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FACULDADE DE ENGENHARIA DE ALIMENTOS

HENRIQUE SILVANO ARRUDA

PHYTOCHEMICAL AND TECHNOLOGICAL PROSPECTION OF THE BRAZILIAN CERRADO FRUIT ARATICUM (*Annona crassiflora* Mart.)

PROSPECÇÃO FITOQUÍMICA E TECNOLÓGICA DO FRUTO DO CERRADO BRASILEIRO ARATICUM (*Annona crassiflora* Mart.)

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Orientadora: Profª Drª Glaucia Maria Pastore

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"Há um tempo em que é preciso abandonar as roupas usadas, que já tem a forma do nosso corpo, e esquecer os nossos caminhos, que nos levam sempre aos mesmos lugares. É o tempo da travessia: e, se não ousarmos fazê-la, teremos ficado, para sempre, à margem de nós mesmos."

Fernando Teixeira de Andrade

RESUMO

Araticum (*Annona crassiflora* Mart.) é uma planta frutífera nativa do Cerrado brasileiro que possui alto potencial nutricional, funcional e econômico. Esta planta tem sido usada desde a antiguidade pela medicina popular para o tratamento de diversas condições patológicas. Tem havido crescente interesse no desenvolvimento de produtos alimentícios à base da polpa de araticum, bem como o aproveitamento de seus subprodutos para obtenção de ingredientes de valor agregado. Compreender a composição química e as atividades biológicas de diferentes partes botânicas do araticum fornece uma base para dar suporte à futuras pesquisas e aplicações. Neste contexto, este estudo tem por objetivo extrair, caracterizar e determinar os fitoquímicos na parte comestível do fruto araticum e em seus subprodutos, bem como propor a utilização da polpa do fruto por meio do desenvolvimento de uma bebida láctea prebiótica enriquecida com inulina. Na primeira etapa do estudo, os compostos fenólicos presentes em diferentes partes do fruto (polpa, casca e sementes) foram fracionados nas formas livre, esterificada, glicosilada e ligada, e caracterizados e quantificados por cromatografia líquida de alta eficiência acoplada à espectrometria de massas com ionização por electrospray. A casca do fruto apresentou os maiores conteúdos de compostos fenólicos e atividades antioxidantes, seguida pela polpa e semente, respectivamente. De forma geral, os compostos fenólicos ligados e esterificados foram as formas predominantes de fenólicos nas partes do fruto araticum e os maiores contribuintes para suas atividades antioxidantes. Catequina e epicatequina foram os fenólicos majoritários da polpa e casca, enquanto as sementes exibiram ácido caféico, catequina e epicatequina como seus principais fenólicos. Baseado nos resultados desta etapa, a casca foi considerada a parte do fruto mais promissora para obtenção de compostos bioativos. Assim, a casca de araticum foi selecionada para um estudo mais detalhado do processo de extração e da composição fitoquímica (segunda etapa). Nesta etapa, foram investigados os efeitos da potência ultrassônica nominal (160-640 W) e do tempo de processo (0,5-5,0 min) sobre a recuperação de compostos fenólicos e atividade antioxidante a partir da casca de araticum. Operando em altas potências ultrassônicas nominais foi possível obter altos rendimentos de compostos fenólicos e atividades antioxidantes em custos tempos de processo (≤5 min). Além disso, cromatografia líquida de alta eficiência acoplada à espectrometria de massas revelou a presença de 142 fitoquímicos no extrato da casca de araticum, 123 dos quais ainda não haviam sido reportados na literatura para esta matéria-prima. Finalmente (terceira etapa), uma bebida láctea prebiótica enriquecida com inulina foi desenvolvida com a polpa de araticum e o impacto de tratamentos térmicos (70 a 100°C por 60 s) sobre a estabilidade química da inulina foi avaliada por cromatografia de troca

aniônica de alta eficiência acoplada ao detector amperométrico pulsado. Nenhuma diferença significativa foi encontrada no conteúdo e perfil dos fruto-oligossacarídeos (GF_2 a GF_{20}) que compõem a inulina entre as bebidas termicamente tratadas e não tratada. Baseado nos dados obtidos, pode-se afirmar que a estrutura da inulina adiciona permaneceu intacta nas bebidas lácteas após os tratamentos térmicos. Através deste estudo, foi possível obter, com sucesso, uma bebida láctea de araticum enriquecida com inulina e termicamente tratada sem prejudicar suas propriedades prebióticas. Portanto, este trabalho abre o caminho para futuros estudos para obtenção de compostos de valor agregado a partir de subprodutos do fruto araticum, especialmente fenólicos antioxidantes, bem como para a potencial aplicação da polpa do fruto em bebidas prebióticas comerciais.

Palavras chave: Bioatividade; Benefícios à saúde; Composição química; Compostos fenólicos; Compostos bioativos.

ABSTRACT

Araticum (*Annona crassiflora* Mart.) is a fruitful tree native to the Brazilian Cerrado biome that holds high nutritional, functional and economic potential. This plant has been used since ancient times by folk medicine for the treatment of several pathological conditions. There has been increasing interest in the development of araticum pulp-based food products as well as the utilization of its by-products to obtain value-added ingredients. Understanding the chemical composition and biological activities of different botanical parts of araticum provides a basis to support future researches and applications. In this context, this study aims to extract, characterize and determine the phytochemicals in the edible part of araticum fruit and its by-products, as well as to propose the utilization of fruit pulp through the development of a prebiotic inulin-enriched araticum whey beverage. In the first stage of the study, phenolic compounds present in the different fruit parts (pulp, peel and seeds) were fractioned in the free, esterified, glycosylated and insoluble-bound forms, and characterized and quantified by high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. The fruit peel showed the highest levels of phenolic compounds and antioxidant activities, followed by pulp and seed, respectively. Overall, insoluble-bound and esterified phenolics were the dominant forms of phenolics from araticum fruit parts and the highest contributors to their antioxidant activities. Catechin and epicatechin were the major phenolics from pulp and peel, whereas seed displayed caffeic acid, catechin and epicatechin as its main phenolics. Based on the results of this stage, the fruit peel was considered the most promising fruit part to obtain bioactive compounds. Thus, araticum peel was chosen for a more detailed study of the extraction process and phytochemical composition (second stage). In this stage, it was investigated the effects of the nominal ultrasonic power (160-640 W) and process time (0.5-5.0 min) on the phenolic compounds recovery and antioxidant activity from araticum peel. Operating at high nominal ultrasonic powers was possible to obtain high phenolic yields and antioxidant activities at short process times ($\leq$5 min). In addition, the high performance liquid chromatography coupled with mass spectrometry analysis revealed that the araticum peel extract possessed 142 phytochemicals, 123 of which had not been reported in the literature for this raw material yet. Finally (third stage), a prebiotic inulin-enriched araticum whey beverage was developed and the impact of thermal treatments (70 to 100°C for 60 s) on chemical stability of the inulin was evaluated by high performance anion exchange chromatography coupled pulsed amperometric detection. No significant differences were found in content and profile of fructo-oligosaccharides (GF_2 to GF_{20}) that make up the inulin between untreated and thermally

treated beverages. Based on the data obtained, it can state that the backbone of the inulin added remained intact in the whey beverages after thermal treatments. Through this study, it was possible to successfully obtain a thermally treated inulin-enriched araticum whey beverage without prejudice to its prebiotic properties. Therefore, this work opens the way to further studies for obtaining value-added compounds from araticum by-products, especially antioxidant phenolics, as well as the potential application of fruit pulp in commercial prebiotic beverages.

Keywords: Bioactivity; Health benefits; Chemical composition; Phenolic compounds; Bioactive compounds.

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GENERAL INTRODUCTION

In recent years, there has been an increasing interest in more natural, healthier and safer products due to the increasing number of people are looking for a natural and healthy lifestyle (Arruda, Pereira, & Pastore, 2017). These changes in the global market have been taking the industries of food, pharmaceutical and cosmetics to look for new natural sources of additives and ingredients to replace the synthetic substances hitherto used (Silva et al., 2018).

Brazil hosts the most biodiversity in the world (15-20% of the total existing on the planet) and it is classified at the top among the world's 17 megadiverse countries. Moreover, it is the second country in terms of species endemism, staying behind only Indonesia (BIOFIN, 2019). These peculiarities offer a broad range of opportunities to search for medicinal plants. For example, in the Brazilian Cerrado biome (second largest biome in Brazil, accounting for approximately 25% of the Brazilian territory) there are more than 600 medicinal plants, among which, it can be highlighted the *Annona crassiflora* Mart. (Arruda, Fernandes, Botrel, & Almeida, 2015; da Costa Oliveira et al., 2018).

A. crassiflora Mart., popularly known as "araticum-do-cerrado", "ariticum", "articum", "marolo", "bruto", "cabeça-de-negro", "cascudo", "panã", "pinha-do cerrado" and "pasmada" (Arruda et al., 2015; Arruda, Pereira, & Pastore, 2018), is a tree native to the Brazilian Cerrado, widely spread throughout the biome spanning across the states of Minas Gerais, São Paulo, Bahia, Mato Grosso do Sul, Mato Grosso, Tocantins, Goiás, Maranhão, Piauí, Pará and Federal District (Cota et al., 2011). This species is one the most consumed in the Cerrado region and it has been used since ancient times as a traditional medicine in preparations for the treatment of several pathological conditions (Arruda & Almeida, 2015). Its leaves and seeds have been traditionally used to prepare infusions with claimed antidiarrhoeic, antitumor and inductor of menstruation properties, and for the treatment of Chagas' disease (Formagio et al., 2015; Morzelle, Souza, Assumpção, & Vilas Boas, 2011). Seeds oil is used against snakebites (Formagio et al., 2015), skin (Luzia & Jorge, 2013) and scalp infections (Roesler, Malta, Carrasco, & Pastore, 2006) in folk medicine. Seeds are also used in traditional medicine in antidiarrheal and insecticide preparations (Pimenta et al., 1999). Leaves infusions are used by oral administration in the treatment of inflammatory and painful ailments such as wounds, snakebites, diarrhoeas, malaria, and rheumatism (da Costa Oliveira et al., 2018). Additionally, fruits are used as tonic and astringent and for treating pain and rheumatism, and

its bark powder has antifungal and antirheumatic properties (Cavéchia & Proença, 2015; Vilar, Ferri, & Chen-Chen, 2011).

Besides being used by folk medicine, araticum tree produces fruits with acceptable sensory characteristics as well as significant nutritional and functional potential (Arruda, Botrel, Fernandes, & Almeida, 2016). Its fruits are highly appreciated by the local population and are among the 20 most used species in the regional foodstuffs (Cavalcante, Naves, Seraphin, & Carvalho, 2008), being consumed either fresh or processed (ice cream, popsicles, jellies, jams and juices). Araticum fruits present unique sensory features such as attractive colour, intense flavour and exotic aroma, as well as high content of nutrients (*e.g.*, dietary fiber, sugars, vitamins A and C, folates and minerals such as copper, manganese, potassium, magnesium and zinc) and bioactive compounds (*e.g.*, phenolic compounds, vitamins and antioxidants) (Bezerra, Pereira, Prado, de Barros Vilas Boas, & Resende, 2018; Cardoso, Oliveira, Bedetti, Martino, & Pinheiro-Sant'Ana, 2013; Schiassi, Souza, Lago, Campos, & Queiroz, 2018). Moreover, recent studies have demonstrated diverse biological properties of extracts from different parts of the araticum fruit, such as the ability to inhibit digestive enzymes and formation glycation products (Justino et al., 2016; Pereira et al., 2017), antibacterial (Silva et al., 2014), antioxidant, hepatoprotective (Justino et al., 2017; Roesler, 2011) and skin healing (de Moura et al., 2018) activities of fruit peel; antiproliferative, anticholinesterase (Formagio et al., 2015), insecticide (Krinski & Massaroli, 2014; Saito et al., 1989; Silva, Pereira, & Turchen, 2013), anti-nematode (Nogueira, Silva, Souza, Duarte, & Martins, 2009) and antioxidant (Luzia & Jorge, 2013; Roesler, 2011) properties of seeds; antioxidant (Arruda et al., 2017, 2018), anti-Alzheimer (Lucas dos Santos, Leite, Alves de Araújo, Giffoni de Carvalho, & Souza, 2018) and antibacterial (Silva et al., 2014) capacities of pulp. These attributes make this fruit a promising ingredient for the development of innovative and healthy products in the food and pharmaceutical industries, whereas its by-products could be potential sources for obtaining value-added compounds. However, araticum fruit and based products are available only in some niche and local markets and its by-products are totally discarded.

The lack of systematic knowledge on the chemical composition, biological activities, processing and quality of processed foods seriously hinders the development of araticum as a sustainable cash crop. In this sense, the main purpose of this study was to obtain, characterize and quantify the main phytochemicals from different parts of araticum fruit (pulp, peel and seeds) to generate interest in the research and development of new technologies and methodologies to valorise this Brazilian Cerrado fruit. Processing araticum fruit for food

product formulations was also proposed by means of the development of a prebiotic inulin-enriched araticum whey beverage in order to increase availability and add even more value to this fruit.

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OBJECTIVES

General objectives

The present research had as objective to carry out an in-depth study on the composition and obtaining of phytochemicals from edible part of araticum fruit (*Annona crassiflora* Mart.) and its by-products, as well as to propose the utilization of fruit pulp through the development of a prebiotic inulin-enriched araticum whey beverage.

Specific objectives

- Fractionate the phenolic compounds from araticum fruit (pulp, peel and seeds) in terms of their respective free, esterified, glycosylated and insoluble-bound forms;
- Characterize and quantify the phenolic compounds present in araticum fruit (pulp, peel and seeds) by HPLC-ESI-MS/MS;
- Determine the antioxidant potential of different phenolic fractions obtained from araticum fruit (pulp, peel and seeds) by DPPH, TEAC and $ORAC_{FL}$ assays;
- Investigate the effects of the ultrasonic power and process time on the phenolic compounds recovery and antioxidant activity from araticum peel using a full factorial experimental design;
- Obtain a greener, faster and safer extraction method for the phenolic compounds recovery from araticum peel using high-intensity ultrasound technology;
- Perform a more deepened characterization regarding the phytochemicals present in the araticum peel by HPLC-ESI-QTOF-MS/MS;
- Develop a prebiotic inulin-enriched araticum whey beverage;
- Evaluate the impact of thermal treatments on chemical stability of the inulin added to araticum whey beverage by HPAEC-PAD;
- Add value to araticum fruit and its by-products.

CHAPTER I

REVIEW ARTICLE

ARATICUM (*Annona crassiflora* Mart.) AS A SOURCE OF NUTRIENTS AND BIOACTIVE COMPOUNDS FOR FOOD AND NON-FOOD PURPOSES: A COMPREHENSIVE REVIEW

Henrique Silvano Arruda and Glaucia Maria Pastore

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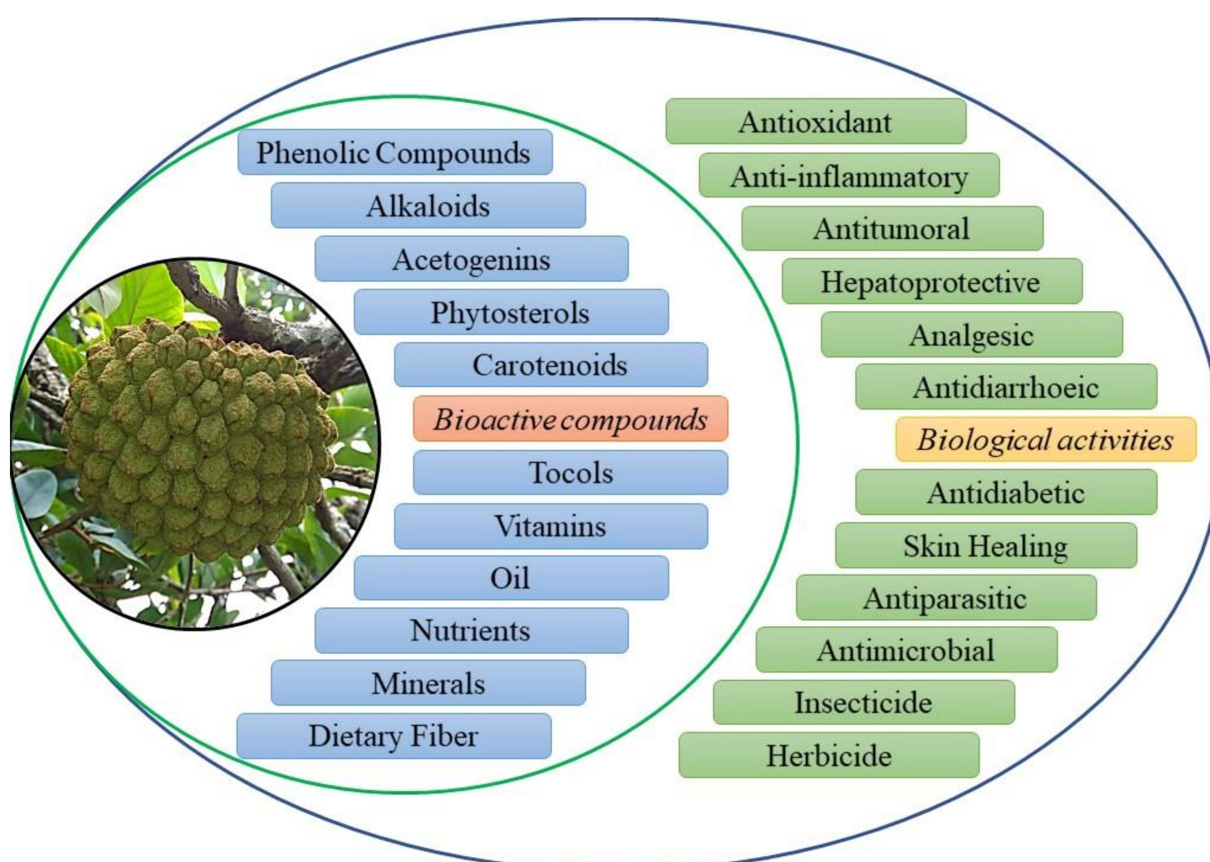
Abstract

Araticum (*Annona crassiflora* Mart.) is a fruitful tree native to the Brazilian Cerrado biome that holds high nutritional, functional and economic potential. This plant has been used since ancient times by folk medicine for the treatment of several pathological conditions. There has been increasing interest in development of pulp-based food product as well as the by-products utilization to obtain value-added ingredients. Understanding the chemical composition and biological activities of different botanical parts of *Annona crassiflora* Mart. provides a basis to support future researches and applications. In this context, this paper carries out an exhaustive review of the scientific literature, on the main phytochemicals of different botanical parts of *Annona crassiflora* Mart. (fruit, leaves, stem and root) and their biological activities, assessing their potential uses for several industrial segments. *Annona crassiflora* Mart. fruits and especially their by-products (peel and seeds) and leaves have been shown a wide range of bioactive compounds such as phenolic compounds, alkaloids, annonaceous acetogenins, tocols, carotenoids, phytosterols, dietary fiber, vitamins, minerals and essential oils. These compounds contribute to various biological activities, including antioxidant, hepatoprotective, anti-

inflammatory, antitumoral, analgesic, antidiabetic, skin healing, antidiarrhoeic, antimicrobial, antiparasitic, insecticide and herbicide activities of *Annona crassiflora* Mart. extracts. Therefore, these findings demonstrate that *Annona crassiflora* Mart. fruit, by-products and leaves can be excellent candidates to be used as functional foods and/or sources for obtaining bioactive compounds for the food, cosmetics and pharmaceutical applications.

Keywords: Biological activity; Health benefits; Nutritional composition; Chemical constituents; Phytochemicals; Nutraceuticals; Marolo; Cerrado fruit.

Graphical abstract



Highlights

- *Annona crassiflora* Mart. has high nutritional, functional and economic value.
- *Annona crassiflora* Mart. contains several bioactive compounds.
- Health promoting effects of *Annona crassiflora* Mart. have been confirmed *in vitro* and *in vivo*.

- *Annona crassiflora* Mart. can be used in the prevention/treatment of chronic degenerative diseases.
- *Annona crassiflora* Mart. has the potential to be exploited by several industrial sectors.

1. Introduction

Brazil is home to one of the greatest biodiversity in the world (20-22% of the total), housing a large number of plant species with medicinal potential (Silva et al., 2018a). For example, in the Brazilian Cerrado biome (second largest biome in Brazil, accounting for approximately 25% of the Brazilian territory) there are more than 600 medicinal plants, among which we can highlight the *Annona crassiflora* Mart. (Arruda, Fernandes, Botrel, & Almeida, 2015; da Costa Oliveira et al., 2018).

Annona crassiflora Mart., popularly known as "araticum-do-cerrado", "ariticum", "articum", "marolo", "bruto", "cabeça-de-negro", "cascudo", "panã", "pinha-do cerrado" and "pasmada" (Arruda et al., 2015; Arruda, Pereira, & Pastore, 2018a), is a tree native to the Brazilian Cerrado, widely spread throughout the biome spanning across the states of Minas Gerais, São Paulo, Bahia, Mato Grosso do Sul, Mato Grosso, Tocantins, Goiás, Maranhão, Piauí, Pará and Federal District (Cota et al., 2011). This species has been used since ancient times as a traditional medicine in preparations for the treatment of several pathological conditions (Arruda & Almeida, 2015). Besides being used by folk medicine, *Annona crassiflora* Mart. tree produces fruits with acceptable sensory characteristics as well as significant nutritional and functional potential (Arruda, Botrel, Fernandes, & Almeida, 2016). Its fruits are highly appreciated by the local population and are among the 20 most used species in the regional foodstuffs (Cavalcante, Naves, Seraphin, & Carvalho, 2008), being consumed either fresh or processed (ice cream, popsicles, jellies, jams and juices). *Annona crassiflora* Mart. fruits present unique sensory features such as attractive colour, intense flavour and exotic aroma, as well as high content of nutrients and bioactive compounds (Bezerra, Pereira, Prado, de Barros Vilas Boas, & Resende, 2018; Cardoso, Oliveira, Bedetti, Martino, & Pinheiro-Sant'Ana, 2013). These attributes make this fruit a promising ingredient for development of innovative and healthy products in the food industry. In addition, fruit by-products and other plant parts have been shown to be potential sources of value-added compounds. However, *Annona crassiflora* Mart. fruit and based products are available only in some niche and local markets and its by-products are totally discarded.

The lack of systematic knowledge on the chemical composition, biological activities, processing and quality of processed foods seriously hinders the development of *Annona crassiflora* Mart. as a sustainable cash crop. So far, there is a lack of review papers presenting systematically information on the occurrence and the concentrations of phytochemical compounds in *Annona crassiflora* Mart., its potential health beneficial effects and other biological properties, as well as on its use for development of food products and obtaining value-added ingredients. Based on the above, the main purpose of this review is to summarize, through an exhaustive compilation of the scientific literature, the main phytochemicals of different botanical parts of *Annona crassiflora* Mart. (fruit, leaves, stem and root) and their biological activities to generate interest in the research and development of new technologies and methodologies to valorise this Brazilian Cerrado plant. Processing *Annona crassiflora* Mart. fruit for food product formulations is also reviewed. Future research directions on how to better utilize the fruit by-products (peel and seeds) and leaves are also suggested. To the best of our knowledge, this is the first review paper that collects and presents all the aforementioned information in one manuscript and can be a reference material to support other researchers in conducting future studies with this plant.

2. Search strategy and studies selection

In the current comprehensive review study, electronic searches were carried out using Scopus, Google Scholar, Science Direct, Web of Science and PubMed databases, to identify relevant studies published in journals and conference abstracts from 1980 to March 2019. In addition, manual searches of the reference lists of studies identified during electronic searches were also undertaken and electronic links to additional related materials were reviewed to identify other studies that were not found in the electronic searches. The search terms were "*Annona crassiflora*", "araticum" and "marolo". The search was not restricted to any specific language. All studies that met title and abstract criteria were selected for full text review. The inclusion criteria were studies that reported results concerning: 1) nutritional composition, 2) phytochemical composition, 3) folk medicine, 4) biological properties and 5) food products. The initial search strategy yielded 768 references for all mentioned databases. After removal of duplicates and further screening in regard to the inclusion criteria, only 105 potential studies were identified to be included for the review.

3. Taxonomy and botanical information

3.1. Taxonomy

Scientific classification of *Annona crassiflora* Mart. taken from the USDA Plant Database (USDA-NRCS, 2019).

Kingdom: Plantae

Subkingdom: Tracheobionta

Super Division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Magnoliidae

Order: Magnoliales

Family: Annonaceae

Genus: *Annona* L.

Species: *Annona crassiflora* Mart.

3.2. Botanical information

Annona crassiflora Mart. is a deciduous, heliophytic, xerophytic, hermaphrodite and medium-sized tree, reaching between 4 and 8 m tall and approximately 4 m crown diameter (**Fig. 1A**) (Cota et al., 2011; Luzia & Jorge, 2013). Generally, its trunk is tortuous with a diameter of 20-30 cm, and covered with a rough and very thick bark resistant to the fire action (**Fig. 1B-C**). Leaves are crasso-membranous, ferruginous-hirsute when young and coriaceous when mature, interchangeably arranged in the horizontal position along the branches (**Fig. 1D**) (Soares, Paiva, Nogueira, Stein, & Santana, 2009). In addition, the leaves are hypostomatic with paracytic stomata, dorsiventral mesophyll, simple trichomes and large amount of sclerenchyma tissue (Corrêa, Chagas, & Pimentel, 2007).

The flowers are hermaphroditic with protogynous self-compatible, where the gynoecium (female organ) matures first that the androecium (male organ) (Soares et al., 2009). They are usually solitary, axillary, actinomorphic with three sepals, six petals and numerous

stamens and carpels. The petals are fleshy of yellowish-green coloration (**Fig. 1E-G**) (Luzia & Jorge, 2013; Pimenta, Rego, Zuffellato-Ribas, Nogueira, & Koehler, 2013). The flowers heat at night (reaching up to 10 °C above the air temperature) and release a scent, which attracts insects, mainly beetles (de Almeida-Júnior et al., 2018).

The fruit is a multiple strobiliform type, syncarpous, glabrous, subglobose shape (**Fig. 1H-J**), composed of cone-shaped buds (90-190 carpels) that contain a single seed (**Fig. 1K**). The fruit dimensions can range from 9.5-18.0 cm long, 10.5-17.8 cm wide and 9.9-16.6 cm thick, weighing from 500 g to 2 kg (Pimenta et al., 2013). The proportions of pulp, peel and seeds in the ripe fruit can vary between 44-49%, 33-41% and 10-19%, respectively (Braga Filho, Naves, Chaves, Pires, & Mazon, 2014). The fruits are composed by mesocarp and endocarp, showing fleshy consistency, coalescing, non drupoid type and arranged on a fleshy and taper receptacle (**Fig. 1J**). The epicarp is glabrous, tubercular and papillose with coloration green when unripe (**Fig. 1H**) and greenish-brown when ripe (**Fig. 1I**) (Pimenta et al., 2013). The pulp is slightly sweet and has a pleasant smell and strong flavour, and coloration raging from white to yellow and yellowish-pink (**Fig. 1J**) (Clerici & Carvalho-Silva, 2011).

The seeds are obovoid flattened with 13.4-22.7 mm long, 9.0-13.6 mm wide and 6.4-11.2 thick (**Fig. 1L**). The integument is glabrous, light brown, opaque, with a smooth texture and a bony consistency. The tegmen is composed of fibrous layers that spread within the endosperm resulting in ruminations. Moreover, seeds have basal aryl of a rough surface and irregular and conspicuous boundary arranged around the hilum and the micropyle. The hilum is basal and miniature, showing irregular shape (between circular and oval) and closing by a fold of buffer. The endosperm is whitish-yellow, ruminated, thick and abundant. The embryo is basal, crude, hyaline and gelatinous, with about 2 mm long (Pimenta et al., 2013).

The flowering of the *Annona crassiflora* Mart. tree occurs predominantly between October and November. The fructification begins in December, with the fruit maturation concentrated between February and April (Silva, Gomes, & Martins, 2009a). The fruit reaches total development at 140 days after anthesis (Silva, Vilas Boas, & Xisto, 2013a). On average, an adult plant produces from 5 to 20 fruits, reaching up to 40 fruits in some cases. Seed dispersal is carried out mainly by animals and occurs during the rainy season, between November and March (Soares et al., 2009).

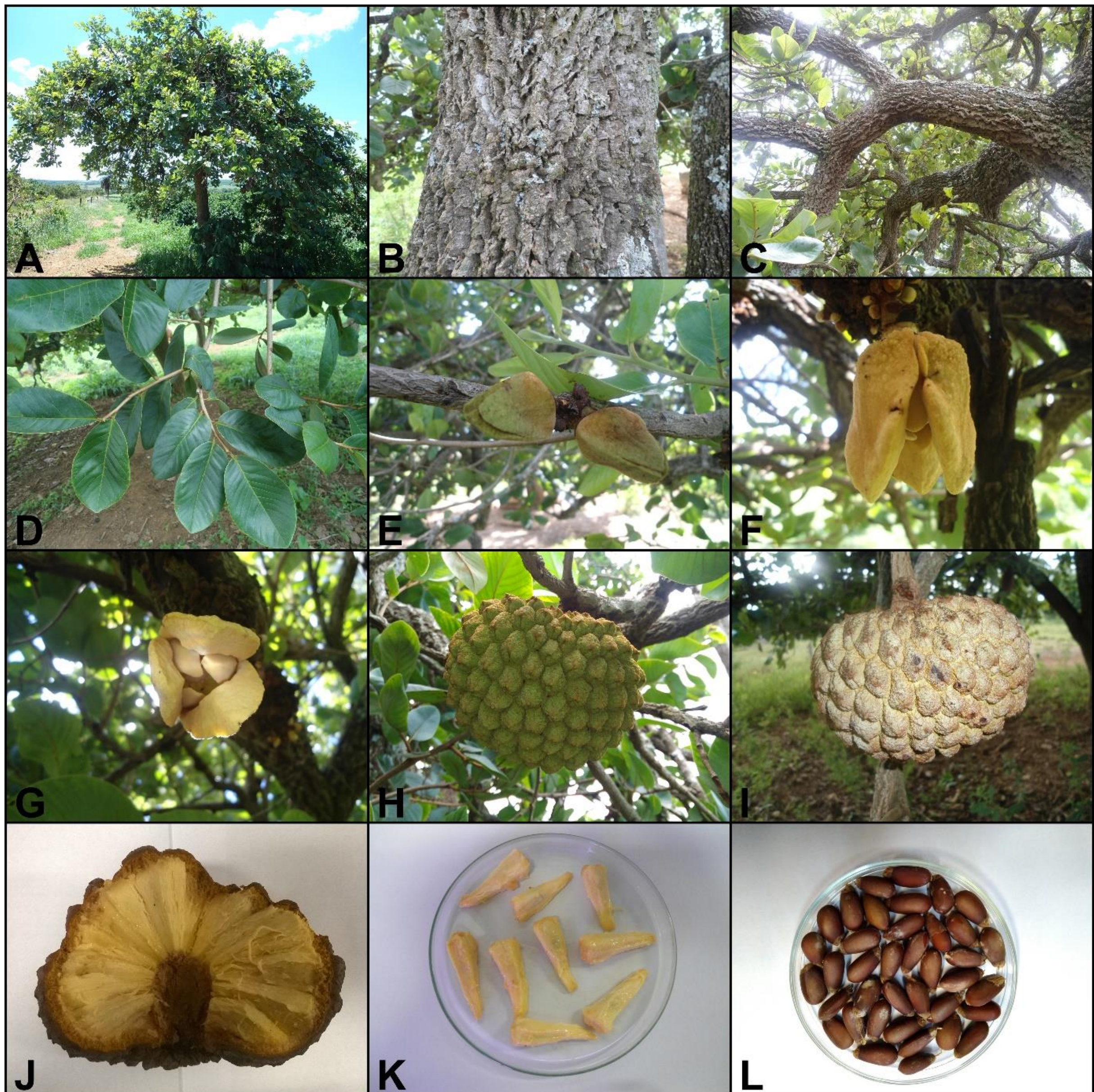


Fig. 1. *Annona crassiflora* Mart. **A**, Tree; **B**, Trunk; **C**, Trunk and branches; **D**, Leaves; **E**, Flower buds; **F**, Flower; **G**, Flower with petals; **H**, Unripe fruit; **I**, Ripe fruit; **J**, Fruit cross-section; **K**, Carpels; **L**, Seeds (**Pictures:** Henrique Silvano Arruda).

4. Nutritional and phytochemical composition of *Annona crassiflora* Mart.

4.1. Physicochemical and proximate composition of Annona crassiflora Mart. fruit

Table 1 lists the physicochemical and nutritional composition of *Annona crassiflora* Mart. fruits. The data indicates that the fruit has reduced total titratable acidity (0.51 g citric acid/100 g) and pH (4.64), and high total soluble solids (13 °Brix) and moisture (74.30%) contents, enabling its use in desserts such as sweets, jams and yogurts. The fruit pulp

contains high contents of carbohydrates (18.65%), lipids (3.78%) and energy value (113.65 kcal/100 g) and relatively low amounts of protein (1.27%).

Table 1 *Annona crassiflora* Mart. fruit pulp composition.

Nutrient/phytochemical	Unit	Value per 100 g	DV (%)[a]	Reference
Physicochemical parameter				
pH	-	4.64±0.00	-	Schiassi et al. (2018a)
Total titratable acidity (TTA)	g citric acid/100 g	0.51±0.01	-	Schiassi et al. (2018a)
Total Soluble Solids (TSS)	°Brix	13.00±0.27	-	Schiassi et al. (2018a)
TSS/TTA ratio	-	25.49	-	Calculated with independent data of Schiassi et al. (2018a)
Proximate				
Moisture	g	74.30±0.60	-	Schiassi et al. (2018a)
Energy value	kcal	113.65±2.48	5.68	Schiassi et al. (2018a)
Protein	g	1.27±0.05	2.54	Schiassi et al. (2018a)
Total lipid (fat)	g	3.78±0.04	5.82	Schiassi et al. (2018a)
Carbohydrate, by difference	g	18.65±0.53	6.22	Schiassi et al. (2018a)
Total dietary fiber	g	21.62±0.64	86.48	Schiassi et al. (2018a)
Insoluble dietary fiber	g	16.03±0.75	-	Schiassi et al. (2018a)
Soluble dietary fiber	g	5.59±0.16	-	Schiassi et al. (2018a)
Total sugars	g	9.43±0.03	-	Schiassi et al. (2018a)
Total pectin	g	1.22±0.14	-	Schiassi et al. (2018a)
Soluble pectin	g	0.39±0.01	-	Schiassi et al. (2018a)
Ash	g	0.68±0.08	-	Schiassi et al. (2018a)
Minerals				
Calcium, Ca	mg	39.26±3.19	3.93	Schiassi et al. (2018a)
Potassium, K	mg	378.69±3.28	10.82	Schiassi et al. (2018a)
Phosphorus, P	mg	22.24±1.26	2.22	Schiassi et al. (2018a)
Magnesium, Mg	mg	31.78±3.05	7.95	Schiassi et al. (2018a)
Iron, Fe	mg	0.65±0.18	3.61	Schiassi et al. (2018a)
Zinc, Zn	mg	0.81±0.02	5.40	Villela et al. (2013)
Copper, Cu	mg	0.92±0.09	46.00	Villela et al. (2013)
Manganese, Mn	mg	0.44±0.02	22.00	Villela et al. (2013)
Sodium, Na	mg	5.47±3.44	0.23	Dragano et al. (2010)
Selenium, Se	µg	0.00±0.00	0.00	Dragano et al. (2010)
Vitamins and Phytochemicals				
Vitamin C	mg	5.23±7.19	8.72	Cardoso et al. (2013)
Vitamin A value[b]	IU	5776.67	115.53	Calculated with independent data of Cardoso et al. (2013)
Total carotenoids	mg	4.98±1.12	-	Cardoso et al. (2013)
α-carotene	mg	2.98±0.78	-	Cardoso et al. (2013)
β-carotene	mg	1.97±0.33	-	Cardoso et al. (2013)
Lycopene	mg	0.02±0.01	-	Cardoso et al. (2013)
Total compounds with Vitamin E activity	µg	494.04±16.80	-	Cardoso et al. (2013)
α-tocopherol	µg	163.11±7.76	-	Cardoso et al. (2013)
α-tocotrienol	µg	332.94±9.61	-	Cardoso et al. (2013)

Vitamin E value[c]	IU	0.393	1.31	Calculated with independent data of Cardoso et al. (2013)
Total folates	μg	27.36±2.31	6.84	Cardoso et al. (2013)
Tetrahydrofolate	μg	21.97±2.11	-	Cardoso et al. (2013)
5-methyltetrahydrofolate	μg	5.39±0.51	-	Cardoso et al. (2013)

IU: International Unit.
[a]DV (%): Percent Daily Values are based on a caloric intake of 2,000 calorie, for adults and children four or more years of age (FDA, 2013). The DV (%) shown are based on a serving of 100 grams of fresh pulp.
[b]Vitamin A value expressed in terms of International Units (UI) was calculated on the basis of the following conversion factors: 1 IU of vitamin A = 0.6 μg of β-carotene or 1.2 μg of other total mixed carotenoids with Vitamin A activity (FAO/INFOODS, 2012).
[c]Vitamin E value expressed in terms of International Units (UI) was calculated on the basis of the following conversion factors: 1 IU of natural vitamin E = 0.67 mg of α-tocopherol or 2.23 mg of α-tocotrienol (FAO/INFOODS, 2012).

The fruit is an excellent source of vitamin A, dietary fiber, copper and manganese, as 100 g of fruit pulp accounts for about 115.53, 86.48, 46.00 and 22.00% of recommended daily intake, respectively. Therefore, *Annona crassiflora* Mart. fruits could be a food alternative for management/prevention of hypovitaminosis A-associated complications and gastrointestinal diseases. The fruit was also shown to be a good source of potassium, magnesium, zinc, vitamin C and folates, contributing with approximately 10.82, 7.95, 5.40, 8.72 and 6.84% of recommended daily intake, respectively. Despite the small contribution of *Annona crassiflora* Mart. fruits to supply the daily recommendations for calcium, iron and vitamin E, it should be noted that this contribution has a significant biological value, since this fruit is consumed mainly by individuals from socially vulnerable families residing in rural areas (Cardoso et al., 2013). Moreover, its very low sodium content (5.47 mg/100 g that represents only 0.23% of recommended daily intake) could benefit persons with hypertension as well as reduce the risk of cardiovascular diseases. Furthermore, in recent years, several phytochemical classes have been identified in *Annona crassiflora* Mart. and studied in detail due to their potential biological effects (**Table 2**). Thus, this fruit could be an important tool to reduce food insecurity, malnutrition and risk for several diseases.

Table 2 Phytochemical composition of *Annona crassiflora* Mart.

Phytochemical	Plant part	Sample form	Major findings	Reference
Volatile compounds	Fruit	The volatile compounds were extracted by headspace solid-phase microextraction.	-CG-MS analysis revealed changes in volatile profile during the fruit development. -β-pinene, ethyl hexanoate, β-linalool, methyl octanoate, ethyl octanoate, bicyclogermacrene, methyl hexanoate, nonanal, β-caryophyllene, methyl decanoate and ethyl decanoate were found.	Silva et al. (2013a)
Volatile compounds	Fruit pulp	The volatile compounds were extracted by headspace solid-phase microextraction.	-GC-MS analysis identified 15 compounds, of which esters were prevalent relative to other compounds. -2,3-butanediol (32.8%), ethyl hexanoate (21.2%), acetic acid (7.9%) and ethyl octanoate (6.8%) were the major volatiles.	Bezerra et al. (2018)
Phenolic compounds	Fruit (peel, seeds and pulp)	Phenolic compounds were extracted with a mixture of methanol-acetone-water (7:7:6) using ultrasound and fractionated in free, esterified, glycosylated and insoluble-bound forms.	-HPLC-MS analysis revealed 10 phenolics (catechin, epicatechin, rutin, quercetin, and protocatechuic, gentisic, chlorogenic, caffeic, *p*-coumaric and ferulic acids) in *Annona crassiflora* Mart. fruit parts. -Peel was found to contain the highest amount of total phenolics (5735.52 μg/g dw), followed by pulp (1792.58 μg/g dw) and seed (164.66 μg/g dw). -Catechin (768.42 and 3526 μg/g dw) and epicatechin (661.81 and 1632.90 μg/g dw) were the major compounds from pulp and peel, whereas seed displayed caffeic acid (45.36 μg/g dw), catechin (33.60 μg/g dw) and epicatechin (25.48 μg/g dw) as its main phenolics. -Phenolics were predominantly present in the insoluble-bound and esterified forms in all of the *Annona crassiflora* Mart. fruit parts.	Arruda et al. (2018b)
Phenolic compounds	Leaves	Dichloromethane and ethyl acetate fractions were combined and fractionated in SiO_2 column.	-NMR analysis revealed 2 flavonoids (quercetin and kaempferol).	Machado et al. (2015)
Phenolic compounds and alkaloids	Leaves	Phytochemicals were extracted using hydroethanolic solution (ethanol 80%).	-HPLC, ESI-MS, UV/Vis spectroscopy and NMR analyses revealed 3 flavonoids ((-)-epicatechin, quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-*O*-α-L-arabinoside and quercetin-3-*O*-β-L-arabinopiranoside) and 1 alkaloid (norstephalagine) in leaves.	da Costa Oliveira et al. (2018)

Phenolic compounds and alkaloids	Leaves	The leaves were extracted with distilled water (10%, w/v) for 48 h at room temperature.	-29 phytochemicals were identified by HPLC-MS analysis, including 10 flavonoids (catechin, epicatechin, quercetin-*O*-dihexoside, quercetin-*O*-hexosyl-pentoside, rutin, quercetin-3-*O*-β-glucopyranoside, kaempferol-*O*-deoxyhexosyl-hexoside, kaempferol-*O*-hexoside, quercetin-*O*-pentoside and kaempferol-*O*-pentoside), 14 procyanidins (4 procyanidin A dimer isomers, 3 procyanidin B dimer isomers, 2 procyanidin A trimer isomers and 5 procyanidin B trimer isomers), 1 phenolic acid (5-*O*-*E*-caffeoylquinic acid) and 4 alkaloids (dimethoxy-dihydroxy-tetrahydroprotoberberine, xylopine, isolaureline and 1 other not identified). -MALDI-MS analysis revealed two polymeric series of proanthocyanidins: polymeric series A was composed only of procyanidin monomers (up to 7 units), and polymeric series B, besides procyanidins (up to 8 units), had 1 prodelphinidin unit.	Ferraz et al. (2017)
Phenolic compounds	Fruit peel	Fruit peel was extracted with ethanol 98% and further fractionated in ethyl acetate and *n*-butanol fractions.	-HPLC-MS analysis revealed 9 (4 organic acids (gluconic, quinic, malic and citric acids), 2 flavonoids (procyanidin B2 and (epi)catechin) and 3 phenolic acids (caffeoyl-glucoside, chlorogenic acid and feruloyl-galactoside)) and 11 (3 organic acids (gluconic, quinic and malic acids), 6 flavonoids (procyanidins B2 and C1, (epi)catechin, quercetin-3-glucoside, kaempferol-3-*O*-rutinoside and kaempferol-7-*O*-glucoside) and 2 phenolic acids (caffeoyl-glucoside and feruloyl-galactoside)) phytochemicals in *n*-butanol and ethyl acetate fractions, respectively.	Justino et al. (2016)
Phenolic compounds	Leaves	Leaves were extracted with hydroethanolic solution (ethanol 80%) and further fractionated and purified on a reverse-phase polyamide column.	-Pure quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-*O*-α-L-arabinoside.	da Costa Oliveira et al. (2017)
Phenolic compounds	Leaves	Leaves were extracted with methanol and further fractionated and purified on a silica gel column chromatography and Sephadex.	-NMR analysis revealed 2 flavonoids (kaempferol-3-*O*-β-glucoside and kaempferol-3-*O*-β-diglucoside).	Rocha et al. (2016)

Phenolic compounds	Leaves	Leaves were extracted with hydroethanolic solution (ethanol 80%) and further fractionated and purified.	-HPLC-MS and NMR analyses revealed 5 flavonoids (quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-*O*-α-L-arabinoside, kaempferol-3-*O*-β-D-galactopyranoside, quercetin-3-*O*-β-D-galactopyranoside, quercetin-3-*O*-β-L-arabinopiranoside and (-)-epicatechin).	Lage et al. (2014)
Phenolic compounds	Seeds	Seeds were extracted with ethanol 75% and further purified on a silica gel column chromatography.	-NMR analysis revealed 2 phenolic compounds (grossamide and *N-trans*-caffeoyltyramine). -Grossamide (12 mg/kg dw) and *N-trans*-caffeoyltyramine (192 mg/kg dw).	Santos et al. (1996a)
Phenolic compounds	Seeds	Seeds were exhaustively extracted by maceration using methanol.	-HPLC analysis showed the presence of rutin (493 µg/mL), caffeic acid (302 µg/mL), sinapic acid (248 µg/mL), ferulic acid (176 µg/mL) and *p*-coumaric acid (106 µg/mL).	Formagio et al. (2015)
Phenolic compounds and organic acids	Fruit (peel, seeds and pulp)	Fruit parts were extracted with ethanol 95%.	-ESI-MS analysis showed the presence of organic acids, sugars and phenolics in *Annona crassiflora* Mart. fruit parts. -Pulp (malic acid and sugars), peel (organic acids: malic, ascorbic and quinic acids; phenolics: caffeic and ferulic acids, xanthoxyline and rutin), seed (organic acids: malic, ascorbic and quinic acids; phenolics: caffeic, ferulic and caffeoyltartaric acids, caffeoylglucose, xanthoxyline, quercetin-*O*-hexosyl-pentoside and rutin).	Roesler et al. (2007)
Several phytochemicals	Fruit peel	Hydroethanolic extract (ethanol 50%) was obtained by high-intensity ultrasound.	-HPLC-MS analysis revealed 142 phytochemicals belonging to different classes, including 5 organic acids, 3 jasmonates, 33 phenolic acids, 73 flavonoids, 6 other phenolics, 21 annonaceous acetogenins and 1 other compound. -123 phytochemicals were reported for the first time in *Annona crassiflora* Mart. peel.	Arruda et al. (2019)
Several phytochemicals	Leaves	Leaves were extracted with KH_2PO_4 buffer solution in D_2O (90 mM, pH = 6.0).	-NMR analysis revealed the presence of amino acids (alanine, threonine, valine), sugars (sucrose, α- and β-glucose), organic acids (formic and γ-aminobutyric acids), phenolics (ferulic acid and quercetin), alkaloid (trigonelline) and choline.	Machado et al. (2015)
Alkaloids	Fruit peel	Fruit peel was extracted with ethanol 98% and further purified by semi-preparative HPLC on RP-C_{18}.	-Pure alkaloid stephalagine (30 mg/kg dw).	Pereira et al. (2017)

Alkaloids	Leaves	Leaves were extracted with acidified ethanol and further fractionated in chloroform and purified by liquid chromatography on column silica gel.	-GC-MS analysis revealed 4 alkaloids (anonaine, annoretine, xylopine and romucosine). -Total alkaloid concentration ranged among plant localization (221.05-2986.89 µg/g dw). -Anonaine was the main alkaloid detected (114.64-2254.33 µg/g dw) followed by annoretine (18.94-559.87 µg/g dw), romucosine (0-300.69 µg/g dw) and xylopine (0-126.46 µg/g dw).	Egydio et al. (2013)
Alkaloids	Stem	Stem was extracted with ethanol and further partitioned in chloroform. The chloroform fraction was partitioned in methanol 90% and purified by on a Sephadex column and thin layer chromatography.	-NMR and ESI-MS analyses revealed the presence of 2 oxaporphynic alkaloids (atherospermidine and liriodenine).	Gonçalves et al. (2006)
Annonaceous acetogenins	Seeds	Seeds were extracted with ethanol 75% and further fractionated on a silica gel column chromatography.	-NMR analysis revealed the presence of 1 annonaceous acetogenin, namely araticulin (32 mg/kg dw).	Santos et al. (1996b)
Annonaceous acetogenins	Seeds	Seeds were extracted with petroleum ether and further fractionated in hydromethanolic solution (methanol 90%) and purified on a silica gel column chromatography.	-NMR analysis revealed the presence of 1 annonaceous acetogenin, namely crassiflorin.	Santos et al. (1994)
Annonaceous acetogenins	Seeds	Seeds were extracted with ethanol 75%.	-Seven pure annonaceous acetogenins were obtained (araticulin, 4-deoxycrassiflorin, crassiflorin, annonin I, almunequin, bullatacin and muricatetrocin B).	Pimenta et al. (1999)
Lipids	Leaves	Oil isolated from the dichloromethane and ethyl acetate fractions.	-GC-MS analysis showed that the major compounds of fatty nature include palmitic acid methyl ester (48.14%), stearic acid methyl ester (11.60%), oleic acid methyl ester (7.74%), palmitic acid ethyl ester (5.20%), γ-dodelactone (3.94%), and 2-isopropyl-5-methylcyclohexanol (2.39%).	Machado et al. (2015)
Lipids	Seeds	Lipid fraction (yield, 28.84% dw) was obtained by extracting	-Phytosterols: campesterol (204.32 mg/kg), stigmasterol (179.25 mg/kg) and β-sitosterol (300.02 mg/kg).	Luzia and Jorge (2013)

		with chloroform, methanol and water (2:1:0.8; v/v/v).	-Tocopherols: α-, β-, γ- and δ-tocopherol (12.20, 3.30, 123.42 and 0.16 mg/kg). -Saturated fatty acids (32.23%): myristic (0.72%), palmitic (18.07%), margaric (0.11%), stearic (11.02%), arachidic (1.59%) and behenic (0.72%) acids. -Monounsaturated fatty acids (50.76%): palmitoleic (1.01%) and oleic (49.75%) acids. -Polyunsaturated fatty acids (17.00%): linoleic (16.29%) and linolenic (0.72%) acids.	
Lipids	Fruit pulp	Cold extraction.	-GC-FID analysis identified 15 fatty acids, corresponding to 98.77% of the oil. -Saturated fatty acids (19.85%): caproic (1.21%), caprylic (1.69%), capric (0.15%), lauric (0.25%), myristic (1.51%), palmitic (9.92%), stearic (4.63%), arachidic (0.37%) and behenic (0.12%) acids. -Monounsaturated fatty acids (75.72%): palmitoleic (0.16%) and oleic (74.83%), vaccenic (0.13%) and gadoleic (0.60%) acids. -Polyunsaturated fatty acids (3.2%): linoleic (0.67%) and linolenic (2.53%) acids.	Lopes et al. (2012)
Lipids	Fruit pulp	Lipid fraction was obtained by extracting with hexane.	-MALDI-MS analysis revealed that *Annona crassiflora* Mart. pulp oil is composed mainly of multiple unsaturated triacylglycerols, such as LLO, OOLn and/or SLLn, OOL and/or LLS, OOO and/or SOL, OOS and/or SSL, PPL and/or POP, PLL, PLO, POO and PPLn (P: palmitic, S: stearic, O: oleic, L: linoleic, Ln: linolenic acid).	Corrêa et al. (2011)
Phytosterols	Seeds	Seeds were extracted with ethanol and further fractionated in ethyl acetate and purified by liquid chromatography on column silica.	-NMR analysis revealed the presence of 2 phytosterols glycosides (stigmasterol and β-sitosterol glycosides).	Inoue et al. (2010a)
Essential oil	Leaves, fruits, and flowers	Hydrodistillation for4 h.	-GC-MS analysis identified 25 compounds which represent over 94% of the oils. -Lavander-lactone, nerolidol, longipinalol, spathulenol, caryophyllene oxide, dihydromyrcene (leaves, flowers and fruit), α-pinene, verbenone, octanol acetate (leaves and fruit), β-elemene	Sirena et al. (2014)

			(leaves and flowers), viridiflorol (fruit and flowers), sabinene. β-pinene, myrcene, pinocarveol, mirtenal, Δ-elemene, β-caryophyllene, α-humulene, germacrene D, bicyclogermacrene, cubebol (leaves), camphor (flowers), epi-longipinalol and hexenyl benzoate (fruit). -Nerolidol was found in the highest concentration in all the oils analysed.	
Essential oil	Leaves	Hydrodistillation for 4 h (yield, 0.06%).	-GC-MS analysis identified 41 compounds, corresponding to 83.2% of the oil contents. -Major compounds included α-amorphene (43.6%), E-caryophyllene (17.7 %), β-germacrene (5.3 %), spirolepechinene (2.2%), β-bourbonene (1.8%) and 7-epi-α-selinene (1.4%).	Oliani et al. (2013)
Oligosaccharides	Fruit pulp	Extracts were produced by hydroethanolic solutions (0-80% ethanol).	-The extract with 50% ethanol had the highest total extractable oligosaccharides content. -HPAEC-PAD analysis showed the presence of 1-kestose (3.00 mg/g dw),1F-β-fructofuranosylnystose (0.25 mg/g dw), maltotriose (0.70 mg/g dw), xylotriose (0.49 mg/g dw), xylopentaose (0.94 mg/g dw) and xylohexaose (1.47 mg/g dw).	Arruda et al. (2017a)

dw: dry weight; HPLC-MS: High-Performance Liquid Chromatography-Mass Spectrometry; GC-MS: Gas Chromatography-Mass Spectrometry; GC-FID: Gas Chromatography-Flame Ionization Detector; HPAEC-PAD: High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection; MALDI-MS: Matrix Assisted Laser Desorption/Ionization-Mass Spectrometry; ESI-MS: Electrospray Ionization-Mass Spectrometry; NMR: nuclear magnetic resonance.

4.2. Carbohydrates

Characterisation and quantification of the sugars from *Annona crassiflora* Mart. pulp were studied by Arruda, Pereira, and Pastore (2017a). Simple sugars dominate the carbohydrate composition of the ripe fruit (357.26 mg/g dw), whereas oligosaccharides account for only a small portion of the sugars (6.84 mg/g dw). Fructose and glucose were the predominant sugars in *Annona crassiflora* Mart. pulp (127.34 and 120.65 mg/g dw, respectively), followed by sucrose (96.77 mg/g dw) and maltose (12.51 mg/g dw). All oligosaccharides (1-kestose, 1F-β-fructofuranosylnystose, maltotriose, xylotriose, xylopentaose and xylohexaose) found in the fruit were in low amounts (values below 3 mg/g dw as can be seen in **Table 2**). Despite the low amounts of prebiotic oligosaccharides (about 0.614 g/100 g dw), *Annona crassiflora* Mart. fruit may contribute to their daily intake. Another interesting feature of this fruit is its glucose/fructose ratio (approximately 1:1) that could benefit its consumption by people suffering from intestinal disorders since it would be better tolerated due to the complete absorption of fructose (Muir et al., 2009).

As described above (section **4.1**), *Annona crassiflora* Mart. fruit is an excellent source of dietary fibers (21.62% of which 5.59% are soluble fibers and 16.03% are insoluble fibers) (**Table 1**). Schiassi, Souza, Lago, Campos, and Queiroz (2018a) analysed the pectin fraction in *Annona crassiflora* Mart. fruit and found low pectin values (1.22% of which 0.39% are soluble pectin), concluding that this fruit could not be used alone as pectin source for food processing. Silva, Abreu, Gonçalves, Damiani, and Vilas Boas (2016a) reported that unripe fruits show high starch content (about 17.5 g/100 g at 120 days after anthesis), whereas in the ripe fruits (145 days after anthesis) this content was drastically reduced (values below 3 g/100 g). The high dietary fibers content, soluble sugars profile (glucose/fructose ratio) and prebiotic oligosaccharides presence can be exploited by persons with restrictive diets (*e.g.*, people suffering from intestinal disorders) or seeking to increase the ingestion of functional carbohydrates from natural sources.

4.3. Minerals

Several studies have determined the mineral content in *Annona crassiflora* Mart. fruit (Damiani et al., 2011; Dragano et al., 2010; Schiassi et al., 2018a; Souza, Pereira, Queiroz, Borges, & Carneiro, 2012; Villela, Batista, & Dessimoni-Pinto, 2013). Although the individual content of each mineral has varied among the studies, potassium was the predominant mineral found in the fruit (177.07-391.48 mg/100 g), followed by calcium (2.18-39.26 mg/100 g),

magnesium (14.23-35.00 mg/100 g) and phosphorous (14.76-22.24 mg/100 g). On the other hand, *Annona crassiflora* Mart. fruit has low levels of iron, manganese, zinc, copper, sodium and selenium (values below 6 mg/100 g). Silva et al. (2016a) reported oscillations on mineral content during fruit development. In addition, factors such as soil, climate, time of the year and genetic variability can also affect the mineral profile (Schiassi et al., 2018a).

4.4. Lipids

The lipid fraction from *Annona crassiflora* Mart. seeds, leaves and fruit pulp has been characterized as can be seen in **Table 2**. Palmitic acid methyl ester (48.14%), stearic acid methyl ester (11.60%), oleic acid methyl ester (7.74%), palmitic acid ethyl ester (5.20%), γ-dodelactone (3.94%), and 2-isopropyl-5-methylcyclohexanol (2.39%) were the major constituents of an oil isolated from the dichloromethane and ethyl acetate fractions of *Annona crassiflora* Mart. leaves (Machado et al., 2015). Some of these compounds exhibit biological properties; for example, palmitic acid methyl ester is a neuro- and cardioprotective agent (Lee et al., 2018), while the 2-isopropyl-5-methylcyclohexanol has nematicidal activity (Machado et al., 2015).

Luzia and Jorge (2013) and Egydio and Santos (2011) analysed fatty acid composition in *Annona crassiflora* Mart. seeds by CG. Both studies reported high oil yields (28.84 and 34.58% dw, respectively). This difference in yield may be related to the extraction method used in each study. Luzia and Jorge (2013) performed a cold extraction using chloroform, methanol and water (2:1:0.8; v/v/v) as extractor solvent, whereas Egydio and Santos (2011) obtained the lipid fraction using *n*-hexane in a Soxhlet apparatus. The lipid fraction from seeds was composed mainly of monounsaturated fatty acids (50.76%), followed by saturated (32.23%) and polyunsaturated fatty acids (17.00%). Oleic acid (49.75%), palmitic acid (18.07%), linoleic acid (16.29%) and stearic acid (11.02%) were found to be the major fatty acids in the seeds (Luzia & Jorge, 2013). High oil content found in *Annona crassiflora* Mart. seeds can be economically attractive for industrial extraction since other oleaginous used worldwide for oils production contain less oil (*e.g.*, corn: 3.1-5.7%; soybean and cottonseed: 18-20; olive: 15-35%) (O'Brien, 2009). Its high concentration of monounsaturated fatty acids is a desired characteristic in food oil due to its increased economic value on the international market, whereas the high proportion of unsaturated fatty acids is useful for paint industries. Moreover, oils rich in oleic acid are of great interest in cosmetics industry (good vehicle for drug solubility-partitioning) (Egydio & Santos, 2011). On the other hand, its polyunsaturated

fatty acids from the omega family are attractive for the food and pharmaceutical industries, as they are essential fatty acids for humans, displaying several biological functions and playing critical roles in the prevention and occurrence of chronic diseases (Saini & Keum, 2018). In addition, this oil presents other functional phytochemicals such as phytosterols, tocopherols and carotenoids (see sections **4.5**, **4.6** and **4.7** for more details) (Luzia & Jorge, 2013), adding even more value to this product. These studies confirm the potential use of *Annona crassiflora* Mart. seeds as a source of oil for food, cosmetic and pharmaceutical applications.

The profile of fatty acids from *Annona crassiflora* Mart. fruit pulp were investigated by Lopes et al. (2012). A total of 15 fatty acids was identified, representing about 99% of the oil content. Pulp oil profile showed predominance of monounsaturated fatty acids (68.05-75.72%), followed by saturated (19.85-26.46%) and polyunsaturated fatty acids (3.20-4.38%). Oleic acid (66.90-74.83%), palmitic acid (9.92-10.78%), stearic acid (4.63-6.83%) and linolenic acid (2.53-2.83%) were the major fatty acids in the *Annona crassiflora* Mart. fruit pulp. Moreover, short chain fatty acids (caproic, caprylic and capric acids) were found in this oil (see **Table 2**), indicating its potential contribution to the formation of typical fruity and buttery aromas of this fruit (Lopes et al., 2012). In another study, Corrêa et al. (2011) evaluated the triacylglycerols present in *Annona crassiflora* Mart. fruit pulp by MALDI Q-TOF MS. This study revealed that *Annona crassiflora* Mart. fruit pulp is composed mainly of multiple unsaturated triacylglycerols, such as LLO, OOLn and/or SLLn, OOL and/or LLS, OOO and/or SOL, OOS and/or SSL, PPL and/or POP, PLL, PLO, POO and PPLn (P: palmitic, S: stearic, O: oleic, L: linoleic, Ln: linolenic acid). The fruit pulp has lipid content (3.78% as shown in **Table 1**) significantly lower than seeds. However, its consumption can contribute to the ingestion of important fatty acids such as oleic and linolenic acids.

4.5. Phytosterols

Phytosterols have been reported in *Annona crassiflora* Mart. leaves and seeds (**Table 2**). Egydio, Valvassoura, and Santos (2013) found β-sitosterol and stigmasterol in leaves. NMR analysis revealed the presence of stigmasterol glycoside and β-sitosterol glycoside in the seeds (Inoue et al., 2010a). Another study showed that seeds oil contains significant phytosterols amounts (683.59 mg/kg). β-sitosterol (300.02 mg/kg) was the major phytosterol found in this oil, followed by campesterol (204.32 mg/kg) and stigmasterol (179.25 mg/kg) (Luzia & Jorge, 2013). Phytosterols have important roles in the areas of foods, nutrition, pharmaceuticals, and cosmetics due to their benefits for human health such as cholesterol-

lowering, anticancer, antimicrobial, anti-inflammatory, antioxidant, anti-atherosclerotic and anti-ulcerative activities (MS et al., 2018). Therefore, the seeds, a fruit by-product, can be a promising source for obtaining phytosterols. In addition, phytosterols composition of the fruit pulp (edible part) and its real contribution to the ingestion of these compounds remain to be studied.

4.6. Tocols

Tocopherols and tocotrienols, collectively referred to as tocols, are vitamin E homologs widespread found in vegetable oils. In addition to acting as a vitamin, these compounds are effective lipid cellular antioxidants and also regulate gene expression, signal transduction, and cell functions. These physiological roles can explain their potential health benefits, which includes, prevention of certain types of cancer, and hypolipidemic, antihypertensive, antiatherogenic, allergic dermatitis suppressive, neuroprotective, nephroprotective and anti-inflammatory activities (Saini & Keum, 2016; Shahidi & de Camargo, 2016). The wide range of health benefits and the importance as antioxidants for several industries have driven the search for new sources of tocols.

Despite their nutritional and economic relevance, there are few studies reporting the tocols composition in *Annona crassiflora* Mart. (**Tables 1** and **2**). Luzia and Jorge (2013) found four tocopherol isomers in seeds oil, totalizing 138.90 mg/kg of tocols. γ-Tocopherol (123.42 mg/kg) was the major tocol in this oil, while the other isomers were detected in low quantities (values below 13 mg/kg). Total tocols in fruit pulp was 494.04 µg/100 g, with α-tocotrienol being the major tocol (332.94 µg/100 g) followed by α-tocopherol (163.11 µg/100 g) (Cardoso et al., 2013). Neither the seed oil nor the fruit pulp can be considered sources of vitamin E, since the consumption of 100 g would correspond to only 5.74 and 1.31% of recommended daily intake, respectively. α-Tocopherol plays the highest biological activity, while the γ- and δ-tocopherol isomers have greater antioxidant activity (Luzia & Jorge, 2013). Thus, the higher content of the γ-tocopherol isomer in the seed oil could contribute significantly to its stability.

4.7. Carotenoids

Carotenoids are a very diverse group of natural pigments (yellow, orange and red) with bioactive properies. These compounds are potent antioxidants and some of them present

pro-vitamin A activity, contributing significantly to the reduction of the incidence of chronic diseases such as cardiovascular diseases, cancer, cataracts, and neural tube defects (Khalid, Saeed-ur-Rahman, Bilal, Iqbal, & Huang, 2019). Reports on the content and composition of carotenoids in *Annona crassiflora* Mart. are scarce in the literature. The seeds oil showed 9.62 µg/g (expressed as β-carotene) of total carotenoids (Luzia & Jorge, 2013). Lycopene, α- and β-carotene were found in fruit pulp, totalizing 4.98 mg/100 g of carotenoids. α- and β-carotene (2.98 and 1.97 mg/100 g) were the major carotenoids in the fruit pulp, whereas lycopene was in low quantity (Cardoso et al., 2013). Silva et al. (2013a) verified that carotenoids content in the fruit pulp increased up to 140 day after anthesis, followed by a decrease at 145 days after anthesis. In addition to contributing to the coloration of *Annona crassiflora* Mart. fruit pulp (yellowish), these carotenoids exert an important nutritional role because they have pro-vitamin A activity (α- and β-carotene). As discussed previously (section **4.1**), 100 g of *Annona crassiflora* Mart. pulp completely supplies the recommended daily intake of vitamin A. However, carotenoids content can be affected by some conservation methods. Silva, Cardoso, and Pinheiro-Sant'Ana (2015a) noted that bleaching (70 °C/2 min) and pasteurization (75 °C/30 min) significantly decreased the α-carotene (about 13.64 and 21.72% reduction) and β-carotene (about 36.08 and 45.57% reduction) contents, and vitamin A values (about 36.67 and 45.65% reduction) in *Annona crassiflora* Mart. pulp fruit. On the other hand, Morais et al. (2017) verified that the pasteurization (85 °C/3 min) of *Annona crassiflora* Mart. pulp reduced only α-carotene content, while the β-carotene value was increased. Therefore, *in natura* fruit consumption would be more interesting both from a nutritional and functional point of view.

4.8. Essential oil

The profile of essential oils obtained from *Annona crassiflora* Mart. fruit, flowers and leaves has been analysed by hydrodistillation and CG-MS analysis (**Table 2**). Oliani et al. (2013) evaluated the composition of essential oil from leaves and identified a total of 41 compounds, corresponding to 83.2% of the oil components. The predominant compounds in the essential oil from leaves were sesquiterpenes (81.7%), followed by monoterpenes (0.8%), aromatic compounds (0.4%) and other constituents (0.3%). α-Amorphene (43.6%), E-caryophyllene (17.7%), β-germacrene (5.3%), spirolepechinene (2.2%), β-bourbonene (1.8%), and 7-epi-α-selinene (1.4%) were the major components in the oil. Sirena et al. (2014) also reported that sesquiterpenes were the predominant compounds in essential oils from *Annona crassiflora* Mart. fruit, flowers and leaves. In this study, 25 compounds were identified,

representing over 94% of the oils content. The season strongly influenced the chemical profile of essential oil from leaves but nerolidol was the main constituent in all seasons (31.1-57.1%), followed by caryophyllene oxide (26.0%) and spathulenol (20.4%) in spring, germacrene D (9.5%) and β-elemene (9.12%) in summer, and α-pinene (12.8%) and β-pinene (12.6) in autumn. Nerolidol (18.7%), spathulenol (14.9%), *epi*-longipinalol (14.7%) and octanol acetate (10%) were the major compounds from fruit essential oil, whereas flower essential oil displayed nerolidol (47.4%), spathulenol (21.2%) and longipinalol (7.8%) as its main components. These studies revealed that the chemical profile of essential oils from *Annona crassiflora* Mart. depends on the geographic region, season and plant organ. Moreover, these phytochemicals have different biological activities. For instance, nerolidol had promising results in therapies for treatment of endometriosis (Melekoglu, Ciftci, Eraslan, Cetin, & Basak, 2018), neurodegenerative diseases (De Carvalho, De Almeida, Campelo, Lellis, & Nunes, 2018) and cancer (Biazi, Zanetti, Baranoski, Corveloni, & Mantovani, 2017), while the spathulenol is an effective antioxidant, anti-inflammatory, antiproliferative and antimycobacterial (do Nascimento et al., 2018), demonstrating the potential use of essential oils from *Annona crassiflora* Mart. by food, pharmaceutical and cosmetic industries.

4.9. Volatile compounds

The volatile components of *Annona crassiflora* Mart. fruit has been profiled by headspace solid-phase microextraction GC-MS analysis (**Table 2**). Silva et al. (2013a) studied the volatile components in *Annona crassiflora* Mart. fruit and verified that volatile profile changed during its development: β-pinene (60, 80, 100 and 120 days after anthesis), β-linalool (60, 80, 100 and 120 days after anthesis), bicyclogermacrene (60, 80 and 100 days after anthesis), nonanal (100 and 120 days after anthesis), β-caryophyllene (120 days after anthesis), methyl hexanoate (100, 120, 140 and 145 days after anthesis), ethyl hexanoate (60, 100, 120, 140 and 145 days after anthesis), methyl octanoate (60, 80, 100, 120, 140 and 145 days after anthesis), ethyl octanoate (60, 80, 100, 120, 140 and 145 days after anthesis), methyl decanoate (140 and 145 days after anthesis) and ethyl decanoate (140 and 145 days after anthesis). In addition, the authors reported having found only compounds belong to the esters group (mainly methyl and ethyl hexanoate, methyl and ethyl octanoate, and methyl and ethyl decanoate) in ripe fruits (145 days after anthesis). Bezerra et al. (2018) also noticed that esters were the prevalent compounds in *Annona crassiflora* Mart. fruit pulp. The most abundant volatile components were 2,3-butanediol (32.8%), ethyl hexanoate (21.2%), acetic acid (7.9%), ethyl

octanoate (6.8%), 1,2-propanediol (5.4%), methyl octanoate (4.7%) and 3-hydroxy-2-butanone (3.3.%). The other compounds identified (methyl hexanoate, ethyl 3-hydroxy-butanoate, octanoic acid, ethyl caproate and/or methyl, propyl hexanoate and hexyl octanoate) were in concentrations below 1.6%. Moreover, unidentified compounds accounted for only 1.2% of the total. Gas chromatography together with olfactometric detection has proven to be a reliable analytical tool to characterize the aroma of volatile compounds. The major volatile compounds found in *Annona crassiflora* Mart. fruit has been reported having odour descriptors from fruity, floral, sweet and buttery (*e.g.*, 2,3-butanediol: fruity, floral, creamy and buttery; ethyl hexanoate and ethyl octanoate: fruity and sweet; methyl octanoate: freshly and fruity; ethyl decanoate: fruity, floral, burnt and sweet; 3-Hydroxy-2-butanone: buttery and milk product; acetic acid: acid and vinegar) (Corsini, Castro, G. Barroso, & Durán-Guerrero, 2019; Isleten Hosoglu, 2018; Nicolli et al., 2018). These studies demonstrate the possible importance of esters for the fruit aroma but the relationship between the chemical composition and sensory aroma profile remains to be studied.

4.10. Organic acids

Organic acids are important flavour contributors to many fruits and vegetables. Malic, citric, tartaric, succinic and quinic acids are the main organic acids responsible by flavour notes for most fruits (Beaulieu & Baldwin, 2002). Some studies have demonstrated the presence of organic acids in *Annona crassiflora* Mart. (**Table 2**). Machado et al. (2015) reported formic and γ-aminobutyric acids in a leaves extract. Malic, citric, quinic, *n*-propylmalic, gluconic and ascorbic acids were found in fruit peel (Arruda et al., 2019; Justino et al., 2016; Roesler, Catharino, Malta, Eberlin, & Pastore, 2007). Malic, ascorbic and quinic acids were also characterized in seeds (Roesler et al., 2007). The predominant organic acids in fruit pulp were reported to be malic and citric acids (958.5 and 294.0 µg/g, respectively) (Damiani et al., 2011), suggesting that these are the main organic acids contributors to *Annona crassiflora* Mart. fruit flavour. In addition, ascorbic acid was found in significant amounts in fruit pulp (5.23 mg/100 g) (Cardoso et al., 2013). From a nutritional point of view, ascorbic acid is the most important organic acid in fruits and vegetables due to its role as vitamin C. Although *Annona crassiflora* Mart. fruit cannot be considered an excellent source of vitamin C, its consumption could contribute significantly to the recommended daily intake of this vitamin (see **Table 1**).

4.11. Phenolic compounds

As can be seen in **Table 2**, several phenolic compounds have been identified in different botanical parts of *Annona crassiflora* Mart., including fruit peel, fruit pulp, seeds and leaves. Sophisticated analytical techniques, including high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) based methods and nuclear magnetic resonance (NMR) spectroscopy have been used for their identification and quantification.

Total phenolic content in *Annona crassiflora* Mart. has been reported depending on the localization of growth, method (*e.g.*, ultrasound, water bath shaker, mixer, etc) and conditions (*e.g.*, particle size, contact time, extraction temperature, solvent polarity and composition, pH, solvent-solid ratio and number of re-extractions, etc) used in extraction. For example, Arruda, Pereira, and Pastore (2017b) showed that the solvent composition, temperature and extraction time significantly impact in the total phenolic content obtained from *Annona crassiflora* Mart. fruit pulp (1.51-4.47 g GAE/100 g dw). In another study, Arruda et al. (2019) reported that the total phenolic content in *Annona crassiflora* Mart. fruit peel were impacted by the ultrasonic power and extraction time (48.90-70.68 mg GAE/g dw). Ripening also influences the total phenolic content in fruit parts (Roesler, Malta, Carrasco, & Pastore, 2006). Silva et al. (2013a) noticed that total phenolic content in the fruit pulp decreased over fruit development. Analysing the fruit parts, Arruda, Pereira, de Morais, Eberlin, and Pastore (2018b) observed that the fruit peel presented the highest values of total phenolics, total flavonoids and condensed tannins (31.65 mg GAE/g dw, 22.05 mg CE/g dw and 17.96 mg CE/g dw, respectively), followed by fruit pulp and seeds (20.49 and 12.68 mg GAE/g dw, 13.51 and 7.33 mg CE/g dw, and 13.67 and 3.08 mg CE/g dw, respectively). Several other studies have also been recorded the total phenolic content in *Annona crassiflora* Mart. fruit parts (peel, pulp and seeds) (Arruda et al., 2018a; Damiani et al., 2011; Justino et al., 2016; Roesler et al., 2007, 2006; Schiassi et al., 2018a; Siqueira, Rosa, Fustinoni, Sant'Ana, & Arruda, 2013; Souza et al., 2012; Villela et al., 2013).

Overall, the phenolics profile and content from different botanical parts *Annona crassiflora* Mart. is very diverse (**Table 2**). For example, study conducted by Arruda et al. (2018b) demonstrated that the fruit peel presented the highest values of total phenolics (5735.52 μg/g dw), followed by fruit pulp and seeds (1792.58 and 164.66 μg/g dw). This study also revealed that catechin and epicatechin were the major phenolics from fruit peel and pulp, whereas seed displayed caffeic acid, catechin and epicatechin as its main phenolics.

Arruda et al. (2018b) showed that fruit pulp contained catechin (768.42 μg/g dw), epicatechin (661.81 μg/g dw), caffeic acid (124.31 μg/g dw), protocatechuic acid (97.92 μg/g dw), ferulic acid (53.71 μg/g dw), chlorogenic acid (43.45 μg/g dw), gentisic acid (14.00 μg/g dw), *p*-coumaric acid (11.86 μg/g dw), rutin (9.31 μg/g dw) and quercetin (7.80 μg/g dw). Santos, Boaventura, de Oliveira, and Cassady (1996a) isolated end identified two phenolic compounds from the seeds, namely grossamide and *N-trans*-caffeoyltyramine. Caffeic acid, ferulic acid, caffeoyltartaric acid, caffeoylglucose, xanthoxyline, quercetin-*O*-hexosyl-pentoside and rutin were reported in seeds ethanolic extract (Roesler et al., 2007). Formagio et al. (2015) found rutin (493 μg/mL), caffeic acid (302 μg/mL), sinapic acid (248 μg/mL), ferulic acid (176 μg/mL) and p-coumaric acid (106 μg/mL) in a seeds methanolic extract. Arruda et al. (2018b) showed that caffeic acid (45.36 μg/g dw), catechin (33.60 μg/g dw), epicatechin (25.48 μg/g dw), protocatechuic acid (22.44 μg/g dw), ferulic acid (16.95 μg/g dw), chlorogenic acid (11.00 μg/g dw), *p*-coumaric acid (3.40 μg/g dw), gentisic acid (3.28 μg/g dw), rutin (1.81 μg/g dw) and quercetin (1.35 μg/g dw) were present in seeds. Roesler et al. (2007) found caffeic acid, ferulic acid, xanthoxyline and rutin in fruit peel. Justino et al. (2016) identified procyanidins B2 and C1, (epi)catechin, quercetin-3-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-7-*O*-glucoside, chlorogenic acid, caffeoyl-glucoside and feruloyl-galactoside in fruit peel. Arruda et al. (2018b) demonstrated that fruit peel was composed by catechin (3526.78 μg/g dw), epicatechin (1632.90 μg/g dw), protocatechuic acid (317.90 μg/g dw), caffeic acid (93.45 μg/g dw), ferulic acid (65.40 μg/g dw), *p*-coumaric acid (46.90 μg/g dw), quercetin (21.83 μg/g dw), chlorogenic acid (13.24 μg/g dw), gentisic acid (8.80 μg/g dw) and rutin (8.30 μg/g dw). Recently, deeper study conducted by Arruda et al. (2019) allowed the identification of 112 phenolic compounds in a hydroethanolic extract (ethanol 50%) from fruit peel, being 73 flavonoids, 33 phenolic acids and 6 other phenolics.

There has been increasing interest in utilization of non-fruit part of *Annona crassiflora* Mart. (**Table 2**). Several phenolic compounds have been identified in *Annona crassiflora* Mart. leaves, including ferulic acid, quercetin, kaempferol (Machado et al., 2015), kaempferol-3-*O*-β-glucoside, kaempferol-3-*O*-β-diglucoside (Rocha et al., 2016), kaempferol-3-*O*-β-D-galactopyranoside, (-)-epicatechin, quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-*O*-α-L-arabinoside, quercetin-3-*O*-β-D-galactopyranoside and quercetin-3-*O*-β-L-arabinopiranoside (da Costa Oliveira et al., 2018; Lage et al., 2014). Ferraz, Silva, Prado, Canabrava, and Bispo-da-Silva (2017) found catechin, epicatechin, quercetin-*O*-dihexoside, quercetin-*O*-hexosyl-pentoside, rutin, quercetin-3-*O*-β-glucopyranoside, kaempferol-*O*-

deoxyhexosyl-hexoside, kaempferol-*O*-hexoside, quercetin-*O*-pentoside and kaempferol-*O*-pentoside, procyanidin A dimer, procyanidin B dimer, procyanidin A trimer, procyanidin B trimer and 5-*O*-E-caffeoylquinic acid in an aqueous extract from leaves. In addition to these compounds, the authors also reported the presence of two polymeric series of proanthocyanidins (up to 8 units) in this extract.

Phenolic compounds have been demonstrated to possess diverse bioactivities or the health benefits, including antitumor, antioxidant, anti-inflammatory, antimicrobial, antihypertensive and hepatoprotective properties (Caleja, Ribeiro, Barreiro, & Ferreira, 2017). Recent studies have reported the ability of phenolic-rich extracts obtained from the *Annona crassiflora* Mart. fruit peel in the prevention, management and/or treatment of some pathological conditions (see section **5** for more details). Therefore, *Annona crassiflora* Mart. fruit pulp can be a novel food ingredient for the development of functional foods, whereas its by-products and leaves seem to offer a potential plant ingredient for novel drug and cosmetic formulations.

4.12. Annonaceous acetogenins

Annonaceous acetogenins constitute a large family of long chained (C35-C37) fatty acids derivatives exclusively found in the *Annonaceae* family (Quílez, Fernández-Arche, García-Giménez, & De la Puerta, 2018). In fact, several annonaceous acetogenins have been identified in *Annona crassiflora* Mart. as shown in **Table 2**. NMR and ESI-MS analyses revealed the presence of annonaceous acetogenins in the plant stem (Pimenta et al., 2011). Seven annonaceous acetogenins, namely araticulin, 4-deoxycrassiflorin, crassiflorin, annonin I, almunequin, bullatacin and muricatetrocin B, have already been properly isolated and characterized from the seeds (Pimenta et al., 1999; Santos, Boaventura, & Oliveira, 1994; Santos, Boaventura, Sun, Cassady, & De Oliveira, 1996b). Moreover, their antitumor activity was investigated in these studies (see section **5.5** for more details). Arruda et al. (2019) also reported the presence of 21 annonaceous acetogenins for the first time in fruit peel. Numerous biological activities have been attributed to annonaceous acetogenins, including antitumor, antiparasitic, immunosuppressive, neurotoxic and pesticidal effects (Liaw, Liou, Wu, Chang, & Wu, 2016). However, among these, their selective cytotoxic potential against several human cell tumour lines has been highlighted by a great number of studies. Therefore, *Annona crassiflora* Mart. fruit by-products (peel and seeds) can potentially be exploited, mainly by the

pharmaceutical industry, as sources of annonaceous acetogenins for drugs development for anticancer therapies.

4.13. Alkaloids

Alkaloids are a group of important secondary metabolites widely found in plant species from *Annonaceae* family (Egydio et al., 2013). As can be seen in **Table 2**, several alkaloids have been found in the leaves, fruit peel and stem of *Annona crassiflora* Mart. Gonçalves, Lara, and Pimenta (2006) isolated two oxaporphynic alkaloids from plant stem, namely atherospermidine and liriodenine. Stephalagine, an aporphine alkaloid, was isolated and characterized for the first time in *Annona crassiflora* Mart. fruit peel by (Pereira et al. (2017). The authors obtained a pure alkaloid stephalagine yield of 30 mg/kg dw and demonstrated its pancreatic lipase inhibitory activity (see section **5.11** for more details). Trigonelline (Machado et al., 2015), norstephalagine (da Costa Oliveira et al., 2018), dimethoxy-dihydroxy-tetrahydroprotoberberine, isolaureline, xylopine, anonaine, annoretine, and romucosine (Egydio et al., 2013; Ferraz et al., 2017) have been identified in leaves. Egydio et al. (2013) reported that alkaloids content and profile from the *Annona crassiflora* Mart. leaves vary according to the geographic region. Total alkaloid concentration in leaves ranged from 221.05 to 2986.89 µg/g dw. Despite geographic variation, anonaine seems to be the main alkaloid present in *Annona crassiflora* Mart. leaves (114.64-2254.33 µg/g dw), followed by annoretine (18.94-559.87 µg/g dw), romucosine (0-300.69 µg/g dw) and xylopine (0-126.46 µg/g dw) (Egydio et al., 2013). Alkaloids exhibit multiple biological activities, and there are already several drugs available on the market produced from natural plant alkaloids, as reviewed in detail by Debnath et al. (2018). Therefore, the fruit peel (a fruit by-product) and leaves can be a potential source for obtaining alkaloids for pharmaceutical industry applications.

5. Biological activities of *Annona crassiflora* Mart. plant and fruits

The presence of diverse bioactive compounds in *Annona crassiflora* Mart. (as summarized above) can contribute to a range of health benefits. *Annona crassiflora* Mart. plant and fruits have been used since ancient times by folk medicine for the treatment of several pathological conditions. Indeed, recent studies have shown that extracts of different parts from *Annona crassiflora* Mart. fruit/plant exhibited a range of biological activities as described in the sections below (**Table 3**).

Table 3 Biological activities of *Annona crassiflora* Mart.

Bioactivity	Plant part	Sample form	Method/model	Major findings	Related compounds	Reference
Antioxidant	Fruit (peel, seeds and pulp)	Phenolic compounds were extracted with a mixture of methanol-acetone-water (7:7:6) and fractionated in free, esterified, glycosylated and insoluble-bound forms.	DPPH, TEAC and ORAC based *in vitro* assays	-Peel was found to contain the highest antioxidant activities by DPPH, TEAC and ORAC assays (189.32, 292.28 and 448.29 µmol TE/g dw), followed by pulp (132.73, 214.38 and 259.26 µmol TE/g dw) and seed (71.66, 94.81 and 260.22 µmol TE/g dw). -Insoluble-bound and esterified phenolics were the highest contributors to antioxidant activities in *Annona crassiflora* Mart. fruit parts.	Phenolic compounds (catechin, epicatechin, rutin, quercetin, and protocatechuic, gentisic, chlorogenic, caffeic, *p*-coumaric and ferulic acids)	Arruda et al. (2018b)
Antioxidant	Fruit peel	Hydroethanolic extract (ethanol 50%)	DPPH, TEAC and ORAC based *in vitro* assays	-The maximum antioxidant activities values were 514.57, 613.76 and 525.41 µmol TE/g dw for DPPH, TEAC and ORAC assays.	Phenolic compounds	Arruda et al. (2019)
Antioxidant	Fruit pulp	Hydroethanolic extract (ethanol 46%)	DPPH, TEAC and ORAC based *in vitro* assays	-The fruit pulp showed 609.58, 546.26 and 1593.72 µmol TE/g dw for DPPH, TEAC and ORAC assays.	Phenolic compounds, mainly flavonoids	Arruda et al. (2017b)
Antioxidant	Fruit (peel, seeds and pulp)	Hydroethanolic extract (ethanol 95%)	*In vitro* lipid peroxidation of rat liver microsomes	-↓Lipid peroxidation. -Seed extract was the most antioxidant followed by peel and pulp extracts (IC_{50} 1.72, 4.44 and 8.62 µg/mL, respectively).	Phenolic compounds	Roesler et al. (2006)
Antioxidant and inhibitory activity against digestive enzymes and protein glycation	Fruit peel	Ethanolic extract and its fractions (hexane, dichloromethane, ethyl acetate, *n*-butanol and aqueous)	*In vitro* antioxidant capacity (DPPH, FRAP and ORAC) and inhibitory activities against glycoside hydrolases (α-amylase and α-	-Ethyl acetate and *n*-butanol fractions showed higher antioxidant capacity (DPPH IC_{50}: 1.5 and 0.8 µg/mL; FRAP: 888 and 921 µmol TE/g; ORAC: 3355 and 2714 µmol TE/g), and inhibitory activities against α-amylase (IC_{50}: 4.5 and 1.7 µg/mL), α-	Phenolic compounds, mainly chlorogenic acid, (epi)catechin, procyanidins, caffeoyl- and feruloyl-glycosides, quercetin- and kaempferol-glycosides	Justino et al. (2016)

			glucosidase) and non-enzymatic glycation	glucosidase (IC_{50}: 554.5 and 787.8 µg/mL) and protein glycation (IC_{50}: 14.3 and 16.0 µg/mL), and lower cytotoxicity.		
Anti-Alzheimer	Fruit pulp	Aqueous extract	*Caenorhabditis elegans*	-Protection of the worms exposed to pro-oxidant agent (juglone), enhancing survival rates. -↓Mean of worms paralyzed after of the 10 hours (13.4% at 1000 µg/mL).	-	Lucas dos Santos et al. (2018)
Antitumor	Seeds	Pure araticulin from *Annona crassiflora* Mart. seeds	*In vitro* antitumor activity against human tumour cell lines A-549, HT-29, MCF-7, RPMI-7951 and U-251	-↓Proliferation of all human tumour cell lines.	Araticulin	Santos et al. (1996b)
Antitumor	Seeds	Pure crassiflorin from *Annona crassiflora* Mart. seeds	*In vitro* antitumor activity against human tumour cell lines A-549, HT-29, MCF-7, RPMI-7951 and U-251	-↓Proliferation of all human tumour cell lines.	Crassiflorin	Santos et al. (1994)
Antitumor	Seeds	Hydroethanolic extract and pure araticulin, crassiflorin and annonin I from seeds	*In vitro* antitumor activity against human tumour cell lines A-549 and RPMI-7951	-↓Proliferation of human tumour cell lines.	Annonaceous acetogenins, mainly araticulin, crassiflorin and annonin I	Pimenta et al. (1999)
Antitumor	Leaves, stem wood, stem bark, root wood and root bark	Hexane and ethanolic extracts	*In vitro* antitumor activity against human tumour cell lines SF-295, HCT-8, MDA-MB-435 and HL-60	-Ethanolic extracts from root bark and root wood inhibited the proliferation of all cancer tumour cells. -Root bark extract (GI_{50} 6.0-14.9 µg/mL) was more active than root wood extract (GI_{50} 13.3-24.9 µg/mL) against all the tumour cell lines.	Annonaceous acetogenins	de Mesquita et al. (2009)

Antitumor	Leaves and seeds	Methanolic extract	*In vitro* antitumor activity against human tumour cell lines UACC-62, MCF-7, NCI/ADR/RES, 786-0, NCI-H460, OVCAR-3, HT-29, K562, UA251, and VERO	-Inhibition/reduction of the proliferation of cancer tumour cells. -Seeds methanolic extract was the most active ($GI_{50} \leq 8.90$ µg/mL against all the tumour cell lines).	Phenolic acids, especially caffeic acid, sinapic acid, ferulic acid and flavonoid	Formagio et al. (2015)
Antitumor	Leaves	Hydroethanolic extract (ethanol 70%) and its hexane fraction	*In vitro* antitumor activity against human cervical cancer cell lines and *in vivo* chorioallantoid membrane assay	-Hydroethanolic extract, and mainly its hexane fraction, significantly reduce cell viability, proliferation and migration of cervical cancer cell lines *in vitro*. -Hexane fraction reduces the cervical tumour growth *in vivo*.	Flavonoids, alkaloids and annonaceous acetogenins	Silva et al. (2018a)
Antitumor	Wood	Annonaceous acetogenins-rich fraction	Ehrlich solid tumour-bearing Swiss mice	-↓Tumours growth in mice both by intratumoral and intravenous administration (38 and 20%, respectively). -Pronounced *in vivo* antitumor effect (single administration at 1.25 mg/kg significantly retarded the growth tumour in both rotes).	Annonaceous acetogenins	Pimenta et al. (2011)
Chemoprotective and antimutagenic	Leaves	Methanolic extract	*Allium cepa* and female Swiss mice	-↓Induced DNA damage in both models. -Act mainly through desmutagenesis (chemical or enzymatical inactivation the mutagen in the extra or intracellular medium) in mammals.	Phenolic compounds, mainly kaempferol glycosides	Rocha et al. (2016)
Antimutagenic	Leaves	Hydroethanolic extract (ethanol 95%)	Male Swiss mice	-↓Micronucleated polychromatic erythrocytes frequencies induced by mitomycin C.	Polyketides, annonaceous acetogenins and flavonoids	Vilar et al. (2008)

Anti-inflammatory	Leaves	Methanolic extract	Female Swiss mice	-Oral treatment with methanolic extract inhibited paw edema, ↓myeloperoxidase activity, ↓total leukocyte counts and protein extravasation in the pleural cavity, and ↓leukocyte migration and plasma extravasation in the air pouch assay, induced by carrageenan.	Phenolic compounds, mainly kaempferol glycosides	Rocha et al. (2016)
Anti-inflammatory and analgesic	Leaves	Filtrate and precipitate from hydroethanolic extract (ethanol 80%)	Male Swiss or Balb/c mice	-Both filtered and precipitated fractions reduced the paw licking time in the second phase (inflammatory pain). -Precipitated fraction significantly inhibited the neutrophilia induced by carrageenan, LPS, or CXCL8, whereas filtered fraction was effective only against the neutrophilia induced by carrageenan.	Flavonoids (quercetin glycosides and (-)-epicatechin) and alkaloids (norstephalagine)	da Costa Oliveira et al. (2018)
Analgesic	Leaves	Pure peltatoside from *Annona crassiflora* Mart. leaves	Male Swiss mice/Carrageenan-induced hyperalgesia	-Peltatoside (100 μg/paw) reversed carrageenan-induced hyperalgesia. -Peltatoside induced peripheral antinociception by activation of the cannabinoid system.	Peltatoside (quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-*O*-α-L-arabinoside)	da Costa Oliveira et al. (2017)
Healing of cutaneous wounds	Fruit peel	Ethyl acetate and *n*-butanol fractions (1:1) from ethanolic extract	Male C57BL/6 mice	-Anti-inflammatory and profibrogenic properties. -↑Skin wound closure and collagen deposition. -↓Neutrophil and macrophage infiltration.	Phenolic compounds, mainly chlorogenic acid, epicatechin, procyanidins B2 and C1, quercetin-glucoside, kaempferol-glucoside, kaempferol-rutinoside, and caffeoyl-glucoside	de Moura et al. (2018)
Antidiarrhoeic	Leaves	Aqueous extract	Male Swiss mice/castor oil-induced diarrhoea	-↓Diarrhoeic faeces without alterations in the total faeces output.	Flavonoids and alkaloids	Ferraz et al. (2017)

				-Antisecretory and/or proabsorptive effects, as well as prokinetic activity on the gastrointestinal tract of mice.		
Hepatoprotective and antioxidant	Fruit peel	*n*-Butanol fraction from ethanolic extract	Male Wistar rats/streptozotocin-induced diabetes	-↓Serum ALT, AST, and ALP activities. -↓Lipid peroxidation, protein carbonylation, and nitration and iNOS content, as well as GPx, SOD, and CAT activities and contents. -↑Total antioxidant capacity and GR activity and GSH levels.	Phenolic compounds, mainly chlorogenic acid, (epi)catechin, procyanidin B2, and caffeoyl-glucoside	Justino et al. (2017)
Hepatoprotective and antioxidant	Fruit peel and seeds	Hydroethanolic extract (ethanol 95%)	Male Wistar rats/CCl_4-induced liver damage	-↓Lipid peroxidation and GSH levels. -Enhancement/maintaining of hepatic antioxidant enzymes activities.	Phenolic compounds, such as caffeic acid, ferulic acid, xanthoxylin, caffeoyltartaric acid, caffeoylglucose, quercetin derivative and rutin	Roesler (2011)
Anti-obesity	Fruit peel	Ethanolic extract, dichloromethane fraction and pure alkaloid stephalagine	*In vitro* lipase inhibition assay and cytotoxicity with Vero cells	-Stephalagine showed the highest pancreatic lipase inhibitory activity (IC_{50} 8.35 µg/mL), followed by ethanolic extract and dichloromethane fraction (IC_{50} 104.50 and 108.10 µg/mL). -Stephalagine showed low cytotoxicity.	Stephalagine	Pereira et al. (2017)
Anticholinesterase	Leaves and seeds	Methanolic extract	*In vitro* acetylcholinesterase inhibition assay	-Inhibition of acetylcholinesterase activity.	Phenolic compounds and tannins	Formagio et al. (2015)
Antimicrobial	Leaves	Hydroethanolic extract (ethanol 80%) and its hydromethanolic	*Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium,*	-Microbial growth inhibition (61-88%).	Flavonoids glycosides and (-)-epicatechin	Lage et al. (2014)

		fraction (methanol 80%)	*Escherichia coli and Candida albicans*			
Antimicrobial	Stem, leaves, and fruit parts (peel, seed and pulp)	Hydroethanolic extract (ethanol 70%)	Oxacillin Resistant *S. aureus* (ORSA) and *S. aureus* ATCC 6538	-MIC for ORSA were 25 mg/mL (stem, leaves and pulp) and 50 mg/mL (seeds and fruit peel). -Stem, fruit peel and pulp extracts were the most active against *S. aureus* ATCC 6538 (MIC 1.56, 6.25 and 12.5 mg/mL, respectively).	Alkaloids, flavonoids, tannins and saponins	Silva et al. (2014a)
Antimicrobial	Leaves	Ethanolic extract	*Escherichia coli* and *Staphylococcus* spp. from cattle	-MIC and MBC of the ethanolic extract from *Annona crassiflora* Mart. leaves were 6.24 mg/mL for all bacterial strains.	Flavonoids and tannins	Ribeiro et al. (2018)
Antimicrobial	Leaves	Hydroethanolic extract (ethanol 95%)	*Candida albicans* (52 strains), *C. tropicalis* (4 strains) and *C. krusei* (3 strains) from human immunodeficiency virus-infected patients with oropharyngeal candidosis	-Antifungal activity against all 59 strains of *Candida* spp. -Leaf extract was more active against *C. tropicalis* strains than *C. krusei* and *C. albicans* (MIC 0.25, 0.50 and 2.00 µg/mL, respectively).	Polyketides and phenolic compounds	Silva et al. (2001)
Antiparasitic	Leaves	Hydroethanolic extract (ethanol 80%) and its fractions (dichloromethane, ethyl acetate, methanol and water)	Nematode *Caenorhabditis elegans*	-↓*C. elegans* larvae mobility (up to 98.13%). -Dichloromethane and ethyl acetate fractions showed to be the most active (98.13 and 89.66%, respectively, at 1000 µg/mL).	Formic acid, γ-aminobutyric acid, and 2-isopropyl-5-methylcyclohexanol	Machado et al. (2015)
Antiparasitic	Leaves and seeds	Aqueous extract	Sheep nematodes	-↓Larval development of sheep trichostrongylides (development reduction of 89.81 and 99.43% for	Annonaceous acetogenins	Nogueira et al. (2009)

				leaves and seeds extract, respectively, at 100 mg/mL).		
Antiparasitic	Root bark	Hexane and ethanolic extracts	*Plasmodium falciparum*	-↓*P. falciparum* growth (LC_{50} 7.6 and 11.9 µg/mL for hexane and ethanolic extracts, respectively).	Annonaceous acetogenins	de Mesquita et al. (2007)
Antiparasitic	Leaves	Ethanolic extract fractions (hexane, chloroform, ethyl acetate and aqueous)	*Plasmodium berghei*-infected mice	-↑Mean survival time of *P. berghei*-infected mice. -↓Parasitaemia (up to 75%).	Alkaloids and flavonoids	Pimenta et al. (2014)
Antiparasitic	Stem wood, stem bark, root wood and root bark	Hexane and ethanolic extracts	*Trypanosoma cruzi* trypomastigotes and *Leishmania donovani* promastigotes	-↓*T. cruzi* and *L. donovani* viability. -Ethanolic extracts from root bark and root wood were the most active against *T. cruzi* (LC_{50} 5.9 and 9.9 µg/mL, respectively) and *L. donovani* (LC_{50} 3.7 and 8.7 µg/mL, respectively).	Alkaloids and annonaceous acetogenins	Mesquita et al. (2005)
Antiparasitic	Leaves	Hydroethanolic extract (ethanol 96%) and alkaloid extract (dichloromethane fraction)	*Trypanosoma cruzi* trypomastigotes and *Leishmania* (L.) *chagasi* promastigotes	-Alkaloid extract reduced *T. cruzi* and *L.* (L.) *chagasi* viability. -Alkaloid extract was very effective against *L.* (L.) *chagasi* (86.1% mortality at 25 µg/mL) and *T. cruzi* (100% mortality at 100 µg/mL).	Isoquinoline alkaloids	Tempone et al. (2005)
Antiparasitic	Leaves	Essential oil	*Leishmania* (L.) *amazonensis*, *L.* (V.) *braziliensis*, *L.* (L.) *infantum chagasi* and *L.* (L.) *major* promastigotes, and *Trypanosoma cruzi* trypomastigotes	-↓*Leishmania* spp. and *T. cruzi* viability. -Essential oil was more active against *T. cruzi* (LC_{50} 5.31 µg/mL) than *Leishmania* spp. (LC_{50} 25.97-39.19 µg/mL).	Terpenes, mainly oxygenated terpenes	Oliani et al. (2013)
Insecticide	Stem	Ethanolic extract	Brine shrimp nauplii (*Artemia salina*)	-100% of nauplii mortality at 1 mg/mL.	Alkaloids and annonaceous acetogenins	Novaes et al. (2016)

				-High insecticide activity (LC_{50} 132.30 µg/mL)		
Insecticide	Seeds, leaves and stem	Hexane, ethanolic and hydroethanolic (ethanol 30%) extracts and ethanolic extract fractions	Housefly (*Musca domestica*), Mediterranean fruit fly (*Ceratitis capitate*), Mexican bean weevil (*Zabrotes subfasciatus*) and boll weevil (*Anthonomus grandis*) adults, and fall armyworm (*Spodoptera frugiperda*) larvae	-Hexane extract from seeds showed insecticide activity against *C. capitate* (62.3% mortality at 10% m/v). -Dichloromethane fraction from seeds was active against *M. domestica* (32.5% mortality at 2% m/v).	-	Saito et al. (1989)
Insecticide	Leaves, stem wood, stem bark, root wood and root bark	Hexane and ethanolic extracts	Mexican bean weevil (*Zabrotes subfasciatus*) and bean weevil (*Acanthoscelides obtectus*) α-amylases	-Ethanolic extract from leaves significantly inhibited the *Z. subfasciatus* and *A. obtectus* α-amylases (86.58 and 68.54% of inhibition, respectively, at 1 mg/mL).	Phenolic compounds	Silva et al. (2009b)
Insecticide	Seeds	Chloroform-methanol (2:1) extract	Nymphs of rice stalk stink bug (*Tibraca limbativentris*)	-Control of second instar nymphs of *T. limbativentris* by topical application (up to 81% mortality).	Annonaceous acetogenins	Krinski and Massaroli (2014)
Insecticide	Seeds	Chloroform-methanol (2:1) extract	Eggs and caterpillars of soybean looper (*Chrysodeixis includens*)	-Control of all instars of *C. includens*, especially by topical application (up to 93.3% mortality).	Annonaceous acetogenins	Massarolli et al. (2017)
Insecticide	Seeds	Hydroethanolic extract (ethanol 75%)	Fall armyworm (*Spodoptera frugiperda*) larvae	-Control of *S. frugiperda* larvae (up to 100% mortality). -Surviving larvae did not complete the biologic cycle.	Annonaceous acetogenins	Pimenta et al. (1999)

Insecticide	Seeds	Methanolic extract	Nymphs of brown stink bug (*Euschistus heros*)	-Control of third instar nymphs of *E. heros*.	Annonaceous acetogenins	Turchen et al. (2014)
Insecticide	Seeds	Methanolic extract	Brown stink bug adults (*Euschistus heros*)	-Population control of *E. heros* in soybean crop after 7 days of application.	-	Silva et al. (2013b)
Insecticide	Root bark, root wood and stem	Hydroethanolic extract (ethanol 95%)	*Aedes aegypti* larvae	-Control of fourth instar larvae of *A. aegypti*. -High insecticide activity (LC_{50} 0.71, 8.94 and 16.1 µg/mL for root bark, root wood and stem, respectively).	Annonaceous acetogenins	Omena et al. (2007)
Insecticide	Leaves, stem wood, stem bark, root wood and root bark	Hexane and ethanolic extracts	*Aedes aegypti* larvae	-Control of third instar larvae of *A. aegypti* (mortality ≥ 65%). -Ethanolic extracts from root bark and root wood were the most active (LC_{50} 23.06 and 26.89 µg/mL, respectively).	Annonaceous acetogenins	Rodrigues et al. (2006)
Insecticide	Seeds	Hexane, chloroform, methanolic (defatted with hexane or dichloromethane) extracts and hexane, hydromethanolic, ethyl acetate and chloroform fractions from methanolic extract defatted with dichloromethane	*Aedes aegypti* larvae	-Hexane, dichloromethane, methanolic (defatted with hexane) extracts and hexane fraction controlled third instar larvae of *A. aegypti* (up to 100% mortality). -Methanolic extract (defatted with hexane) was the most active followed by dichloromethane extract, hexane fraction and hexane extract (LC_{50} 0.100, 0.185, 0.433 and 0.507 mg/mL, respectively).	Mainly annonaceous acetogenins	Costa et al. (2013)

Molluscicide	Root wood, root bark, stem, fruit peel and pulp+seed	Hydroethanolic extract (ethanol 90%)	Snails (*Biomphalaria glabrata*) adults and their egg masses	-Control of adult snails and snail egg masses. -Stem, root bark and pulp+seed extracts were the most active extracts against adult snails (LC_{90} 2.34, 3.79 and 13.21 µg/mL, respectively) and egg masses (LC_{90} 1 µg/mL for all extracts).	Annonaceous acetogenins	dos Santos and Sant'Ana (2001)
Herbicide	Stem and leaves	Ethanolic extract	Etiolated wheat coleoptiles (*Triticum aestivum*), lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.) and onion (*Allium cepa* L.) diasporas	-Inhibition of wheat coleoptile elongation (only seeds). -Inhibition of lettuce (only leaves) and tomato (stem and leaves) germination. -↓Shoot and root development of tomato and onion.	Alkaloids and annonaceous acetogenins	Novaes et al. (2016)
Herbicide	Leaves and seeds	Ethanolic extract	Seeds of lettuce (*L. sativa* cv. Salad Bowl)	-Inhibition of germination (up to 85.81 and 48.25% for seed and leaf extracts at 1.2 mg/L, respectively). -↓Root elongation (IC_{50} 0.1 and 0.6 mg/L for leaf and seed extracts, respectively).	Alkaloids, catechins, tannins, depsides, depsidones, coumarin derivatives, steroids and triterpenoids	Ribeiro et al. (2013)
Herbicide	Seeds, leaves and stems	Hydroethanolic extract (ethanol 70%)	Weeds (*Brachiaria brizantha*, *Euphorbia heterophylla* and *Ipomoea grandifolia*)	-Inhibition of germination (up to 100%) and root and hypocotyl development (up to 97.9 and 100%, respectively) of the weeds. -Seeds extract was the most active against all weeds. -Ethyl acetate fraction from seeds showed the highest inhibitory effect on weeds, but no effect on soybean development.	-	Inoue et al. (2010b)
Herbicide	Seeds	Steroids glycosides from seeds	Weeds (*Euphorbia heterophylla* and	-↓Root and hypocotyl development of *E. heterophylla*.	Stigmasterol and β-sitosterol glycosides	Inoue et al. (2010a)

			Ipomoea grandifolia)			
Herbicide	Seeds, leaves, and branches	Aqueous extract	Weed (*Brachiaria brizantha*)	-↓Seed germination and root development of *B. brizantha*. -Seeds extract was the most active against *B. brizantha*.	-	Inoue et al. (2009)
Anti-ophidic	Seeds	Ethyl acetate fraction from hydroethanolic extract (ethanol 80%)	Guinea-pig isolated ileum	-Inhibition of drug-induced (histamine, acetylcholine and bradykinin) contractions of guinea-pig ileum. -Inhibitory effect was dose-dependent. -↓Membrane permeability to calcium.	-	Weinberg et al. (1993)

↑: increase; ↓: reduction; *GI_{50}:* growth inhibitory activity (extract concentration that resulted in a 50% reduction in the cellular growth relative to the untreated control cells); *A-549:* lung tumour cells; *RPMI-7951:* melanoma tumour cells; *U-251:* glioma tumour cells; *UACC-62:* melanoma tumour cells; *MCF-7:* breast tumour cells; *NCI/ADR/RES:* ovarian tumour cells expressing the phenotype of multiple drug resistance; *786-0:* renal tumour cells; *NCI-H460:* lung tumour, non-small cells; *OVCAR-3:* ovarian tumour cells; *HT-29:* colon tumour cells; *K562:* leukaemia tumour cells; *UA251:* glioma tumour cells; *VERO:* green monkey kidney tumour cells; *SF-295:* brain tumour cells; *HCT-8:* human colon carcinoma cells; *MDA-MB-435:* melanoma tumour cells; *HL-60:* leukaemia tumour cells; *MIC:* minimum inhibitory concentration; *MBC:* minimum bacterial concentration; *DPPH:* DPPH radical scavenging activity; *TEAC:* Trolox equivalent antioxidant capacity; *ORAC:* oxygen radical absorbance capacity; *FRAP:* ferric reducing antioxidant power; *TE:* Trolox equivalents; *dw:* dry weight; *IC_{50}:* extract concentration that resulted in a 50% reduction in the enzymatic activity to the untreated control; *LC_{50}* or *LC_{90}:* lethal concentration (extract concentration which causes 50 or 90% mortality); *ALT:* alanine aminotransferase; *AST:* aspartate aminotransferase; *ALP:* alkaline phosphatase; *iNOS:* inducible nitric oxide synthase; *GPx:* glutathione peroxidase; *SOD:* superoxide dismutase; *CAT:* catalase; *GR:* glutathione reductase; *GSH:* reduced glutathione.

5.1. Traditional use

Since ancient times, plants have been used as a drugs source for the illnesses and diseases treatment (Vilar, Ferreira, Ferri, Guillo, & Chen Chen, 2008). *Annona crassiflora* Mart. fruit, as well as other plant parts, have long been used in the Brazilian local and traditional medicine. The traditional use of this plant includes the treatment of fever (Mesquita et al., 2005), wounds, venereal diseases, snakebites, louses and, furthermore, it can act as an antimicrobial, antidiarrheal, antirheumatic (Arruda et al., 2015; Silva et al., 2014a), stimulant, astringent (Rocha et al., 2016), sudorific, analgesic and antimalarial (Lage et al., 2014).

Annona crassiflora Mart. leaves and seeds have been traditionally used to prepare infusions with claimed antidiarrhoeic, antitumor and inductor of menstruation properties, and for the treatment of Chagas' disease (Formagio et al., 2015; Morzelle, Souza, Assumpção, & Vilas Boas, 2011). Seeds oil is used against snakebites (Formagio et al., 2015), skin (Luzia & Jorge, 2013) and scalp infections (Roesler et al., 2006) in folk medicine. Seeds are also used in traditional medicine in antidiarrheal and insecticide preparations (Pimenta et al., 1999). Leaves infusions are used by oral administration in the treatment of inflammatory and painful ailments such as wounds, snakebites, diarrhoeas, malaria, and rheumatism (da Costa Oliveira et al., 2018). Additionally, fruits are used as tonic and astringent, and its bark powder has antifungal and antirheumatic properties (Vilar, Ferri, & Chen-Chen, 2011). Cavéchia and Proença (2015) studied the traditional knowledge from native Cerrado plants used by folk medicine in Distrito Federal region, Brazil. *Annona crassiflora* Mart. fruit was cited by the interviewees as tonic and for treating pain and rheumatism. Decoction and infusions of seeds are reported as a medicine against chronic diarrhoea by local population from region of the Alto Rio Grande, Minas Gerais, Brazil (Rodrigues & Carvalho, 2001).

5.2. Antioxidant activity

Antioxidants are defined as any molecules capable of inhibiting, reducing or delaying the oxidation of other molecules (Gülçin, 2012). These compounds can be act both in chemical or biochemical systems by several ways, such as scavenging reactive oxygen and/or nitrogen species, chelating transition metals, donating hydrogen atoms, inhibiting enzymes involved in oxidative stress, and upregulating and/or protecting endogenous defence systems (Arruda et al., 2019).

In vitro and *in vivo* antioxidant activities of extracts/fractions from different botanical parts of *Annona crassiflora* Mart. (fruit and plant) have been studied (**Table 3**). These studies showed that the extracts from different parts of *Annona crassiflora* Mart. fruit were potent antioxidants. Recent study showed that the *Annona crassiflora* Mart. fruit pulp had high antioxidant activity (DPPH IC_{50} of 93.76 µg/mL, and 231.79 and 902.27 µmol TE/g dw for TEAC and ORAC assays, respectively) (Arruda et al., 2018a). Hydroethanolic extracts from *Annona crassiflora* Mart. fruit pulp and peel showed strong antioxidant activities with 609.58 and 514.57, 683.65 and 613.76, and 525.41 and 1593.72 µmol TE/g dw for DPPH, TEAC and ORAC assays, respectively (Arruda et al., 2017a, 2019). In another recent study, Arruda et al. (2018b) reported that *Annona crassiflora* Mart. fruit peel had the highest antioxidant activities by DPPH, TEAC and ORAC assays (189.32, 292.28 and 448.29 µmol TE/g dw), followed by pulp (132.73, 214.38 and 259.26 µmol TE/g dw) and seed (71.66, 94.81 and 260.22 µmol TE/g dw). In addition, the authors demonstrated that insoluble-bound and esterified phenolic fractions were the major contributors to antioxidant activity. Ethanolic extract from *Annona crassiflora* Mart. fruit peel and its organic fractions were recently evaluated for their antioxidant activity by different methods and the results demonstrated that ethyl acetate and *n*-butanol fractions showed the strongest antioxidant activities (DPPH IC_{50} of 1.5 and 0.8 µg/mL, ORAC 3355 and 2714 µmol TE/g, and FRAP 888 and 921 µmol TE/g) (Justino et al., 2016).

Rat liver microsomes were also used to investigate the potential of ethanolic extracts from *Annona crassiflora* Mart. fruit pulp, peel and seeds of inhibiting the lipid peroxidation. The seeds extract exhibited the highest lipid peroxidation capacity inhibition of microsomes with IC_{50} value (1.72 µg/mL) very close to the positive controls (1.01 and 1.96 µg/mL for gallic acid and catechin, respectively), followed by the fruit peel and pulp extracts (IC_{50} 4.44 and 8.62 µg/mL), respectively (Roesler et al., 2006). The *in vivo* antioxidant activity of *Annona crassiflora* Mart. fruit peel and seeds extracts was tested on male Wistar rats (Justino et al., 2017; Roesler, 2011). The oral administration of ethanolic extracts from *Annona crassiflora* Mart. fruit peel and seeds during 14 consecutive days to the male Wistar rats treated with CCl_4 (single oral dose) decreased the lipid peroxidation, induced cytochrome b5 and enhanced/maintained the activity of hepatic antioxidant enzymes (*e.g.*, catalase, glutathione peroxidase and glutathione reductase). Furthermore, the extracts did not cause any clinical signs or alterations attributable to hepatic toxicity or other organ damage (Roesler, 2011). In another recent study, Justino et al. (2017) observed that the oral administration of a phenolic-rich fraction obtained from ethanolic extract of *Annona crassiflora* Mart. fruit peel to the diabetic

male Wistar rats (induced by streptozotocin) during 30 days was able to reduce the oxidative stress biomarkers (hepatic lipid peroxidation, protein carbonylation and nitration, inducible nitric oxide synthase level, and activities and expressions of glutathione peroxidase, superoxide dismutase, and catalase), besides improving the glutathione defence system (increased the antioxidant capacity and glutathione reductase activity, and reduced glutathione level).

The potent antioxidant activity of the *Annona crassiflora* Mart. extracts have been attributed mainly to the presence of phenolic compounds (Arruda et al., 2019, 2018b; Justino et al., 2016). Moreover, the particular combination of these phenolics with each other and with other bioactive compounds can be significantly increasing their antioxidant activity (Arruda et al., 2018a,b).

5.3. Hepatoprotective activity

The liver is a key organ in the detoxification and metabolism processes, thus being essential for the maintenance of homeostasis of the human organism. However, hepatotoxic substances from xenobiotics (*e.g.*, viruses, bacteria, metabolites and drugs) can lead the liver to produce excessive reactive species, inducing hepatic disorders, inflammation, fibrosis and cirrhosis (Xia, Wang, Yu, Liang, & Kuang, 2019). The drugs currently used to treat liver damage are generally of low efficacy and promote many adverse effects (Wang, Jiang, Ren, Shen, & Xie, 2019). Thus, the discovery of effective, accessible, and safer hepatoprotective agents from natural sources becomes an attractive approach to the treatment and control of liver damage. Recent studies have demonstrated that *Annona crassiflora* Mart. fruit by-products exhibit *in vivo* hepatoprotective effects (**Table 3**).

Roesler (2011) studied the effect of ethanolic extracts from *Annona crassiflora* Mart. fruit peel and seeds on CCl_4-induced liver damage in rats and observed that the oral administration of extracts from *Annona crassiflora* Mart. fruit peel and seeds during 14 days were able to prevent/inhibit the hepatic lipid peroxidation and enhance/maintain the activity of hepatic antioxidant enzymes. In another study, Justino et al. (2017) investigated a phenolic-rich fraction from ethanolic extract of *Annona crassiflora* Mart. fruit peel against hepatic oxidative and nitrosative stress in streptozotocin-induced diabetic rats. They observed that the treatment with this fraction during 30 days decreased the serum biochemical parameters associated with diabetes complication (serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities), besides reducing the oxidative stress biomarkers improving the

defence system as described in section **5.2** above. In addition, these extracts caused no clinical signs of hepatotoxicity or liver damage. The hepatoprotective effect is mainly attributed to the phenolic compounds that can modulate various biochemical parameters (*e.g.*, scavenge reactive species, lipid peroxidation reduction, endogenous antioxidants production/regulation, defence system regulation and drug detoxification activation) (Justino et al., 2017; Roesler, 2011).

5.4. Anti-inflammatory activity

Chronic inflammation is a critical factor in triggering many diseases such as cancer, diabetes, cardiovascular disease, neurodegenerative diseases and degenerative joint diseases (Lucas, Russell, & Keast, 2011). Anti-inflammatory activity of extracts from the leaves and fruit peel of *Annona crassiflora* Mart. has been proven *in vivo* (**Table 3**). Oral treatment with methanolic extract from leaves (100-300 mg/kg) inhibited important inflammatory parameters carrageenan-induced in mice, such as paw edema (47-53% inhibition), myeloperoxidase activity (60-63% inhibition), total leukocyte count (78-90% inhibition), protein extravasation in the pleural cavity (approximately 100% inhibition) and leukocyte migration into the pouch (43% inhibition). In addition, these results were very close to those of the positive control (dexamethasone) (Rocha et al., 2016). da Costa Oliveira et al. (2018) studied the *in vivo* anti-inflammatory property of filtered and precipitated hydroethanolic fractions (ethanol 80%) from *Annona crassiflora* Mart. leaves in carrageenan-, zymosan-, LPS- and CXCL8-induced inflammation. Precipitated fraction (0.053-530, 53 and 53 mg/kg, respectively) significantly inhibited the carrageenan-, LPS-, or CXCL8-induced neutrophil recruitment to the pleural cavity, whereas filtered fraction (587 mg/kg) was effective only against the carrageenan-induced neutrophilia. In another study performed in a *in vivo* wound-healing model, an ointment preparation containing phenolic-rich fraction from fruit peel (2, 4 and 6%) was able to reduce neutrophil and macrophage infiltration in cutaneous wounds (de Moura et al., 2018). These extracts are rich in phenolic compounds and several of them have already been identified as can be observed in **Table 2**. The anti-inflammatory activity of *Annona crassiflora* Mart. extracts can be mainly associated with these compounds, since many previous studies showed that different phenolic compounds have anti-inflammatory activities in animal models. Phenolic compounds may exert anti-inflammatory effects through radical scavenging, inhibition of enzymes involved in the proinflammatory process (*e.g.*, cyclooxygenase-2, lipoxygenase and inducible nitric oxide synthase), inhibition of nuclear factor-kappa B and the activating protein-

1, and activation of phase-II antioxidant detoxifying enzymes, protein kinase-C, mitogen activated protein kinase and nuclear factor erythroid 2-related factor (Hussain et al., 2016).

5.5. Antitumoral and antimutagenic activities

Cancer, a group of chronic diseases characterized by uncontrolled cell growth, represents a significant public health problem worldwide (Mohammadi, Mianabadi, & Mehrad-Majd, 2019). Cancer is the second leading cause of death worldwide, accounting for 1 in 6 deaths annually. Moreover, it is estimated there are around 14 million new cases and 9 million cancer-related deaths every year (Gao, Zhang, Wang, & Xiao, 2019). Therefore, there is a growing search for new sources of phytotherapeutics for the cancer treatment or adjuvants in conventional anticancer therapies. In line with this, several extracts, fractions and isolated compounds from *Annona crassiflora* Mart. have been tested both *in vitro* and *in vivo* against many types of cancer (**Table 3**).

Anticancer property of crassiflorin, an annonaceous acetogenin, isolated from *Annona crassiflora* Mart seeds was studied *in vitro* against the lung carcinoma (A-549), colon adenocarcinoma (HT-29), breast carcinoma (MCF-7), melanoma (RPMI-7951) and central nervous system carcinoma (U-251) cells (Santos et al., 1994). Crassiflorin was highly cytotoxic against all human tumour cell lines tested (ED_{50}, 0.003-0.02 µg/mL) and more effective than the positive control (adriamycin; ED_{50}, 0.3-2.0 µg/mL). In another study, the same authors investigated the antiproliferative effect of a hydroethanolic extract (ethanol 75%) and isolated compounds from *Annona crassiflora* Mart seeds on same cancer cell lines (Santos et al., 1996b). The extract exhibited significant cytotoxicity to human lung carcinoma and melanoma cells. Moreover, the authors demonstrated that the araticulin, an annonaceous acetogenin, isolated from this extract showed significant activities against all human tumour cell lines evaluated in this study (ED_{50}, 0.001-0.6 µg/mL) when compared with adriamycin (positive control; ED_{50}, 0.002-0.01 µg/mL). Also, Pimenta et al. (1999) isolated seven annonaceous acetogenin (see **Table 2**) from *Annona crassiflora* Mart seeds and evaluated their antiproliferative activity. Among the isolated compounds, araticulin, crassiflorin and annonin I showed significant activities against human tumour cell lines.

de Mesquita et al. (2009) investigated the cytotoxic potential of hexane and ethanolic extracts from several botanical plant parts of *Annona crassiflora* Mart. against tumour cell lines (brain: SF-295; human colon carcinoma: HCT-8; melanoma: MDA-MB-435; and

leukaemia: HL-60). Only the ethanolic extracts from root bark and root wood demonstrated a substantial antiproliferative effect against one or more cell lines. Leukaemia cells showed a higher extracts sensitivity (GI_{50}, 6.0-13.4 µg/mL) when compared to the other tumour cell lines (GI_{50}, 13.3-24.9 µg/mL). The results also indicated that root bark extract (GI_{50}, 6.0-14.9 µg/mL) was more active than root wood extract (GI_{50}, 13.3-24.9 µg/mL) against all the tumour cell lines. In another recent study the methanolic extract from *Annona crassiflora* Mart. leaves and seeds revealed that both extracts manifested significant antiproliferative activity against 10 human tumour cell lines (see **Table 3**). Seeds extract was more effective than the leaves extract against all the tumour cell lines, with GI_{50} values below 8.9 µg/mL. The strongest antiproliferative effect was detected against UA251 (glioma), NCI-H460 (ovarian expressing the phenotype of multiple drug resistance), and HT-29 (colon) tumour cells with GI_{50} values between 0.01 and 0.06 µg/mL. The seeds extract contained mainly rutin, caffeic acid and sinapic acid.

The potential anticancer of a hydroethanolic extract (ethanol 70%) and its hexane fraction obtained from *Annona crassiflora* Mart. leaves was verified by Silva et al. (2018a). Hydroethanolic extract, and mainly its hexane fraction, significantly reduced cell viability, proliferation and migration of cervical cancer cell lines *in vitro*, showing IC_{50} values of 3.40-39.88 µg/mL and 0.18-14.71 µg/mL, respectively. The researchers demonstrated that hexane fraction modulate different cell responses in cervical cancer cells, such as DNA damage (H2AX activity), apoptosis via intrinsic pathway (cleavage of caspase-9, caspase-3, poly (ADP-ribose) polymerase (PARP) and mitochondrial membrane depolarization) and decreased p21 expression by ubiquitin proteasome pathway. Furthermore, the anticancer effect of the hexane fraction was also investigated by *in vivo* chorioallantoid membrane model. Compared with control group, the hexane fraction (dose of 15 µg/mL) inhibited the cervical tumour growth (59.8±32.1% reduction) but did not decrease the number of blood vessels recruited to the tumours, indicating that in this chorioallantoid membrane model, the hexane fraction effect is anti-neoplastic rather than anti-angiogenic. Several bioactive compounds were found in this fraction like flavonoids, annonaceous acetogenins and alkaloids (see **Table 2**). Another *in vivo* study showed the antitumor property of an annonaceous acetogenins-rich fraction from *Annona crassiflora* Mart. wood using Ehrlich solid tumour-bearing Swiss mice model (Pimenta et al., 2011). This fraction exhibited a pronounced *in vivo* antitumor effect both by intratumoral and intravenous routs, given that a single administration (1.25 mg/kg) reduced the tumour growth by 38 and 20%, respectively.

Moreover, other studies revealed that extracts from *Annona crassiflora* Mart. leaves manifested significant *in vivo* antimutagenic activity (Rocha et al., 2016; Vilar et al., 2008). Vilar et al. (2008) evaluated the mutagenic, antimutagenic and cytotoxic effects of hydroethanolic extract (ethanol 95%) from *Annona crassiflora* Mart. leaves by micronucleus test in mice. The authors reported that this extract exhibited antimutagenic effect (20-100 mg/kg) and low cytotoxicity (non-cytotoxic at concentrations below 50 mg/kg), besides no mutagenicity (10-160 mg/kg). The antimutagenic effect of a methanolic extract from *Annona crassiflora* Mart. leaves was also evaluated by micronucleus test in mice peripheral blood in the study by Rocha et al. (2016). The treatment with this extract (15 mg/kg) was chemoprotective and antimutagenic, reducing the induced DNA damage by 75.00 and 64.58% for the pre-treatment and simultaneous treatment, respectively. In addition, no mutagenic effect was observed. According to the authors, the antimutagenic effect of the extract is mainly due to its desmutagenic activity, inactivating chemically and/or enzymatically the mutagen in the extra or intracellular medium. In this extract, the potential antimutagenic compounds were flavonoids, since its fractionation led to the isolation of kaempferol 3-*O*-β-glucoside and kaempferol 3-*O*-β-diglucoside.

5.6. Analgesic activity

Pain is the most frequently reported symptom by patients in medical appointments, being associated with several pathological conditions (da Costa Oliveira et al., 2018). Moreover, pathological pain (neuropathic pain, inflammatory pain and cancer pain) is a chronic pain lasting for more than 3 months clinically that significantly affects the quality of life and welfare of the patient (Sun et al., 2018). In this context, some extracts and isolated compounds from *Annona crassiflora* Mart. leaves have been test in animal models (**Table 3**).

Antinociceptive effect of filtered and precipitated hydroethanolic fractions (ethanol 80%) from *Annona crassiflora* Mart. leaves was evaluated *in vivo* using a mouse model (da Costa Oliveira et al., 2018). The results of this study showed that oral administration of both fractions reduced inflammatory response (see section **5.4**) and paw licking time in the second phase (corresponding to inflammatory pain; 53-530 and 1000 mg/kg for precipitated and filtered fractions, respectively), whereas no effect was observed in paw licking time in the first phase (corresponding to neuropathic pain), latency of the tail withdrawal and motor performance. Thus, the authors suggested that the antinociceptive effect may be due to anti-inflammatory property (inhibiting the synthesis and/or action of inflammatory mediators) of

bioactive compounds from these fractions (see **Table 2**). In another study, da Costa Oliveira et al. (2017) analysed the analgesic potential of peltatoside isolated from *Annona crassiflora* Mart. leaves in a mouse model. The intraplantar administration of peltatoside exerted a dose-dependent antinociceptive effect. Moreover, this study demonstrated that peltatoside reversed carrageenan-induced hyperalgesia (increased responsiveness to noxious stimulus) 20 min after peripheral administration (100 µg/paw), and it showed local (100 µg/paw) and systemic (200 µg/paw) antinociceptive effect. The authors suggested that peltatoside induces analgesia through the activation of peripheral cannabinoid-1 receptors with endocannabinoids involvement.

5.7. Management of diabetes and its associated complications

Diabetes mellitus (a chronic metabolic disorder characterized by persistent hyperglycaemia) and its associated complications are one of the major causes of mortality and morbidity worldwide (Laddha & Kulkarni, 2019). Several natural compounds like phenolic compounds and alkaloids have been reported in the management of diabetes and its associated complications. The treatment with a phenolic-rich fraction (*n*-butanol fraction) from ethanolic extract of *Annona crassiflora* Mart. fruit peel during 30 days was not able to control hyperglycaemia in streptozotocin-induced diabetic rats (Justino et al., 2017). However, *in vitro* and *in vivo* studies have demonstrated that phenolic-rich extracts/fractions from *Annona crassiflora* Mart. fruit peel can become an attractive approach for the prevention and therapy of diabetes complications, such as vascular complications, since they had high inhibitory activities against digestive enzymes (IC_{50} for α-amylase and α-glucosidase were 1.71-75.50 and 554.5-5103.0 µg/mL, respectively) and blocked the formation of glycation products *in vitro* (IC_{50} ranging from 14.3-84.6 µg/mL) (Justino et al., 2016), and decreased the serum biochemical parameters associated with diabetes complications (serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities) *in vivo* (dose of 25-100 mg/kg/day) (Justino et al., 2017). These effects are mainly due to the presence of phenolic compounds that reduce the oxidative stress, interfere in the enzymatic action of digestive enzymes by binding to a site other than the active site of enzyme-substrate complex and suppress the formation of advanced glycation end-products (Justino et al., 2017, 2016).

5.8. Skin healing activity

Skin, the largest organ of the human organism, plays multiple key functions in the body, including a protective barrier against environmental aggressors, immunological activity and acting as a neuro-endocrine organ (Kur-Piotrowska et al., 2018). The skin wounds can be naturally repaired and healed by the body, but in some cases (*e.g.*, patients with some pathological conditions like diabetes and skin wounds induced by burns, traumas and chronic disorders), skin wound-healing can become a lengthy process, resulting in the impairment of the life quality and in prolonged hospitalization period (Silva et al., 2018b). Therefore, there is a growing search for new natural compounds with healing-promoting characteristics for the development of skin healing cosmetics and medicines. In a recent study, the effect of the topical application of ointment preparations containing phenolic-rich fraction from *Annona crassiflora* Mart. fruit peel (2, 4 and 6%) was evaluated in a skin wound-healing model in mice for 7 days (de Moura et al., 2018). The results of this study showed that the topical application of these ointment preparations reduced inflammatory response (see section **5.4**), improved skin wound closure (up to 84% after 7 days of treatment) and collagen deposition in cutaneous wounds. Several phenolic compounds were identified in this fraction, such as chlorogenic acid, epicatechin, procyanidins B2 and C1, quercetin-glucoside, kaempferol-rutinoside, kaempferol-glucoside, and caffeoyl-glucoside (see **Table 2**). Phenolic compounds can be aid tissue repair process by several mechanisms, such as reducing inflammatory response and cellular oxidative stress, modulating expression of some essential chemokines in the healing process *(e.g.*, interleukin 8, monocyte-1 chemotactic protein, interferon gamma-induced protein of 10 kDa, and interferon gamma via epidermal growth factor receptor), regulating growth factors (e.g., vascular endothelial growth factor, transforming growth factor beta, fibroblast growth factor, platelet-derived growth factor and epidermal growth factor), stimulating collagen deposition, and acting as antimicrobial, among others (de Moura et al., 2018; Działo et al., 2016; Pastore et al., 2012).

5.9. Antidiarrhoeic activity

Diarrhoea is an important gastrointestinal disorder which can be triggered by various agents, including intestinal inflammation, microbial pathogens infection, food allergy and drug intolerance. Diarrhoea may represent an occasional inconvenience but can be a fatal ailment, especially for malnourished children or individuals with compromised immune systems (Motlhatlego, Mfotie Njoya, Abdalla, Eloff, & McGaw, 2018). The antidiarrhoeic

properties of an aqueous extract from *Annona crassiflora* Mart. leaves were investigated using a mouse model (Ferraz et al., 2017). Oral administration of the extract at a dose of 1000 µg/kg significantly reduced the diarrhoeic faeces induced by castor oil as well as increased the distance travelled by charcoal meal in the intestine. Moreover, the intraperitoneal administration of the extract was able to decrease the enteropooling (intestinal fluid accumulation) produced by castor oil administration. Several bioactive compounds were identified in this extract, including flavonoids, alkaloids and proanthocyanidins (see **Table 2**), and some of them have shown antidiarrhoeic properties. The authors associated the antidiarrhoeic effect of the *Annona crassiflora* Mart. leaves extract to its ability to inhibit intestinal secretion and/or to increase intestinal absorption.

5.10. Anti-Alzheimer disease potential

Alzheimer disease is an age-related neurodegenerative disorder characterized by the classical hall markers of abnormal extracellular amyloid-β peptide deposition and tau-pathology (intraneuronal accumulation of neurofibrillary tangles) that lead to synapse and neuron loss, causing progressive cognitive and memory impairment (Ambrad Giovannetti & Fuhrmann, 2019). Preliminary study carried out by Lucas dos Santos, Leite, Alves de Araújo, Giffoni de Carvalho, and Souza (2018) demonstrated that aqueous extract from *Annona crassiflora* Mart. fruit pulp had therapeutic potential in combating to oxidative stress, and age-related diseases associated with protein misfolding and accumulation (*e.g.*, Alzheimer). The authors found that the treatment with this extract (1000 µg/mL) was able to protect the worms exposed to pro-oxidant agent (32.2% survival when compared to the control group 5.5%) and decrease the β-Amyloid-Induced Paralysis (13.4% reduction in worms expressing the Aβ1-42 peptide) in *Caenorhabditis elegans* model. In another study, Formagio et al. (2015) demonstrated that methanolic extracts from leaves and seeds of *Annona crassiflora* Mart. had *in vitro* anticholinesterase property. Extracts from leaves (2 mg/mL) and seeds (1.5 mg/mL) inhibited the acetylcholinesterase activity by 22 and 45%, respectively. The seed extract was rich in phenolic compounds, mainly rutin, caffeic acid, sinapic acid, ferulic acid and p-coumaric acid (see **Table 2**) that could explain, at least in part, its higher anticholinesterase activity, since several studies have demonstrated the anticholinesterase potential of these compounds (Formagio et al., 2015). These results suggest that *Annona crassiflora* Mart. and its active compounds could be promising therapeutics for Alzheimer disease.

5.11. Anti-obesity potential

It is estimated that about 13% of the adult world population is obese, making this condition one the most important global public health problem since obesity is associated with several health problems such as increased risk for heart disease, type 2 diabetes, hypertension, various types of cancer and numerous adverse health outcomes (Silvester, Aseer, & Yun, 2019). Thus, much attention has been focused on the search of natural substances with anti-obesity potential. In this context, Pereira et al. (2017) studied the effect of extract, fractions and isolated compounds from *Annona crassiflora* Mart fruit peel on pancreatic lipase activity using *in vitro* model. Ethanolic extract, dichloromethane fraction and pure alkaloid stephalagine inhibited the pancreatic lipase, showing IC_{50} values of 104.50, 108.10 and 8.35 µg/mL, respectively. This study showed that the stephalagine had high pancreatic lipase inhibitory activity and low cytotoxicity, suggesting its use as a new potential anti-obesity drug.

5.12. Anti-ophidic potential

Snakebite envenoming is an important public health problem, especially in tropical and subtropical countries, due to its high morbidity and mortality rates, thus being considered a neglected disease by the World Health Organization (Corrêa et al., 2019). *Annona crassiflora* Mart. seeds has been traditionally used in folk medicine to treat snake bites (Santos et al., 1996b). Weinberg, Pires, Weinberg, and Oliveira (1993) showed that ethyl acetate fraction from *Annona crassiflora* Mart. fruit seeds inhibited the drug-induced (histamine, acetylcholine and bradykinin) contractions of guinea-pig ileum in a dose-dependent manner, as well as decreased the membrane permeability to calcium. The authors showed that the seeds extract antagonizes the chemical mediators action liberated by snake venom which could be alleviate the snakebites effects.

5.13. Antiparasitic activity

Parasitic diseases are a cause of considerable mortality and morbidity around the world, affecting millions of people every year. It is estimated that between 25 and 30% of the world population is infected at least one parasite, thus representing important public health and economic concerns (Blanco et al., 2018). *Annona crassiflora* Mart. has been a target for antiparasitic research, as can be seen in the studies reported below.

5.13.1. Antimalarial activity

Malaria, an infectious disease caused by pathogens from the genus *Plasmodium*, is the most important endemic parasitic infection in humans, accounting for about 216 million new cases and approximately 445 thousand deaths in 2016 (Scarim et al., 2019). Antimalarial activity of *Annona crassiflora* Mart. plant has been proved *in vitro* and *in vivo* (**Table 3**). de Mesquita, Grellier, Mambu, de Paula, and Espindola (2007) evaluated the effect of hexane and ethanolic extracts from different botanical parts of *Annona crassiflora* Mart. against chloroquine-resistant strain of *P. falciparum*. Among all extracts tested, only extracts obtained from the root bark showed significant antiplasmodial activity, with LC_{50} values of 7.6 and 11.9 µg/mL for hexane and ethanolic extracts, respectively. On the other hand, antimalarial activity of ethanolic extract fractions from *Annona crassiflora* Mart. plant was determined *in vivo* using *P. berghei*-infected mice model (intraperitoneal administration at 12.5 mg/kg/day) (Pimenta et al., 2014). According to the results, the wood fractions showed no significant antimalarial activity, whereas some leaves fractions were able to reduce the parasitaemia and/or increase the mean survival time of *P. berghei*-infected mice. Hexane, chloroform II and ethyl acetate fractions from leaves significantly increased the mean survival time of infected mice (18.2, 21.0 and 19.8 days, respectively) compared with the control group (12.4 days). Moreover, parasitaemia was reduced by all tested leaves fractions at day 4 post-infection (maximum reduction of 75% for basic aqueous fraction) while only solid ethyl acetate fraction did not differ from the control group at day 7 post-infection. The potential active compounds were flavonoids and alkaloids.

5.13.2. Antileishmanial and trypanocidal activities

Leishmaniasis and American trypanosomiasis (Chagas' disease) are among the most prevalent neglected tropical diseases. Leishmaniasis are a group of clinical diseases caused by more than 20 species of parasites from the genus *Leishmania* that affect about 12 million people worldwide, with approximately 1 million new cases and 20 to 30 thousand reported deaths annually. Meanwhile, Chagas' disease is provoked by protozoan *Trypanosoma cruzi*, infecting about 7 million people around the world and causing more than 7500 reported deaths each year (Costa et al., 2018; Scarim et al., 2019). However, the available drugs for the treatment of these diseases have been limited due to several factors such as drug resistance, availability, high costs and toxicity, impelling a large number of researches to discover new bioactive natural compounds (Costa et al., 2018).

The effect of the hexane and ethanolic extracts from the several botanical parts of *Annona crassiflora* Mart. on *T. cruzi* trypomastigotes and *L. donovani* promastigotes was evaluated by Mesquita et al. (2005). The LC_{50} of the extracts ranged from 3.7-45.9 µg/mL. *L. donovani* showed a higher extracts sensitivity (LC_{50}, 3.7-12.4 µg/mL) when compared to *T. cruzi* (LC_{50}, 5.9-45.9 µg/mL). Ethanolic extracts from root bark and root wood were the most active against *T. cruzi* (LC_{50}, 5.9 and 9.9 µg/mL, respectively) and *L. donovani* (LC_{50}, 3.7 and 8.7 µg/mL, respectively). Tempone et al. (2005) carried out experiments on *T. cruzi* trypomastigotes, and *L.* (L.) *chagasi* promastigotes and amastigotes using hydroethanolic extract (ethanol 96%) and alkaloid extract (dichloromethane fraction) from *Annona crassiflora* Mart. leaves. In the tests, only the alkaloid extract was effective against *L.* (L.) *chagasi* promastigotes (LC_{50}, 24.89 µg/mL), *L.* (L.) *chagasi* amastigotes (86.1% mortality at 25 µg/mL) and *T. cruzi* trypomastigotes (100% mortality at 100 µg/mL). In another study, Oliani et al. (2013) investigated the essential oil from *Annona crassiflora* Mart. leaves against *L.* (L.) *amazonensis*, *L.* (V.) *braziliensis*, *L.* (L.) *infantum chagasi* and *L.* (L.) *major* promastigotes, and *T. cruzi* trypomastigotes. The authors observed that *T. cruzi* (LC_{50}, 5.31 µg/mL) was more sensitive to essential oil compared to *Leishmania* spp. (LC_{50}, 25.97-39.19 µg/mL). In addition, the essential oil was about 9 times more effective against *T. cruzi* than benznidazole (reference drug; LC_{50} value of 45.02 µg/mL). The essential oil contained mainly α-amorphene (43.6%), E-caryophyllene (17.7%) and β-germacrene (5.3%).

5.13.3. Anthelmintic activity

Helminth parasites can infect humans, animals and plants negatively affecting human life quality, livestock and agriculture, thereby causing significant public health and economic concern (Blanco et al., 2018). *Annona crassiflora* Mart. has shown its potential as sources of metabolites to be used in nematode control (Machado et al., 2015; Nogueira, Silva, Souza, Duarte, & Martins, 2009). Nogueira et al. (2009) found that aqueous extracts from *Annona crassiflora* Mart. leaves and seeds had anthelmintic activity against sheep nematodes. Extracts from leaves and seeds effectively inhibited the larval development of sheep trichostrongylides reducing the larvae number by 89.81 and 99.43%, respectively, at a concentration of 100 mg/mL. In another work, Machado et al. (2015) explored nematicidal property of *Annona crassiflora* Mart. leaves using nematode *Caenorhabditis elegans* as a model. The results of their study showed that hydroethanolic extract (ethanol 80%) and its fractions (dichloromethane, ethyl acetate, methanol and water) were able to immobilize the *C.*

elegans larvae. Dichloromethane and ethyl acetate fractions showed to be the most active fractions (98.13 and 89.66% larvae immobility, respectively, at 1000 µg/mL), presenting similar efficiency to the positive control (ivermectin; 100% larvae immobility at 1000 µg/mL). In addition, the authors combined these two fractions and isolated an oil that was chemically and functionally characterized. The authors verified that the oil and 2-isopropyl-5-methylcyclohexanol (one of the compounds identified in the oil) dose-dependently reduced the larvae mobility with ED_{50} values of 350 and 113 µg/mL, respectively. They also observed that 2-isopropyl-5-methylcyclohexanol caused damage to cell membrane integrity and interfered in the cellular process of exogenous molecule exclusion, suggesting a potential nematicidal utility.

The results catalogued in this review highlight that several extracts obtained from different botanical parts of *Annona crassiflora* Mart. exhibit antiparasitic activity against diverse parasites. This activity could be readily attributed to different classes of bioactive compounds found in *Annona crassiflora* Mart. with recognized antiparasitic potential, such as phenolic compounds, alkaloids, annonaceous acetogenins and terpenes (see section **4** above).

5.14. Insecticide activity

Insects and their larvae can be important vectors of viruses transmitted to humans and can also cause reduction of production and quality of vegetable crops. Different investigations have demonstrated that extracts from several parts of *Annona crassiflora* Mart. plant and fruit have shown significant activity against some larvae and insects (**Table 3**).

5.14.1. Insect crop pest control

It is estimated that insects and diseases can cause losses of 20-40% in crop production, and insect pests represent a significant portion of this loss. Broad-spectrum chemical insecticides have been the main strategy used to control insects in several crops (Douglas, 2018). However, the increasing resistance of insects and the greater regulatory burden linked to environmental toxicity and concerns for human health have driven the search for new insecticides derived from natural sources. In this sense, *Annona crassiflora* Mart. plant and fruit have been the subject of many studies.

Silva et al. (2009b) tested hexane and ethanolic extracts obtained from diferente botanical parts of *Annona crassiflora* Mart. against Mexican bean weevil (*Zabrotes*

subfasciatus) and bean weevil (*Acanthoscelides obtectus*) α-amylases. Only the ethanolic extract from leaves significantly inhibited the *Z. subfasciatus* and *A. obtectus* α-amylases (86.58 and 68.54% of inhibition, respectively, at 1 mg/mL) with IC_{50} values of 802.67 and 546.25 µg/mL, respectively. The authors attributed this effect to phenolic compounds. In fact, several studies have shown that *Annona crassiflora* Mart. leaves are rich in phenolic compounds (see **Table 2**).

Saito et al. (1989) investigated the insecticide activity of several extracts and extract fractions from *Annona crassiflora* Mart. leaves, seeds and stem against housefly (*Musca domestica*), Mediterranean fruit fly (*Ceratitis capitate*), Mexican bean weevil (*Zabrotes subfasciatus*) and boll weevil (*Anthonomus grandis*) adults, and fall armyworm (*Spodoptera frugiperda*) larvae. The researchers found a low or no activity for most of the extracts and fractions tested. Only hexane extract and dichloromethane fraction from seeds exhibited significant effect against *C. capitate* (62.3% mortality at 10% m/v) and *M. domestica* (32.5% mortality at 2% m/v), respectively. Indeed, most of the studies investigating the insecticide activity of *Annona crassiflora* Mart. have been carried out with seed extracts (Krinski & Massaroli, 2014; Massarolli, Pereira, & Foerster, 2017; Pimenta et al., 1999; Silva, Pereira, & Turchen, 2013b; Turchen, Golin, Butnariu, & Pereira, 2014).

When testing the hydroethanolic extract (ethanol 75%) from *Annona crassiflora* Mart. seeds against *S. frugiperda* larvae at the concentrations from 2000 to 10000 µg/mL, Pimenta et al. (1999) verified a significant increase in larvae mortality in a dose-dependent manner, with larvae mortality varying from 40 to 100% mortality. Moreover, surviving larvae did not complete the biologic cycle. The potential active compounds in this extract were annonaceous acetogenins (see **Table 2**).

Methanolic extracts from *Annona crassiflora* Mart. seeds were active against nymphs and adults of brown stink bug (*Euschistus heros*) (Silva et al., 2013b; Turchen et al., 2014). Turchen et al. (2014) observed a dose-dependent effect of the methanolic extract from *Annona crassiflora* Mart. seeds (0.5-8.0% m/v) on mortality of third instar nymphs of *E. heros*. On the other hand, Silva et al. (2013b) evaluated the effect of spraying a methanolic extract from *Annona crassiflora* Mart. seeds (2% m/v) against *E. heros* adults in soybean crop. This study showed that seeds extract was able to reduce the *E. heros* population in soybean crop after 7 days of application when compared to control.

Chloroform-methanol (2:1 v/v) extract from *Annona crassiflora* Mart. seeds was also investigated against nymphs of rice stalk stink bug (*Tibraca limbativentris*) and eggs and caterpillars of soybean looper (*Chrysodeixis includens*). Krinski and Massaroli (2014) verified that the treatment with this extract (0.5-8.0% m/v) dose-dependently killed the second instar nymphs of *T. limbativentris* reaching up to 81% mortality 120 h after application. In another study, Massarolli et al. (2017) evaluated the effect of this extract (0.5-8.0% m/v) on *C. includens* in different stages of larval development using two strategies: by ingestion of treated soybean leaves and direct contact. Chloroform-methanol extract from *Annona crassiflora* Mart. seeds significantly increased the mortality rate of *C. includens* caterpillars of first, third, and fifth instars, especially by topical application (up to 93.3% mortality for the highest concentration).

5.14.2. Dengue, yellow fever, chikungunya, and Zica vector control

Arbovirus diseases such as dengue, yellow fever, Zika, and chikungunya are re-emerging worldwide with increasing prevalence and/or severity, representing major international public health concerns. These arboviruses have great impact on human health, and can cause from high fever lasting 4-14 days and joint pain or even neurological diseases and death (Achee et al., 2019; Souza-Neto, Powell, & Bonizzoni, 2019). These four arboviruses have as primary transmitter the *Aedes aegypti* mosquito, stressing the importance of developing effective strategies for its control. In this context, the *Annona crassiflora* Mart. has been also evaluated.

Rodrigues, De Paula, Degallier, Molez, and Espíndola (2006) explored larvicidal activity of hexane and ethanolic extracts from leaves, stem wood, stem bark, root wood and root bark of *Annona crassiflora* Mart. against third instar larvae of *A. aegypti*. Among the extracts tested, hexane and ethanolic extracts from root wood and root bark, and hexane extract from stem bark presented larvicidal activity (mortality ≥ 65% at 500 μg/mL after 24 h). In addition, ethanolic extracts from root bark and root wood were the most active with LC_{50} values of 23.06 and 26.89 μg/mL, respectively. High larvicidal activity against *A. aegypti* larvae was also reported by Omena et al. (2007) in ethanolic extracts (ethanol 95%) from root bark, root wood and stem. Root bark extract had the highest activity (LC_{50}, 0.71 μg/mL) followed by root wood and stem extracts (LC_{50}, 8.94 and 16.1 μg/mL, respectively). Extracts and fractions obtained from *Annona crassiflora* Mart. seeds were also tested against third instar larvae of *A. aegypti* by Costa et al. (2013). The results this study demonstrated that hexane, dichloromethane, methanolic (defatted with hexane) extracts and hexane fraction killed up to

100% of larvae at 1 mg/mL. Methanolic extract (defatted with hexane) was the most active followed by dichloromethane extract, hexane fraction and hexane extract (LC_{50}, 0.100, 0.185, 0.433 and 0.507 mg/mL, respectively).

The insecticide and larvicidal activities of extracts from *Annona crassiflora* Mart. plant and fruit parts could be due the presence of secondary metabolites, mainly annonaceous acetogenins. Indeed, annonaceous acetogenins have been found in various parts of this plant as described in section **4.12** above. Annonaceous acetogenins are potent insecticides due to their interference in the hormonal activity of same insects, and especially for their strong inhibitory action on the mitochondrial complex I (NADH ubiquinone oxidoreductase) in insects that reduce the ATP production, inducing apoptosis (programmed cell death) (Massarolli et al., 2017).

5.15. Schistosomiasis vector control

Human schistosomiasis, a neglected tropical disease caused by infection with one of the five species of schistosomes: *Schistosoma mansoni*, *S. japonicum*, *S. haematobium*, *S. intercalatum* and *S. mekongi*, affects more than 250 million people worldwide, particularly in tropical and subtropical countries. Among the five main species infecting humans, *S. mansoni* is responsible for the majority of hepatointestinal schistosomiasis, and is transmitted through the host snail *Biomphalaria glabrata* (Chuah, Gobert, Latif, Heo, & Leow, 2019; Stensgaard, Vounatsou, Sengupta, & Utzinger, 2019). Thus, controlling the population of host snails becomes a viable strategy. In this context, dos Santos and Sant'Ana (2001) carried out a research with hydroethanolic extracts (ethanol 90%) from different botanical parts of *Annona crassiflora* Mart. against adult forms and egg masses of *B. glabrata*. The majority of analysed extracts showed molluscicide activity against this snail, some of them with significant LC_{90} values (< 20 μg/mL), as shown for stem, root bark and pulp+seed extracts (LC_{90}, 2.34, 3.79 and 13.21 μg/mL, respectively). Moreover, these extracts presented additional toxicity toward snail egg masses with LC_{90} of 1 μg/mL.

5.16. Antimicrobial activity

The advances of the analytical tools have allowed the discovery of plants and their active principles that possess an effect on pathogenic, opportunistic commensal and spoilage microorganisms. In this context, extracts from several parts of *Annona crassiflora* Mart. plant

and fruit have been targets for antifungal and antibacterial research, as can be seen in the studies reported below.

5.16.1. Antifungal activity

Hydroethanolic extract (ethanol 95%) from *Annona crassiflora* Mart. leaves were investigated against 59 strains of *Candida* spp. (52 strains of *C. albicans*, 4 strains of *C. tropicalis* and 3 strains of *C. krusei*) isolated from human immunodeficiency virus-infected patients with oropharyngeal candidosis. The tested extract showed antifungal activity against all strains of *Candida* spp., being more effective against *C. tropicalis* strains (MIC, 0.25 μg/mL) than *C. krusei* and *C. albicans* strains (MIC, 0.50 and 2.00 μg/mL, respectively). In addition, the leaves extract was more active than the positive controls (MIC, 8-16, 8-128 and 8 μg/mL for ketoconazole, fluconazole and itraconazole, respectively) (Silva et al., 2001). In another study, hydroethanolic extract (ethanol 80%) from leaves and its hydromethanolic fraction (methanol 80%) exhibited high antifungal activity against *C. albicans* with growth inhibition rates of 80 and 86%, respectively, at 500 μg/mL (Lage et al., 2014).

5.16.2. Antibacterial activity

Silva et al. (2014a) used hydroethanolic extracts (ethanol 70%) from stem, leaves, and fruit parts (peel, seed and pulp) of *Annona crassiflora* Mart. against 60 samples of Oxacillin Resistant *Staphylococcus aureus* (ORSA). In the disk diffusion assay, the extracts (100 mg/mL) from stem, leaves, pulp, and fruit peel were actives against ORSA and *S. aureus* ATCC 25923 (standard strain) with inhibition zones diameters ranging from 5 to 15 mm and 10 to 20 mm, respectively. In the MIC assay, extracts from stem, pulp and leaves were the most active against ORSA (25 mg/mL) followed by the extracts from fruit peel and seed (50 mg/mL), whereas stem extract showed the highest bacterial activity against and *S. aureus* ATCC 25923 (1.56 mg/mL).

Lage et al. (2014) tested the antibacterial activity of the hydroethanolic extract (ethanol 80%) from *Annona crassiflora* leaves and its hydromethanolic fraction (methanol 80%). Both the extract and fraction (500 μg/mL) showed activity against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *Escherichia coli* with growth inhibition rates between 61 and 88%. Moreover, (-)-epicatechin (one of the compounds isolated from the extract) was highly effective against *S. aureus* (86% inhibition at 250 μg/mL).

Aqueous and ethanolic extracts from *Annona crassiflora* Mart. leaves were evaluated against three *Staphylococcus* (S178: *S. aureus*; S135 and S182: *S. haemolyticus*) and two *E. coli* (E2 and E3) isolated from cows with mastitis, besides *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 isolated from human clinical (reference strains). Disk diffusion assay demonstrated that ethanolic extract was more active than aqueous extract against all bacteria strains with inhibition zones ranging from 9.3 to 19.8 mm. Both the MIC and MBC of the ethanolic extract were 6.24 mg/mL for all bacterial strains. In addition, the authors verified no antibacterial effect after removal of the tannins, suggesting that these compounds are the main active antibacterial agents present in this extract (Ribeiro et al., 2018).

The antimicrobial activity of extracts from *Annona crassiflora* Mart. fruit and plant parts could be due the presence of bioactive compounds such as phenolic compounds and alkaloids as described in sections **4.11** and **4.13** above. Indeed, recent studies have proven that phenolic compounds and alkaloids isolated from plant extracts are potent antimicrobial agents (Chen, Huo, Hu, Xu, & Zhang, 2018; Ng, Lyu, Mark, & Chen, 2019; Nzogong et al., 2018; Tavares et al., 2014).

5.17. Herbicide activity

Weeds compete with crops leading to the qualitative and quantitative reduction in production, stressing the importance of their control (Sharma, Jha, & Reddy, 2018). Herbicides are chemical substances used to control weeds and correspond to the method most used by farmers for this purpose. However, the intensive and indiscriminate use of herbicides can entail negative implications for the environment, human and animal health, as well as selecting tolerant and resistant biotypes (Inoue, Santana, Pereira, Possamai, & Azevedo, 2009). Thus, extracts and natural substances can be viable alternatives for weeds management, minimizing environmental problems and ensuring an ecosystem equilibrium. Extracts and isolated compounds from different botanical parts of *Annona crassiflora* Mart. have demonstrated herbicide potential (**Table 3**).

The effect of the ethanolic extracts from *Annona crassiflora* Mart. leaves and seeds on germination and early growth of lettuce seedlings (*Lactuca sativa* L.) was evaluated by Ribeiro et al. (2013). Extracts from leaves and seeds significantly reduced the germination percentage of lettuce seeds with up to 48.25 and 85.81% reduction at 1.2 mg/L, respectively. Moreover, both extracts inhibited the root-tip elongation in a dose-dependent manner with IC_{50}

values of 0.1 and 0.6 mg/L for leaves and seeds extracts, respectively. Additionally, Formagio et al. (2010) showed that the methanolic extract from *Annona crassiflora* Mart. aerial parts (1% w/v) also significantly inhibited the radicle and hypocotyls growth of lettuce. In another study, the ethanolic extracts from leaves and stem of *Annona crassiflora* Mart. were also tested against wheat (*Triticum aestivum*), lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.) and onion (*Allium cepa* L.) (Novaes, Torres, & dos Santos, 2016). This study demonstrated that these extracts were able to inhibit the wheat coleoptile elongation (only seeds extracts at ≥ 0.4 mg/mL), lettuce (only leaves extract at 0.8 mg/mL) and tomato (stem and leaves extracts at 0.8 mg/mL) germination, and shoot and root development of tomato and onion (stem and leaves extracts generally at 0.8 mg/mL).

Also, positive effects of other extracts and isolated compounds from *Annona crassiflora* Mart. have been reported in weeds. Inoue et al. (2009) evaluated the efficacy of aqueous extracts from seeds, leaves and branches of *Annona crassiflora* Mart. on marandu grass (*Brachiaria brizantha*) and soybean (*Glycine max*) germination and development. The results showed that all extracts (20-100 g/L) significantly reduced the seed germination and root development of *B. brizantha*, practically without affecting soybean germination and development. In addition, seeds extract was the most active against *B. brizantha* with inhibition of up to 90.32% germination and 81.63% root development at 100 g/L. Inoue et al. (2010b) also performed experiments on weeds (*Brachiaria brizantha, Euphorbia heterophylla* and *Ipomoea grandifolia*) and soybean using an hydroethanolic extract (70% ethanol) and its fractions obtained from seeds, leaves and stem of *Annona crassiflora* Mart. Hydroethanolic extract from seeds (2% v/v) was found to have the greatest effect against all weeds, inhibiting up to 100% germination, and 97.9 and 100% root and hypocotyl development, respectively. Among the fractions tested (methanolic, ethyl acetate, dichloromethane and petroleum ether), ethyl acetate fraction from seeds (1-4% v/v) exhibited the most promising results, showing the highest inhibitory effect on weeds, but no effect on soybean development. In another study, Inoue et al. (2010a) isolated two steroids glycosides (stigmasterol glycoside and β-sitosterol glycoside) from *Annona crassiflora* Mart. seeds and evaluated their effect on weeds (*Euphorbia heterophylla* and *Ipomoea grandifolia*) germination and development. The results indicated that the fraction containing these substances did not inhibit germination and germination velocity index of weeds but reduced radicle and hypocotyl development of *E. heterophylla* (20-100 mg/L).

These studies display the herbicide potential of several extracts obtained from different botanical parts of *Annona crassiflora* Mart. for weeds control. Alkaloids and annonaceous acetogenins seem to be the main compounds associated with herbicide activity in the *Annonaceae* family (Novaes et al., 2016). However, for the development of agricultural biodefensives for sustainable weeds management, it is necessary to carry out future researches in order to isolate and identify the compounds responsible for its herbicide potential.

6. Potential applications of *Annona crassiflora* Mart. plant, fruit and by-products

The use of natural bioactive compounds in the development of new products has become increasing in several industrial sectors due to the health-conscious consumers. *Annona crassiflora* Mart. is a source of high value-added compounds with several industrial applications, including phenolic compounds, alkaloids, annonaceous acetogenins, and other bioactive compounds. The potential applications *Annona crassiflora* Mart. fruit and by-products, extracts and value-added compounds are presented in **Fig. 2**.

As previously shown, *Annona crassiflora* Mart. fruit pulp hold attractive sensorial features as well as high nutritional and functional potential. However, this fruit present a very short production cycle and is highly perishable, making its consumption possible only at certain times of the year and in limited regions (Botrel, Rodrigues, Souza, & Fernandes, 2016). One way to increase availability and add even more value to this fruit is to process its pulp and/or create new products. Studies demonstrate that the use of *Annona crassiflora* Mart. fruit pulp in the formulation of new products, including bread, food bars, yogurt, nectar, juice, jams, ice-cream, fermented dairy beverages and candy, is an interesting option for the food industry, since these products have a good sensorial acceptance and purchase intent by the consumer (**Table 4**). In addition, the incorporation of *Annona crassiflora* Mart. fruit pulp in food products can improve their nutritional and functional characteristics. For example, *Annona crassiflora* Mart. fruit juice had high antioxidant activity and phenolic compounds content (Schiassi et al., 2018b), whereas its incorporation in ice-cream provided a dietary fiber increment in final food product (Morzelle, Lamounier, Souza, Salgado, & Vilas-Boas, 2012). Lima et al. (2016) reported an increase in protein, lipid and mineral contents in fermented dairy beverages added with *Annona crassiflora* Mart. pulp. Incorporation of *Annona crassiflora* Mart. pulp flour in food bars provided a food product with substantial improvement in contents of dietary fiber, vitamin C, carotenoids, antioxidant activity and minerals such as calcium and magnesium

(Silva, Siqueira, Lago, Rosell, & Vilas Boas, 2014b; Silva et al., 2018c). These aspects could be well exploited by the field of functional foods.

There has been increasing interest in utilization of fruit by-products and non-fruit part of *Annona crassiflora* Mart. Extracts from fruit peel, seeds, leaves, stem and root have been shown to possess a wide range of biological activities (**Fig. 2.**), suggesting that these extracts could be further used by food, packaging, medical, pharmaceutical and cosmetic industries. Moreover, these parts can be used to obtain value-added ingredients such as phenolics, alkaloids, annonaceous acetogenins, oils and essential oils.

Fruit by-products and leaves are potential sources for obtaining phenolic compounds that could be used as functional ingredients in the preparation of functional foods, replacers of synthetic antioxidants in food products, antimicrobial and antioxidant agents both in food and in packaging and coatings for foods, additives of biomaterials for tissue engineering purposes, active ingredients in the production of cosmetics, drugs and nutraceuticals, among others. Fruit by-products can be exploited as a source of annonaceous acetogenins, while alkaloids can be purified from fruit peel and leaves. These classes of bioactive compounds exhibit a variety of biological activities, including antitumoral, insecticide, antiparasitic and herbicide activities. Thereby, these by-products can be used to obtain alkaloids and annonaceous acetogenins for pharmaceutical, medical, veterinary and agricultural applications.

Annona crassiflora Mart. seeds can also be a promising source for obtaining oil due to high oil yields (28.84-34.58% dw) (Egydio & Santos, 2011; Luzia & Jorge, 2013). *Annona crassiflora* Mart. seeds oil presents desired characteristic for food and pharmaceutical industries such as high concentration of monounsaturated fatty acids, which are able to reduce the blood levels of undesirable LDL and increase the levels of beneficial HDL, besides presence of polyunsaturated fatty acids from the omega family and functional phytochemicals, including phytosterols, tocopherols and carotenoids (Araújo, Rodriguez-Jasso, Ruiz, Pintado, & Aguilar, 2018; Luzia & Jorge, 2013). Moreover, its high content of oleic acid (about 50% of the oil) is of great interest in cosmetics industry (good vehicle for drug solubility-partitioning), whereas the high proportion of unsaturated fatty acids (almost 68% of the oil) is useful for paint industries (Egydio & Santos, 2011).

Annona crassiflora Mart. fruit peel was successfully utilized as a substrate for lipase production by *Lichtheimia ramosa* (de Andrade Silva, Lacerda, Leite, & Fonseca, 2014). The results of this study showed protein enrichment (143.31%) and high lipase production (0.58

U/g) in *Annona crassiflora* Mart. fruit peel substrate cultured with *L. ramosa* in solid-state bioprocessing. Therefore, the biotechnological industry can exploit the *Annona crassiflora* Mart. fruit peel as a substrate to produce lipase for food, drugs and detergent obtaining, biological wastewater treatment and accelerated biodegradation of polymers, among others (de Andrade Silva et al., 2014).

Overall, there is great potential to utilize *Annona crassiflora* Mart. for a broad applications range. Different botanical parts (fruit, leaves, stem and root) and products from fractionation and processing can be used for development of innovative products in food, packaging, feed, agricultural, cosmetic, pharmaceutical and medical industries.

7. Conclusion

Annona crassiflora Mart. fruit and related products have attracted increasing interest of consumers and researchers due to its sensorial, nutritional and bioactive potential. Different botanical parts of *Annona crassiflora* Mart. (fruit pulp, fruit peel, seeds, leaves, stem and root) contain a variety of bioactive compounds such as phenolics, alkaloids, annonaceous acetogenins, carotenoids, tocols and phytosterols. *Annona crassiflora* Mart. fruit is a good source of dietary fiber, sugars, vitamins A and C, phenolic compounds, folates and minerals such as copper, manganese, potassium, magnesium and zinc. Other plant parts (especially the leaves) and mainly fruit by-products (peel and seeds) are also important parts of *Annona crassiflora* Mart. because they are potential sources of value-added compounds such as oils (seeds), essential oils (leaves), phenolics (fruit peel and leaves), alkaloids (fruit peel and leaves) and annonaceous acetogenins (seeds and fruit peel). These bioactive compounds found in *Annona crassiflora* Mart. exhibit various biological activities and have been evaluated through *in vitro* and *in vivo* tests. The claimed biological activities of extracts from different botanical parts of *Annona crassiflora* Mart. include antioxidant, hepatoprotective, anti-inflammatory, antitumoral, analgesic, antidiabetic, skin healing, antidiarrhoeic, antimicrobial, antiparasitic, insecticide and herbicide activities. These findings suggest that *Annona crassiflora* Mart. can be a potential source of value-added ingredients for use in pharmaceutical, medical, cosmetic, agricultural, packaging, feed and food industry applications.

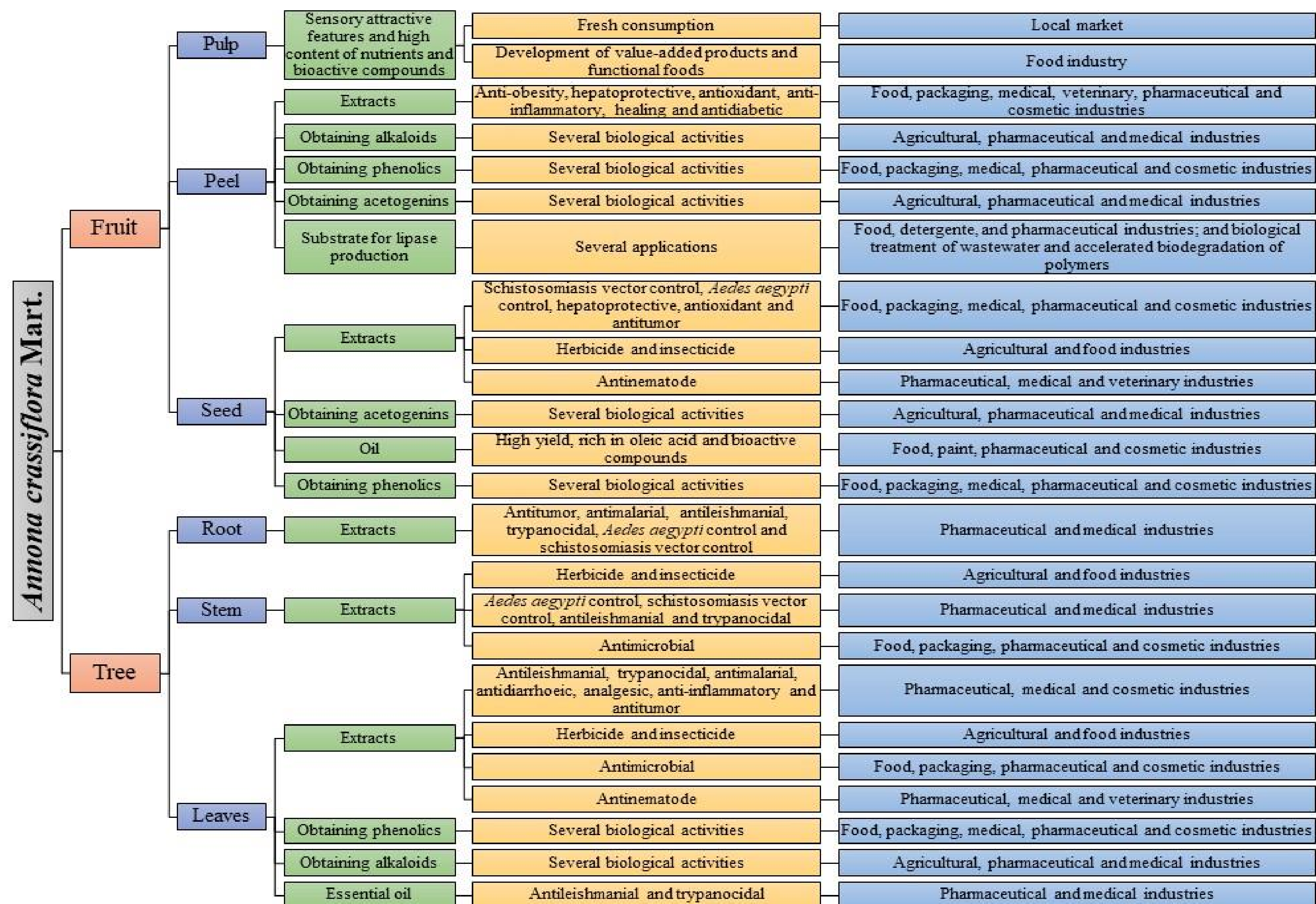


Fig. 2. Potential applications of *Annona crassiflora* Mart. fruit and by-products.

Table 4 Food products developed using *Annona crassiflora* Mart. fruit pulp.

Product	Formulation	Major findings	Reference
Juice	40% of *Annona crassiflora* Mart. pulp	-The juice produced using *Annona crassiflora* Mart. pulp presented a great acceptance by consumers, and contributed to high antioxidant activity and phenolic compounds content.	Schiassi et al. (2018b)
Nectar	Mixed nectar of *Annona crassiflora* Mart. fruit and passion fruit	-Two formulations were developed: F1 (50% *Annona crassiflora* Mart. pulp + 50% passion fruit pulp) and F2 (70% *Annona crassiflora* Mart. pulp + 30% passion fruit pulp). -Both formulations had high acceptance (close to 8, corresponding to the term "I like very much") and purchase intent scores (average of 4.5, which is between the terms "I would probably buy" and "I would certainly buy"), suggesting high market potential.	Morzelle et al. (2011)
Food bar	*Annona crassiflora* Mart. pulp flour (20, 30, 40, and 50% replacement of oatmeal)	-The food bars developed with *Annona crassiflora* Mart. pulp flour demonstrated nutritional and commercial potential and can be considered as product rich in dietary fiber, having considerable amounts of vitamin C, total carotenoids, and antioxidant activity, besides showing to be rich in minerals such as calcium and magnesium. -The food bar with 50% *Annona crassiflora* Mart. pulp flour showed the best results for the physical, chemical, and nutritional analyses as well as the best consumer acceptance.	Silva et al. (2018c)
Food bar	*Annona crassiflora* Mart. pulp flour (5, 10, 15 and 20% replacement of corn starch cake)	-The replacement of part of corn starch biscuit for *Annona crassiflora* Mart. flour improved the sensory acceptability of the food bars.	Silva, Siqueira, Damiane, and Vilas Boas (2016b)
Snack bar	*Annona crassiflora* Mart. pulp flour (5, 10, 15 and 20% replacement of corn starch biscuit)	-Incorporation of *Annona crassiflora* Mart. flour in snack bars provides a food product with substantial improvement in contents of dietary fiber, vitamin C, minerals and antioxidant activity, besides improves the sensory properties of the snack bars.	Silva et al. (2014b)
Pulp powder	Inulin (0, 20 and 30%) as drying aid	-Making available of this fruit as an ingredient in the development of new powdered products in the food industry (*e.g.*, candies, ice cream, yogurt, and others). -Adding value to the product by use of inulin (prebiotic and sensory properties).	Botrel et al. (2016)
Jam	Mixed jam (40% of *Annona crassiflora* Mart. pulp, 35% of soursop pulp and 25% of sweet passion fruit pulp)	-Mixed jam had high acceptance scores (close to 7) for colour, appearance, smell, taste and overall liking.	de Souza et al. (2012)

Jam	Mixed jam (50% of *Annona crassiflora* Mart. pulp and 50% of araça pulp)	-Mixed jam was stable over one-year of storage without the addition of additives.	Damiani et al. (2012)
Jam	*Annona crassiflora* Mart. pulp	-Two formulations were developed: conventional extra jam type and *light* jam. -Both jams were physiochemically and microbiologically stable over 6 months of storage.	Arévalo-Pinedo et al. (2013)
Jam	Extra jam type (50% of *Annona crassiflora* Mart. pulp)	-*Annona crassiflora* Mart. jam was physicochemically and microbiologically stable over one-year of storage (parameters modifications were within the standards established by legislation). -*Annona crassiflora* Mart. jam had high acceptance scores (note 8) for appearance, colour, flavour, and aroma in 0, 6 and 12 months of storage.	Damiani et al. (2017)
Milk caramel	16.7% of *Annona crassiflora* Mart. pulp	-Milk caramels with *Annona crassiflora* Mart. pulp and the control formulation (milk caramel) were equally accepted by children. -Milk caramels with *Annona crassiflora* Mart. pulp were well accepted by children, showing average acceptance scores between the terms “I like moderately” and “I like very much”, and acceptability indexes > 80%.	Arruda et al. (2016)
Bread	*Annona crassiflora* Mart. pulp flour (10 and 20% replacement of wheat flour)	-Breads with *Annona crassiflora* Mart. pulp flour and the control formulation were equally accepted by consumers. -Breads with *Annona crassiflora* Mart. pulp flour were well accepted by consumers, showing average acceptance scores between the terms “I like” and “I like very much”, and acceptability indexes > 80%.	Villela et al. (2013)
Fermented dairy beverage	*Annona crassiflora* Mart. pulp (5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0% w/v)	-Proteins, lipids and ash increased as the *Annona crassiflora* Mart. pulp concentration increased. -All formulations were equally preferred by consumers, regardless of the *Annona crassiflora* Mart. pulp concentration.	Lima et al. (2016)
Fermented milk drink	*Annona crassiflora* Mart. pulp (4, 8, 12 and 16%)	-All formulations were equally accepted by panellists regarding parameters of colour, aroma, flavour, acidity, viscosity and appearance. -*Annona crassiflora* Mart. fermented milk drink showed high purchase intent (73% of panellists reported: “yes, I would buy the product”).	Silva et al. (2015b)
Yogurt	*Annona crassiflora* Mart. pulp (20 and 30%)	-Traditional (sucrose) and *diet/light* (sodium cyclamate/saccharine (2:1)) yogurts were developed. -All formulations, except the formulation with 1.9% sodium cyclamate/saccharin + 20% *Annona crassiflora* Mart. pulp, were well accepted by panellists, showing average acceptance scores between the terms “I like moderately” and “I like very much”.	Della Lucia et al. (2012)

Yogurt	*Annona crassiflora* Mart. pulp (0, 12.5, 25 and 50%)	-Yogurt formulations with 12.5 and 25% of *Annona crassiflora* Mart. pulp and the control formulation were equally accepted by consumers, showing average acceptance scores between the terms "I like slightly" and "I like moderately".	Oliveira et al. (2008)
Yogurt	*Annona crassiflora* Mart. marmalade (20, 25 and 30%)	-Yogurt with 25% of *Annona crassiflora* Mart. marmalade was the preferred sample, showing high acceptance score (average of 7.4, which is between the terms "I would eat this often" and "I would eat this very often").	Rocha, Cobucci, Maitan, and Silva (2008)
Ice-cream	*Annona crassiflora* Mart. pulp	-Incorporation of *Annona crassiflora* Mart. pulp in ice-cream provides a dietary fiber increment in final food product (1.21 g/100 g). -*Annona crassiflora* Mart. ice-cream had high acceptance scores (3.28-4.51 on the 5-point hedonic scale) for colour, appearance, aroma, taste and texture.	Morzelle et al. (2012)

8. Future research directions

According to the literature, *Annona crassiflora* Mart. represent a rich source of nutrients and phytochemicals, and its phytochemical content is highly correlated with their biological properties. Although there are numerous studies investigating the occurrence of the total amount of phytochemicals in *Annona crassiflora* Mart., there is limited information for the profile and content of specific phytochemicals found in *Annona crassiflora* Mart. fruit, as well as in other botanical plant parts. Thus, more research should be carried out to determine the complete profiles of phytochemical compounds as well as their contents in different botanical parts of *Annona crassiflora* Mart. Likewise, the study of more adequate extraction and recovery techniques of the bioactive compounds and knowledge of extracted phytochemicals stability is essential.

A large number of research studies have demonstrated that *Annona crassiflora* Mart. exhibits high nutritional value, several bioactive compounds and biological properties. However, for *Annona crassiflora* Mart. to become a sustainable cash crop is necessary agronomic and genetic studies in order to develop technical plantations to increase productivity and fruit availability at different times of the year. Suitable fruit pulp conservation methods and development of novel food fruit pulp-based products are also topics of special interest. Conservation methods (such as drying processes, thermal and non-thermal pasteurization processes) for increasing shelf life of *Annona crassiflora* Mart. fruits and/or pulp-based food products and their effects on preserving the nutritional and phytochemical contents are important issues that should be investigated in depth.

Having in mind the presence of several phytochemicals in the *Annona crassiflora* Mart. extracts and their association with the biological properties, the potential applications of these extracts as functional ingredients of food products should be examined. Another topic that needs further clarification is the medicinal application of the leaf, peel and seed extracts in pharmacology and cosmetology. The positive effects of *Annona crassiflora* Mart. extracts on management and/or treatment of several diseases have been demonstrated by *in vitro* studies and animal experiments. However, toxicological, pre-clinical and clinical trials should be carried out to further confirm these effects in human health and assure the safety and well-being of the consumers. Likewise, future clinical and nutritional trials could allow a deeper understanding of beneficial effects of *Annona crassiflora* Mart. fruit consumption in human health.

Finally, the use and valorisation of the by-products (peel and seeds) produced during *Annona crassiflora* Mart. fruit processing should be further investigated, since this could offer new source of value-added ingredients and solve environmental issues on the sustainable management of these materials.

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Conflict of interest

The authors report no conflict of interest.

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CHAPTER II

RESEARCH ARTICLE

DETERMINATION OF FREE, ESTERIFIED, GLYCOSYLATED AND INSOLUBLE-BOUND PHENOLICS COMPOSITION IN THE EDIBLE PART OF ARATICUM FRUIT (*Annona crassiflora* Mart.) AND ITS BY-PRODUCTS BY HPLC-ESI-MS/MS

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Abstract

Phenolics present in the free, esterified, glycosylated and insoluble-bound forms of araticum pulp, peel and seed were for the first time characterized and quantified using HPLC-ESI-MS/MS. Levels of total phenolics, flavonoids, condensed tannins and antioxidant activities from araticum fruit followed the order peel > pulp > seed. Overall, insoluble-bound and esterified phenolics were the dominant forms of phenolics from araticum fruit parts and the highest contributors to their antioxidant activities. Extracts were found to contain contrasting levels of phenolics that were specific to each fruit part. From 10 phenolics quantified in araticum fruit, catechin and epicatechin were the major ones from pulp and peel, whereas seed displayed caffeic acid, catechin and epicatechin as its main phenolics. Araticum fruit was found to provide a good source of phenolics, and the full exploitation of this fruit may find applications in the food, cosmetic and pharmaceutical industries.

Keywords: Brazilian Cerrado fruit; Antioxidant activity; Bioactive compounds; Natural phenolic antioxidants; Extraction procedure.

Highlights

- First report on different phenolic forms in araticum fruit.
- Insoluble-bound and esterified phenolics were the major contributors to total phenolics.
- Six phenolic acids and four flavonoids were quantified in araticum fruit.
- Catechin, epicatechin, caffeic and protocatechuic acids were the main phenolics.
- Araticum fruit and its by-products are promising sources of natural phenolic antioxidants.

Chemical compounds studied in this article

Catechin (PubChem CID: 9064); Epicatechin (PubChem CID: 72276); Rutin (PubChem CID: 5280805); Quercetin (PubChem CID: 5280343); Protocatechuic acid (PubChem CID: 72); Gentisic acid (PubChem CID: 3469); Chlorogenic acid (PubChem CID: 1794427); Caffeic acid (PubChem CID: 689043); *p*-Coumaric acid (PubChem CID: 637542); Ferulic acid (PubChem CID: 445858)

1. Introduction

Araticum or marolo (*Annona crassiflora* Mart.) is an exotic fruit of Brazilian Cerrado belonging to the *Annonaceae* family. This fruit has oval or rounded shapes, tomentose surfaces of green colour when developing and brown when ripe with dimensions ranging from 9-15 cm in length per 10-15 cm diameter, and weighing from 0.5-4.5 kg. Its seeds (70-190 per fruit) are dark-brown with obovate-flattened shape, measuring from 10-13 mm per 20-27 mm, whereas its pulp is lightly sweet with a pleasant aroma, and colour ranging from white to yellow (Arruda & Almeida, 2015). Araticum is one the most consumed fruits in the Cerrado region (within the top 20) (Arruda, Pereira, & Pastore, 2017a). Apart from its unique sensory features such as colour, flavour and aroma, the araticum fruit holds high nutritional value (1.52 g proteins, 3.50 g lipids, 14.39 g carbohydrates, 0.47 g ash, 6.80 g dietary fibre and 95.12 kcal per 100 grams of pulp) and a great diversity of phytochemicals such as carotenoids, tocopherols, folates, some vitamins (Cardoso, Oliveira, Bedetti, Martino, & Pinheiro-Sant'Ana, 2013), and mainly phenolic constituents (Arruda, Pereira, & Pastore, 2017b; Roesler, Catharino, Malta, Eberlin, & Pastore, 2007). The rich profile of nutrients and phytochemicals in the araticum fruit

has attracted growing interest of researchers, consumers and food, cosmetic and pharmaceutical industries.

Recent studies have demonstrated diverse biological properties of extracts from different parts of the araticum fruit, such as the ability to inhibit digestive enzymes and formation glycation products (Justino et al., 2016; Pereira et al., 2017), and antioxidant (Roesler, 2011), antibacterial (Silva et al., 2014) and hepatoprotective (Justino et al., 2017) activities of fruit peel; antiproliferative, anticholinesterase (Formagio et al., 2015), insecticidal (Krinski & Massaroli, 2014), larvicidal (Pimenta, Pinto, Takahashi, Silva, & Boaventura, 2003) and antioxidant (Luzia & Jorge, 2013; Roesler, 2011) properties of seeds; antioxidant (Arruda et al., 2017b) and antibacterial (Silva et al., 2014) capacities of pulp. These biological activities have been associated with the presence of bioactive constituents, mainly phenolics (Justino et al., 2016). Beneficial effects of phenolic compounds have been attributed mainly to their antioxidant activity which can be mediated by multiple mechanisms such as scavenging or reducing reactive species, chelating transition metal ions, inhibiting enzymes involved in oxidative stress, and up-regulating or protecting antioxidant defence (Cheynier, 2012; Dai & Mumper, 2010). There is therefore great interest to discover new sources of natural antioxidants, since they have been used in different industry sectors as dietary supplements, ingredients in functional foods or cosmetics and synthetic antioxidants replacers (Arruda et al., 2017b).

Little information is available related to the profile and contents of phenolic compounds in the araticum fruit. Previous studies have also been performed only with its soluble phenolic compounds (extractable fraction: phenolics that can be extracted using different solvents), ignoring the insoluble-bound phenolics (non-extractable fraction: phenolics that are bound to cell wall polysaccharides or proteins forming insoluble stable complexes). It is therefore likely that to the phenolic constituents of the araticum fruit have been sub-characterized, and the total phenolics content hence their actual contribution on biological activities underestimated. Although the insoluble-bound phenolics are not extracted by organic solvents, when consumed they can undergo the action of intestinal enzymes or colonic microbiota releasing free and more soluble phenolics and metabolites that are then absorbed (Wang et al., 2016). The aim of this study was therefore to investigate more comprehensively the phenolic profile of the araticum fruit. For that, the fruit was fractionated and the phenolic compounds present in the araticum pulp, peel and seed were characterized and quantified by ultra-high performance liquid chromatography coupled with electrospray ionization tandem

mass spectrometry (HPLC-ESI-MS/MS) in terms of their respective free, esterified, glycosylated and insoluble-bound forms. The antioxidant potential of each extract was also evaluated by different antioxidant methods.

2. Material and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2- diphenyl-1-picrylhydrazil (DPPH), 2,2′-azobis(2-methylamidinopropane)-dihydrochloride (AAPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS), fluorescein, Folin-Ciocalteu reagent, methanol and formic acid grade HPLC, quinic acid and all phenolic compound standards (gallic acid, protocatechuic acid, catechin, chlorogenic acid, 4-hydroxybenzoic acid, epicatechin, caffeic acid, gentisic acid, *p*-coumaric acid, sinapic acid, ferulic acid, rutin, quercetin, naringenin, and apigenin) with a purity of ≥96% were purchased from Sigma-Aldrich (St. Louis, USA). The others solvents and reagents used in this study were of analytical grade. All solutions were prepared with ultrapure water (18 MΩ cm^{-1}) obtained from a Milli-Q water purification system (Millipore, Bedford, USA).

2.2. Plant material and sample preparation

The mature araticum fruits (*Annona crassiflora* Mart.) were collected in natural areas of the Cerrado Biome located in the municipality of Carmo do Paranaíba (19°00’03” south latitude, 46°18’58” west longitude and 1061 m altitude), Minas Gerais, Brazil, where the climatic conditions (average temperature and rainfall) during the period of fruit growth (December to March) vary from 18-26°C and 105-245 mm, respectively. A voucher specimen (UEC 197249) has been deposited in the Herbarium of the Institute of Biology of the University of Campinas, Brazil (Herbarium UEC) (**Annex 1**). The morphologically perfect and completely mature fruits were collected during harvest season (March 2016), after naturally falling from the trees. The collection area was divided into three sub-areas and two kilograms of fruits were collected in each sub-area. The fruits were transported from the collection site to the laboratory up to 48 h after collection. The araticum fruits were washed with distilled water and manually separated in three parts (pulp, peel and seeds). Then, the samples were freeze-dried (LIOTOP, model L101, São Carlos, Brazil) and ground using a knife grinder (Marconi, model MA340, Piracicaba, Brazil). The powders obtained were stored at -80°C until analysis.

2.3. Extraction of phenolic fractions

2.3.1. Preparation of crude araticum pulp, peel and seed extracts

Soluble (free, esterified and glycosylated) and insoluble-bound phenolic compounds from the araticum pulp, peel and seed were extracted and fractionated according to the method described by Wang et al. (2016), with slight modifications (**Fig. 1**). Freeze-dried samples (1 g) of araticum fruit (pulp, peel and seed) were extracted with 15 mL of a mixture of methanol-acetone-water (7:7:6, v/v/v) according to Ayoub, de Camargo, and Shahidi (2016). These mixtures were ultrasonicated (UNIQUE, model UCS-2850, 25 kHz, 120 W, Brazil) for 30 minutes at room temperature, and then centrifuged at 4000 *g* for 5 minutes at 5°C (Hettich Zentrifugen, model Rotanta 460R, Tuttlingen, Germany). After centrifugation, the supernatants were collected and the residues were re-extracted two more times under the same conditions. The supernatants were combined and used for the fractionation of soluble phenolic compounds in three fractions (free, esterified and glycosylated), and the solid residue was stored for determination of insoluble-bound phenolic compounds.

2.3.2. Obtaining the free phenolic compounds

Free phenolic compounds were obtained according to the method described by Wang et al. (2016). The combined supernatants obtained as previously described were evaporated under vacuum at 35°C (Rotavapor model RII, Büchi Labortechnik, Flawil, Switzerland) to remove the organic solvents, and the aqueous phase was acidified to pH 2 using 6 M HCl and centrifuged (4000 *g*, 5 minutes, 5°C) to remove any precipitates. Then, the clear supernatant was extracted 3 times with an equal volume of hexane to remove interfering lipids. The free phenolic compounds were extracted 3 times with diethyl ether-ethyl acetate (1:1, v/v) at a solvent to aqueous phase ratio of 1:1 (v/v). The organic phases were combined, dehydrated and filtered through anhydrous sodium sulphate using a No. 1 Whatman filter paper, and evaporated to dryness under vacuum at 35°C. The dry residues (free phenolic compounds fraction) were dissolved in 5 mL of HPLC grade methanol.

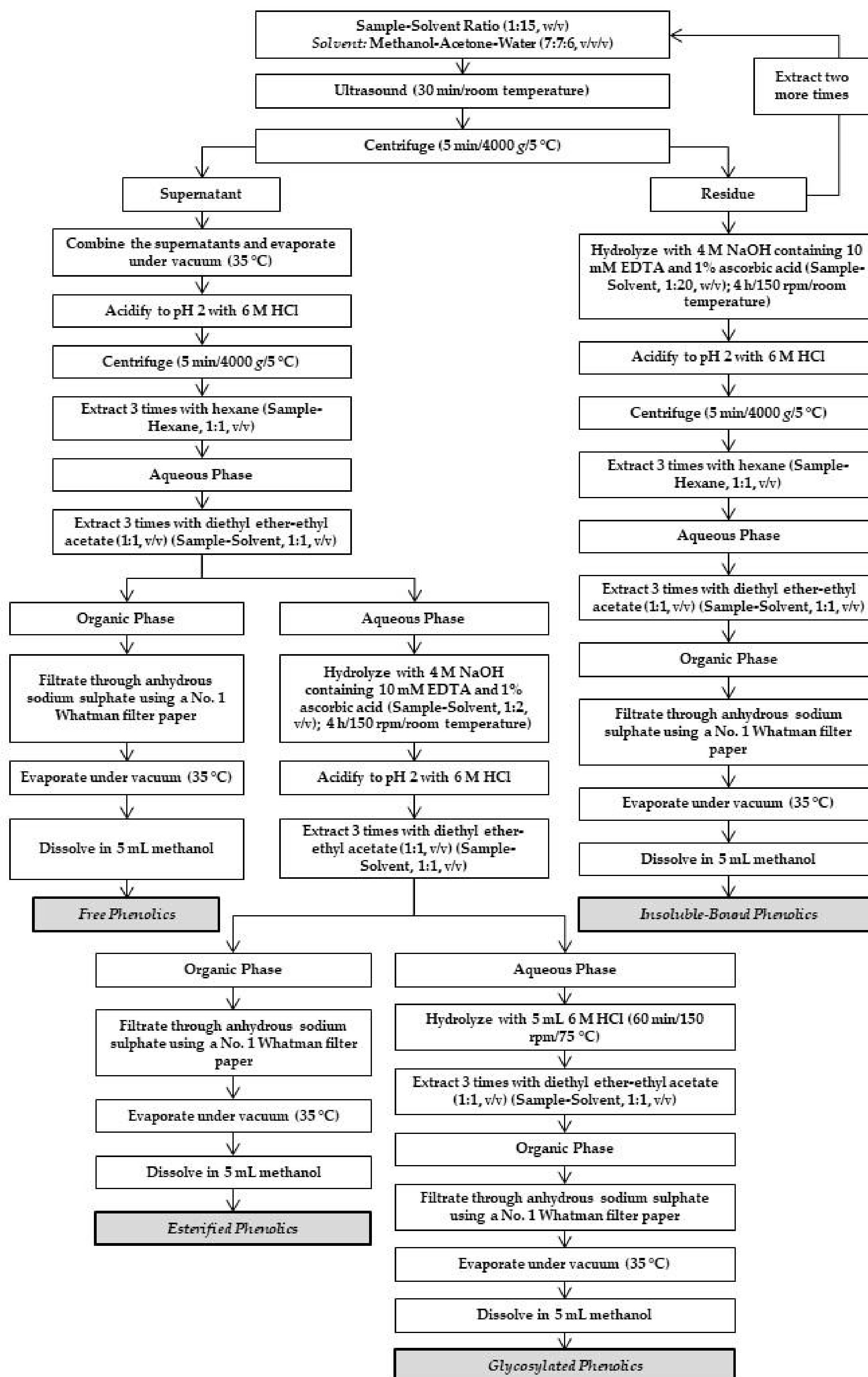

Fig. 1. Flowchart describing the procedure adopted for extraction and fractionation of phenolic compounds from araticum pulp, peel and seed.

2.3.3. Obtaining the soluble esters of phenolic compounds

Soluble esters of phenolic compounds were obtained according to the method described by Wang et al. (2016). The remaining aqueous phase after the free phenolics extraction was hydrolysed with 4 M NaOH containing 10 mM EDTA and 1% ascorbic acid (aqueous phase to solvent ratio of 1:2, v/v) for 4 h at room temperature using a water bath shaker (150 rpm) (New Brunswick Scientific Classic Series, model C76, NJ, USA) to release esterified phenolics. The pH of the hydrolysate was adjusted to 2 using 6 M HCl and the phenolic compounds released from soluble esters were extracted 3 times as described for free phenolics.

2.3.4. Obtaining the soluble glycosides of phenolic compounds

Soluble glycosides of phenolic compounds were obtained according to the method described by Wang et al. (2016). The remaining aqueous phase after the esterified phenolics extraction was hydrolysed with 5 mL 6 M HCl for 60 minutes at 75°C using a water bath shaker (150 rpm) to release glycosylated phenolics. Phenolic compounds released from soluble glycosides were extracted 3 times as described for free phenolics.

2.3.5. Obtaining the insoluble-bound phenolic compounds

Insoluble-bound phenolic compounds were obtained according to the method described by Wang et al. (2016). The solid residue obtained after the soluble phenolics extraction was hydrolysed with 4 M NaOH containing 10 mM EDTA and 1% ascorbic acid at a solid to solvent ratio of 1:20 (w/v) for 4 h at room temperature using a water bath shaker (150 rpm) to release insoluble-bound phenolics. The pH of the mixture was adjusted to 2 using 6 M HCl and centrifuged at 4000 *g* for 5 minutes at 5°C. Then, the supernatant was extracted 3 times with an equal volume of hexane to remove interfering lipids. Phenolic compounds released from insoluble-bound phenolics were extracted 3 times as described for free phenolics.

2.4. Determination of total phenolic content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu colorimetric method according to Singleton and Rossi (1965), with slight modifications as described by Arruda et al. (2017b). The diluted extract (100 µL) was mixed with 100 µL of 50% (v/v) Folin-

Ciocalteu reagent in a microtube, and 800 µL of 5% (w/v) sodium carbonate were added to each microtube. The mixtures were allowed to stand for 20 minutes at room temperature and the absorbance was measured at 760 nm against a blank on a spectrophotometer (Beckman, model DU600, CA, USA). Gallic acid was used for the standard curve and the results were expressed as mg gallic acid equivalents per gram of freeze-dried plant material (mg GAE/g fdw).

2.5. Determination of total flavonoid content (TFC)

Total flavonoid content was determined using the aluminium chloride colorimetric assay as described by Zhishen, Mengcheng, and Jianming (1999), with slight modifications (Arruda et al., 2017b). In a microtube, 100 µL of diluted extract was mixed with 400 µL of ultrapure water, and 30 µL of 5% (w/v) $NaNO_2$ were added to each microtube. After 5 minutes, 30 µL of 10% (w/v) $AlCl_3$ were added to the reaction mixture and allowed to stand for 6 minutes. Finally, 200 µL of 1 M NaOH and 240 µL of ultrapure water were added and mixed, and the absorbance was measured at 510 nm against a blank on a spectrophotometer. Total flavonoid content was calculated using a standard curve prepared for catechin and the results were expressed as mg catechin equivalents per gram of freeze-dried plant material (mg CE/g fdw).

2.6. Determination of condensed tannin (proanthocyanidin) content (CTC)

Condensed tannin content was determined according to method described by Julkunen-Tiitto (1985), with slight modifications. Briefly, 30 µL of diluted extract, 900 µL of 4% (w/v) vanillin prepared in methanol and 450 µL of concentred HCl were mixed and incubated at room temperature for 20 minutes. Then, the absorbance was measured at 500 nm against a blank on a spectrophotometer. Catechin was used to make the standard curve and the results were expressed as mg catechin equivalents per gram of freeze-dried plant material (mg CE/g fdw).

2.7. Antioxidant activity assays

2.7.1. DPPH scavenging assay

The antioxidant activity by DPPH scavenging assay was determined according to Brand-Williams, Cuvelier, and Berset (1995), with slight modifications (Arruda et al., 2017b).

An aliquot (200 μL) of diluted extract was mixed with 1 mL of 0.004% (w/v) DPPH in ethanol. The reaction mixture was kept at room temperature for 30 minutes, and the absorbance of the remaining DPPH was measured at 517 nm against a blank on a spectrophotometer. The results were expressed as micromoles of Trolox equivalents per gram of freeze-dried plant material (μmol TE/g fdw).

2.7.2. Trolox equivalent antioxidant capacity (TEAC) assay

The Trolox equivalent antioxidant capacity of araticum extracts was determined based on the methods described by Re et al. (1999) and modified by Arruda et al. (2017b). The radical cation $ABTS^{\bullet+}$ was prepared overnight in the dark mixing 5 mL of 7 mM ABTS solution and 88 μL of a 145 mM potassium persulfate solution. Subsequently, the radical cation $ABTS^{\bullet+}$ was diluted with ultrapure water until reaching an absorbance of 0.70±0.02 at 734 nm. Phenolic extracts (200 μL) were mixed with 1 mL of $ABTS^{\bullet+}$ solution and the reaction mixture was allowed to stand at room temperature for 6 minutes. The absorbance of the remaining $ABTS^{\bullet+}$ was measured at 734 nm against a blank on a spectrophotometer. The results were expressed as micromoles of Trolox equivalents per gram of freeze-dried plant material (μmol TE/g fdw).

2.7.3. Oxygen radical absorbance capacity (ORACFL) assay

The $ORAC_{FL}$ assay was carried out on the basis of the method described by Dávalos, Gómez-Cordovés, and Bartolomé (2004). In a 96 well dark microplate, 20 μL of diluted extract, blank or Trolox, 120 μL of fluorescein in potassium phosphate buffer (0.378 μg/mL, pH 7.4) and 60 μL of AAPH (108 mg/mL) were mixed. The fluorescence was determined and recorded every minute for 80 minutes at 37°C on a NovoStar Microplate reader (New Brunswick Scientific Classic Series, model C76, NJ, USA) with fluorescence filters (excitation and emission wavelengths of 485 and 520 nm, respectively). Trolox were used to prepare the standard curve and the results were expressed as micromoles of Trolox equivalents per gram of freeze-dried plant material (μmol TE/g fdw).

2.8. HPLC-ESI-MS/MS analysis of the phenolic compounds

The phenolic compounds of araticum fruit extracts were characterized and quantified using an HPLC system connected to a triple quadrupole mass spectrometer (LCMS

8040; Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source according to the method described by Bataglion, da Silva, Eberlin, and Koolen (2015) with slight modifications. Chromatographic separation of phenolic compounds was carried out on a Shim-pack XR-ODS III column (150 × 2.0 mm i.d., particle size 2.2 μm, Shimadzu, Kyoto, Japan) in a thermostated oven at 40°C. The autosampler temperature was maintained at 10°C and the injection volume was 10 μL. Mobile phases consisted of 0.1% formic acid in water (eluent A) and methanol (eluent B) at a flow rate of 0.40 mL/min. The elution conditions were as follows: 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 ESI source was operated in the negative ion mode with the following main parameters: capillary voltage of 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) at 224 kPa. For each standard, the deprotonated molecule $[M-H]^-$ was used as the precursor ion for the MS/MS scans and the two most abundant product ions were chosen for quantification and confirmation via multiple reaction monitoring (MRM). MRM events (**Table 1**) were optimized using Labsolution software.

Phenolic compounds were characterized by comparison of their HPLC-ESI(-)-MS/MS dissociation patterns and retention time with those of authentic standards. The quantification of the characterized phenolic compounds was performed by comparison with the calibration curve of each standard. Stock solutions of each standard compound (1 mg/mL) were prepared in methanol and stored. An intermediate solution containing all standards (1.25 μg/mL) was prepared in 0.1% formic acid in water and dilutions from this solution were done at 9 different levels for calibration curves (20-1000 ng/mL). As the food matrix affects the ionization of analytes, mainly when working with electrospray ionization, the calibration curves were prepared a synthetic matrix (sugars mixture: glucose, fructose, arabinose, xylose and sucrose at final concentration of 20 ng/mL of each sugar). Data were acquired and processed by Labsolution software (version 5.53 SP2, Shimadzu). Results were expressed as μg per gram of freeze-dried plant material (μg/g fdw).

Table 1. Mass spectrometry parameters for MRM transitions (Q1 Pre Bias voltage, Q3 Pre Bias voltage and collision energy (CE)) and analytical parameters of the quantification method (retention time (r.t.), linearity (r), slope, intercept, limit of detection (LOD) and limit of quantification (LOQ)) for the analysed compounds.

Code	Compound	Transitions (m/z)*	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)	r.t. (min)	r	Slope	Intercept	LOD (ng/mL)	LOQ (ng/mL)
1	Quinic acid	191.10>84.90	20	22	30	1.05	0.9990	76.15	-16.14	0.530	1.768
		191.10>92.90	12	27	27						
2	Gallic acid	169.00>125.05	30	17	19	2.85	0.9969	91.64	-72.51	0.595	1.984
		169.00>78.95	30	21	29						
3	Protocatechuic acid	153.00>109.00	30	15	17	3.72	0.9981	322.22	224.90	0.803	2.677
		153.00>108.15	30	30	18						
4	Catechin	289.10>151.00	18	22	24	3.99	0.9990	18.18	0.09	1.504	5.013
		289.10>123.00	19	27	28						
5	Chlorogenic acid	353.10>191.10	16	20	18	4.22	0.9996	384.56	65.91	0.373	1.243
		353.10>85.15	16	39	27						
6	4-Hydroxybenzoic acid	137.00>93.10	26	16	14	4.33	0.9966	1076.33	942.99	0.087	0.291
		137.00>65.05	26	28	23						
7	Epicatechin	289.10>244.90	21	13	24	4.37	0.9972	40.24	-30.94	1.583	5.277
		289.10>108.90	13	23	15						
8	Caffeic acid	179.10>135.05	11	15	24	4.48	0.9988	631.95	326.90	0.426	1.418
		179.10>134.10	11	25	23						
9	Gentisic acid	153.00>108.10	29	23	17	4.58	0.9963	81.25	-71.40	0.792	2.641
		153.00>109.10	29	15	19						
10	*p*-Coumaric acid	163.10>119.10	10	14	21	4.89	0.9975	1220.34	912.27	0.146	0.486
		163.10>93.10	10	34	14						
11	Sinapic acid	223.00>208.05	25	14	20	4.95	0.9993	183.98	43.45	0.695	2.318
		223.00>164.00	25	16	28						
12	Ferulic acid	193.10>134.00	12	17	21	4.95	0.9995	208.94	46.11	0.516	1.720
		193.10>178.00	12	11	30						
13	Rutin	609.00>299.95	28	48	28	5.05	0.9996	196.89	17.52	0.282	0.940
		609.00>301.05	28	35	28						
14	Quercetin	301.00>151.00	20	23	24	5.82	0.9984	913.93	187.11	0.054	0.180
		301.00>178.95	20	19	30						
15	Naringenin	270.90>151.00	18	17	29	5.92	0.9986	617.27	313.09	0.192	0.641
		270.90>119.10	18	29	18						
16	Apigenin	269.00>117.10	18	34	20	6.59	0.9964	667.56	937.84	1.017	3.390
		269.00>149.00	18	25	13						

*First transition used for quantitation. Second transition used for identification.

2.9. Statistical analysis

The results are subjected to one-way analysis of variance (ANOVA) and differences between means were located using Tukey's HSD test. Correlation analyses between phenolics and antioxidant activity were carried out using standard Pearson correlation. All statistical analyses were performed at a significance level of 5% ($p \leq 0.05$) using STATISTICA software (Statsoft, Oklahoma, USA) version 12.0. The data are reported as mean values ± standard deviation of three replicates.

3. Results and discussion

3.1. Total phenolic content (TPC)

The total free, esterified, glycosylated and insoluble-bound phenolics were determined for the first time in araticum fruit. TPC of araticum fruit extracts ranged from 1.20-18.20 mg GAE/g fdw (**Table 2**). Among all extracts, araticum peel esterified extract showed the highest TPC (18.20 mg GAE/g fdw), whereas araticum seed esterified (1.20 mg GAE/g fdw) and pulp free (1.41 mg GAE/g fdw) extracts had the lowest TPC. In general, the TPC was higher in the fractions containing esterified or insoluble-bound phenolics. Araticum pulp had higher TPC in its insoluble-bound form (9.04 mg GAE/g fdw), whereas in araticum peel the highest TPC value was found in its esterified fraction (18.20 mg GAE/g fdw), and in araticum seed the TPC was predominant in free form (7.31 mg GAE/g fdw). In all araticum fruit parts, soluble phenolic content (sum of free, esterified and glycosylated fractions) was higher than of the insoluble-bound fraction. These results are according to a previous study conducted with common fruits, in which the soluble phenolic fraction also comprised most of phenolics in these samples (62-96%) (Sun, Chu, Wu, & Liu, 2002). Although phenolics in fruits are mainly in soluble form, the phenolics extracted from the insoluble-bound fraction accounted for 19.94, 20.74 and 44.12% of TPC in araticum peel, seed and pulp, respectively. These findings demonstrate that the contents of insoluble-bound phenolics remaining in the samples were not negligible, especially from araticum pulp (44.12%).

Table 2. Total contents of phenolics, flavonoids and condensed tannins, and antioxidant activity by DPPH, TEAC and ORACFL methods in four phenolic fractions of araticum pulp, peel and seed.

Assay	Araticum Pulp				
	Free	**Esterified**	**Glycosylated**	**Insoluble-bound**	**Total**
Total phenolics (mg GAE/g fdw)	1.41±0.00 d	7.12±0.03 b	2.93±0.04 c	9.04±0.04 a	20.49±0.08
Total flavonoids (mg CE/g fdw)	1.22±0.02 c	4.42±0.12 b	1.11±0.03 c	6.76±0.08 a	13.51±0.04
Condensed tannins (mg CE/g dw)	1.65±0.05 d	4.33±0.07 b	1.83±0.00 c	5.87±0.03 a	13.67±0.15
DPPH (µmol TE/g fdw)	5.87±0.07 d	53.85±0.64 b	17.44±0.08 c	55.57±0.33 a	132.73±0.73
TEAC (µmol TE/g fdw)	12.83±0.13 d	77.58±0.33 b	24.89±0.12 c	99.07±0.72 a	214.38±1.05
$ORAC_{FL}$ (µmol TE/g fdw)	24.68±0.15 d	71.08±1.36 b	44.31±1.04 c	119.19±1.56 a	259.26±3.64
Assay	**Araticum Peel**				
	Free	**Esterified**	**Glycosylated**	**Insoluble-bound**	**Total**
Total phenolics (mg GAE/g fdw)	1.79±0.05 d	18.20±0.41 a	5.35±0.14 c	6.31±0.06 b	31.65±0.32
Total flavonoids (mg CE/g fdw)	1.68±0.05 d	13.29±0.30 a	2.51±0.08 c	4.57±0.09 b	22.05±0.37
Condensed tannins (mg CE/g fdw)	2.01±0.03 c	11.82±0.08 a	1.06±0.01 d	3.06±0.07 b	17.96±0.05
DPPH (µmol TE/g fdw)	11.65±0.14 d	101.55±2.39 a	34.75±0.10 c	41.37±0.42 b	189.32±2.85
TEAC (µmol TE/g fdw)	19.79±0.08 d	163.32±1.36 a	46.14±0.23 c	63.03±0.66 b	292.28±0.85
$ORAC_{FL}$ (µmol TE/g fdw)	40.89±2.34 d	221.72±4.96 a	68.39±2.38 c	117.28±3.73 b	448.29±10.63
Assay	**Araticum Seed**				
	Free	**Esterified**	**Glycosylated**	**Insoluble-bound**	**Total**
Total phenolics (mg GAE/g fdw)	7.31±0.04 a	1.20±0.03 d	1.53±0.06 c	2.63±0.01 b	12.68±0.08
Total flavonoids (mg CE/g fdw)	5.30±0.05 a	0.27±0.01 c	0.15±0.00 d	1.61±0.01 b	7.33±0.06
Condensed tannins (mg CE/g dw)	0.40±0.01 c	1.06±0.02 a	0.52±0.01 b	1.10±0.04 a	3.08±0.05
DPPH (µmol TE/g fdw)	33.66±0.19 a	10.41±0.02 d	12.88±0.04 c	14.71±0.08 b	71.66±0.30
TEAC (µmol TE/g fdw)	48.52±0.76 a	10.34±0.12 d	13.97±0.07 c	21.99±0.05 b	94.81±0.82
$ORAC_{FL}$ (µmol TE/g fdw)	169.19±7.80 a	14.71±1.08 c	16.93±0.41 c	59.39±2.61 b	260.22±9.32

Data represent mean values for each sample ± standard deviations ($n = 3$). Means followed by the same lowercase letters in a row are not significantly different ($p > 0.05$).

The localization of a phenolic compound within a tissue reflects its physiological role for the plant. The plants often store the phenolic compounds at strategically important sites

where they act as a signalling or in plant defence mechanisms (Ewané, Lepoivre, De Lapeyre de Bellaire, & Lassois, 2012). Phenolic compounds from araticum fruit were localized primarily in its peel (48.83%), demonstrating their role in fruit defence against adverse biotic (*e.g.*, pathogens, insects and herbivores attack) and abiotic (*e.g.*, UV radiation and temperature) factors. The total phenolic content (sum of free, esterified, glycosylated and insoluble-bound phenolics) of araticum fruit parts followed the decreasing order of peel (31.65 mg GAE/g fdw) > pulp (20.49 mg GAE/g fdw) > seed (12.68 mg GAE/g fdw). These findings lead support to the results obtained using HPLC-ESI-MS/MS (**Table 3**), where the peel contained the highest total phenolics, followed by pulp and seed. Previous studies also reported high TPC values in araticum pulp (Arruda et al., 2017b), peel (Justino et al., 2016; Roesler et al., 2007) and seed (Roesler et al., 2007). Roesler et al. (2007) reported distinct distribution trend of TPC in crude extracts of araticum fruit parts compared to our data (seed > peel > pulp). This discrepancy can be due to extraction method employed, extraction conditions, extraction solvent, among others (Arruda et al., 2017b; Chen, Zhang, Chen, Han, & Gao, 2017). In addition, non-phenolic reducing substances like sugars, amino acids, vitamin C, and other organic acids may be present in crude extracts and could also react with Folin-Ciocalteu reagent, leading to discrepancies (Abeywickrama, Debnath, Ambigaipalan, & Shahidi, 2016).

3.2. Total flavonoid (TFC) and condensed tannins (proanthocyanidins) (CTC) contents

Araticum fruit extracts showed TFC and CTC values ranging from 0.15-13.29 and 0.40-11.82 mg CE/g fdw, respectively (**Table 2**). TFC and CTC exhibited the same trend as that observed for TPC. In general, soluble TFC and CTC were higher than that found for the fraction containing insoluble-bound phenolics. Araticum pulp had higher TFC and CTC in its insoluble-bound form, whereas in araticum peel the highest TFC and CTC values were found in its esterified fraction. Araticum seed had higher TFC in its free form, and similar amounts of CTC in the insoluble-bound and esterified forms. Among all extracts, araticum peel esterified extract showed the highest TFC and CTC followed by araticum pulp insoluble-bound extract, whereas araticum seed glycosylated and free extracts had the lowest TFC (0.15 mg CE/g fdw) and CTC (0.40 mg CE/g fdw), respectively. Generally, the higher TFC and CTC contents were found in the insoluble-bound or esterified phenolic fractions. These findings are accordance with the HPLC-ESI-MS/MS data (**Table 3**), where the insoluble-bound fraction contained the highest total flavonoids value in araticum pulp and seed, whereas araticum peel presented the highest total flavonoids content in the esterified fraction.

Table 3. Content of phenolic compounds in four phenolic fractions of araticum pulp, peel and seed.

Class	Sub-class	Compound	Concentration (µg/g fdw)				
			Araticum Pulp				
			Free	**Esterified**	**Glycosylated**	**Insoluble-bound**	**Total**
Flavonoids	Flavanols	Catechin	26.87±0.56 Dc	336.01±7.45 Ab	n.d.	405.54±3.41 Aa	768.42±4.42 A
		Epicatechin	307.38±2.55 Aa	115.11±2.95 Bc	n.d.	239.32±9.55 Bb	661.81±11.93 B
		ΣFlavanols	334.24±2.51 c[1]	451.12±10.00 b[1]	n.d.	644.86±11.98 a[1]	1430.23±12.70 [1]
	Flavonols	Rutin	4.09±0.10 Eb	5.21±0.19 Ea	n.d.	n.d.	9.31±0.29 F
		Quercetin	0.25±0.01 Fd	3.80±0.11 Ea	2.12±0.04 Db	1.62±0.02 Dc	7.80±0.11 F
		ΣFlavonols	4.34±0.11 b[3]	9.02±0.29 a[4]	2.12±0.04 c[3]	1.62±0.02 d[3]	17.11±0.37 [4]
		ΣFlavonoids	338.59±2.42 c	460.14±9.87 b	2.12±0.04 d	646.49±11.96 a	1447.34±12.33
Phenolic acids	Hydroxybenzoic acids	Protocatechuic acid	1.28±0.03 Fd	25.36±0.34 Db	8.38±0.44 Cc	62.89±0.36 Ca	97.92±0.16 D
		Gentisic acid	n.d.	1.20±0.25 Eb	11.70±0.52 Ba	1.10±0.40 Db	14.00±0.43 F
		ΣHydroxybenzoic acids	1.28±0.03 d[3]	26.57±0.23 b[3]	20.08±0.08 c[2]	63.98±0.39 a[2]	111.92±0.59 [3]
	Hydroxycinnamic acids	Chlorogenic acid	43.45±1.24 B	n.d.	n.d.	n.d.	43.45±1.24 E
		Caffeic acid	35.00±0.52 Cb	63.28±0.31 Ca	22.85±0.13 Ac	3.18±0.10 Dd	124.31±0.94 C
		p-Coumaric acid	5.25±0.15 Eb	5.84±0.07 Ea	0.28±0.02 Ec	0.48±0.02 Dc	11.86±0.04 F
		Ferulic acid	24.82±0.06 Da	24.60±1.04 Da	2.19±0.09 Db	2.10±0.10 Db	53.71±1.06 E
		ΣHydroxycinnamic acids	108.52±1.70 a[2]	93.72±1.20 b[2]	25.32±0.22 c[1]	5.76±0.03 d[3]	233.32±2.27 [2]
		ΣPhenolic acids	109.81±1.71 b	120.28±1.40 a	45.40±0.27 d	69.75±0.41 c	345.24±2.07
		ΣPhenolic compounds	448.39±4.05 c	580.42±9.04 b	47.53±0.25 c	716.23±11.94 d	1792.58±14.05
Class	**Sub-class**	**Compound**	**Araticum Peel**				
			Free	**Esterified**	**Glycosylated**	**Insoluble-bound**	**Total**
Organic acid	-	Quinic acid	1.27±0.09 D	n.d.	n.d.	n.d.	1.27±0.09 G
Flavonoids	Flavanols	Catechin	31.58±1.03 Bc	2682.85±31.30 Aa	n.d.	812.36±16.03 Ab	3526.78±15.45 A
		Epicatechin	298.53±7.78 Ac	1007.05±12.12 Ba	n.d.	327.31±7.85 Bb	1632.90±23.03 B
		ΣFlavanols	330.11±7.84 c[1]	3689.90±28.33 a[1]	n.d.	1139.67±15.07 b[1]	5159.68±26.38 [1]
	Flavonols	Rutin	3.33±0.08 Db	4.27±0.42 Da	n.d.	0.71±0.17 Fc	8.30±0.41 G
		Quercetin	0.20±0.03 Dd	10.79±0.12 Da	10.20±0.26 Cb	0.65±0.02 Fc	21.83±0.31 FG
		ΣFlavonols	3.52±0.07 c[3]	15.06±0.52 a[3]	10.20±0.26 b[3]	1.36±0.17 d[4]	30.14±0.64 [4]
		ΣFlavonoids	333.63±7.89 c	3704.96±28.05 a	10.20±0.26 d	1141.03±15.02 b	5189.82±26.95
Phenolic acids	Hydroxybenzoic acids	Protocatechuic acid	2.85±0.01 Dd	163.07±5.33 Ca	26.93±1.65 Ac	125.06±0.93 Cb	317.90±7.85 C

		Gentisic acid	n.d.	n.d.	7.36±0.09 Da	1.44±0.29 Fb	8.80±0.36 G
		ΣHydroxybenzoic acids	2.85±0.01 d^{3}	163.07±5.33 a^{2}	34.29±1.57 c^{1}	126.51±0.66 b^{2}	326.71±7.50 ^{2}
	Hydroxycinnamic acids	Chlorogenic acid	13.24±0.39 C	n.d.	n.d.	n.d.	13.24±0.39 G
		Caffeic acid	25.59±0.57 Ba	23.25±0.62 Db	22.01±0.67 Bb	22.60±0.75 Eb	93.45±1.43 D
		p-Coumaric acid	1.37±0.06 Dc	4.46±0.05 Db	0.31±0.02 Ed	40.76±0.57 Da	46.90±0.56 EF
		Ferulic acid	12.31±0.43 Cc	15.52±1.62 Db	0.87±0.02 Ed	36.70±1.38 DEa	65.40±2.08 E
		ΣHydroxycinnamic acids	52.51±1.05 b^{2}	43.23±1.04 c^{3}	23.20±0.70 d^{2}	100.06±1.49 a^{3}	219.00±0.52 ^{3}
		ΣPhenolic acids	55.36±1.06 c	206.30±5.32 b	57.49±2.09 c	226.57±1.43 a	545.71±7.19
		ΣPhenolic compounds	388.99±8.68 c	3911.26±23.08 a	67.68±1.85 d	1367.60±13.74 b	5735.52±24.32

Class	**Sub-class**	**Compound**	**Araticum Seed**				
			Free	**Esterified**	**Glycosylated**	**Insoluble-bound**	**Total**
Flavonoids	Flavanols	Catechin	1.55±0.12 DEb	n.d.	n.d.	32.05±0.49 Aa	33.60±0.48 B
		Epicatechin	11.55±0.67 Bb	n.d.	n.d.	13.93±1.21 Ba	25.48±0.70 C
		ΣFlavanols	13.09±0.57 b^{2}	n.d.	n.d.	45.98±0.76 a^{1}	59.07±0.23 ^{2}
	Flavonols	Rutin	0.73±0.07 Fb	1.08±0.13 Da	n.d.	n.d.	1.81±0.05 H
		Quercetin	n.d.	0.04±0.01 Fb	1.22±0.04 Ca	0.09±0.00 Eb	1.35±0.04 H
		ΣFlavonols	0.73±0.07 b^{4}	1.12±0.12 a^{3}	1.22±0.04 a^{3}	0.09±0.00 c^{4}	3.16±0.09 ^{4}
		ΣFlavonoids	13.82±0.52 b	1.12±0.12 c	1.22±0.04 c	46.07±0.76 a	62.23±0.15
Phenolic acids	Hydroxybenzoic acids	Protocatechuic acid	2.12±0.13 Dc	3.03±0.25 Cb	2.17±0.09 Bbc	15.12±0.60 Ba	22.44±0.52 D
		Gentisic acid	n.d.	n.d.	0.94±0.37 Cb	2.33±0.16 Da	3.28±0.22 G
		ΣHydroxybenzoic acids	2.12±0.13 b^{3}	3.03±0.25 b^{2}	3.11±0.46 b^{2}	17.45±0.62 a^{2}	25.72±0.66 ^{3}
	Hydroxycinnamic acids	Chlorogenic acid	11.00±0.12 B	n.d.	n.d.	n.d.	11.00±0.12 F
		Caffeic acid	14.88±0.15 Ab	15.69±0.09 Aa	8.26±0.32 Ac	6.54±0.17 Cd	45.36±0.22 A
		p-Coumaric acid	1.06±0.06 EFb	0.55±0.01 Ec	n.d.	1.78±0.07 Da	3.40±0.05 G
		Ferulic acid	5.19±0.13 Cb	4.57±0.09 Bc	1.00±0.07 Cd	6.18±0.15 Ca	16.95±0.26 E
		ΣHydroxycinnamic acids	32.13±0.31 a^{1}	20.82±0.09 b^{1}	9.26±0.33 c^{1}	14.50±0.18 d^{3}	76.71±0.14 ^{1}
		ΣPhenolic acids	34.25±0.43 a	23.85±0.33 c	12.37±0.75 d	31.96±0.62 b	102.43±0.74
		ΣPhenolic compounds	48.07±0.11 b	24.97±0.44 c	13.60±0.76 d	78.02±1.31 a	164.66±0.89

Data represent mean values for each sample ± standard deviations ($n = 3$). Means followed by the same capital letters within a column part are not significantly different ($p > 0.05$) within the same sample for individual phenolic content. Means followed by the same lowercase letters in a row are not significantly different ($p > 0.05$). Means followed by the same superscript numbers within a column are not significantly different ($p > 0.05$) within the same sample for sub-class phenolics. n.d.: not detected.

Araticum peel showed the highest total flavonoids and condensed tannins contents (sum of phenolic fractions), followed by pulp and seed, respectively (**Table 2**). In agreement with this finding, Justino et al. (2016) also reported high TFC and CTC in araticum peel extracts, besides, this study demonstrated that flavonoids (catechin, epicatechin, quercetin-3-glucoside, kaempferol-3-*O*-rutinoside and kaempferol-7-*O*-glucoside) and tannins (procyanidins B2 and C1) were the main phenolics identified in peel extracts (7 of 10 phenolics identified). In addition, most of the flavonoids and condensed tannins of araticum fruit were found in its peel (51.41 and 51.74%, respectively). This trend is expected, since flavonoids and tannins participate in plant defence against UV radiation and herbivores, fungi and viruses attack (Cheynier, 2012). These compounds tend therefore to concentrate on the outermost layer of the fruit.

3.3. Antioxidant activity assays

Phenolic compounds display several functional properties, mainly due to their antioxidant capacity, acting as reducing agents, hydrogen donors, transition metal chelators, reactive oxygen and/or nitrogen species (ROS/RNS) quenchers, inhibitors of enzymes involved in oxidative stress, and upregulators and/or protectors of endogenous defence systems (Arruda et al., 2017b; Cheynier, 2012; Dai & Mumper, 2010). Considering this variety of antioxidant mechanisms, the antioxidant activities of the araticum extracts were determined using several assays (**Table 2**), including DPPH, TEAC and $ORAC_{FL}$.

Antioxidant activities from araticum fruit extracts by the DPPH, TEAC and $ORAC_{FL}$ assays ranged from 5.87-101.55, 10.34-163.32 and 14.71-221.72 µmol TE/g fdw, respectively. Overall, araticum extracts having higher antioxidant activities were supportive to the results for TPC, TFC, CTC and total phenolics by HPLC-ESI-MS/MS. In all araticum fruit parts, antioxidant activities of soluble phenolic fraction were higher than those found for the fraction containing insoluble-bound phenolics. Antioxidant activities from araticum pulp were also mainly due to its insoluble-bound phenolic fraction, whereas in the araticum peel the esterified phenolic fraction displayed the highest antioxidant values. On the other hand, free fraction exhibited the highest antioxidant powers in araticum seed. Among all extracts, the fraction containing esterified phenolics from araticum peel had the highest antioxidant activities (up to 17.30-, 15.79- and 15.07-fold higher than DPPH, TEAC and $ORAC_{FL}$ values found for remaining fractions, respectively), while esterified and glycosylated fractions from araticum seed were the least antioxidants. In general, it can be concluded that insoluble-bound and

esterified phenolics are the dominant forms of phenolics from araticum fruit parts and the highest contributors to their antioxidant activities.

Combining the four phenolic fractions, the total antioxidant activities of the araticum fruit parts were in the following order: peel > pulp > seed. These results are also supported by the HPLC-ESI-MS/MS data (**Table 3**), in which extracts with higher phenolics content showed the highest antioxidant activities, thus confirming that phenolics were the major contributors to the antioxidant activities from araticum fruit extracts. Phenolics from araticum fruit parts also proved to be potent antioxidants: seed (71.66, 94.81 and 260.22 µmol TE/g fdw for the results from total DPPH, TEAC and $ORAC_{FL}$, respectively), pulp (132.73, 214.38 and 259.26 µmol TE/g fdw for the results from total DPPH, TEAC and $ORAC_{FL}$, respectively) and peel (189.32, 292.28 and 448.29 µmol TE/g fdw for the results from total DPPH, TEAC and $ORAC_{FL}$, respectively). Previous studies have also demonstrated strong antioxidant activities for araticum pulp (Arruda et al., 2017b), peel (Justino et al., 2016; Roesler et al., 2007) and seed extracts (Roesler et al., 2007).

Phenolics act as antioxidants by deactivating free radicals through two main mechanisms: hydrogen atom transfer (ability to quench free radicals by hydrogen donation) and single electron transfer (ability to transfer one electron to reduce any compound, including radicals, metals and carbonyls) (Prior, Wu, & Schaich, 2005). The araticum extracts were also found to show the highest antioxidant activity for the $ORAC_{FL}$ assay (except for esterified phenolics in pulp), followed by TEAC and DPPH. $ORAC_{FL}$ assay measures the capacity of a compound to inhibit peroxyl radicals (Gülçin, 2012). Peroxyl radicals are highly reactive species that may cause damage to cellular components, such as DNA, proteins and polyunsaturated fatty acids in the membranes. Thus, these radicals are involved in the generation and/or aggravation of several pathological conditions like cancer development, inflammatory processes, and heart disease (Ayoub et al., 2016). The effective capacity of the araticum fruit parts as peroxyl radicals scavengers found here points to their potential to prevent and/or treat oxidative-related diseases.

3.4. Characterization and quantification of phenolic compounds from araticum pulp, peel and seed by HPLC-ESI-MS/MS

The chromatographic and mass spectrometry parameters were optimized to ensure a good peaks resolution and the best isomers separation (**Table 1**). The method exhibited very

good linearity with R^2 values above 0.99, as well as the limits of detection (LOD = $3s/S$; s is the standard deviation of the intercept and S is the calibration curve slope) and quantification (LOQ = $10s/S$). The HPLC-ESI-MS/MS method provided indeed low LODs and LOQs for all compounds analysed with values ranging from 0.054-1.583 and 0.180-5.277 ng/mL, respectively. After the optimization, the HPLC-ESI-MS/MS method was used to characterize and quantify the predominant phenolic compounds present in araticum samples (**Table 3**).

To the best of our knowledge, this is the first study reporting the characterization and quantification of phenolic compounds in the araticum fruit including its pulp, seed and peel in terms of free, esterified, glycosylated and insoluble-bound phenolic profiles. A total of 10 phenolic compounds were characterized and quantified in araticum extract fractions, including 2 flavanols (catechin and epicatechin), 2 flavonols (rutin and quercetin), 2 hydroxybenzoic acids (protocatechuic and gentisic acids), and 4 hydroxycinnamic acids (chlorogenic, caffeic, *p*-coumaric and ferulic acids). Our findings show that the profiles and contents of phenolic compounds are quite contrasting depending on the araticum fruit part as well as the specific phenolic fraction considered.

Insoluble-bound phenolics were the main phenolic fraction in araticum pulp (39.96%) and seed (47.38%), whereas esterified phenolics accounted for most of the araticum peel phenolics (68.19%). In addition, glycosylated phenolics were the minority fraction in all araticum fruit parts (2.65, 1.18 and 8.26% for the results from pulp, peel and seed, respectively). Phenolics liberated from the insoluble-bound fraction comprised 23.84, 39.96 and 47.38% of total phenolics in araticum peel, pulp and seed, respectively. Insoluble-bound phenolics cannot be generally extracted by organic solvents, but these compounds are very important in health benefits from the nutritional viewpoint and for the extraction yield from the industrial viewpoint. When the insoluble-bound phenolics are consumed as part of a normal food, they will be likely released from the bound-forms by the action of intestinal enzymes or colonic microbiota and then be absorbed, contributing to the overall biological effects of the food consumed (Wang et al., 2016). The use of extraction methods that provide the release of insoluble-bound phenolics from raw material can also increases the extraction yields, reducing industrial costs. These findings demonstrate the importance of evaluating the insoluble-bound phenolics in food plants, since they comprise a significant portion of their phenolic compounds. Ignoring this fraction in analytical procedures would therefore inevitably lead to substantial underestimation of their functional and industrial potentials.

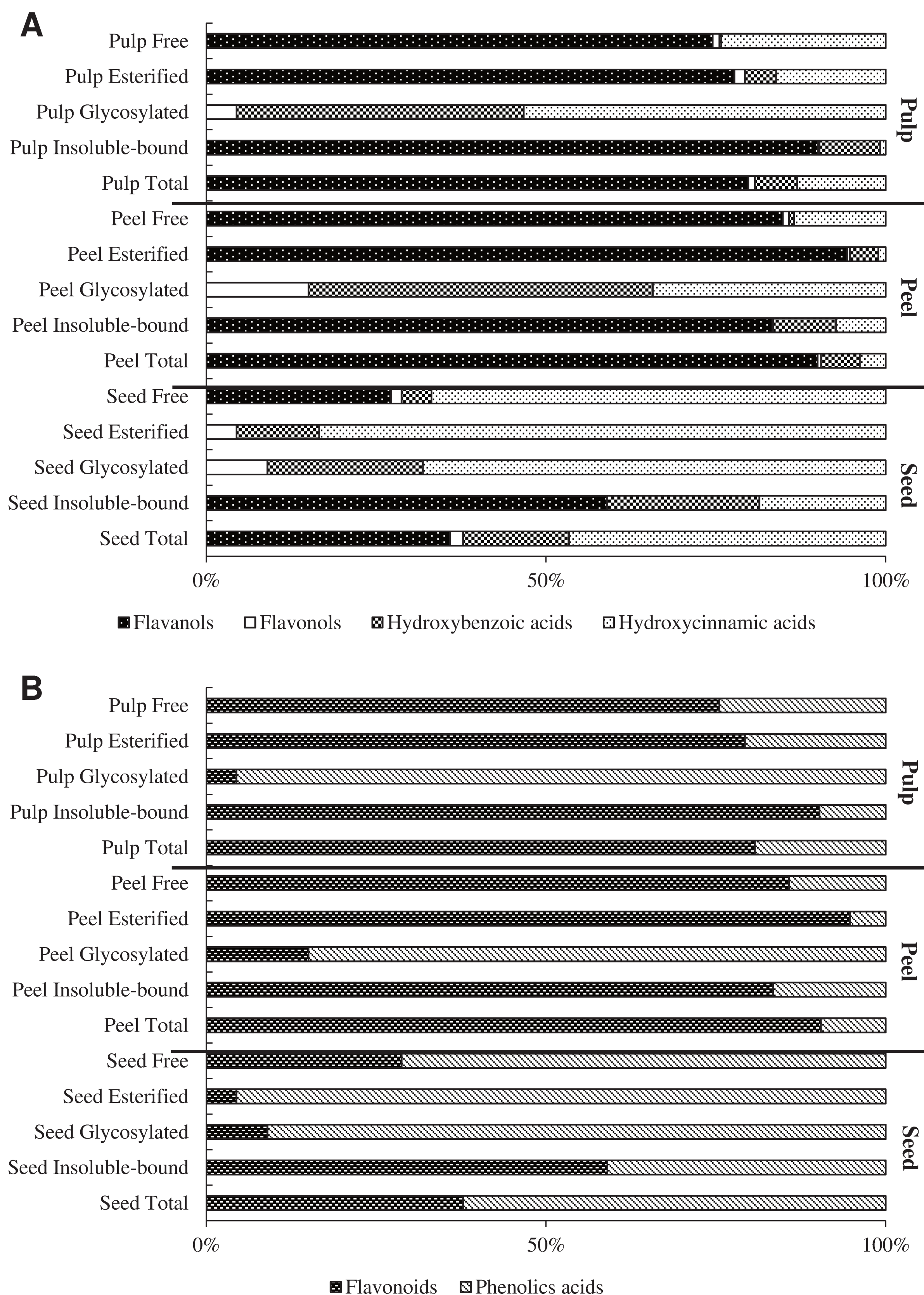

Fig. 2. Profile of phenolics sub-classes (A) and classes (B) in four phenolic fractions of araticum pulp, peel and seed.

As **Fig. 2A** shows, flavanols are the predominant phenolic sub-class found in the araticum pulp and peel as well as in their free, esterified and insoluble-bound fractions. Similar results were found in another fruits of the *Annona* genus, namely cherimoya (*Annona cherimola*), in which the most important phenolic sub-class present in cherimoya pulp and peel was also the flavanols (García-Salas, Gómez-Caravaca, Morales-Soto, Segura-Carretero, & Fernández-Gutiérrez, 2015). Hydroxycinnamic acids also comprised the most phenolics from araticum seed and their esterified and glycosylated fractions as well as glycosylated fraction from araticum pulp. Hydroxybenzoic acids were dominant only in the glycosylated fraction from araticum peel; whereas flavonols were the minority phenolic sub-class in all analysed fractions. Generally, hydroxybenzoic acids are found in low concentrations in food plants (Abeywickrama et al., 2016).

Regarding phenolic classes, glycosylated fractions contained the most phenolic acids, and the other fractions (except for the free and esterified fractions from seed) contained the most flavonoids (**Fig. 2B**). Phenolic acids are rarely found in the free forms, generally occurring bound to other compounds in the cell walls, glycosylated to simple sugar or as soluble esters (Abeywickrama et al., 2016). Overall, flavonoids were the predominant phenolic class from araticum pulp (80.74%) and peel (90.49%); whereas in the seeds were the phenolic acids (62.21%). Arruda et al. (2017b) reported that flavonoids were the most plentiful phenolic compounds (~86%) in crude araticum pulp extract. A previous study has shown that the araticum peel presents mainly phenolics belonging to the flavonoids class (procyanidins B2 and C1, (epi)catechin, quercetin and kaempferol-based structures) (Justino et al., 2016); whereas in araticum seed, most phenolics previously identified belong to the phenolic acids class (caffeic acid-based structures, ferulic, *p*-coumaric and sinapic acids) (Formagio et al., 2015; Roesler et al., 2007).

Quantification of phenolic compounds using HPLC-ESI-MS/MS (**Table 3**) revealed that caffeic acid was the major phenolic present in the araticum seed phenolic fractions (30.95, 62.84 and 60.74% for free, esterified and glycosylated fractions, respectively), except for the insoluble-bound fraction which had catechin as the major compound (41.08%). Catechin was also the major phenolic present in the esterified (57.89 and 68.59% from pulp and peel, respectively) and insoluble-bound (56.62 and 59.40% from pulp and peel, respectively) fractions from araticum pulp and peel; whereas epicatechin was the predominant compound in the free fractions (68.55 and 76.74% for the results from pulp and peel, respectively). In addition, caffeic and protocatechuic acids were the main phenolics in the glycosylated fractions

from araticum pulp and peel, respectively. In general, araticum pulp and peel displayed the flavonoids catechin (42.87 and 61.49%) and epicatechin (36.91 and 28.47%) as their major phenolic compounds. The study established the following order of main phenolics in araticum pulp: catechin > epicatechin > caffeic acid > protocatechuic acid > ferulic acid > chlorogenic acid > gentisic acid > *p*-coumaric acid > rutin > quercetin. The araticum pulp is known to contain a high content of phenolic compounds (Arruda et al., 2017b; Siqueira, Rosa, Fustinoni, Sant'Ana, & Arruda, 2013; Souza, Pereira, Queiroz, Borges, & Carneiro, 2012), but there are no data yet regarding the phenolic profile in araticum pulp. In addition to catechin and epicatechin, araticum peel contained quercetin, rutin, and protocatechuic, caffeic, ferulic, *p*-coumaric, chlorogenic and gentisic acids. Catechin, epicatechin, rutin, chlorogenic acid, quercetin derivative (quercetin-3-glucoside), and caffeic (caffeic acid and caffeoyl-glucoside) and ferulic (ferulic acid and feruloyl-galactoside) acids derivatives were previously characterized in araticum peel (Justino et al., 2016; Roesler et al., 2007). Protocatechuic, *p*-coumaric and gentisic acids were however identified for the first time in this study. For araticum seed, the phenolic acid caffeic acid (27.55%) was found as the main phenolic constituent, followed by catechin (20.41%), epicatechin (15.47%), protocatechuic acid (13.63%) and ferulic acid (10.29%). Roesler et al. (2007) and Formagio et al. (2015) also reported the presence of caffeic acid and derivatives (caffeoyltartaric acid and caffeoyl-glucose), ferulic and *p*-coumaric acids, quercetin and rutin in araticum seed. On the other hand, catechin, epicatechin, and protocatechuic, chlorogenic and gentisic acids were identified here for the first time.

As also determined through the Folin-Ciocalteu assay, araticum peel was found to contain the highest amount of total phenolics (5735.52 µg/g fdw), followed by pulp (1792.58 µg/g fdw) and seed (164.66 µg/g fdw). Total phenolic content from araticum seed was 11.9 and 34.8-fold lower than the values found for the pulp and peel, respectively. Despite presenting relatively low phenolic contents, the araticum seed extracts showed significant antioxidant activity (**Table 2**). Araticum seed had a distinct phenolic profile (several phenolics in similar amounts), thus, although they are in small amounts, these compounds can interact with each other synergistically, significantly increasing their antioxidant activity.

Araticum pulp and peel showed to be expressive sources of phenolic compounds, especially the flavan-3-ol monomers catechin (768.42 and 3526.78 µg/g fdw for the pulp and peel, respectively) and epichatechin (661.81 and 1632.90 µg/g fdw for the pulp and peel, respectively) (**Table 3**). The major phenolic compounds found in araticum fruit and its by-products have been reported to exhibit numerous biological effects. Greater catechin and

epicatechin intakes were associated with decreased pancreatic cancer risk in male smokers not consuming supplemental α-tocopherol and/or β-carotene (Bobe et al., 2008). Higher dietary intakes of flavan-3-ol monomers (catechin and epicatechin) and their polymers were associated with a significant reduction in oxidative stress biomarkers (Cassidy et al., 2015). High intakes of flavanols and flavan-3-ols monomers were also inversely associated to type 2 diabetes risk (Zamora-Ros et al., 2013). Vermeulen et al. (2013) reported a significant inverse association between esophageal cancer risk and flavanols in current smokers. Recently, Dower, Geleijnse, Hollman, Soedamah-Muthu, and Kromhout (2016) reported that the epicatechin intake is inversely related to coronary heart disease (CHD) mortality in elderly men and to cardiovascular diseases (CVD) mortality in prevalent cases of CVD, wherein a median epicatechin intake of 22 mg/day reduced by 38% the CHD mortality risk and 46% the long-term CVD mortality risk compared to subjects with a median intake of 8 mg/day. A daily intake of 22 mg flavan-3-ol monomers (catechin plus epicatechin) is equivalent to ~50 g fresh araticum pulp. In addition, only 9 g fresh araticum peel would be needed to extract 22 mg flavan-3-ol monomers. Our study, together with the cited studies, suggest that these compounds contribute to the reported health-promoting effects of araticum fruit and points to the use of this Cerrado fruit by the food and pharmaceutical industries. We propose the araticum pulp as a novel food ingredient for the development of functional foods, whereas the araticum peel seems to offer a potential plant ingredient for novel medicine and cosmetic formulations.

3.5. Correlation between phenolic compounds and antioxidant activity

Table 4 shows the correlations between phenolic compounds and antioxidant activities of araticum pulp, peel and seed. The results from araticum pulp show that the antioxidant activities (DPPH, TEAC and $ORAC_{FL}$) were correlated significantly ($p < 0.01$) with the TPC (r = 0.993-0.999), TFC (r = 0.985-0.996), CTC (r = 0.988-0.995) and the sum of detected phenolic content by HPLC-ESI-MS/MS (r = 0.945-0.955). Positive high linear correlations ($p < 0.01$) were also observed between the antioxidant activities and TPC (r = 0.990-0.999), TFC (r = 0.987-0.997), CTC (r = 0.969-0.984) and the sum of detected phenolic content by HPLC-ESI-MS/MS (r = 0.965-0.977) for the araticum peel. The results from araticum seed also showed positive linear correlations (p < 0.01) between the antioxidant activities and TPC (r = 0.987-0.993), TFC (r = 0.940-0.995), CTC (r = 0.683-0.823) and the sum of detected phenolic content by HPLC-ESI-MS/MS (r = 0.842-0.896). The positive relationships between antioxidant activities and phenolic compounds content in araticum fruit

extracts were in accordance with other studies (Ayoub et al., 2016; Chen et al., 2017; Tan, Chang, & Zhang, 2017). These results suggest that the phenolic compounds are the main responsible for the antioxidant activity in araticum fruits. The TPC had also significant and positive correlations ($p < 0.01$) with the total detected phenolic content by HPLC-ESI-MS/MS (r = 0.954, 0.976 and 0.864 for the araticum pulp, peel and seed, respectively), indicating that the method used for the phenolic compounds extraction was effective to eliminate potential non-phenolic interfering substances (such as ascorbic acid, sugars, amino acids and peptides, organic acids and metal complexes) that react with Folin-Ciocalteu reagent.

Phenolic compounds are recognized for being highly effective as reducing agents and scavenging free radicals (Chen et al., 2017). There is however no information about the contribution of individual phenolics to their overall antioxidant activity in the araticum fruit. The correlation analysis was therefore established to determinate the major phenolic compounds contributors to the antioxidant activity of the phenolic extracts of araticum pulp, peel and seed (**Table 4**).

The main phenolics contributors to antioxidant activities in araticum pulp seem to be catechin > protocatechuic acid > quercetin > epicatechin > caffeic acid > *p*-coumaric acid > ferulic acid > rutin. Concerning araticum peel, it may be highlighted that the main phenolics contributors to antioxidant activities are in the following order: protocatechuic acid > epicatechin > catechin > quercetin > caffeic acid > rutin > ferulic acid > *p*-coumaric acid. It can also be noted that both in the pulp and in the peel, the chlorogenic acid appear to not contribute to the antioxidant activity for the DPPH (r = 0.298 and 0.315 for the results from pulp and peel, respectively), TEAC (r = 0.316 and 0.319) and $ORAC_{FL}$ assays (r = 0.372 and 0.359, respectively); whereas the gentisic acid was not a significant contributor to TEAC assay (r = 0.509 and 0.497). Regarding phenolics in araticum seed, the main contributors to antioxidant activities are ferulic acid > caffeic acid > chlorogenic acid > epicatechin > *p*-coumaric acid > rutin > protocatechuic acid. Unlike the pulp and peel, the quercetin was not significant contributor to TEAC and $ORAC_{FL}$ assays (r = 0.467 and 0.328, respectively) in araticum seed; whereas the catechin and gentisic acid did not contribute to $ORAC_{FL}$ assay (r = 0.514 and 0.512, respectively). Chen et al. (2017) reported that epicatechin, catechin, rutin, isoquercitrin, quercitrin, quercetin, and gallic, *p*-coumaric, ferulic and protocatechuic acids were the main contributors to antioxidant activities (DPPH, TEAC and FRAP assays) in 14 subtropical fruit leaves; whereas Sumczynski, Kotásková, Orsavová, and Valášek (2017) verified that epigallocatechin, epicatechin, rutin, kaempferol, quercetin, and ferulic, vanillic, ellagic, sinapic,

syringic, caffeic, *o*-coumaric, *p*-hydroxybenzoic, protocatechuic, gallic, cinnamic and *p*-coumaric acids were the main phenolics related to the DPPH and TEAC assays in wild rices. Moreover, vanillic and *p*-coumaric acids, luteolin, apigenin and diosmetin were significantly correlated to the DPPH and TEAC assays in olive oils (Kelebek, Selli, & Kola, 2017).

Our findings demonstrate therefore that each part of the araticum fruit presents different phenolics contributors (structural type and contribution order) to their antioxidant activities. Considering only the four major phenolics contributors in each sample analysed, it was observed that the flavonoids (epicatechin, catechin and quercetin) were the main contributors to antioxidant activities from pulp and peel, whereas for the seed were the phenolic acids (ferulic, caffeic and chlorogenic acids). The antioxidant activity of each phenolic compound depends largely on the number of aromatic and hydroxyl groups, and the specific positioning of these groups (Sumczynski et al., 2017). The antioxidant activity of a phenolic compound has been found to directly correlate with the degree of hydroxylation. Phenolic acids with the phenyl ring trihydroxylated and flavonoids with the B ring trihydroxylated have a high antioxidant activity (Cartea, Francisco, Soengas, & Velasco, 2011). The substitution of hydrogen atoms by ethyl or *n*-butyl groups at the *para*-position of the phenyl ring improves the antioxidant activity of phenolic acids; whereas the antioxidant activity decreases with the presence of chain or branched alkyl groups in this position (Shahidi, Janitha, & Wanasundara, 1992). Flavonoids hold high antioxidant activity due to the presence of one or more of the following structural elements that contribute to the antioxidant activity: (i) an *ortho*-dihydroxy structure in the B ring (3’, 4’-OH), (ii) 2,3-double bond coupled with a 4-oxo function in the C ring, and (iii) hydroxyl groups at positions 3 and 5 (Chen et al., 2017). Glycosylation of flavonoids reduces however their antioxidant activity compared with corresponding aglycones, probably due to steric hindrance caused by the crowding sugar moieties (Cartea et al., 2011). Antioxidant activity of phenolics is also affected by intermolecular interactions, which can be either synergistic or antagonistic, depending on the conditions and compounds under study (Arruda et al., 2017b). The contribution of a phenolic compound to antioxidant activity in a food or extract will therefore depend on its concentration and chemical features, matrix composition, and medium conditions.

Table 4. Pearson's correlation coefficients (r) between phenolic compounds and antioxidant activity of araticum pulp, peel and seed.

Parameters	**Pulp**	**Peel**	**Seed**
Total phenolics (Folin-Ciocalteu) *vs.* DPPH	0.996**	0.998**	0.987**
Total phenolics (Folin-Ciocalteu) *vs.* TEAC	0.999**	0.999**	0.996**
Total phenolics (Folin-Ciocalteu) *vs.* $ORAC_{FL}$	0.993**	0.990**	0.993**
Total flavonoids *vs.* DPPH	0.985**	0.993**	0.940**
Total flavonoids *vs.* TEAC	0.996**	0.997**	0.964**
Total flavonoids *vs.* $ORAC_{FL}$	0.989**	0.987**	0.995**
Condensed tannins *vs.* DPPH	0.988**	0.975**	0.823**
Condensed tannins *vs.* TEAC	0.994**	0.984**	0.792**
Condensed tannins *vs.* $ORAC_{FL}$	0.995**	0.969**	0.683**
Total phenolics (UHPLC-MS) *vs.* Total phenolics (Folin-Ciocalteu)	0.954**	0.976**	0.864**
Total phenolics (UHPLC-MS) *vs.* DPPH	0.945**	0.967**	0.893**
Total phenolics (UHPLC-MS) *vs.* TEAC	0.955**	0.977**	0.896**
Total phenolics (UHPLC-MS) *vs.* $ORAC_{FL}$	0.951**	0.965**	0.842**
Catechin *vs.* DPPH	0.979**	0.954**	0.551*
Epicatechin *vs.* DPPH	0.779**	0.957**	0.869**
Rutin *vs.* DPPH	0.710**	0.860**	0.779**
Quercetin *vs.* DPPH	0.936**	0.905**	0.522*
Protocatechuic acid *vs.* DPPH	0.945**	0.963**	0.702**
Gentisic acid *vs.* DPPH	0.528*	0.534*	0.618*
Chlorogenic acid *vs.* DPPH	0.298 ns	0.315 ns	0.851**
Caffeic acid *vs.* DPPH	0.802**	0.878**	0.936**
p-Coumaric acid *vs.* DPPH	0.734**	0.597*	0.859**
Ferulic acid *vs.* DPPH	0.723**	0.786**	0.919**
Catechin *vs.* TEAC	0.984**	0.965**	0.569*
Epicatechin *vs.* TEAC	0.804**	0.968**	0.897**
Rutin *vs.* TEAC	0.680**	0.872**	0.748**
Quercetin *vs.* TEAC	0.905**	0.891**	0.467 ns
Protocatechuic acid *vs.* TEAC	0.968**	0.965**	0.704**
Gentisic acid *vs.* TEAC	0.509 ns	0.497 ns	0.614*
Chlorogenic acid *vs.* TEAC	0.316 ns	0.319 ns	0.877**
Caffeic acid *vs.* TEAC	0.766**	0.868**	0.906**
p-Coumaric acid *vs.* TEAC	0.710**	0.589*	0.870**
Ferulic acid *vs.* TEAC	0.701**	0.783**	0.912**
Catechin *vs.* $ORAC_{FL}$	0.953**	0.946**	0.514 ns
Epicatechin *vs.* $ORAC_{FL}$	0.832**	0.961**	0.890**
Rutin *vs.* $ORAC_{FL}$	0.648**	0.871**	0.705**
Quercetin *vs.* $ORAC_{FL}$	0.886**	0.872**	0.328 ns
Protocatechuic acid *vs.* $ORAC_{FL}$	0.969**	0.976**	0.623*
Gentisic acid *vs.* $ORAC_{FL}$	0.580*	0.522*	0.512 ns
Chlorogenic acid *vs.* $ORAC_{FL}$	0.372 ns	0.359 ns	0.938**
Caffeic acid *vs.* $ORAC_{FL}$	0.747**	0.902**	0.832**
p-Coumaric acid *vs.* $ORAC_{FL}$	0.689**	0.659**	0.830**
Ferulic acid *vs.* $ORAC_{FL}$	0.687**	0.840**	0.856**

ns, non-significant.
** Significant at $p < 0.01$.
* Significant at $p < 0.05$.

4. Conclusion

For the first time, phenolics from araticum fruit parts (pulp, peel and seed) were fractioned into their respective free, esterified, glycosylated and insoluble-bound forms and then characterized and quantified and their antioxidant properties were evaluated. The results indicate that the profiles of free, esterified, glycosylated and insoluble-bound phenolics and their antioxidant activities of araticum fruit parts are quite contrasting. Ten phenolic compounds were properly characterized and quantified by HPLC-ESI-MS/MS. Flavonoids made the highest contribution for the araticum pulp and peel, whereas phenolic acids comprised the most phenolics from araticum seed. Catechin and epicatechin were the major compounds from araticum pulp and peel, whereas seed displayed caffeic acid, catechin and epicatechin as its main phenolics. Phenolics were predominantly present in the insoluble-bound and esterified forms in all of the araticum fruit parts. The important contribution of insoluble-bound phenolics, especially for araticum pulp (39.96%) and seed (47.38%), demonstrated that simple extraction of soluble phenolics might lead to underestimation of phenolic contents and their actual contribution on the biological activities. In addition, phenolic fractions showed scavenging activity against DPPH, ABTS and peroxyl radicals. Our findings clearly reveal that araticum fruit and its by-products offer promising sources of natural antioxidants for further development of nutraceuticals, functional ingredients, foods, cosmetics, drugs or value-added products.

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Conflict of interest

The authors report no conflict of interest.

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CHAPTER III

RESEARCH ARTICLE

EFFECTS OF HIGH-INTENSITY ULTRASOUND PROCESS PARAMETERS ON THE PHENOLIC COMPOUNDS RECOVERY FROM ARATICUM PEEL

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Abstract

In this work, we investigated the effects of the nominal ultrasonic power (160-640 W) and process time (0.5-5.0 min) on the phenolic compounds recovery and antioxidant activity from araticum peel. The individual and synergistic effects of the process variables on the phenolic recovery were estimated using a full factorial experimental design. Operating at high nominal ultrasonic powers was possible to obtain high phenolic yields and antioxidant activities at short process times (≤ 5 min). The HPLC-ESI-QTOF-MS/MS analysis revealed that the araticum peel sample possessed 142 phytochemicals, 123 of which had not been reported in literature for this raw material yet. The most abundant phenolic compounds recovered were epicatechin, rutin, chlorogenic acid, catechin and ferulic acid. Thus, high-intensity ultrasound technology proved to be a simple, efficient, fast and low environmental impact method for obtaining phenolic compounds from araticum peel. In addition, araticum peel showed to be a promising source bioactive natural phenolics for further applications in the food, nutraceutical, cosmetic and pharmaceutical industries.

Keywords: Emerging technology; Ultrasonic power; Bioactive compounds; By-products; HPLC-ESI-MS/MS; *Annona crassiflora* Mart.

Highlights

- Effect of ultrasonic power (160-640 W) and process time (0.5-5.0 min) on phenolics recovery was evaluated.
- Ultrasonic process intensification significantly improved the phenolics recovery.
- 142 phytochemical compounds were tentatively identified with HPLC-ESI-QTOF-MS/MS.

1. Introduction

Phenolic compounds are secondary metabolites ubiquitously distributed in the plant kingdom. These plant metabolites are characterized by possessing at least one aromatic ring containing one or more hydroxyl groups substituents, called phenol groups, which are derived from L-phenylalanine or tyrosine. In addition, their structures can be associated with carbohydrates, organic acids, lipids, amines and cell wall components [1]. Currently, more than 8000 phenolic structures have been described and isolated from natural sources, and among them, over 4000 are flavonoids [2]. Phenolic compounds from plant materials present a wide variety of applications in several industry sectors, such as food and beverage, pharmaceutical and cosmetic industries. These compounds have been effectively used as dietary supplements, ingredients in functional foods and cosmetics, and replacers for synthetic antioxidants [3]. Moreover, numerous studies have shown that the consumption of natural phenolic compounds can prevent, slow or reverse the development of chronic diseases, such as cardiovascular diseases [4,5], metabolic syndrome [6], cancer [7,8], and type 2 diabetes [9].

Annona crassiflora Mart., a fruitful plant native from Brazilian Cerrado belonging to the *Annonaceae* family, produces fruits (popularly known as “araticum” or “marolo”) that are very much appreciated in this region (within the top 20 more consumed) [10]. Since the peel accounts for about 40% of the whole fruit weight [11], very large amounts of by-products are formed after its consumption or processing. Araticum peel extracts have demonstrated to exhibit several biological properties, such as potent antioxidant capacity [10,12,13], ability to inhibit digestive enzymes and formation of glycation products [14,15], antibacterial [16] and

hepatoprotective [12,13] activities. These beneficial effects have been associated with the bioactive compounds present in araticum peel like phenolics [10,14] and alkaloids [15]. The araticum peel has been recently shown by our group as a source of phenolics [10]. Therefore, araticum peel can be a potential material to recover highly valuable biomolecules, mainly phenolics, for further applications in the food, nutraceutical and pharmaceutical industries, and consequently add more value to the Cerrado fruits industry.

The choice of extraction procedure is one the most important steps in the recovery and purification of phenolic compounds from plant matrices and it should be based on simplicity, versatility, cost and the capacity to both extract and preserve these compounds [3]. Thus, several emerging extraction methods, mainly those based on high-energy techniques, have been used for recovery of phenolic compounds from plants instead of conventional methods due to the fast extraction rate, the high recovery yield, the reduced detrimental effect on the extracted compounds, the preservation of thermosensitive compounds, the lower processing temperatures, and the use of ‘‘recognized as safe” organic solvents [17]. Among these methods, ultrasound-assisted extraction has been gaining increasing attention because it is a simple, rapid, less expensive, effective and eco-friendly method with high reproducibility, high recovery yield in short time, lower energy consumption, reduced solvent consumption, applicability to diversified samples, ease of implementation by food and pharmaceutical industries and rapid return of investment [18–20].

Phenolic compounds from plants can found both free and conjugated to proteins, sugars, organic acids, cell wall carbohydrates and polysaccharides [3], thus complicating their recovery by conventional extraction methods. In addition, to increase recovery efficiency, conventional methods use toxic solvents, present high solvent consumption and long extraction time [21]. In addition to overcoming these disadvantages, ultrasound-assisted extraction enhances phenolic recovery by disrupting the plant cell walls based on the cavitation phaenomena [18]. Although ultrasound-assisted extraction has been used in the phenolic recovery from diverse plant matrices such as mandarin peel [22], pecan nut shell [21], waste spent coffee grounds [19], mangosteen hull [23], yarrow [24], bovine pennyroyal leaves [25], carrot pomace [26], blueberry wine pomace [27], and olives [20], the process parameters remain still poorly explored and their individual and synergistic effects on the recovery of these compounds unclear.

To effectively recover phenolic compounds from plant matrices is necessary to understand the effect of process parameters on their extraction. However, no information

concerning the effects of ultrasonic parameters on the phenolic compounds recovery from araticum peel is available in the literature. In this context, high-intensity ultrasound was employed as a simple, fast and of low environmental impact method to recover phenolics antioxidants from araticum peel, a food industry by-product. A full factorial experimental design was used to evaluate the effects of applied nominal ultrasonic power (W) and extraction time (min) on the phenolics recovery and antioxidant activities. Moreover, the profile of phenolics and other polar compounds were characterized more deeply using HPLC-ESI-QTOF-MS/MS.

2. Material and methods

2.1. Chemicals and reagents

Folin-Ciocalteu reagent, fluorescein, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2- diphenyl-1-picrylhydrazil), AAPH [2,2′-azobis(2-methylamidinopropane)-dihydrochloride], ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt], methanol, acetonitrile and formic acid grade HPLC, and all phenolic compound standards with a purity of ≥ 96% were purchased from Sigma-Aldrich (St. Louis, USA). The others solvents and reagents used in this study were of analytical grade. All solutions were prepared with ultrapure water (18 MΩ cm^{-1}) obtained from a Milli-Q water purification system (Millipore, Bedford, USA). The standards (pyrogallol, gallic acid, gallocatechin, protocatechuic acid, epigallocatechin, catechin, chlorogenic acid, 4-hydroxybenzoic acid, epicatechin, vicenin-2, caffeic acid, gentisic acid, epicatechin gallate, vanillin, ethyl gallate, *p*-coumaric acid, vitexin, sinapic acid, ferulic acid, naringin, rutin, propyl gallate, ellagic acid, myricetin, quercetin, naringenin, luteolin, hesperetin, kaempferol, apigenin, chrysin) were prepared as a stock solution (1 mg/mL in methanol 100%), stored in a ultra-freezer (-80°C) and used to prepare the calibration curves by appropriate dilution from the mixture.

2.2. Plant material and sample preparation

The araticum fruits (*Annona crassiflora* Mart.) were collected in natural areas of the Cerrado Biome located in the municipality of Carmo do Paranaíba, Minas Gerais, Brazil. A voucher specimen (UEC 197249) has been deposited in the Herbarium of the Institute of Biology of the University of Campinas, Brazil (Herbarium UEC) (**Annex 1**). In addition,

following Law n° 13.123/2015 and its regulations, the activity of access to genetic heritage was regimented by the Board of the Genetic Heritage Management under number AA9DDF7 (**Annex 2**).

The morphologically perfect and completely mature fruits were washed with distilled water and manually peeled. Then, the peels were freeze-dried (LIOTOP, model L101, São Carlos, Brazil), ground using a knife grinder (Marconi, model MA340, Piracicaba, Brazil) to obtain a powder and stored at -20°C until analysis.

2.3. Ultrasound-assisted solid-liquid extraction (USLE)

19 kHz ultrasonic generator with a 13 mm probe (Unique, Disruptor, 800 W, Indaiatuba, Brazil) was employed for sonicating samples. Hydroethanolic solution (1:1, v/v) was selected as the extractor solvent because ethanol is a GRAS (Generally Recognized As Safe) solvent widely used for the extraction of phenolic compounds and its combination with the water increases the phenolic extraction efficiency, mainly, glycosylated fraction [3].

USLE was performed by mixing 2 g of dried araticum peel with 20 mL of hydroethanolic solution (1:1, v/v) in a 50 mL Falcon tube. Ultrasonic probe was dipped 10 mm in the sample to provide direct contact with the sample. The mixtures were subjected to sonication under different independent variable conditions (nominal ultrasonic power and extraction time). The operating extraction conditions with the different nominal ultrasonic power and extraction time combinations are shown in **Table 1**. An ice bath was used in all experiments to prevent samples overheating during the extraction aiming to protect the bioactive compounds. The maximum temperature of the sample, as measured at the outlet of the system, was 40.4°C. Energy density (J/mL), the energy input per unit volume, transferred from ultrasound equipment to the extraction system was calculated according to **Eq. (1)**:

$$ED\ \left(\frac{J}{mL}\right) = \frac{Nominal\ ultrasonic\ power\ (W) \times Extraction\ time\ (s)}{Sample\ volume\ (mL)} \quad (1)$$

After extraction, the extracts were centrifuged at 4000*g* for 11 min at 5°C (Hettich Zentrifugen, model Rotanta 460R, Tuttlingen, Germany). The supernatants were collected, filtered through a 0.22 µm PTFE filter and stored at -80°C until further analyses.

Table 1. Influence of the nominal ultrasonic power (W) and extraction time (min) on the content of total phenolic compounds (TPC) and antioxidant activities (DPPH, TEAC and $ORAC_{FL}$) from araticum peel extracts.

Nominal ultrasonic power (W)	Extraction time (min)	TPC (mg GAE/g fdw)	DPPH (µmol TE/g fdw)	TEAC (µmol TE/g fdw)	$ORAC_{FL}$ (µmol TE/g fdw)
160	0.5	51.82±1.39	431.17±6.99	467.33±3.57	415.36±27.46
	1.0	51.17±1.15	388.00±12.34	494.39±4.29	376.46±4.97
	3.0	50.57±1.72	368.54±2.75	496.47±8.21	315.89±18.63
	5.0	48.90±0.12	376.01±5.05	453.93±7.31	363.16±10.41
280	0.5	58.88±1.15	434.18±15.86	538.41±8.31	450.68±18.37
	1.0	55.02±0.97	413.86±8.31	508.41±5.47	391.23±6.48
	3.0	59.59±0.65	443.14±21.97	548.34±5.98	450.69±5.19
	5.0	60.42±0.90	460.78±9.60	572.77±10.28	437.41±4.44
400	0.5	57.12±0.50	428.76±10.08	529.43±9.60	415.17±9.61
	1.0	58.98±0.50	450.84±10.10	571.85±4.65	392.60±19.81
	3.0	61.48±0.37	464.58±8.39	574.65±4.13	358.96±19.06
	5.0	61.23±1.86	449.62±3.94	558.92±5.67	396.34±5.57
520	0.5	60.75±0.45	450.15±5.14	556.79±8.96	404.59±16.07
	1.0	57.78±1.35	435.60±14.02	559.65±4.72	440.79±6.69
	3.0	61.37±0.12	465.52±18.15	569.17±3.45	511.58±1.16
	5.0	70.68±1.51	513.49±11.12	600.10±15.98	481.88±10.92
640	0.5	57.29±1.23	420.99±15.64	537.88±6.43	450.81±17.00
	1.0	64.45±0.19	453.14±14.26	593.03±4.52	473.62±12.42
	3.0	69.61±1.90	502.62±10.74	613.76±1.21	525.41±15.27
	5.0	69.11±0.66	514.57±11.49	613.47±5.35	465.27±25.75

2.4. Experimental design

The effects of nominal ultrasonic power (160-640 W) and extraction time (0.5-5.0 min) on the phenolic compounds recovery and antioxidant activities from araticum peel were investigated using a full factorial experimental design (5 × 4), resulting in 20 total experimental runs (**Table 1**). The experiments were conducted in random order, and the experimental data were fitted to the second-order polynomial regression model as shown in **Eq. (2)**.

$$Y = a_0 + \sum_{i=1}^{k} a_i X_i + \sum_{i=1}^{k} a_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} a_{ij} X_i X_j + \varepsilon \quad (2)$$

where: Y is the dependent variable; a_0 is the constant of the model; a_i, a_{ii} and a_{ij} are the model coefficients (linear, quadratic and interaction terms, respectively); X_i and X_j are de values of the independent variables (nominal ultrasonic power and extraction time, respectively); and ε is the experimental error.

Contour plots were generated from the regression coefficients of the polynomial equations. The contour plots were used to show the relationship between the response variables (phenolic compounds extraction and antioxidant activities) and the levels of each independent variable (nominal ultrasonic power and extraction time). The statistical significance of each regression coefficient was determined using Student's t test. The polynomial regression models were evaluated by analysis of variance (ANOVA). In addition, the goodness of fit of the polynomial regression models were statistically verified by the determination coefficient (R^2), adjusted determination coefficient (R^2_{adj}), predicted determination coefficient ($R^2_{prediction}$) and the predicted error sum of squares (PRESS).

2.5. Determination of total phenolic content (TPC)

Total phenolic content was quantitatively analysed using Folin-Ciocalteu colorimetric method as illustrated by Arruda et al. [10]. Briefly, 100 μL of diluted extract was mixed with 100 μL of Folin-Ciocalteu reagent (1:1, v/v) and 800 μL of sodium carbonate (5%, w/v). Then, the reaction solution was kept in the dark for 20 min at room temperature and the absorbance was measured at 760 nm against a blank on a spectrophotometer (Beckman, model DU600, CA, USA). Gallic acid was employed as standard to quantify TPC and the results were expressed as mg gallic acid equivalents per gram of freeze-dried peel (mg GAE/g fdw).

2.6. Antioxidant activity assays

2.6.1. DPPH scavenging assay

The DPPH scavenging assay was performed according to a method as described by Brand-Williams et al. [28], with slight modifications [10]. An aliquot (200 μL) of diluted extract was mixed with 1 mL of DPPH in ethanol (0.004%, w/v). The reaction mixture was incubated for 30 min at room temperature in darkness, and the absorbance of the remaining DPPH was read at 517 nm against a blank. The results were expressed as micromoles of Trolox equivalents per gram of freeze-dried peel (μmol TE/g fdw).

2.6.2. Trolox equivalent antioxidant capacity (TEAC) assay

Trolox equivalent antioxidant capacity was evaluated according to Re et al. [29] with some modifications [10]. The radical cation $ABTS^{\bullet +}$ was prepared overnight at room temperature in darkness mixing 5 mL ABTS solution (7 mM in water) with 88 μL of potassium

persulfate solution (145 mM). For the sample analysis, the radical cation $ABTS^{•+}$ solution was adjusted with ultrapure water to achieve an absorbance value of 0.70 ± 0.02 at 734 nm. Araticum peel extracts (200 μL) were reacted with 1 mL of $ABTS^{•+}$ solution and incubated for 6 min at room temperature. The absorbance of the remaining $ABTS^{•+}$ was measured at 734 nm against a blank, and the results were expressed as micromoles of Trolox equivalents per gram of freeze-dried peel (μmol TE/g fdw).

2.6.3. Oxygen radical absorbance capacity ($ORAC_{FL}$) assay

The $ORAC_{FL}$ assay was carried out on the basis of the method described by Dávalos et al. [30]. In a 96 well dark microplate, 20 μL of diluted extract, blank or Trolox, and 120 μL of fluorescein in potassium phosphate buffer (0.378 μg/mL, pH 7.4) were pipetted into each working well of the microplate. Then, 60 μL of AAPH (108 mg/mL) were added, and the fluorescence (excitation and emission wavelengths 485 and 520 nm, respectively) was read every minute for 80 min at 37°C on a NovoStar Microplate reader (New Brunswick Scientific Classic Series, model C76, NJ, USA). Results were calculated based on differences in areas under the fluorescence decay curve between the blank, samples and standards. Trolox were used to prepare the standard curve and the results were expressed as micromoles of Trolox equivalents per gram of freeze-dried peel (μmol TE/g fdw).

2.7. Determination of phenolic compounds by HPLC-ESI-MS/MS

HPLC-ESI-MS/MS analysis of the phenolic compounds in the araticum peel extracts was performed on a LCMS-8040 tandem quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Shimadzu, Kyoto, Japan) according to the method described by Arruda et al. [10]. Chromatographic separation was achieved in a Shim-pack XR-ODS III column (150 × 2 mm i.d., particle size 2.2 μm, Shimadzu, Kyoto, Japan). The extracts were diluted in 0.1% formic acid in water (v/v) and injected into the column using an auto-sampler. The column temperature was set at 40°C and the injection volume of the samples was 10 μL. The analytes were eluted from the column using gradient elution at a constant flow rate of 0.40 mL/min. The solvent gradient consisted of 0.1% formic acid in water (eluent A) and methanol (eluent B) with the following proportions: 0-1 min, 5% B; 1-4 min, from 5% to 60% B; 4-7 min, from 60% to 70% B; 7-10 min, from 70% to 100% B; 10-10.50 min, 100% B; 10.50-11 min, from 100% to 5% B; 11-15 min, 5% B.

The ESI source was operated in the negative ion mode and the optimum values of the source parameters were: capillary voltage of 3.5 kV; drying and nebulizing gas (N_2) flow rates of 20 and 3 L/min, respectively; collision induced dissociation gas pressure (Ar) of 224 kPa; and the heat block and desolvation line temperatures of 300 and 250°C, respectively. The phenolic compounds were identified by comparison of their retention time and HPLC-ESI-MS/MS dissociation patterns with those of authentic standards. Quantification of individual phenolics was carried out by integrating the peak areas and using the calibration curves (20-1000 ng/mL) obtained with the corresponding standards. Acquisition was performed in multiple reaction monitoring (MRM) mode, and the Labsolution software (version 5.53 SP2, Shimadzu) was used for data acquisition and processing. Results were expressed as µg per gram of freeze-dried peel (µg/g fdw).

2.8. Profile of phenolics and other polar compounds by HPLC-ESI-QTOF-MS/MS

Profile of phenolic compounds and other polar compounds were performed using a HPLC system (Agilent Technologies 1290 series Infinity System LC, Santa Clara, CA, USA) coupled to a Q-ToF iFunnel (Agilent Technologies 6550) mass spectrometer fitted with an electrospray ionization (ESI) source. Separation of compounds was achieved on a Poroshell 120 SB-Aq column (100 × 2.1 mm i.d., particle size 2.7 µm, Agilent Technologies, Santa Clara, CA, USA) maintained at 40°C. The mobile phase consisted of 0.1% formic acid in water (eluent A) and acetonitrile containing 0.1% formic acid (v/v) (eluent B). The samples were eluted with a flow rate of 0.45 mL/min with the following gradient: 0-1 min, 5% B; 1-10 min, from 5% to 18% B; 10-13 min, from 18% to 70% B; 13-15 min, from 70% to 100% B; 15-17 min, 100% B; and 3 min of post time at 5% B to column re-equilibration. MS analyses were carried out with electrospray ionization interface (ESI) in negative ionization mode under the following conditions: VCap 3000 V; fragmentor voltage at 150 V; OCT 1RF Vpp at 750 V; gas temperature at 290°C; sheath gas temperature at 350°C; drying gas flow at 12 L/min. Mass spectra were acquired in profile mode and the acquisition range was 100-1500 m/z. Agilent MassHunter Qualitative Analysis software version B.07.00 was used to acquire and process the data. The molecular formula proposed by the MassHunter software for the different signals obtained in the MS experiments were compared with previously reported phenolics and other polar compounds found in fruits, and a maximum error of 8 ppm was accepted. For MS/MS experiments, the auto MS/MS acquisition mode was used; the main fragments were compared

with the fragmentation patterns reported in available references for phenolics and other polar compounds.

2.9. Statistical analysis

All experiments were carried out in triplicate and all values were reported as mean values ± standard deviation. The results are subjected to one-way analysis of variance (ANOVA) and differences between means were located using Tukey's HSD test. Correlation analyses between phenolics and antioxidant activity were carried out using standard Pearson correlation.

3. Results and discussion

3.1. Synergic effect of the nominal ultrasonic power and extraction time on phenolics recovery

The synergistic effects of the ultrasonic parameters on phenolics recovery and antioxidant activity were evaluated by the full factorial experimental design. In this study, we aimed to understand how interactions between the nominal ultrasonic power (W) and extraction time (min) affect phenolic compounds recovery in order to identify the lower level of each process parameter capable of propitiating the highest phenolics yields recovery from araticum peel. In the phenolics recovery studies, the optimal conditions that result in the greater phenolics recovery are associated with the process intensification. Therefore, we did not employ the full factorial experimental design aiming to find the optimized conditions of the ultrasonic phenolics extraction instead we focused on the synergic effects among the variables.

3.1.1. Fitting the models for the total phenolic content (TPC) and antioxidant activity

Table 1 shows the total phenolic content (TPC) and antioxidant activities (DPPH, TEAC and $ORAC_{FL}$) of araticum peel extracts found after ultrasonic extraction under different experimental conditions using full factorial experimental design. TPC, DPPH, TEAC and $ORAC_{FL}$ values from araticum peel extracts ranged from 48.90 to 70.68 mg GAE/g fdw, 368.54 to 514.57 μmol TE/g fdw, 453.93 to 613.76 μmol TE/g fdw and 315.89 to 525.41 μmol TE/g fdw, respectively. The experimental data were fitted to a quadratic polynomial model and the regression coefficients determined (**Table 2**). The statistical significance of each regression coefficient and quadratic polynomial models are given in **Table 2**.

Table 2. Regression coefficients and evaluation of the mathematical models for TPC, antioxidant activities and main individual phenolic compounds from araticum peel extracts.

Model parameters	**TPC**	**DPPH**	**TEAC**	**$ORAC_{FL}$**	**Total Phenolics**	**Epicatechin**	**Rutin**	**Chlorogenic Acid**	**Catechin**	**Ferulic Acid**
Regression coefficients										
a_0 $(Constant)$	44.98***	367.7***	398.7***	383.8***	129.6***	76.3***	30.62***	5.88***	6.36***	6.19***
a_1 $(Ultrasonic\ power)$	0.049**	0.32**	0.523***	0.042^{ns}	0.0476^{ns}	-0.0642^{ns}	0.0642***	0.02221**	0.01162^{ns}	0.00008^{ns}
a_2 $(Extraction\ time)$	-0.67^{ns}	-16.3^{ns}	13.4^{ns}	-9.7^{ns}	4.05^{ns}	1.94^{ns}	0.76^{ns}	0.598^{ns}	0.256^{ns}	-0.114^{ns}
a_1^2	-0.000045*	-0.000365**	-0.000475**	0.000095^{ns}	0.000008^{ns}	0.000112^{ns}	-0.000059**	-0.000020*	-0.000012^{ns}	-0.000002^{ns}
a_2^2	-0.119^{ns}	0.28^{ns}	-3.30^{ns}	-0.62^{ns}	-2.14^{ns}	-1.44^{ns}	-0.281^{ns}	-0.123^{ns}	-0.088^{ns}	-0.087^{ns}
a_1a_2	0.00615**	0.0556***	0.0281*	0.0375^{ns}	0.03723***	0.02564***	0.00440**	0.001588*	0.002225**	0.002163***
Polynomial model										
Regression (p-value)	<0.0001	<0.0001	<0.0001	0.0450	<0.0001	0.0001	<0.0001	<0.0001	0.0004	0.0086
R^2	0.87	0.85	0.85	0.52	0.89	0.82	0.88	0.85	0.77	0.64
R_{adj}^2	0.82	0.79	0.80	0.35	0.85	0.76	0.83	0.79	0.69	0.50
$R_{prediction}^2$	0.71	0.65	0.66	0.00	0.73	0.53	0.76	0.66	0.54	0.20
PRESS	2.59	18.07	20.34	42.62	12.69	10.32	2.42	1.07	1.24	0.98

^{ns} Non-significant.
*** Significant at p<0.01.
** Significant at p<0.05.
* Significant at p<0.1.

Linear and quadratic effects of nominal ultrasonic power, and the interaction effect between nominal ultrasonic power and extraction time was significant ($p \leq 0.1$) for the TPC, DPPH and TEAC assays. In contrast, no parameter was significant for the $ORAC_{FL}$ assay ($p > 0.1$). Analysis of variance (ANOVA) showed that the obtained quadratic polynomial models were highly significant ($p < 0.0001$) except for $ORAC_{FL}$ assay ($p = 0.045$), confirming the good fit of the models and their capability to elucidate the variation in responses through regression equations. The accuracy of the models and degree of agreement between the experimental and predicted values were assessed based on the determination coefficient (R^2), adjusted determination coefficient (R^2_{adj}) and predicted determination coefficient ($R^2_{prediction}$) [19,31]. Thus, the high values obtained for R^2 (≥ 0.85), R^2_{adj} (≥ 0.79) and $R^2_{prediction}$ (≥ 0.65) indicate that the models could adequately represent the effects of the nominal ultrasonic power and extraction time on the TPC, DPPH and TEAC responses. In addition, the low PRESS values (excluding $ORAC_{FL}$ assay) suggested that the adjusted quadratic polynomial models are suitable for predictive applications [32].

3.1.2. Fitting the models for the individual phenolic compounds extraction

In order to evaluate the effect of ultrasonic extraction parameters on recovery of individual phenolic compounds, the HPLC-ESI-MS/MS method was used to quantify the predominant phenolic compounds present in araticum peel extracts based on authentic phenolic standards. As can be seen in **Fig. 1**, 14 phenolic compounds were positively identified and quantified in araticum peel extracts by HPLC-ESI-MS/MS. **Table 3** shows the concentration of each phenolic compound obtained from araticum peel extracts after ultrasonic extraction under different experimental conditions using full factorial experimental design. Total phenolic content (sum of detected phenolic content by HPLC-ESI-MS/MS) from araticum peel extracts ranged from 126.23 to 242.52 µg/g fdw. Epicatechin, rutin, chlorogenic acid, catechin and ferulic acid were found as main phenolic compounds from araticum peel extracts with contents ranging from 54.46 to 136.47, 38.02 to 58.53, 9.11 to 16.83, 7.38 to 15.23, and 5.18 to 10.96 µg/g fdw, respectively. Thus, besides the sum of detected phenolic content by HPLC-ESI-MS/MS, these 5 main phenolics were also used to evaluate the effect of nominal ultrasonic power and extraction time on their individual phenolic recovery from araticum peel.

Table 3. Influence of the nominal ultrasonic power (W) and extraction time (min) on the individual phenolic compounds content found in the araticum peel extracts.

		Concentration (μg/g fdw)							
Nominal ultrasonic Power (W)	**Extraction time (min)**	**Epicatechin**	**Rutin**	**Chlorogenic Acid**	**Catechin**	**Ferulic Acid**	**Vicenin-2**	**Vanillin**	**Naringenin**
160	0.5	79.32±2.38	39.89±0.22	10.16±0.05	10.18±0.13	6.20±0.15	1.48±0.06	1.58±0.17	1.19±0.14
	1.0	75.65±3.41	40.20±0.98	9.11±0.24	7.38±0.35	5.50±0.78	1.60±0.28	1.10±0.26	1.11±0.06
	3.0	54.46±1.40	39.87±0.38	9.56±0.42	8.60±0.33	6.20±0.37	1.82±0.15	1.45±0.09	1.20±0.02
	5.0	63.59±2.32	38.02±0.62	9.23±0.20	8.62±0.72	5.18±0.31	1.84±0.34	1.71±0.21	1.00±0.02
280	0.5	79.06±3.30	47.87±1.02	11.57±0.51	9.52±0.28	7.18±0.28	2.44±0.14	1.60±0.25	1.22±0.06
	1.0	71.67±1.21	45.44±0.60	11.08±0.11	8.08±0.89	6.20±0.56	1.87±0.11	1.15±0.11	1.24±0.04
	3.0	92.42±0.59	48.19±0.34	13.27±0.50	9.52±0.17	7.04±0.47	2.60±0.30	1.91±0.09	1.39±0.08
	5.0	87.75±0.08	49.60±0.10	14.46±0.19	11.77±1.40	6.27±0.64	1.99±0.07	1.62±0.26	1.58±0.06
400	0.5	65.57±4.18	46.43±1.23	11.47±0.35	8.04±0.04	5.70±0.36	2.48±0.70	1.33±0.31	1.38±0.04
	1.0	72.33±2.73	49.91±0.13	13.21±0.15	11.18±0.50	7.89±0.50	2.15±0.21	1.91±0.56	1.72±0.05
	3.0	84.95±0.58	55.07±1.21	14.84±0.26	13.15±1.15	8.82±0.33	2.77±0.46	1.90±0.21	1.73±0.04
	5.0	89.12±1.59	52.46±1.25	14.63±0.50	13.45±0.61	7.73±0.11	2.95±0.32	1.97±0.10	1.62±0.03
520	0.5	82.01±3.80	46.97±1.80	12.34±0.46	11.31±0.48	6.41±0.37	2.30±0.13	2.05±0.73	1.48±0.04
	1.0	85.67±3.67	48.32±1.45	12.62±0.38	10.38±1.99	5.75±0.43	1.38±0.15	1.97±0.27	1.39±0.05
	3.0	108.66±3.64	50.99±1.61	13.71±0.52	12.56±1.01	6.58±0.45	2.46±0.36	3.16±0.90	1.60±0.18
	5.0	119.45±0.10	55.14±0.98	15.42±0.24	12.57±0.65	7.47±0.25	2.44±0.08	3.47±0.55	1.85±0.05
640	0.5	81.99±0.44	48.78±1.57	11.81±0.63	8.85±0.69	5.71±0.24	2.02±0.21	2.00±0.42	1.45±0.10
	1.0	105.82±3.56	55.21±2.03	15.35±0.44	11.08±0.56	7.95±0.42	2.40±0.04	2.17±0.63	1.60±0.06
	3.0	136.47±10.02	55.75±0.42	16.27±1.15	14.10±1.19	8.26±0.69	2.49±0.06	3.04±0.51	1.90±0.14
	5.0	123.07±5.32	58.53±0.66	16.83±0.25	15.23±1.28	10.96±0.92	2.58±0.34	2.85±0.45	2.15±0.07

Table 3. (Continued)

Nominal ultrasonic Power (W)	Extraction time (min)	Concentration (µg/g fdw)						
		Protocatechuic Acid	**Caffeic Acid**	**Luteolin**	***p*-Coumaric Acid**	**4-Hydroxybenzoic Acid**	**Vitexin**	**Total phenolics**
160	0.5	1.20±0.12	0.38±0.08	0.49±0.06	0.33±0.06	0.25±0.01	0.12±0.04	152.77±1.98
	1.0	1.09±0.08	0.56±0.06	0.55±0.00	0.23±0.04	0.20±0.01	0.08±0.03	144.37±3.83
	3.0	1.28±0.02	0.68±0.13	0.51±0.03	0.25±0.03	0.21±0.01	0.13±0.02	126.23±1.19
	5.0	1.17±0.13	0.65±0.05	0.55±0.02	0.13±0.02	0.19±0.02	0.08±0.01	131.97±1.80
280	0.5	1.20±0.05	0.49±0.12	0.58±0.05	0.30±0.00	0.25±0.01	0.14±0.01	163.41±3.17
	1.0	1.03±0.03	0.56±0.13	0.54±0.01	0.26±0.06	0.27±0.03	0.14±0.01	149.54±1.58
	3.0	1.53±0.16	0.71±0.10	0.60±0.02	0.41±0.04	0.29±0.03	0.15±0.01	180.04±1.34
	5.0	1.36±0.09	0.93±0.05	0.52±0.06	0.39±0.03	0.41±0.03	0.18±0.01	178.82±1.75
400	0.5	1.44±0.04	0.72±0.07	0.69±0.07	0.34±0.05	0.35±0.01	0.14±0.03	146.08±4.13
	1.0	1.42±0.14	0.75±0.11	0.60±0.05	0.33±0.02	0.25±0.03	0.15±0.02	163.79±2.78
	3.0	1.48±0.00	0.98±0.12	0.70±0.08	0.46±0.01	0.41±0.01	0.13±0.02	187.39±0.61
	5.0	1.37±0.04	1.00±0.14	0.72±0.17	0.42±0.02	0.42±0.03	0.15±0.01	188.01±2.41
520	0.5	1.28±0.05	0.71±0.05	0.65±0.12	0.26±0.01	0.35±0.02	0.17±0.01	168.31±3.36
	1.0	1.37±0.08	0.62±0.10	0.64±0.10	0.40±0.03	0.31±0.01	0.15±0.00	170.98±1.20
	3.0	1.36±0.10	0.80±0.12	0.76±0.05	0.37±0.02	0.33±0.02	0.17±0.02	203.51±5.16
	5.0	1.32±0.02	0.93±0.21	0.82±0.03	0.42±0.01	0.49±0.02	0.16±0.06	221.96±1.63
640	0.5	1.31±0.06	0.63±0.05	0.79±0.06	0.27±0.01	0.33±0.01	0.14±0.00	166.07±2.52
	1.0	1.47±0.08	1.13±0.19	0.83±0.14	0.39±0.02	0.40±0.04	0.18±0.01	205.98±2.41
	3.0	1.51±0.11	0.74±0.02	0.91±0.15	0.44±0.04	0.45±0.07	0.18±0.04	242.52±11.07
	5.0	1.64±0.07	1.22±0.26	0.86±0.09	0.49±0.02	0.46±0.01	0.14±0.02	237.03±5.67

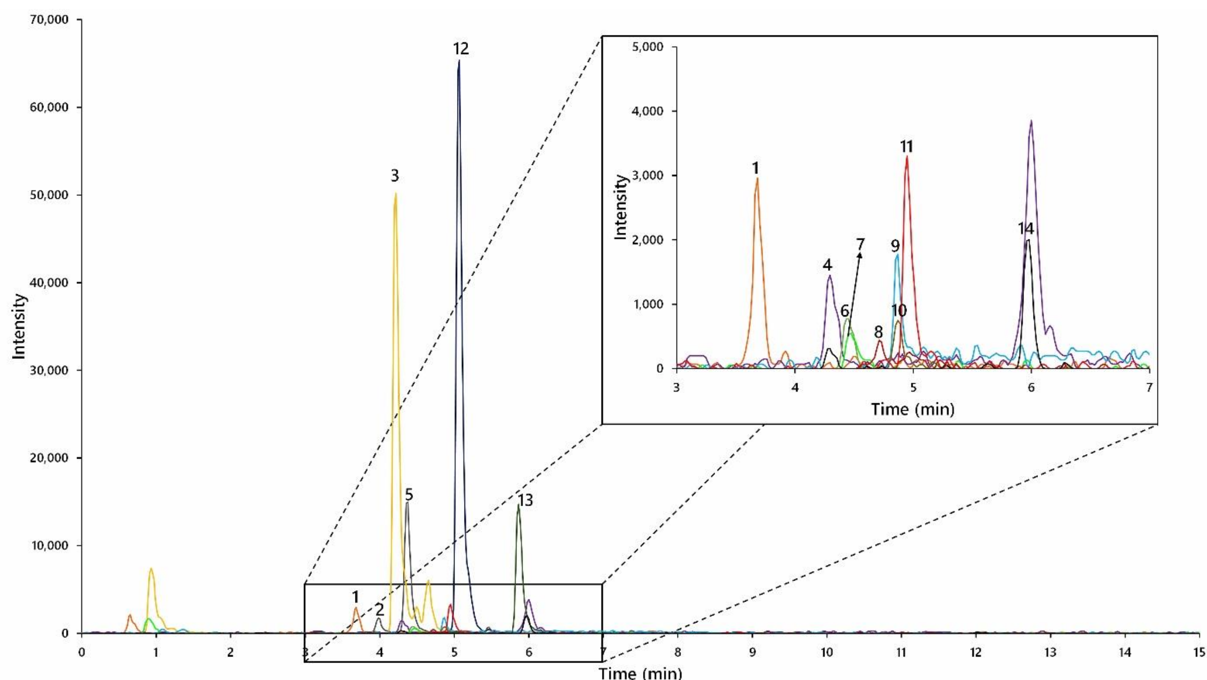

Fig. 1. HPLC-ESI-MS/MS chromatogram showing the phenolic compounds profile in the araticum peel extract. *Legend:* (1) Protocatechuic acid, (2) Catechin, (3) Chlorogenic acid, (4) 4-Hydroxybenzoic acid, (5) Epicatechin, (6) Vicenin-2, (7) Caffeic acid, (8) Vanillin, (9) *p*-Coumaric acid, (10) Vitexin, (11) Ferulic acid, (12) rutin, (13) Naringenin, and (14) Luteolin.

The experimental data were fitted to a quadratic polynomial model and the regression coefficients determined. The estimated regression coefficients of the multiple regression models, the probability (p-values) of each regression coefficient and quadratic polynomial models, R^2, R^2_{adj}, $R^2_{prediction}$ and PRESS of the response variables are shown in **Table 2**. Linear and quadratic effects of nominal ultrasonic power, and the interaction effect between nominal ultrasonic power and extraction time was significant ($p \leq 0.1$) for the rutin and chlorogenic acid contents. On the other hand, only the interaction effect between nominal ultrasonic power and extraction time was significant ($p \leq 0.1$) for the sum of detected phenolic content by HPLC-ESI-MS/MS, epicatechin, catechin and ferulic acid. All obtained quadratic polynomial models were highly significant with $p \leq 0.0086$ and showed low PRESS values ($\leq$ 12.69), which implied that model equations were adequate for predicting the effects of process parameters on the ultrasonic extraction of phenolics from araticum peel [20,32]. In addition, exception for ferulic acid, the obtained quadratic polynomial models exhibited R^2 ($\geq$ 0.77), R^2_{adj} ($\geq$ 0.69) and $R^2_{prediction}$ ($\geq$ 0.54) values relatively high, demonstrating that the

experimental results were well adjusted to the polynomial models proposed by full factorial experimental design [31].

3.1.3. Effect of the ultrasonic extraction parameters on the phenolics content and antioxidant activity

The effects of the independent variables (nominal ultrasonic power and extraction time) and their interactions on the phenolics content and antioxidant activity are shown in the contour plots (**Fig. 2** and **3**). As can be seen in **Fig. 2** and **3**, the nominal ultrasonic power factor has a significant synergistic effect with extraction time. TPC, individual phenolic contents, and antioxidant activities by the DPPH, TEAC and $ORAC_{FL}$ assays increased as the applied nominal ultrasonic power and extraction time increased. These results agree with previously reported data [18,23], which showed a significant increase in the phenolics/antioxidants recovery with increasing nominal ultrasonic power and extraction time. However, some others studies have reported that the phenolics/antioxidants recovery increases with ultrasonication process intensification up to a certain point, then begins to decrease [19,22]. Prolonged exposure of the extracted phenolics/antioxidants to the high ultrasonic powers can result in the degradation and structural destruction of extracted phenolics/antioxidants due to higher pressure and temperature created by the intense collapse of cavitation microbubbles, leading to lower recovery efficiency [19,22]. This trend was not noticed in the present study mainly due to two factors: i) lower extraction times compared to other studies (≤ 5min), thus reducing the exposure of the extracted phenolics/antioxidants to the intense process conditions; and ii) cooling of the sample throughout the extraction procedure, avoiding sample overheating during the extraction (sample temperature ≤ 40.4°C).

According to **Fig. 2** and **3**, we verified that the nominal ultrasonic power presents an important effect on the phenolic compounds recovery and antioxidant activity. However, from the literature, we identified that few studies evaluated the effect synergistic between nominal ultrasonic power and other process variables in high-intensity ultrasound-assisted extraction of phenolics/antioxidants compounds from plant matrices [18,19,23]. The results reported in the literature are mostly based on the effects of temperature, pH, solid to liquid ratio, solvent concentration and extraction time [24–27]. Therefore, detailed studies evaluating diverse levels of nominal ultrasonic power and its interaction with other process variables can contribute to advances in obtaining of bioactive compounds by using high-intensity ultrasound-assisted extraction.

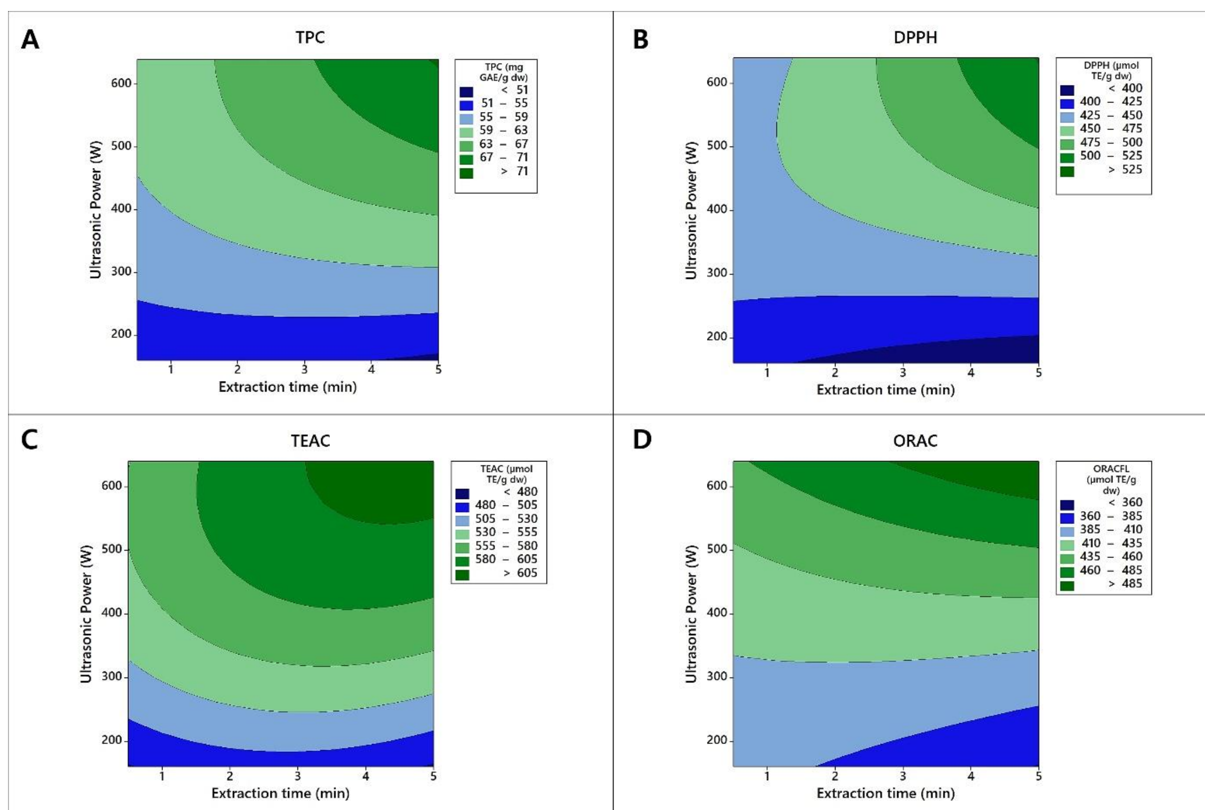

Fig. 2. Contour plots showing the effects of the nominal ultrasonic power and extraction time on the (A) total phenolic compounds (TPC) and antioxidant activities by (B) DPPH, (C) TEAC and (D) ORACFL assays from araticum peel extracts.

Our findings demonstrated that the ultrasonication process intensification had a positive effect on the phenolic compounds recovery and antioxidant activities values from araticum peel. The increase of the phenolics recovery and antioxidant activities can be attributed to the increase in energy density (**Eq. (1)**), since the intensity of the acoustic cavitation is increased as energy density increases. Acoustic cavitation phenomenon caused by the propagation of ultrasound pressure waves promotes the formation and implosion of microbubbles on the matrix surface resulting in micro-jetting which generates diverse effects such as surface peeling, fragmentation, erosion, sonocapillary effect, sonoporation, local shear stress and destruction/detexturation of plant structures. In addition, the implosion of cavitation microbubbles in a liquid media leads to macro-turbulences and micro-mixing. These combined effects result in the collapse of plant matrix, enabling solvent penetration into the plant material and improving the release of intracellular substances into the solvent, consequently increasing extraction rates [17,33].

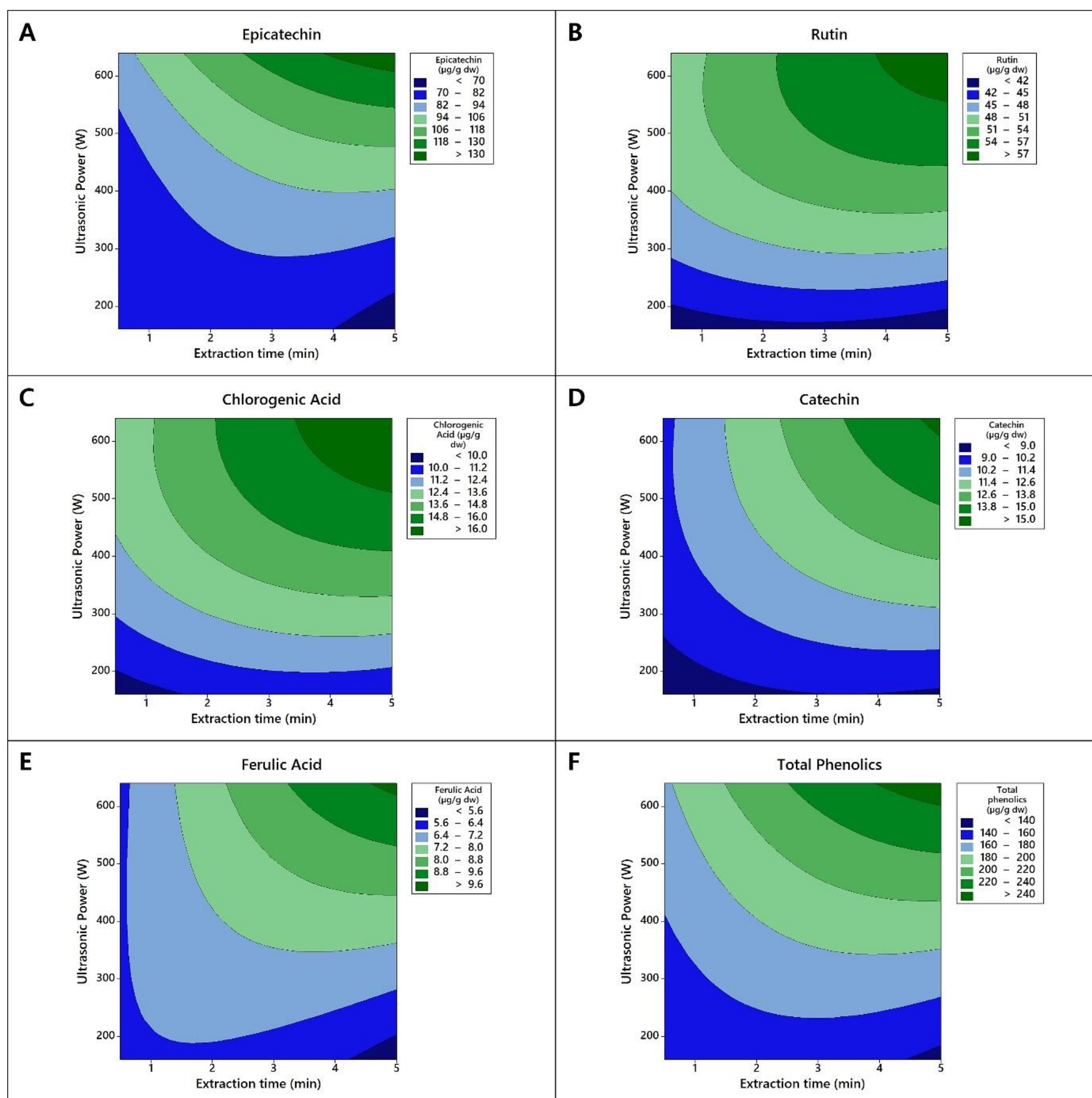

Fig. 3. Contour plots showing the effects of the nominal ultrasonic power and extraction time on the content of (A) epicatechin, (B) rutin, (C) chlorogenic acid, (D) catechin, (E) ferulic acid, and (F) total phenolics found in araticum peel extracts by HPLC-ESI-MS/MS.

During cavitation microbubbles collapse, an enormous concentration of energy is released leading to intense local heating [34]. During ultrasound-assisted extraction of phenolics from araticum peel, the samples temperature increased proportionally ($r = 0.80$; $p < 0.0001$) to the input of the ultrasound energy (energy density). These hotspots may have contributed to the increased recovery of phenolic compounds by the softening and swelling of the plant tissues; the weakening of phenol-macromolecules interactions; the increase in solubility of the compounds in the extraction system; the reduction in viscosity and surface

tension of the solvent; and the increase of the molecular mobility. These combined effects make the plant matrix more permeable and facilitate the penetration of the solvent into the matrix, accelerating the mass transfer rate [3,35]. It is worth emphasizing that for the most intense process conditions, the sample temperature at the end of extraction was no more than 40.4°C due to cooling of the samples during the extraction process. This avoided the samples overheating that could lead to the chemical and/or thermal degradation of certain phenolic compounds [36]. Thus, despite the recovery enhancement by ultrasound is mainly due to the cavitation phenomena, effects thermal derived from cavitation microbubbles collapse can also collaborate for the increase in phenolics recovery.

3.2. Araticum peel phenolic-rich extracts characterization

As can be observed in **Table 1**, the maximum TPC and antioxidant activities values obtained from araticum peel by using high-intensity ultrasound-assisted extraction were 70.68 mg GAE/g fdw, and 514.57, 613.76 and 525.41 μmol TE/g fdw for DPPH, TEAC and $ORAC_{FL}$ antioxidant assays, respectively. Previous studies also reported high TPC values and strong antioxidant activities in araticum peel extracts [10,14,37]. However, these studies used different extraction techniques/equipment (mixer [37], mixer combined with maceration [14], and ultrasonic bath [10]) and did not evaluate the effects of process variables. Moreover, in most cases, the quantification was performed based on extract mass instead of the raw material, making these data not comparable to the ones of the present study. Nevertheless, the efficacy of an extraction process is not only shown through improved target compounds extraction yield but also for its ability of reducing the extraction time. Evaluating the interaction of the nominal ultrasonic power with the extraction time (**Fig. 2** and **3**), it was found that the recovery of a high phenolic antioxidants content could be achieved at short extraction times (≤ 5 min) when operating with higher nominal ultrasonic powers, whereas the previous studies took from 40 minutes to 6 days to obtain the phenolic-rich extracts [10,14,37]. Thus, our findings demonstrated that the use of high-intensity ultrasound was able to recover high phenolic contents significantly reducing extraction time.

The biological and technological properties of phenolic compounds are mainly due to their antioxidant capacity. Phenolic compounds can act as antioxidants in chemical or biochemical systems by several ways, such as donating hydrogen atoms, chelating transition metals, scavenging reactive oxygen and/or nitrogen species (ROS/RNS), inhibiting enzymes involved in oxidative stress, and upregulating and/or protecting endogenous defence systems

[10]. Therefore, Pearson correlation analysis was conducted to evaluate the correlation between antioxidant activity and phenolic compounds in araticum peel extracts and the results are presented in **Table 4**. Antioxidant activities (DPPH, TEAC and $ORAC_{FL}$) were high and positively correlated with the TPC ($r \geq 0.72$; $p \geq 0.0003$) and the sum of detected phenolic content by HPLC-ESI-MS/MS ($r \geq 0.78$; $p > 0.0001$). These results indicate that the phenolic compounds are the main responsible for the antioxidant activity in araticum peel extracts, although more in-deep studies are needed.

Table 4. Correlations between the phenolic compounds content and the antioxidant activities in araticum peel extracts.

Parameters	Pearson's correlation coefficients (r)	p-value
TPC *vs.* DPPH	0.95	<0.0001
TPC *vs.* TEAC	0.95	<0.0001
TPC *vs.* $ORAC_{FL}$	0.72	0.0003
TPC *vs.* Total Phenolics (HPLC-MS)	0.95	<0.0001
Total Phenolics (HPLC-MS) *vs.* DPPH	0.93	<0.0001
Total Phenolics (HPLC-MS) *vs.* TEAC	0.89	<0.0001
Total Phenolics (HPLC-MS) *vs.* $ORAC_{FL}$	0.78	<0.0001

Phenolic compounds can deactivate free radicals by two main mechanisms: single electron transfer (ability to transfer one electron to reduce any compound, including radicals, metals and carbonyls) and hydrogen atom transfer (ability to quench free radicals by hydrogen donation). TEAC and DPPH assays are based both on hydrogen atom transfer and single electron transfer, whereas $ORAC_{FL}$ is hydrogen atom transfer based assay [38]. The higher correlations found between phenolic contents and DPPH ($r = 0.952$ and 0.93 for the results from TPC and the sum of detected phenolic content by HPLC-ESI-MS/MS, respectively) and TEAC ($r = 0.95$ and 0.89 for the results from TPC and the sum of detected phenolic content by HPLC-ESI-MS/MS, respectively) values as compared with the ones found for $ORAC_{FL}$ ($r = 0.72$ and 0.78 for the results from TPC and the sum of detected phenolic content by HPLC-ESI-MS/MS, respectively) values suggests that phenolic compounds from araticum peel extracts act more efficiently through mixed mechanism than by hydrogen atom transfer.

The HPLC-ESI-MS/MS phenolic profile of araticum peel extracts obtained by using high-intensity ultrasound technology is shown in **Fig. 1**. Between the 31 phenolic compounds tested, 14 were positively identified in the araticum peel extracts, including 7

flavonoids (3 flavones: vicenin-2, luteolin and vitexin; 2 flavan-3-ols: catechin and epicatechin; 1 flavonol: rutin; and 1 flavanone: naringenin), 6 phenolic acids (4 hydroxycinnamic acids: chlorogenic, caffeic, *p*-coumaric and ferulic acids; and 2 hydroxybenzoic acids: protocatechuic and 4-hydroxybenzoic acids) and 1 hydroxybenzaldehyde (vanillin). As can be seen in **Table 3**, flavonoids were the predominant phenolic class from araticum peel extracts, accounting for approximately 86% of all quantified phenolics. These results agree with previously reported data by Arruda et al. [10], who found that flavonoids were the most plentiful phenolic compounds (90.49%) in araticum peel extracts. The predominance of flavonoids in the araticum peel may explain the high antioxidant activity presented by the extracts (**Table 1**), since flavonoids possess several structural elements that contribute for their antioxidant activity as previously discussed by Arruda et al. [10].

Among the 14 phenolic compounds positively identified and quantified by HPLC-ESI-MS/MS, 8 (epicatechin, catechin, rutin, and chlorogenic, caffeic, ferulic, protocatechuic and *p*-coumaric acids) were previously characterized in araticum peel extracts [10,14,37], whereas vicenin-2, vanillin, naringenin, luteolin, 4-hydroxybenzoic acid and vitexin were identified and quantified here for the first time. Quantification of phenolic compounds (**Table 3**) revealed that epicatechin was the major phenolic present in the araticum peel extracts, followed by rutin, chlorogenic acid, catechin and ferulic acid, respectively. These compounds have been reported to exhibit numerous health benefit effects [4–9].

3.3. Araticum peel extract characterization by HPLC-ESI-QTOF-MS/MS

In order to obtain a more deepened characterization regarding the phytochemicals present in the araticum peel extract obtained using high-intensity ultrasound technology, this was submitted to a profile analysis by HPLC-ESI-QTOF-MS/MS. An overview of all the phytochemicals tentatively identified in the araticum peel extract is given in **Table 5**. Peaks identification was performed on the basis of their accurate mass (error $\leq$ 8 ppm) and fragmentation pattern together with the information previously reported in the literature and databases for plant phytochemicals. Moreover, reported phytochemical information from other parts of araticum plant and *Annona* genus were used as an indicative information to assist the identification of compounds due to the analogue of compound composition of plant belonging to the same species and genus. Some peaks were analysed only for their accurate mass, as their relative intensity was much lower than that of the major peaks, making it impossible their fragmentation.

Table 5. Identified or tentatively identified phytochemical compounds from the araticum peel extract by HPLC-ESI-QTOF-MS/MS under negative ion mode.

No.	Proposed compound	r.t. (min)	m/z experimental	Calculated mass	MS/MS fragments	Molecular formula	Error (ppm)
Organic acids							
1	Malic acid	0.69	133.0418 $[M-H]^-$	134.0221	115	$C_4H_6O_5$	-4.40
2	Quinic acid	0.73	191.0203 $[M-H]^-$	192.0276	191/160/129/111	$C_6H_8O_7$	2.62
3	Citric or quinic acid derivative	0.77	405.0245 $[M-H]^-$	406.0320	405/191/173/111	$C_{21}H_{10}O_9$	-2.58
11	*n*-Propylmalic acid	1.47	175.0614 $[M-H]^-$	176.0687	131/115/113	$C_7H_{12}O_5$	-1.09
28	Citric acid	2.88	191.0202 $[M-H]^-$	192.0274	191/160/129/111	$C_6H_8O_7$	1.72
Jasmonates							
39	Tuberonic acid hexoside isomer 1	3.67	387.1669 $[M-H]^-$	388.1741	387/207	$C_{18}H_{28}O_9$	0.35
43	Tuberonic acid hexoside isomer 2	4.07	387.1658 $[M-H]^-$	388.1732	387/207	$C_{18}H_{28}O_9$	-2.07
56	Tuberonic acid hexoside isomer 3	4.68	387.1670 $[M-H]^-$	388.1742	387/207	$C_{18}H_{28}O_9$	0.52
Phenolic acids							
5	Syringic acid hexoside isomer 1	1.03	359.0995 $[M-H]^-$	360.1068	359/239/197/182/153/123	$C_{15}H_{20}O_{10}$	3.26
6	Feruloyl hexoside isomer 1	1.08	401.1097 $[M+COOH]^-$	356.1114	355/175/160	$C_{16}H_{20}O_9$	0.17
8	Protocatechuic acid hexoside	1.32	315.0744 $[M-H]^-$	316.0817	153/152/109/108	$C_{13}H_{16}O_9$	-7.33
9	Feruloyl hexoside isomer 2	1.32	401.1102 $[M+COOH]^-$	356.1111	355/175/160	$C_{16}H_{20}O_9$	-0.47
12	Hydroxybenzoic acid hexoside	1.54	345.0828 $[M+COOH]^-$	300.0865	299/263/137	$C_{13}H_{16}O_8$	6.69
13	Feruloyl hexoside isomer 3	1.58	401.1088 $[M+COOH]^-$	356.1104	355/175/160	$C_{16}H_{20}O_9$	-2.39
15	Caffeoyl dihexoside isomer 1	1.72	503.1412 $[M-H]^-$	504.1492	503/341/323/179/161/137	$C_{21}H_{28}O_{14}$	1.79
17	Dihydroxybenzoic acid pentoside	2.01	285.0624 $[M-H]^-$	286.0724	225/152/121/108	$C_{12}H_{14}O_8$	-2.51
20	Vanillic acid hexoside isomer 1	2.14	329.0889 $[M-H]^-$	330.0959	167/123	$C_{14}H_{18}O_{10}$	-3.53
21	4-hydroxybenzoic acid	2.18	137.0249 $[M-H]^-$	138.0349	137/108	$C_7H_6O_3$	-3.86
22	Caffeoyl dihexoside isomer 2	2.24	503.1414 $[M-H]^-$	504.1486	503/341/323/179/161/137	$C_{21}H_{28}O_{14}$	0.67
23	Caffeoyl hexoside isomer 1	2.36	341.0884 $[M-H]^-$	342.0958	341/179/135	$C_{15}H_{18}O_9$	1.43
24	Syringic acid hexoside isomer 2	2.51	359.0981 $[M-H]^-$	360.1049	359/239/197/182/153/123	$C_{15}H_{20}O_{10}$	-2.02
26	Syringic acid	2.79	197.0464 $[M-H]^-$	198.0537		$C_9H_{10}O_5$	-4.51

27	Syringic acid hexoside isomer 3	2.83	359.0991 [M-H]⁻	360.1064	359/239/197/182/153/123	$C_{15}H_{20}O_{10}$	2.29
30	Vanilic acid	3.05	167.0356 [M-H]⁻	168.0429		$C_8H_8O_4$	-3.78
31	Vanillic acid hexoside isomer 2	3.06	329.0885 [M-H]⁻	330.0958	329/167/123	$C_{14}H_{18}O_9$	2.07
32	Caffeoyl hexoside isomer 2	3.41	341.0879 [M-H]⁻	342.0952	341/179/135	$C_{15}H_{18}O_9$	-0.21
38	Vanillic acid hexoside isomer 3	3.58	375.0946 [M+COOH]⁻	330.0963	329/167/123	$C_{14}H_{18}O_9$	3.78
40	Caffeic acid	3.70	179.0357 [M-H]⁻	180.0431	179/135	$C_9H_8O_4$	1.48
41	Vanillic acid hexoside isomer 4	3.95	329.0889 [M-H]⁻	330.0962	329/167/123	$C_{14}H_{18}O_9$	3.19
45	Feruloyl hexoside isomer 4	4.13	401.1104 [M+COOH]⁻	356.1121	355/175/160	$C_{16}H_{20}O_9$	2.11
46	Chlorogenic acid	4.18	353.0869 [M-H]⁻	354.0945	191/179/135	$C_{16}H_{18}O_9$	1.65
50	Feruloyl hexoside isomer 5	4.41	355.1033 [M-H]⁻	356.1104	355/175/160	$C_{16}H_{20}O_9$	-2.43
54	*p*-Coumaric acid isomer 1	4.66	163.0403 [M-H]⁻	164.0473		$C_9H_8O_3$	0.45
63	Caffeoylquinic acid	5.02	353.0886 [M-H]⁻	354.0960		$C_{16}H_{18}O_9$	1.41
66	*p*-Coumaric acid isomer 2	5.35	163.0408 [M-H]⁻	164.0481		$C_9H_8O_3$	-4.38
67	Feruloyl hexoside isomer 6	5.36	355.1037 [M-H]⁻	356.1110	355/175/160	$C_{16}H_{20}O_9$	-0.86
78	Protocatechuic acid	6.39	153.0196 [M-H]⁻	154.0269		$C_7H_6O_4$	-1.63
79	Caffeoylshikimic acid	6.48	335.0780 [M-H]⁻	336.0853	335/179/161/135	$C_{16}H_{16}O_8$	-2.22
80	Ferulic acid	6.69	193.0506 [M-H]⁻	194.0580		$C_{10}H_{10}O_4$	-0.49
88	Caffeoyltyramine isomer 1	8.11	344.1146 [M+COOH]⁻	299.1164	298/178/161/135	$C_{17}H_{17}NO_4$	-1.27
106	Caffeoyltyramine isomer 2	10.50	298.1087 [M-H]⁻	299.1159	298/178/161/135	$C_{17}H_{17}NO_4$	-2.89
Flavonoids							
4	Procyanidin B tetramer isomer 1	1.03	1153.2572 [M-H]⁻	1154.2649	1153/863/577/489/433/326/287/243	$C_{60}H_{50}O_{24}$	-3.72
10	Kuwanon G isomer 1	1.42	727.1943 [M+Cl]⁻	692.2253		$C_{40}H_{36}O_{11}$	-0.38
16	Kuwanon G isomer 2	1.97	727.1934 [M+Cl]⁻	692.2246		$C_{40}H_{36}O_{11}$	-1.50
18	Isorhamnetin	2.10	315.0521 [M-H]⁻	316.0594		$C_{16}H_{12}O_7$	-3.35
19	Procyanidin A dimer isomer 1	2.14	575.1204 [M-H]⁻	576.1278		$C_{30}H_{24}O_{12}$	1.75
25	Procyanidin A dimer isomer 2	2.64	575.1206 [M-H]⁻	576.1278		$C_{30}H_{24}O_{12}$	1.69
29	Procyanidin A trimer isomer 1	3.00	863.1833 [M-H]⁻	864.1905		$C_{45}H_{36}O_{18}$	0.36
33	Catechin	3.41	289.0723 [M-H]⁻	290.0796	289/245/203/125	$C_{15}H_{14}O_6$	-1.78
34	Procyanidin B dimer isomer 1	3.44	577.1359 [M-H]⁻	578.1431	577/451/425/407/381/289/273/161/125	$C_{30}H_{26}O_{12}$	-0.37
35	(Epi)gallocatechin	3.49	305.0672 [M-H]⁻	306.0744		$C_{15}H_{14}O_7$	-1.59

36	Procyanidin B dimer isomer 2	3.56	613.1122 [M+Cl]$^-$	578.1433	577/451/425/407/381/289/273/161/125	$C_{30}H_{26}O_{12}$	-0.08
37	Procyanidin A dimer isomer 3	3.58	575.1197 [M-H]$^-$	576.1278		$C_{30}H_{24}O_{12}$	1.80
42	Procyanidin B trimer isomer 1	4.05	865.1979 [M-H]$^-$	866.2049	865/695/577/405/287/289	$C_{45}H_{38}O_{18}$	-1.04
44	Kuwanon G isomer 3	4.07	737.2264 [M+COOH]$^-$	692.2281		$C_{40}H_{36}O_{11}$	3.57
47	Procyanidin A dimer isomer 4	4.19	575.1201 [M-H]$^-$	576.1274		$C_{30}H_{24}O_{12}$	1.00
48	Procyanidin B trimer isomer 2	4.24	865.2004 [M-H]$^-$	866.2077	865/695/577/405/287/289	$C_{45}H_{38}O_{18}$	0.14
49	Quercetin hexoside pentoside isomer 1	4.41	631.1066 [M+Cl]$^-$	596.1372	595/300/301/271	$C_{26}H_{28}O_{16}$	-0.91
51	Procyanidin B tetramer isomer 2	4.42	1153.2606 [M-H]$^-$	1154.2679	1153/863/577/489/433/326/287/243	$C_{60}H_{50}O_{24}$	-1.14
52	Procyanidin B dimer isomer 3	4.45	577.1357 [M-H]$^-$	578.1429	577/451/425/407/381/289/273/161/125	$C_{30}H_{26}O_{12}$	-0.66
53	Procyanidin A trimer isomer 2	4.62	863.1830 [M-H]$^-$	864.1902		$C_{45}H_{36}O_{18}$	0.02
55	Quercetin hexoside pentoside isomer 2	4.66	631.1045 [M+Cl]$^-$	596.1371	595/300/301/271	$C_{26}H_{28}O_{16}$	-1.00
57	Epicatechin	4.71	289.0731 [M-H]$^-$	290.0804	289/245/203/125	$C_{15}H_{14}O_6$	1.10
58	Procyanidin B tetramer isomer 3	4.76	1153.2604 [M-H]$^-$	1154.2743	1153/863/577/489/433/326/287/243	$C_{60}H_{50}O_{24}$	4.42
59	Procyanidin B dimer isomer 4	4.77	577.1353 [M-H]$^-$	578.1428	577/451/425/407/381/289/273/161/125	$C_{30}H_{26}O_{12}$	-0.91
60	Procyanidin B trimer isomer 3	4.79	865.1988 [M-H]$^-$	866.2060	865/695/577/405/289/287	$C_{45}H_{38}O_{18}$	0.25
61	Procyanidin A tetramer	4.92	1151.2450 [M-H]$^-$	1152.2528		$C_{60}H_{48}O_{24}$	-0.71
62	Procyanidin B trimer isomer 4	4.94	865.1986 [M-H]$^-$	866.2058	865/695/577/405/287/289	$C_{45}H_{38}O_{18}$	-0.01
64	Procyanidin B tetramer isomer 4	5.07	1153.2582 [M-H]$^-$	1154.2655	1153/863/577/489/433/326/287/243	$C_{60}H_{50}O_{24}$	-3.22
65	Procyanidin A trimer isomer 3	5.09	863.1820 [M-H]$^-$	864.1893		$C_{45}H_{36}O_{18}$	-1.07
68	Procyanidin B trimer isomer 5	5.36	901.1729 [M+Cl]$^-$	866.1996	865/695/577/405/289/287	$C_{45}H_{38}O_{18}$	-7.12
69	Quercetin diglucoside isomer 1	5.44	625.1396 [M-H]$^-$	626.1480		$C_{27}H_{30}O_{17}$	0.55
70	Procyanidin B tetramer isomer 5	5.49	1153.2614 [M-H]$^-$	1154.2686	1153/863/577/489/433/326/287/243	$C_{60}H_{50}O_{24}$	-0.54
71	Kaempferol 3-O-di-*p*-coumaroyl hexoside isomer 1	5.57	739.1664 [M-H]$^-$	740.1735		$C_{39}H_{32}O_{15}$	-1.85
72	Procyanidin B trimer isomer 6	5.57	865.1983 [M-H]$^-$	866.2063	865/695/577/405/289/287	$C_{45}H_{38}O_{18}$	0.56
73	(epi)Afzelechin-(epi)catechin	5.70	561.1393 [M-H]$^-$	562.1466	435/407/271/245/125	$C_{30}H_{26}O_{11}$	1.65
74	Procyanidin A dimer isomer 5	5.99	621.1254 [M+COOH]$^-$	576.1277		$C_{30}H_{24}O_{12}$	1.63
75	Quercetin hexoside pentoside isomer 3	6.06	595.1307 [M-H]$^-$	596.1386	595/300/301/271	$C_{26}H_{28}O_{16}$	1.55
76	Procyanidin B dimer isomer 5	6.20	577.1353 [M-H]$^-$	578.1426	577/451/425/407/381/289/273/161/125	$C_{30}H_{26}O_{12}$	-1.19
77	Procyanidin B trimer isomer 7	6.31	865.1991 [M-H]$^-$	866.2062	865/695/577/405/289/287	$C_{45}H_{38}O_{18}$	0.51

81	Procyanidin B tetramer isomer 6	6.77	1153.2638 $[M-H]^-$	1154.2709	1153/863/577/489/433/326/287/243	$C_{60}H_{50}O_{24}$	1.47
83	Procyanidin B trimer isomer 8	6.92	865.1983 $[M-H]^-$	866.2059	865/695/577/405/289/287	$C_{45}H_{38}O_{18}$	0.06
84	Vicenin-2	7.05	593.1504 $[M-H]^-$	594.1604	593/503/473/353/321/234	$C_{27}H_{30}O_{15}$	1.47
85	Dihydroxyquercetin (taxifolin)	7.30	303.0507 $[M-H]^-$	304.0580	151/125	$C_{15}H_{12}O_{7}$	1.12
86	Neocarthamin	7.73	449.1093 $[M-H]^-$	450.1165	449/269/151	$C_{21}H_{22}O_{11}$	-0.63
87	Quercetin diglucoside isomer 2	7.82	625.1414 $[M-H]^-$	626.1487		$C_{27}H_{30}O_{17}$	-0.64
89	Quercetin hexoside pentoside isomer 4	8.19	595.1304 $[M-H]^-$	596.1376	595/300/301/271	$C_{26}H_{28}O_{16}$	-0.21
92	Quercetin hexoside pentoside isomer 5	8.47	595.1300 $[M-H]^-$	596.1372	595/300/301/271	$C_{26}H_{28}O_{16}$	-0.81
94	Procyanidin B dimer isomer 6	8.74	577.1354 $[M-H]^-$	578.1427	577/451/425/407/381/289/273/161/125	$C_{30}H_{26}O_{12}$	-0.97
95	Rutin	8.90	609.1486 $[M-H]^-$	610.1586	609/300	$C_{27}H_{30}O_{16}$	3.84
96	Vitexin	9.08	431.0992 $[M-H]^-$	432.1060		$C_{21}H_{20}O_{10}$	-0.77
97	Luteolin hexoside pentoside	9.08	579.1352 $[M-H]^-$	580.1424	579/284/151	$C_{26}H_{28}O_{15}$	0.77
98	Quercetin hexoside	9.21	463.0873 $[M-H]^-$	464.0945	463/301/300/179/151	$C_{21}H_{20}O_{12}$	2.04
99	Procyanidin B trimer isomer 9	9.28	865.1984 $[M-H]^-$	866.2057	865/695/577/405/289/287	$C_{45}H_{38}O_{18}$	-0.06
100	Procyanidin B tetramer isomer 7	9.43	1153.2616 $[M-H]^-$	1154.2687	1153/863/577/489/433/326/287/243	$C_{60}H_{50}O_{24}$	-0.39
101	Procyanidin A trimer isomer 4	9.87	899.1598 $[M+Cl]^-$	864.1990		$C_{45}H_{36}O_{18}$	10.22
102	Kaempferol hexoside deoxyhexoside	9.89	593.1499 $[M-H]^-$	594.1571	593/285/284/227/151/125	$C_{27}H_{30}O_{15}$	2.24
103	Kaempferol hexoside isomer 1	10.09	447.0926 $[M-H]^-$	448.0998	447/284	$C_{21}H_{20}O_{11}$	-1.76
104	Quercetin pentoside	10.20	433.0775 $[M-H]^-$	434.0849	433/301/300/271/179/151	$C_{20}H_{18}O_{11}$	0.07
105	Kaempferol hexoside isomer 2	10.34	447.0937 $[M-H]^-$	448.1010	447/284	$C_{21}H_{20}O_{11}$	0.85
107	Catechin dihexoside	10.57	593.1507 $[M-H]^-$	594.1580	593/285	$C_{27}H_{30}O_{15}$	0.81
108	Isorhamnetin hexoside deoxyhexoside isomer 1	10.72	623.1622 $[M-H]^-$	624.1694	623/315/314/151	$C_{28}H_{32}O_{16}$	-0.57
109	Procyanidin A dimer isomer 6	10.75	575.1203 $[M-H]^-$	576.1277		$C_{30}H_{24}O_{12}$	1.54
110	Kaempferol hexoside isomer 3	10.83	447.0946 $[M-H]^-$	448.1018	447/284	$C_{21}H_{20}O_{11}$	2.73
111	Isorhamnetin hexoside deoxyhexoside isomer 2	11.03	623.1609 $[M-H]^-$	625.1681	623/315	$C_{28}H_{32}O_{16}$	1.55
112	Isorhamnetin hexoside isomer 1	11.07	477.1034 $[M-H]^-$	478.1106		$C_{22}H_{22}O_{12}$	-3.69
113	Isorhamnetin hexoside isomer 2	11.24	477.105 $[M-H]^-$	478.1124		$C_{22}H_{22}O_{12}$	-0.08
114	Kaempferol hexoside isomer 4	11.60	447.0926 $[M-H]^-$	448.0998	447/284	$C_{21}H_{20}O_{11}$	-1.75

115	Quercetin	11.94	301.0360 $[M-H]^-$	302.0434	273/151/107	$C_{15}H_{10}O_7$	-2.32
116	Luteolin	12.10	285.0413 $[M-H]^-$	286.0513	225/151/133	$C_{15}H_{10}O_6$	-2.00
117	Procyanidin B dimer isomer 7	12.12	623.1412 $[M+COOH]^-$	578.1431	577/451/425/407/381/289/273/161/125	$C_{30}H_{26}O_{12}$	-0.32
118	Kaempferol *p*-coumaroyl hexoside	12.17	593.1301 $[M-H]^-$	594.1375	593/285/163	$C_{30}H_{26}O_{13}$	-0.22
119	Naringenin	12.19	271.0618 $[M-H]^-$	272.0718	152/119	$C_{15}H_{12}O_5$	2.43
120	Kaempferol 3-O-di-*p*-coumaroyl hexoside isomer 2	12.68	739.1679 $[M-H]^-$	740.1749		$C_{39}H_{32}O_{15}$	0.07
Other phenolics							
7	Vanillin	1.28	151.0397 $[M-H]^-$	152.0473		$C_8H_8O_3$	0.59
14	3-β-glucopyranosyloxy-2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one	1.68	373.1141 $[M-H]^-$	374.1214	343/193/150	$C_{16}H_{22}O_{10}$	-0.16
82	Lariciresinol hexoside isomer 1	6.82	521.2034 $[M-H]^-$	522.2105		$C_{26}H_{34}O_{11}$	-0.82
90	Lariciresinol hexoside isomer 2	8.25	521.2037 $[M-H]^-$	522.2110		$C_{26}H_{34}O_{11}$	-1.61
91	Lariciresinol hexoside isomer 3	8.43	521.2017 $[M-H]^-$	522.2090		$C_{26}H_{34}O_{11}$	2.13
93	Lariciresinol hexoside isomer 4	8.69	521.2012 $[M-H]^-$	522.2084		$C_{26}H_{34}O_{11}$	3.22
Annonaceous acetogenins							
121	Annohexocin or Murihexocin isomer 1	13.37	627.4488 $[M-H]^-$	628.4561		$C_{35}H_{64}O_9$	-1.65
123	Annohexocin or Murihexocin isomer 2	13.63	627.4471 $[M-H]^-$	628.4544		$C_{35}H_{64}O_9$	1.07
124	Annohexocin or Murihexocin isomer 3	13.71	627.4483 $[M-H]^-$	628.4559		$C_{35}H_{64}O_9$	-1.44
125	Annonisin isomer 1	13.76	655.4413 $[M+COOH]^-$	610.4459		$C_{35}H_{62}O_8$	2.47
126	Annonisin isomer 2	13.89	609.4366 $[M-H]^-$	610.4440		$C_{35}H_{62}O_8$	-0.68
127	Squamostatin A isomer 1	13.93	637.4688 $[M-H]^-$	638.4760		$C_{37}H_{66}O_8$	-0.40
128	Squamostatin A isomer 3	13.93	637.4678 $[M-H]^-$	638.4752		$C_{37}H_{66}O_8$	0.84
129	Annomuricin A	14.02	611.4534 $[M-H]^-$	612.4606		$C_{35}H_{64}O_8$	0.60
130	Annoglaucin	14.03	673.4463 $[M+Cl]^-$	638.4811		$C_{37}H_{66}O_8$	2.62
131	Squamostatin A isomer 2	14.06	637.4685 $[M-H]^-$	638.4757		$C_{37}H_{66}O_8$	0.08
132	9-oxo-asimicinone	14.06	635.4540 $[M-H]^-$	636.4609		$C_{37}H_{64}O_8$	-1.26
133	Annonisin isomer 3	14.15	609.4364 $[M-H]^-$	610.4482		$C_{35}H_{62}O_8$	6.17
134	Squamostatin E or Squamocin F isomer 1	14.23	621.4719 $[M-H]^-$	622.4804		$C_{37}H_{66}O_7$	0.74
135	Montanacin A	14.23	639.4846 $[M-H]^-$	640.4919		$C_{37}H_{68}O_8$	-0.73

136	Mosinone A isomer 1	14.35	665.4624 [M+COOH]$^{-}$	620.4642		$C_{37}H_{64}O_7$	-2.93
137	Squamoxinone or Annomutacin isomer 1	14.38	669.4936 [M+COOH]$^{-}$	624.4953		$C_{37}H_{68}O_7$	-3.28
138	Squamostatin E or Squamocin F isomer 2	14.41	621.4724 [M-H]$^{-}$	622.4799		$C_{37}H_{66}O_7$	1.60
139	Squamosinin A	14.54	621.4378 [M-H]$^{-}$	622.4451		$C_{36}H_{62}O_8$	-0.98
140	Annoreticuin	14.58	595.4588 [M-H]$^{-}$	596.4661		$C_{35}H_{64}O_7$	-1.46
141	Mosinone A isomer 2	14.64	619.4579 [M-H]$^{-}$	620.4666		$C_{37}H_{64}O_7$	0.91
142	Squamoxinone or Annomutacin isomer 2	14.79	623.4894 [M-H]$^{-}$	624.4966		$C_{37}H_{68}O_7$	-1.26
Other compounds							
122	*p*-Decycloxybenzoic acid	13.37	277.1824 [M-H]$^{-}$	278.1897	233/215	$C_{17}H_{26}O_3$	-5.36

A total of 142 phytochemicals belonging to different classes were tentatively identified in the araticum peel extract, including 5 organic acids, 3 jasmonates, 33 phenolic acids, 73 flavonoids, 6 other phenolics, 21 annonaceous acetogenins and 1 other compound. To the best of our knowledge, the most part of these compounds (123 of them) were reported here for the first time in araticum peel extract. Only quinic acid, citric acid, malic acid, catechin, epicatechin, quercetin, rutin, protocatechuic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, caffeoyl-glucoside (caffeoyl hexoside derivative), feruloyl-galactoside (feruloyl hexoside derivative), quercetin-3-glucoside (quercetin hexoside derivative), kaempferol-3-O-rutinoside (kaempferol hexoside deoxyhexoside derivative), kaempferol-7-O-glucoside (kaempferol hexoside derivative), procyanidin B dimer and procyanidin B trimer had previously been identified in araticum peel extracts [10,14,37].

A total of 112 different phenolic compounds were found in araticum peel extract, including monomeric compounds, oligomeric compounds, conjugated phenolic compounds with sugar moieties, and polymeric compounds. Qualitatively, flavonoids (a total of 73) represent the main phenolic class in the araticum peel extract, followed by phenolic acids and other phenolics. The antioxidant activity of an extract depends strongly on the phenolic content and their chemical features (*e.g.*, number of aromatic and hydroxyl groups, and the specific positioning of these groups; substitution of hydrogen atoms by other groups and glycosylation degree) [10]. In addition, flavonoids can also interact synergistically with phenolic acids, increasing the total antioxidant activity [39]. Therefore, the presence of a large number of phenolic compounds in araticum peel extract may explain the high antioxidant activity demonstrated for this extract.

Twenty-one annonaceous acetogenins were tentatively identified, whereby all them characterized here for the first time in araticum peel. Although there are no previous reports in the literature regarding the identification of annonaceous acetogenins in araticum peel, numerous annonaceous acetogenins have been isolated and identified from various parts in other plants in the family *Annonaceae* [40,41]. Annonaceous acetogenins constitute a large family of fatty acid derived natural products found almost exclusively from *Annonaceae* species. These compounds have a broad spectrum of biological activities, such as antineoplastic, antiparasitic, cytotoxic, immunosuppressive, neurotoxic and pesticidal effects [41], attracting increasing attention of chemists and biologists.

Our results demonstrated that the use of high-intensity ultrasound technology associated with HPLC-ESI-QTOF-MS/MS analysis allowed the recovery and characterization

of a large number of phytochemicals from araticum peel, many of which have not been previously reported in the literature for this raw material.

4. Conclusion

High-intensity ultrasound was employed for phenolic antioxidants recovery from araticum peel, a food industry by-product. The results demonstrated that the process parameters evaluated (nominal ultrasonic power: 160-640 W and extraction time: 0.5-5.0 min) had significant effect on phenolics recovery. Ultrasonication process intensification significantly increased the phenolics antioxidants recovery. This work demonstrated that high phenolic yields and antioxidant activities values can be achieved at short extraction times, while operating in high nominal ultrasonic powers due to synergic effect between these process parameters. From HPLC-ESI-MS/MS analysis, 14 phenolic compounds were found and quantified in araticum peel extracts, being epicatechin, rutin, chlorogenic acid, catechin and ferulic acid the most abundant compounds. Moreover, 142 phytochemical compounds were tentatively identified in araticum peel extracts by using HPLC-ESI-QTOF-MS/MS, of which 123 were reported here for the first time. These results contribute to strengthening the high-intensity ultrasound technology as a simple, fast and of low environmental impact process to recover high-added value compounds like phenolics antioxidants from by-products derived from food processing. In addition, araticum peel showed to be a promising source to recover phenolic compounds with high antioxidant capacity for further applications in the food, nutraceutical, cosmetic and pharmaceutical industries.

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Conflict of interest

The authors report no conflict of interest.

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CHAPTER IV

RESEARCH ARTICLE

EFFECT OF THE TEMPERATURE ON INULIN STABILITY IN PREBIOTIC CARBOHYDRATE-ENRICHED ARATICUM WHEY BEVERAGE

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Abstract

Industrial processes can cause structure changes in prebiotic carbohydrates affecting their health benefits and technological properties, but these aspects have received little attention. Therefore, the impact of thermal treatments (70 to 100°C for 60 s) on chemical stability of inulin in a dairy system, araticum flavoured whey beverage, was evaluated in this study for the first time. Compared with untreated whey beverage, the thermally treated whey beverages did not exhibit any changes in total soluble solids, pH, ζ-potential, FTIR spectra and soluble sugars (glucose, fructose, sucrose and lactose). The chromatographic analysis displayed no significant differences in content and profile of inulin (average DP = 10) between untreated and thermally treated beverage, showing that inulin had strong ability to resist thermal degradation in a dairy system. Based on the data obtained, we can state that the backbone of the inulin remained intact in the dairy system after thermal treatments. Therefore, commercial dairy beverages thermally treated can be successfully enriched with inulin without prejudice to their prebiotic properties.

Keywords: Fructo-oligosaccharides; Carbohydrate chemistry; Functional beverage; Dairy system; *Annona crassiflora* Mart.

Highlights

- Inulin was added in an araticum whey beverage thermally processed.
- Inulin thermal stability was evaluated in a dairy system for the first time.
- Inulin structure was thermally stable (70-100°C/ 60 s) in the dairy system.
- Inulin can be added successfully in commercial whey beverages thermally treated.

1. Introduction

The increasing human risk factors to various deadly diseases and the growing interest of modern consumers in foods that promote health and well-being have led various industrial segments to invest in innovations in their products. Thus, there is a strong global trend for the development of foods naturally rich in bioactive compounds or enriched/fortified with these substances, boosting the segment of functional foods in the food industry (Caleja, Ribeiro, Barreiro, & Ferreira, 2017; Monteiro et al., 2018; Sardarodiyan & Mohamadi Sani, 2016). Among the various functional food categories commercially available, beverages segment is the most attractive due to: (i) convenience and more likely to meet consumer demands for size, shape, content and appearance; (ii) ease of distribution and better storage for refrigerated and shelf-stable products; and (iii) possibility to add desirable nutrients and bioactive compounds (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014).

The dairy industry has played a leading role in the development of functional foods, and dairy-based beverages account for approximately 43% of the functional beverage market. Among current developments in dairy beverages, new ready-to-drink beverages (drinks containing combinations of dairy and fruit constituents with added bioactive compounds) have become very popular in the global dairy market due to being interesting vehicles for bioactive food ingredients and adding convenience value by mixing with fruits that meets consumer needs (Mikkola & Colantuono, 2017), besides contributing to mitigate environmental problems caused by cheese whey pollution (Guimarães et al., 2018).

Dairy beverages are considered excellent vehicles for prebiotic delivery, as they have attractive sensory characteristics, making them well accepted and often consumed by a significant portion of the population. Prebiotics are currently defined as a substrate that: (i) resits to host digestion; (ii) is metabolized by gut microbiota; and (iii) selectively stimulates the growth/activity of gut bacteria associated with health and well-being (Arruda et al., 2017).

Several fermentable carbohydrates have been reported as prebiotic, but the non-digestive oligosaccharides fructans (inulin and fructo-oligosaccharides) and galactans are the dietary prebiotics most extensively reported to have health beneficial in humans (Gibson et al., 2017).

Inulin is one of the most studied and commercially available prebiotics, and it has successfully been added to functional beverages. Inulin is a water-soluble carbohydrate found in several edible vegetables and fruits, consisting mainly, if not exclusively, of repeating fructose units linked together by β-(2←1) linkages, usually with an (1↔2) α-*D*-glucose end group (Roberfroid, 2007). The inulin prebiotic capacity is explained by its chemical structure. The β-(2←1) fructosyl-fructose glycosidic linkages from inulin cannot be hydrolysed by human digestive enzymes, allