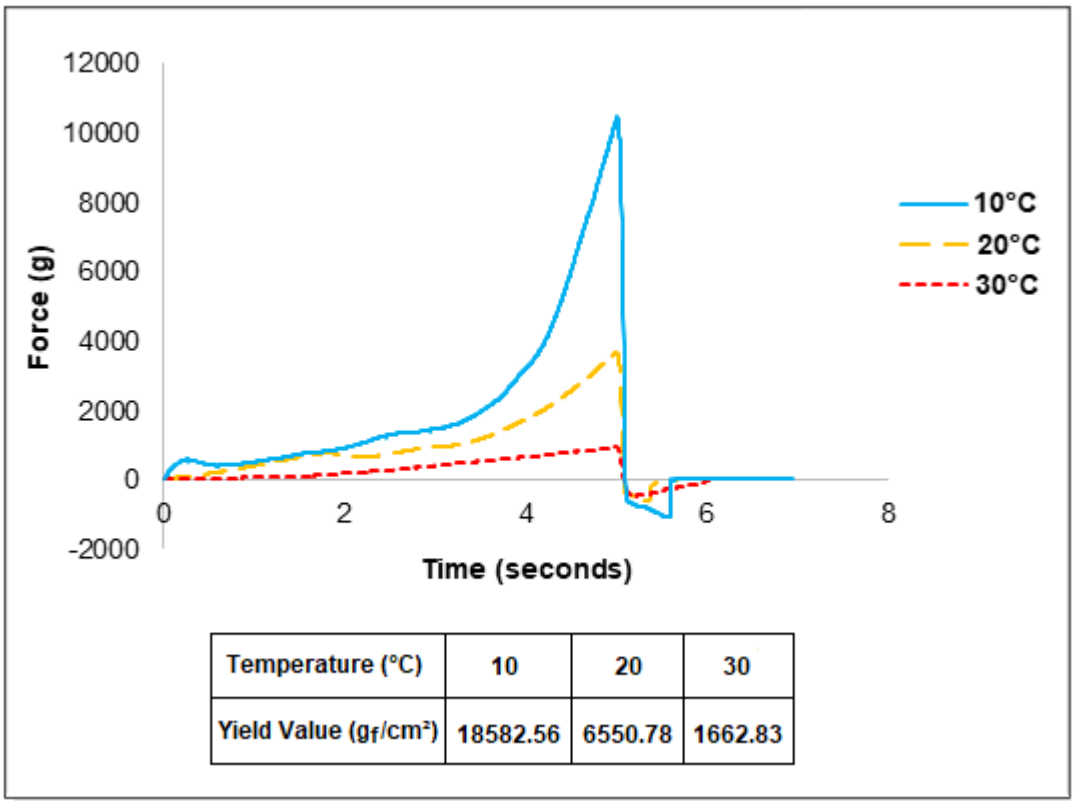


Figure 3. Polarised light microphotographs images obtained by static crystallization of monguba oil at 20 and 30 °C, 24 hours after melting. A, 20x lens at 20°C (scale bars represent 100 µm) and B, 40x lens at 30°C (scale bars represent 50 µm).

It was evident the slow crystallization at 20°C, cooperated with the isothermal data via NMR. High POP content in MO may have contributed to the slow crystallization, and this same behavior was observed on palm oil (Mello et al., 2020). MO crystals consisted mainly of disaturated and trisaturated triacylglycerols, which represent the medium and high-melting lipid, respectively. Shi et al. (2005) reported that the morphology and aggregates of crystal elements are dominated by the high-melting lipid, or its triacylglycerol saturation level and the characteristics of the crystals are affected by the nature of the low-melting lipid. Thereby, the textural properties of a lipid system is influenced by the SFC and microstructural factors (Braipson-Danthine & Deroanne, 2004).

Although the texturometer method is used to evaluate the texture, it is possible to estimate the brittleness of a lipid system from the irregularities of the curve obtained by the penetration test (Braipson-Danthine & Deroanne, 2004). These irregularities were observed in the consistency curves of MO (Figure 4). The oil from monguba seed stored at 30°C showed 3.9x lower consistency value than those stored at 20°C, whereas at 10 °C was 2.8x lower compared with the oil stored at 20 °C (Figure 4). This effect agrees with solid fat content values, which can be observed the slow reduction of solids content from 10 °C to 30 °C. Shi et al. (2005) reported that systems containing high and low-melting classes are harder and brittle, being in agreement with the data found in the consistency analysis.



Temperature (°C)	10	20	30
Yield Value (g_f/cm^2)	18582.56	6550.78	1662.83

Figure 4. Consistency curve and yield value of monguba oil conditioned at different temperatures.

Palm stearin stored at 25 °C using a cone with a 40° angle had a yield value of 8051 g/cm^2 (Mello et al., 2021), a higher value than that found for MO at 20°C (6550.78 g/cm^2) using a cone with a 60° angle. Cocoa butters of industrial blends and produced in different Brazilian regions showed consistency values ranging from 14413.72 to 20877.39 g/cm^2 and 557.44 to 1950.03 g/cm^2 at 20 °C and 30 °C, respectively (Ribeiro et al., 2012). At 20°C, MO showed a yield value of 6550.78 g/cm^2, while at 30 °C was 1662.83 g/cm^2.

Haighton (1959) classifies fats from very soft to very hard, as follow: <50: very soft, to just pourable; 100-200: soft, but already spreadable; 200-800: satisfactory plastic and spreadable; 1000-1500: too hard, limit of spreadability; >1500: too hard. Considering this ranges, MO showed a very hard consistency (> 1500 g/cm^2) and was brittle at all tested temperatures (10, 20 and 30 °C) using a cone with a 60° angle.

Despite its hard consistency, MO can be added to cocoa butter as a modulator of the physical properties of cocoa butter according to a study conducted by Ribeiro et al. (2013).

3.6. Biological activity

Preclinical trial of the cytotoxic activity of MO was evaluated against SK-Mel-103 (melanoma) human tumor cell lines, and the 3T3 (fibroblast) as control cell line (Table 4). MO showed good cytotoxic activity (IC_{50} < 50 µg/mL) against SK-Mel-103 cell line, on the other hand MO was inactive against 3T3 cell line (IC_{50} > 50 µg/mL). Comparing with doxorubicin (positive control), MO was less active against SK-Mel-103 cell line. However, the chemotherapeutic drug (doxorubicin), despite lower IC_{50} values compared to MO, had higher cytotoxic to 3T3 cell line. These data reinforce the harmful effects of oncolytic agents for the long-term treatment of patients. In preclinical *in vivo* trial was not observed anormal changes in vital and reproductive organs in animals treated orally for 28 days with MO obtained by cold pressing (Marcelino et al., 2020). In this *in vivo* assay was identified alterations in biochemical parameters, however Marcelino et al. (2020) considered that these results were normal for the specie and they concluded that was not found signs indicating toxic association at the doses tested for histopathological, biochemical, and/or hematological studies (Marcelino et al., 2020).

Table 4. Cytotoxic activity of monguba oil against human tumor cell (SK-Mel-103) and control cell (3T3) lines.

Cell line	IC_{50} (µg/mL)[1]	
	Control[2]	Monguba oil
3T3	0.54 ± 0.14	112.80 ± 0.91
SK-Mel-103	4.028 ± 0.08	30.65 ± 0.09

[1]Data presented as means ± standard deviation of triplicate determination.

[2]Doxorubicin.

Photoprotective effect of MO was also evaluated against UV irradiation on 3T3 cell line (Figure 5). The results of this bioassay showed that all concentrations of MO maintained the cell viability of UVB-irradiated 3T3 cells above 70%. Hence, the best concentration was 1 µg/mL (110.94%) which curiously increased the cell viability. In addition, when non-UVB-irradiated 3T3 cell lines were exposed to MO, it was observed that the highest tested concentration (1000 µg/mL) significantly

decreased the cell viability (25.78%, respectively) compared with non-UVB-irradiated 3T3 cell without MO addition (100% cell viability). Although the medium (100 µg/mL) and lower (10 and 1 µg/mL) concentrations also reduced the cell viability (54.69%, 65.38% and 71.88%, respectively) of non-UVB-irradiated 3T3 cell. In general terms, the results of 3T3 cell viability for concentrations at 1-100 µg/mL could be considered normal because showed low cytotoxic effects in the cytotoxic bioassay (IC_{50} = 112.80 µg/mL for 3T3 cell line).

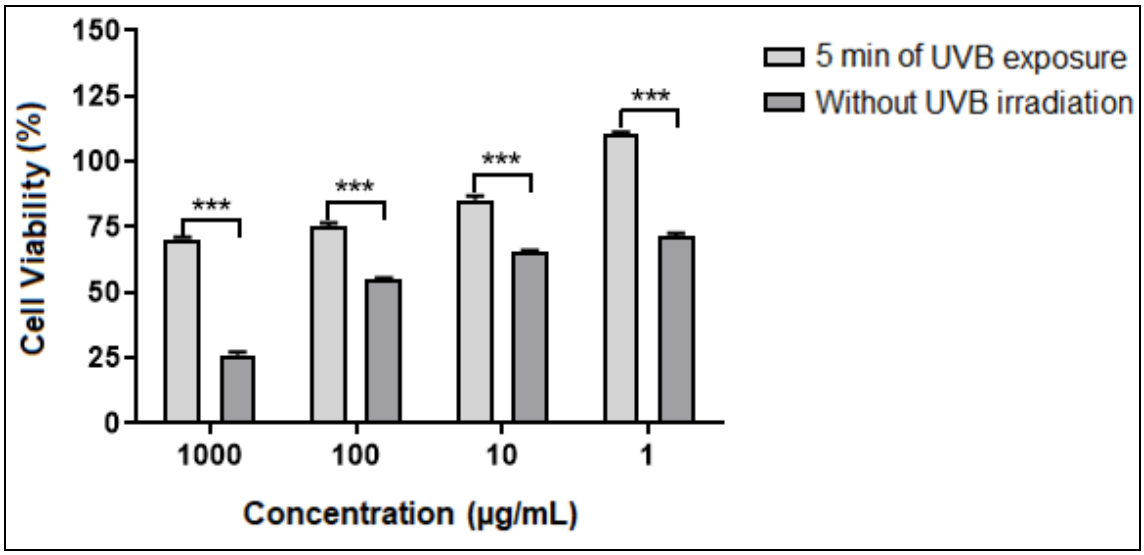


Figure 5. Photoprotective effect of monguba oil on 3T3 (fibroblasts) cell line UVB-irradiated. Data are shown as average ± standard error of the mean from triplicate determination.

*** indicates significant differences ($p < 0.001$) regarding treatment with and without UVB irradiation from each concentration.

It is known that reactive oxygen species (ROS) produced due to UVB irradiation trigger an inflammatory effect. A study of Varma et al. (2019) showed concentration-dependent protection of virgin coconut oil against intracellular ROS produced by UVB irradiation in anti-inflammatory action by inhibiting the various levels of cytokines, including TNF-a, IFNg, IL-6, IL-5 and IL -8 and improves skin barrier function by regulating AQP-3, filaggrin and involucrin mRNA expression.

Sachdeva et al. (2005) observed that γ-tocopherol had slightly cytotoxic in 3T3 cell line. Previously studies have demonstrated that some tocopherols are cytotoxic in some cell types and their cytotoxicity correlates with the degree of

methylation of the chroman ring, depending on the dose, the incubation time, and the cell line (McCormick & Parker, 2004; Sachdeva et al., 2005). The compounds present in MO have already been greatly defined by literature, and it is known that its oil has significant concentrations of γ-tocopherol (Rodrigues & Pastore, 2021). Therefore, the slightly decrease cell viability in 3T3 cell line may have been caused by this compound. It is important to highlight that the tested concentrations of MO exhibited cellular viability upper than 50%, except for the concentration of 1000 µg/mL non-UVB-irradiated. However, when the 3T3 cells received UV irradiation with the highest concentration of oil, an increase upper than 2.5x in cell viability was observed. And this phenomenon was observed at all tested concentrations.

Based on these results, the concentration at 1 µg/mL did not cause phototoxic damages to the cells and was able to reduce the damages caused by the UV radiation. This effect was possible because the concentration of bioactive compounds present in MO was succeeded in the cells defense against the oxidative stress generated by UV radiation, without causing a pro-oxidant activity. Besides, we suggest a new investigation to understand the UV-induced growth that occurred when added MO in 3T3 cells. Comprising this result MO may be used for application in pharmaceutical fields.

4. Conclusion

This study presents a comprehensive evaluation of the chemical composition and their effects on physical properties on MO. High amounts of palmitic acid and unsaturated FAs (oleic and linoleic acids) in MO contributed to the main TAG species being composed of disaturated TAGs. MO showed polymorphic β′ conformation and maintained stability for 28 days. The curve consistency data revealed that the oil from monguba seeds was very hard and brittle at all tested temperatures.

Considering the results obtained in this study, we can cite some possible applications for MO. Firstly, as a modulator of cocoa butter or palm oil to change the physical properties of these lipid matrices, thus improving and increasing quality and stability, and reducing costs. Secondly, as observed in our study, MO showed a polymorphic form for application in the formulation of ice cream, whipped cream, confectionery fillings, and cosmetic formulations. Moreover, MO had good cytotoxic

activity against SK-Mel-103 cell line and the concentration of 1 µg/mL increased cell viability as well as reduced damage caused by UV radiation. Although the sensory properties of MO have not been evaluated, it has been observed that it is non-sticky and gives a soft characteristic to the skin. Therefore, monguba seed oil can also be employed as a base for ointment/cream because of its consistency. However, further research is needed to study these possibilities.

Conflict of interest

The authors report no conflict of interest.

Acknowledgements

The authors are thankful to Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support offered through fellowships (financial code 001)) and to National Council for Scientific and Technological Development (CNPq), including grant processes #311166/2016-4; #427859/2018-2; #304266/2017-5. This work was also supported by the São Paulo Research Foundation (FAPESP) (process numbers: 2015/50333-1; 2015/03726-8, and 2016/06407-3).

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General discussion

After an advanced research about the monguba plant (*Pachira aquatica* Aubl.), we have observed a lack in the literature, regarding the bioactive compounds present in the seeds of *P. aquatica* (Chapter I). Literature data showed that the seeds presented flavonoids, anthocyanins and small concentrations of β-carotene and lycopene. Hence, we investigated the profile of phenolic compounds of monguba seeds by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS) and the antioxidant activity of phenolic compounds. In addition, the proximate composition of the seeds and physicochemical composition of the oil and seeds of *P. aquatica* were evaluated (Chapter II).

Based on the results, we observed that the seeds of monguba can be considered a source of fiber, minerals, proteins, and lipids. Although the seeds of monguba are not popularly consumed, they could be placed on the market in their natural, roasted, whole or ground form. Besides, some phenolic compounds were quantified and identified in the seeds. The group of phenolic acids was predominant, especially the caffeic, 4-hydroxybenzoic, and ferric acids. In the in vitro antioxidant tests, it was observed that the phenolic fraction containing the highest concentration of caffeic and ferric phenolic acids showed the best antioxidant activity. Some studies showed that some parts of the monguba plant such as the bark and seeds showed antimicrobial actions, confirming the reports found about the use of these parts in the treatment of vaginal infections in traditional medicine. Therefore, the monguba seeds have potential for food and biological applications (Chapter I). We also have observed that geographic changes influence the profile of fatty acids and tocopherol concentrations in monguba oil (Chapter II).

The third part of this work was dedicated to obtaining the oil from the monguba seeds using supercritical CO_2 extraction (SC-CO_2) and to evaluate the physicochemical composition of the oil in relation to the conventional extraction methodology (Chapter III). As monguba being rich in lipids, we extracted the oil from its seeds under different process conditions using SC-CO_2 extraction and we have compared these data with the conventional extraction technique (Soxhlet). The results showed that the studied process conditions (pressure and temperature) did not affect the quality of the oil using SC-CO_2 extraction. Moreover, for all the

conditions tested the monguba oil showed concentrations of γ-tocopherol above 48 mg/kg and minimum antioxidant activity of 470.17 µmol/g, these values are higher than those found for extraction using Soxhlet which obtained 25.14 mg/kg and 95.96 µmol/g, respectively. Therefore, it was possible to obtain a good quality oil using SC-CO_2 extraction with similar yield to the Soxhlet extraction technique (Chapter III).

A very important factor, which has not been found in the literature, is the determination of the triacylglycerols (TAGs) of monguba oil. Determining the composition of TAGs is essential to understand the rheology, polymorphic conformation, solid fat content (SFC), melting point and microstructure. Thus, more accurately directing the application of monguba oil in the development of new products. Therefore, in the last part of the thesis (Chapter IV) we evaluated the composition and crystallization properties of monguba oil obtained via SC-CO_2 extraction, besides to its biological activity.

The TAGs composition of monguba oil is predominantly composed of unsaturated TAGs, followed by trisaturated and monosaturated. As reported in the literature, monguba oil showed similar melting point (41.4°C, SFC = 4%). The data of the thermal behavior during melting showed that the oil is melting completely at 51.40°C, which agrees with the value observed in the SFC analysis. It was also possible to notice that monguba oil has a slow crystallization, which is affected by the composition of TAG molecules and the fatty acids that form these molecules and the minority compounds present that make monguba oil a very heterogeneous crude material. The polymorphic conformation of monguba oil remained stable at 20°C throughout the evaluated storage time (1, 7, 14, 21 and 28 days) showing peaks corresponding to the β′ form, which are characteristic of long-chain unsaturated TAGs. Due to the predominance of unsaturated TAGs, the texture of monguba oil at the evaluated temperatures (10, 20 and 30°) showed to be brittle and very hard using the 60° cone.

Evaluating the cytotoxic and photoprotective activities of monguba oil, it was observed that this oil showed good cytotoxic activity against melanoma (skin cancer) cells, without affecting the cell viability of fibroblast cells. It was interestingly to note that the fibroblast cells irradiated with UVB light in the presence of monguba oil, for all tested concentrations, showed a significant increase in cell viability, and at the concentration of 1000 µg/mL this phenomenon is quite remarkable. Therefore,

monguba oil presents a potential for usage in the development of food and pharmaceutical cosmetic products (Chapter IV).

General conclusion

The thesis data clearly shows that monguba seeds have great potential to be explored by food, pharmaceutical and cosmetics industries as a source of bioactive and high-added value compounds, as well in the product development. We have demonstrated herein that monguba seeds and oil have great content of bioactive compounds, and its oil can be full recovered by using SC-CO_2 extraction. This oil was deep characterized and showed photoprotective activity in vitro assay, which brings new insights for next studies regarding its biological activities and technological properties. Finally, the execution of this thesis allowed us to discover many interesting gaps about the monguba seed and its oil and, consequently, to establish future perspectives about it, namely:

❖ To evaluate the primary metabolites composition of monguba seeds, such as soluble and insoluble fiber content.

❖ To evaluate and identify the phytochemical composition of monguba seeds regarding the secondary metabolites that were not evaluated herein, such as alkaloids.

❖ Optimization to obtain the oil and bioactive compounds of monguba seeds by using green technologies.

❖ To evaluate biological properties of monguba seeds and oil through in vivo assays, such as benefits to human health, and harmful effects.

❖ To develop new products from monguba seeds.

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Annexs

Annex 1: License to reuse the content of the research article published to Journal of Food Composition and Analysis in the doctoral thesis (Chapter 1)

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A review of the nutritional composition and current applications of monguba (Pachira aquatica Aubl.) plant

Author: Alexsandra Pereira Rodrigues,Glaucia Maria Pastore

Publication: Journal of Food Composition and Analysis

Publisher: Elsevier

Date: Jun 1, 2021

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Annex 2: Review article published in Journal of Food Composition and Analysis

Journal of Food Composition and Analysis 99 (2021) 103878

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Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Critical Review

A review of the nutritional composition and current applications of monguba *(Pachira aquatica* Aubl.*)* plant

Alexsandra Pereira Rodrigues *, Glaucia Maria Pastore

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ARTICLE INFO

Keywords:
Munguba
Chestnut
Phytochemical
Nutritional value
Health benefits
Functional food
Oilseed

ABSTRACT

The monguba (*Pachira aquatica* Aubl.) tree is distributed from southern Mexico to South America. This plant has a variety of applications, including handicraft works, the consumption of its seeds as an alternative to coffee consumption, and the use of monguba bark and seeds in the treatment of vaginal infections in folk medicine. This review aims to draw the attention of researchers to the nutraceutical potential, physicochemical and nutritional composition, and bioactive compounds in the monguba plant for applications in the food, and other industries. After searching for publications containing '*Pachira aquatica*', 'monguba' or 'munguba' in titles and abstracts from 1878 to 2021 in six databases, the resulting publications were analyzed, and 54 full-text articles were included in this review. We observed that monguba has not been much explored and it is not widely known, though it is a novel oilseed with nutritional potential since it is rich in oils, proteins, minerals, fibers and phytochemicals. However, in vivo studies are required to verify the current uses of monguba in folk medicine by analyzing potential effects of the fruit, in addition to the compounds related to such bioactivity.

1. Introduction

The *Pachira aquatica* Aubl. plant is often found on wetlands near lakes and rivers. It is native to the tropical regions, and it is distributed from southern Mexico to South America (Janick and Paull, 2006; Robyns, 1964). *Pachira aquatica* is also known as monguba, castanheira d'água, munguba, castanheira do Maranhão, among other names (de Oliveira et al., 2007; Peixoto and Escudeiro, 2002). *Pachira aquatica* is a fast-growing tree that grows around 30-meter high. Moreover, monguba is used for city urbanization and as an ornamental plant (Infante, 2004; Janick and Paull, 2006; Lorenzi, 2008; Silva et al., 2012). Monguba has been known and used by healers, indigenous, and midwives for the treatment of some diseases, which use has been adopted by the local community. There are also reported antimicrobial activities against some pathogenic microorganisms.

Monguba seeds are consumed by animals such as swine and cattle, and by people, who consume them boiled, raw or baked (Janick and Paull, 2006; Lorenzi, 2008). The main components of monguba seeds are lipids, proteins, carbohydrates, calcium, magnesium, and potassium, in addition to bioactive compounds such as tocopherols, phenolic compounds and significant amounts of the essential amino acids threonine and tryptophan (Jorge and Luzia, 2012; Oliveira et al., 2000; Rodrigues et al., 2019).

Currently, there has been a growing interest in the health potential of bioactive compounds from plant-based foods. Therefore, in this review, we expect to draw the attention of nutrition and bioactive compound researchers to the potential of *Pachira aquatica* for further studies and applications. The nutritional properties and the phytochemical composition of monguba are the focus of this review. In addition, we discuss the botanical and toxicity information of this plant, as well as the antioxidant and biological properties of this plant phytochemicals, and the use of monguba as a food, a cosmetic, and therapeutic.

2. Strategy and criteria for selecting of publications

The data collection for this review started by searching for the keywords '*Pachira aquatica*', 'monguba' and 'munguba' in publications from 1878 to 2021 in six databases: Scopus, Science Direct, Google Scholar, Scielo, Medline (PubMed), and Web of Science. All studies that mentioned at least one of these words in the title and abstract were

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https://doi.org/10.1016/j.jfca.2021.103878
Received 9 September 2020; Received in revised form 2 March 2021; Accepted 3 March 2021
Available online 5 March 2021

Annex 3: Corrigendum article "A review of the nutritional composition and current applications of monguba (*Pachira aquatica* Aubl.) plant"

Journal of Food Composition and Analysis 100 (2021) 103919

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Corrigendum

Corrigendum to "A review of the nutritional composition and current applications of monguba (Pachira aquatica Aubl.) plant" [J. Food Compos. Anal. 99 (2021) 103878]

Alexsandra Pereira Rodrigues *, Glaucia Maria Pastore

The authors regret that Table 2 has become disfigured for the Topic: Oil in the Section: Phytosterols after publication in the journal.

We wish to make the following corrections:

- **Section 4. Correction to the text of Table 2 of content (Topic: Oil; Section: Phytosterols):**

Table 2. The main compounds from monguba (*Pachira aquatica* Aubl.) plant.

	Compound	Value	Reference
Seeds			
Carbohydrates (g/100 g DW)			
	Glucose	0.29	(Rodrigues et al., 2019)
	Fructose	0.23	
	Sucrose	3.72	
	Maltose	n.d.	
	Raffinose	0.23	
	Stachyose	0.41	
	Verbascose	n.d.	
Minerals (mg/100 g)			
	Calcium	55.89–156.38	(Becker et al., 2018; Janick and Paull, 2006; Rodrigues et al., 2019)
	Iron	0.44–4.0	
	Phosphorus	302.3	
	Potassium	700–1461.84	
	Sodium	1.14–76.1	
	Magnesium	87.53–303.99	
	Manganese	0.20–1.01	
	Zinc	0.99–2.58	
	Cupper	0.75–2.26	
Phenolic compounds (μg/g DW)			
	Gallocatechin	1.08	(Rodrigues et al., 2019)
	Quercetin	0.34	
	Protocatechuic acid	4.17	
	4-Hydroxybenzoic acid	118.88	
	Gentisic acid	1.05	
	Chlorogenic acid	58.82	
	Caffeic acid	445.54	
	p-Coumaric acid	26.33	
	Vanillic acid	2.90	
	Ferulic acid	116.07	
Oil			
Fatty acids (%)			
	Palmitic acid	44.90–76.19	(Dourado et al., 2015; Jorge & Luzia, 2012; Rodrigues et al., 2019; Spitzer, 1991; Sunday, Gillian, & John, 2019; Lopes et al., 2020; Raiser et al., 2020; De Bruin et al., 1963)
	Stearic acid	1.77–8.68	
	Malvalic acid	1.63	
	Oleic acid	6.62–39.30	
	Linoleic acid	5.00–11.35	
	Linolenic acid	0.24–0.46	
	Sterculic acid	8.16–26.5	
	Saturated fatty acids (SFA)	46.67–84.87	
	Monounsaturated fatty acids (MUFA)*	6.62–39.30	
	Polyunsaturated fatty acids (PUFA)	5.24–11.81	
Tocopherols (mg/kg oil)			
	α-tocoferol	15.23	(Jorge & Luzia, 2012; Rodrigues et al., 2019; Lopes et al., 2020)
	β-tocoferol	1.13	
	γ-tocoferol	34.66–820.8	
	δ-tocoferol	0.26	
Phytosterols (mg/100 g oil)			
	Campesterol	**10.11–14.19**	**(Lopes et al., 2020)**
	Stigmasterol	**11.01–17.00**	
	β-sitosterol	**89.70–130.47**	

n.d., not detected.
DW, Dry weight.
*Malvalic and Sterculic acid values not included.

The authors would like to apologise for any inconvenience caused.

DOI of original article: https://doi.org/10.1016/j.jfca.2021.103878.
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https://doi.org/10.1016/j.jfca.2021.103919

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Chemical Composition and Antioxidant Activity of Monguba (Pachira aquatica) Seeds

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Publication: Food Research International

Publisher: Elsevier

Date: July 2019

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Annex 5: Research article published in Food Research International

Food Research International 121 (2019) 880–887

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Chemical Composition and Antioxidant Activity of Monguba (*Pachira aquatica*) Seeds

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ARTICLE INFO

Keywords:
Carbohydrates
Fatty Acids
Mineral Composition
Phenolic Compounds
Tocopherols
Bioactive Compounds

Chemical compounds studied in this article:
4-hydroxybenzoic acid (PubChem CID: 135)
Caffeic acid (PubChem CID: 689043)
Ferulic acid (PubChem CID: 445858)
Magnesium (PubChem CID: 5462224)
Potassium (PubChem CID: 5462222)
Zinc (PubChem CID: 23994)
Stachyose (PubChem CID: 439531)
Sucrose (PubChem CID: 5988)
Palmitic acid (PubChem CID: 985)
γ-tocopherol (PubChem CID: 92729)

ABSTRACT

Monguba fruit has a seed with a chestnut-like flavor that can be consumed boiled, fried, and roasted. These nutritious seeds also have been used in popular medicine to treat several diseases. Nevertheless, the nutritional and functional potential of monguba seed is still underexploited. In this sense, we investigated the nutritional and functional components of monguba seeds. These seeds showed high total content of sugars, mainly sucrose, whereas the content of the raffinose family oligosaccharides was low. The mineral assay showed high amount of minerals, namely potassium, calcium, magnesium and zinc, which indicate that monguba seeds can be a new source of these minerals. UHPLC-ESI-MS/MS analysis showed caffeic, ferulic and 4-hydroxybenzoic acids as the main phenolic compounds, mainly in the esterified form, in these seeds. Monguba seed showed high lipid content, in which the main compounds were palmitic acid and γ-tocopherol. The soluble and insoluble phenolic fractions from monguba seeds showed high antioxidant activity measured by the oxygen radical absorption capacity (ORAC) and the trolox equivalent antioxidant capacity (TEAC) assays. Therefore, the monguba seeds have great potential to be explored by food, pharmaceutical and cosmetic industries due to their chemical composition.

1. Introduction

Pachira aquatica Aubl is a tree belonging to the Bombacaceae family, and is found from Southern Mexico to Guyana, and in Northeastern Brazil. This plant was also introduced in Guangdong, Southern Yunnan, and Taiwan as a cultivated plant (Cheng et al., 2017; Jorge & Luzia, 2012; Oliveira et al., 2000; Silva, Azevedo, & Azevedo, 2015). It is known as Malabar chestnut, French peanut, Guiana chestnut, monguba (Brazil), false cocoa, or pumpo (Guatemala), and it is commercially sold under the name 'money tree' (Cheng et al., 2017; Silva et al., 2015). Monguba can be found frequently in wetlands, from which comes its scientific name 'aquatica', however its adaptability to different climates and soil conditions has enabled its cultivation as an ornamental plant in different regions of Brazil (Peixoto & Escudeiro, 2002; Santana, dos Santos, Silva, & das Virgens, 2016).

Monguba fruits are football-shaped and surrounded by a brown wooden peel containing large seeds (see Fig. S1A-B in the Supplementary material). These seeds show a chestnut-like flavor and eventually are consumed boiled, fried or roasted, and can also be ground into flour for baking bread (Bailey, Bailey, & Bailey Hortorium, 1976; Jorge & Luzia, 2012; Silva, Amaral, Braga, Sousa, & Figueiredo, 2014).

Some studies have shown that monguba seeds present high amount of lipids (44%), 12.9% of protein, and that they could contribute to the recommended daily intake of fibers and minerals (de Bruin, Heesterman, & Mills, 1963; Oliveira et al., 2000). Also, Oliveira et al. (2000) observed high amount of essential amino acids such as tryptophan, threonine and phenylalanine/tyrosine. Leterme, Buldgen, Estrada, and Londoño (2006) found the mineral content in monguba seeds ranging from 3.44 to 3.69% and containing more phosphate,

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https://doi.org/10.1016/j.foodres.2019.01.014
Received 2 May 2018; Received in revised form 29 December 2018; Accepted 7 January 2019
Available online 09 January 2019
0963-9969/

Annex 6: Supplementary data "Chemical Composition and Antioxidant Activity of Monguba (*Pachira aquatica*) Seeds"

Supplementary Material

Chemical Composition and Antioxidant Activity of Monguba (*Pachira aquatica*) Seeds

Alexsandra Pereira Rodrigues[a]*, Gustavo Araujo Pereira[a], Pedro Henrique Ferreira Tomé[b], Henrique Silvano Arruda[a], Marcos Nogueira Eberlin[c], Glaucia Maria Pastore[a]

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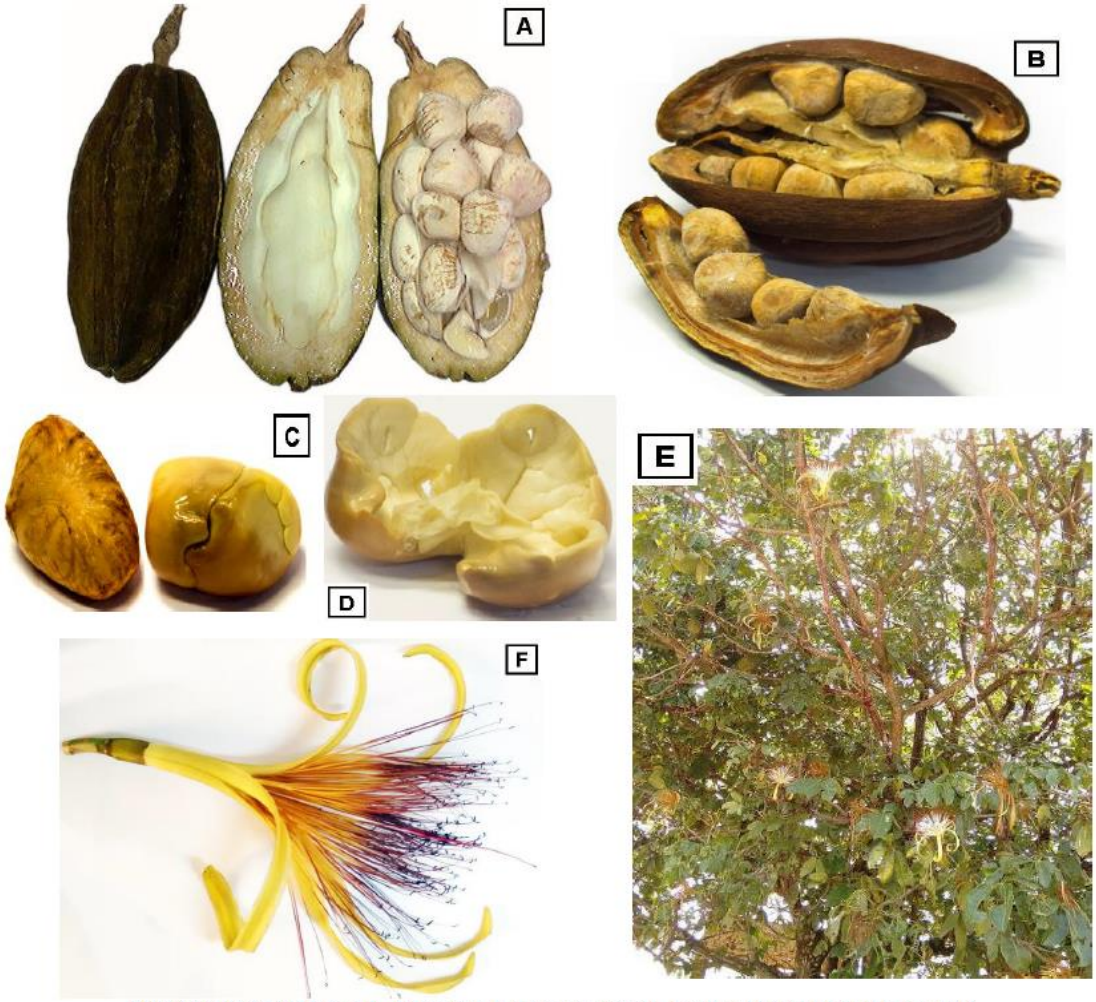

Supplement S1 (Figure S1). Monguba (*Pachira aquatica*). A) Green fruit and fruit cut in half, cross section showing seeds, B) Ripe fruit and seeds, C) Seeds surrounded by the endocarp (left) and without endocarp (right), D) Open seed, E) Tree with leaves and flowers, F) Flower in partially senescent phase

Supplement S2 (Table S2). Means value quantification (g/100g DW) of sugars and raffinose family oligosaccharides (RFOs) from commonly consumed seeds, grain and cereals.

Samples	Glucose	Fructose	Sucrose	Maltose	Total Mono- and Disaccharides	Raffinose	Stachyose	Verbascose	Total RFOs
Soybean	0.72±0.05 c	0.56±0.05 b	3.90±0.32 a	n.d.	5.18±0.04 a	0.77±0.03 a	3.72±0.04 a	0.26±0,01 a	4.75±0.06 a
Black bean	0.48±0.01 d	0.23±0.02 d	3.00±0.29 ab	n.d.	3.72±0.32 b	0.46±0.03 b	0.04±0.00 e	0.30±0.00 a	0.79±0.04 b
White bean	0.39±0.12 de	0.19±0.05 de	2.98±1.13 ab	n.d.	3.56±1.30 bc	0.20±0.07 d	0.05±0.02 e	0.18±0.07 b	0.42±0.16 c
Pinto bean	0.38±0.04 def	0.16±0.03 def	2.24±0.33 bcd	n.d.	2.77±0.41 bcd	0.30±0.03 cd	0.05± 0.01 e	0.17±0.00 b	0.51±0.04 c
Corn	0.22±0.02 fgh	0.12±0.00 ef	0.46±0.00 ef	0.32±0.03	1.12±0.05 ef	0.17±0.01 d	n.d.	n.d.	0.17±0.01 d
Rice	0.13±0.01 h	0.03±0.00 g	0.12±0.02 f	n.d.	0.29±0.04 f	n.d.	n.d.	n.d.	n.d.
White sesame	0.38±0.00 de	0.22±0.00 d	1.44±0.02 cde	n.d.	2.04±0.03 cde	n.d.	0.18±0.00 c	n.d.	0.18±0.00 d
Black sesame	0.17±0.03 gh	0.07± 0.02 fg	0.30±0.06 f	n.d.	0.54±0.10 f	n.d.	0.11±0.03 d	0.03±0..00 c	0.14±0.04 d
Brown linseed	0.30±0.04 fge	0.13±0.01 ef	0.95±0.05 def	n.d.	1.37±0.10 def	0.39±0.04 bc	n.d.	n.d.	0.39±0.04 c
Golden linseed	0.37±0.04 de	0.11±0.01 ef	1.13±0.01 def	n.d.	1.61±0.05 def	0.71±0.10 a	n.d.	n.d.	0.71±0.10 b
Quinoa	0.99±0.01 b	0.48±0.01 c	1.64±0.03 cde	n.d.	3.10±0.06 bc	n.d.	n.d.	n.d.	n.d.
Chia	2.27±0.04 a	0.86±0.01 a	2.40±0.03 bc	n.d.	5.52±0.07 a	n.d.	0.39±0.01 b	n.d.	0.39±0.01 c

n.d., not detected.

Data presented as mean ± standard deviation for the triplicate determination (n = 3). Means of quantification sugars with different lowercase letters within a column were significantly different ($p < 0.05$).

Annex 7: Corrigendum article "Chemical Composition and Antioxidant Activity of Monguba (*Pachira aquatica*) Seeds"

Food Research International 137 (2020) 109203

Contents lists available at ScienceDirect

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Corrigendum

Corrigendum to 'Chemical Composition and Antioxidant Activity of Monguba (*Pachira aquatica*) Seeds' [Food Res. Int. 121 (2019) 880–887]

Alexsandra Pereira Rodrigues[a,*], Gustavo Araujo Pereira[a], Pedro Henrique Ferreira Tomé[b], Henrique Silvano Arruda[a], Marcos Nogueira Eberlin[c], Glaucia Maria Pastore[a]

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The authors regret that tocopherol values and some sentences of the text were wrong. We wish to make the following corrections:

Section 3.1. Correction to the value and text of 8th paragraph:

- 1st line value 513.5 mg/kg to be corrected with 51.35 mg/kg.
- 5th line text Λ lower value was found to be corrected with Λ similar value of total tocopherols was found.
- 12th line text tocopherols content higher to be corrected with to-copherols content lower.

Section 3.1. Correction to the value and text in Table 3:

- 14th line text γ-tocoferol and value 513.5 ± 9.00 to be corrected with γ-tocopherol and 51.35 ± 9.00

Section 3.3. Correction to the legend of Table 5:

- 3rd line text different ($p > .05$) to be corrected with different ($p > 0.05$).

The authors would like to apologize for any inconvenience caused to the readers.

DOI of original article: https://doi.org/10.1016/j.foodres.2019.01.014
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https://doi.org/10.1016/j.foodres.2020.109203

Available online 08 April 2020

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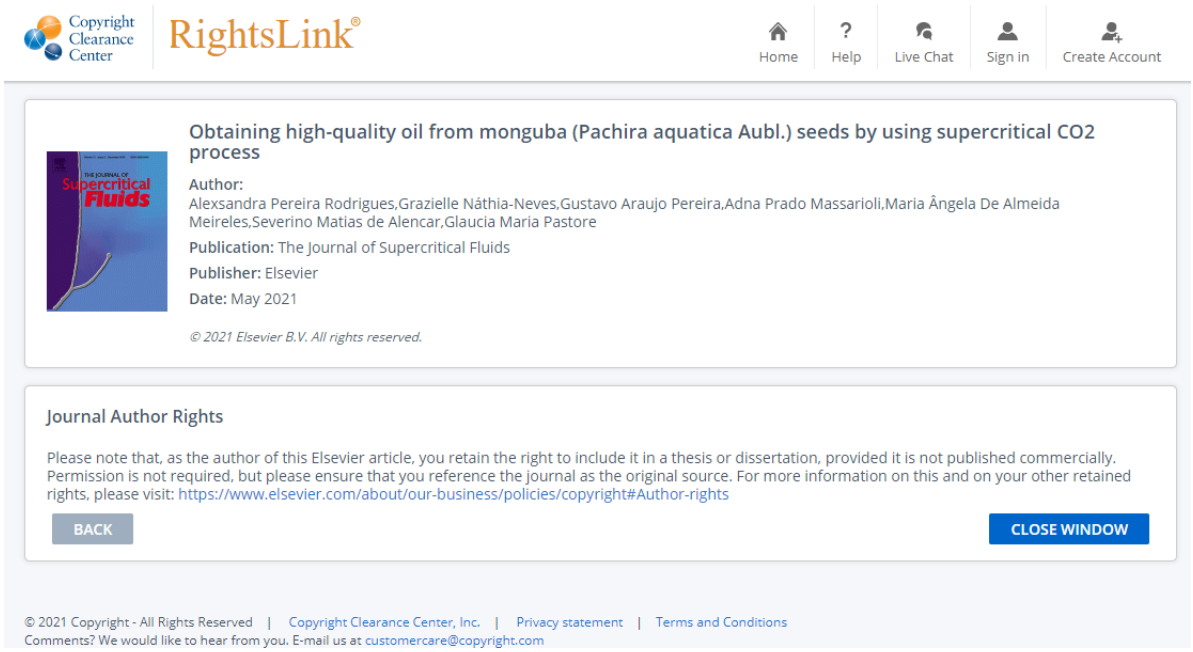

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Obtaining high-quality oil from monguba (Pachira aquatica Aubl.) seeds by using supercritical CO2 process

Author:
Alexsandra Pereira Rodrigues,Grazielle Náthia-Neves,Gustavo Araujo Pereira,Adna Prado Massarioli,Maria Ângela De Almeida Meireles,Severino Matias de Alencar,Glaucia Maria Pastore

Publication: The Journal of Supercritical Fluids

Publisher: Elsevier

Date: May 2021

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Annex 9: Research article published in The Journal of Supercritical Fluids

The Journal of Supercritical Fluids 171 (2021) 105192

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Obtaining high-quality oil from monguba (*Pachira aquatica* Aubl.) seeds by using supercritical CO_2 process

Alexsandra Pereira Rodrigues[a,*], Grazielle Náthia-Neves[b], Gustavo Araujo Pereira[a,d], Adna Prado Massarioli[c], Maria Ângela De Almeida Meireles[b], Severino Matias de Alencar[c], Glaucia Maria Pastore[a]

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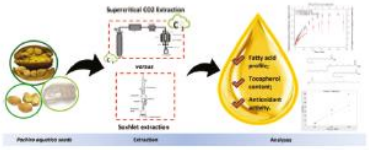
HIGHLIGHTS

- The highest global yield was obtained at 35 MPa and 60 °C.
- Palmitic acid was the main fatty acid in all process settings studied.
- Pressure and temperature did not affect the γ-tocopherol content and the antioxidant activity.
- High-quality monguba oil was extracted by using supercritical CO_2.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Emerging technology
Fatty acids
Kinetic
Antioxidant activity
Tocopherol

ABSTRACT

Monguba seeds show high content of extractable oil, approximately 54% (w/w). Literature data have reported the recovery of monguba seed oil using conventional methods. However, the use of emerging and environment-friendly methods should be applied, especially the supercritical fluid process. Therefore, we evaluated herein the effect of supercritical CO_2 process (15–35 MPa and 40–60 °C) on yield, fatty acid profile, tocopherols content, and antioxidant activity of monguba oil. The highest global yield (39.64 g/100 g) was obtained by using 35 MPa and 60 °C. Palmitic acid was the main fatty acid, which partially justifies the highest global yield observed at 60 °C. Supercritical process operated at 60 °C, 35 MPa for 160 min showed global yield equal to the Soxhlet method. The use of supercritical CO_2 allows the recovery of high content of high-quality monguba oil without affecting the antioxidant activity and the γ-tocopherol content.

1. Introduction

The *Pachira aquatica* Aubl. tree is found from Southern Mexico to South America [1,2]. It belongs to the Malvaceae family due to its genetic similarity to the species of this family [3]. The *Pachira aquatica* plant is popularly known as monguba, munguba, and Guiana chestnut [4,5]. Monguba seeds show high content of oil (approximately 54%) in which palmitic acid and total unsaturated fatty acids (*e.g.* oleic and linoleic acids) stand out in values of 44.90–76.19% and 13.42–51.11%, respectively. Other important properties of the monguba oil include high content of bioactive lipid compounds, such as vitamin E and phytosterols. Monguba seeds have also been reported as a source of proteins, amino acids (essential and non-essential), minerals, and fiber [1,4,6–8].

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https://doi.org/10.1016/j.supflu.2021.105192
Received 4 September 2020; Received in revised form 2 February 2021; Accepted 4 February 2021
Available online 10 February 2021

Annex 10: Supplementary data “Obtaining high-quality oil from monguba (Pachira aquatica Aubl.) seeds by using supercritical CO_2 process”

Supplementary Table 1 (Table S1). Effect of the variables on extraction yield, γ-tocopherol, fatty acids profile, and antioxidant activity of monguba oil obtained by supercritical CO_2 method.

	Effect	Standard error	Value-P
Yield			
Pressure (MPa)			
15	-12.899	0.550	0.001**
25	-0.465	0.550	0.436
35	13.364	0.550	0.001**
Temperature (°C)			
40	-2.994	0.389	0.001**
60	2.994	0.389	0.001**
Pressure (MPa)*Temperature (°C)			
15/40	3.394	0.550	0.002**
25/40	-0.032	0.550	0.956
35/40	-3.362	0.550	0.002**
15/60	-3.394	0.550	0.002**
25/60	0.032	0.550	0.956
35/60	3.362	0.550	0.002**
γ-Tocopherol purity			
Pressure (MPa)			
15	-1.29	2.31	0.602
25	2.38	2.31	0.351
35	-1.09	2.31	0.657
Temperature (°C)			
40	-0.26	1.63	0.880
60	0.26	1.63	0.880
Pressure (MPa)*Temperature (°C)			
15/40	0.86	2.31	0.724
25/40	-0.93	2.31	0.704
35/40	0.07	2.31	0.979
15/60	-0.86	2.31	0.724
25/60	0.93	2.31	0.704
35/60	-0.07	2.31	0.979
ORAC			
Pressure (MPa)			
15	-23.2	83.3	0.792
25	99.3	83.3	0.287
35	-76.0	83.3	0.403
Temperature (°C)			
40	-137.7	58.9	0.066
60	137.7	58.9	0.066

Pressure (MPa)*Temperature (°C)			
15/40	-170.6	83.3	0.096
25/40	46.2	83.3	0.603
35/40	124.4	83.3	0.195
15/60	170.6	83.3	0.096
25/60	-46.2	83.3	0.603
35/60	-124.4	83.3	0.195
Palmitic acid (C 16:0)			
Pressure (MPa)			
15	0.483	0.413	0.295
25	-0.907	0.413	0.080
35	0.423	0.413	0.353
Temperature (ºC)			
40	-0.805	0.292	0.040**
60	0.805	0.292	0.040**
Pressure (MPa)*Temperature (ºC)			
15/40	-0.175	0.413	0.690
25/40	-0.690	0.413	0.156
35/40	0.865	0.413	0.091
15/60	0.175	0.413	0.690
25/60	0.690	0.413	0.156
35/60	-0.865	0.413	0.091
Stearic acid (18:0)			
Pressure (MPa)			
15	-0.020	0.012	0.157
25	-0.080	0.012	0.001**
35	0.100	0.012	0.000**
Temperature (ºC)			
40	-0.071	0.009	0.000**
60	0.071	0.009	0.000**
Pressure (MPa)*Temperature (ºC)			
15/40	0.003	0.012	0.793
25/40	-0.042	0.012	0.018**
35/40	0.038	0.012	0.024**
15/60	-0.003	0.012	0.793
25/60	0.042	0.012	0.018**
35/60	-0.038	0.012	0.024**
Oleic acid (18:1)			
Pressure (MPa)			
15	-0.245	0.207	0.290
25	0.458	0.207	0.078
35	-0.212	0.207	0.352

Temperature (ºC)			
40	0.416	0.146	0.036**
60	-0.416	0.146	0.036**
Pressure (MPa)*Temperature (ºC)			
15/40	0.092	0.207	0.676
25/40	0.334	0.207	0.167
35/40	-0.426	0.207	0.095
15/60	-0.092	0.207	0.676
25/60	-0.334	0.207	0.167
35/60	0.426	0.207	0.095
Linoleic acid (18:2)			
Pressure (MPa)			
15	-0.203	0.214	0.385
25	0.522	0.214	0.058
35	-0.318	0.214	0.196
Temperature (ºC)			
40	0.449	0.151	0.031**
60	-0.449	0.151	0.031**
Pressure (MPa)*Temperature (ºC)			
15/40	0.063	0.214	0.779
25/40	0.403	0.214	0.117
35/40	-0.467	0.214	0.081
15/60	-0.063	0.214	0.779
25/60	-0.403	0.214	0.117
35/60	0.467	0.214	0.081
Sum saturated fatty acid			
Pressure (MPa)			
15	0.382	0.490	0.471
25	-0.946	0.490	0.112
35	0.564	0.490	0.302
Temperature (ºC)			
40	-0.902	0.347	0.048**
60	0.902	0.347	0.048**
Pressure (MPa)*Temperature (ºC)			
15/40	-0.223	0.490	0.668
25/40	-0.706	0.490	0.209
35/40	0.929	0.490	0.117
15/60	0.223	0.490	0.668
25/60	0.706	0.490	0.209
35/60	-0.929	0.490	0.117
Sum unsaturated fatty acid			
Pressure (MPa)			

15	-0.260	0.580	0.673
25	0.705	0.580	0.279
35	-0.445	0.580	0.478
Temperature (ºC)			
40	0.780	0.410	0.116
60	-0.780	0.410	0.116
Pressure (MPa)*Temperature (ºC)			
15/40	0.345	0.580	0.578
25/40	0.465	0.580	0.459
35/40	-0.810	0.580	0.222
15/60	-0.345	0.580	0.578
25/60	-0.465	0.580	0.459
35/60	0.810	0.580	0.222

**Significant effect considering a significance of 95%.

Annex 11: Declaration regarding access to the Brazilian genetic heritage

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 A50A9D1

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:	**A50A9D1**
Usuário:	**Alexsandra Pereira Rodrigues**
CPF/CNPJ:	**418.758.968-64**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Pachira aquatica

Título da Atividade:	**Caracterização das sementes e óleo da Pachira aquatica**

Equipe

Alexsandra Pereira Rodrigues	**UNICAMP**
Glaucia Maria Pastore	**Unicamp**

Data do Cadastro:	**05/09/2018 08:12:56**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **0:22** de **16/06/2021**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN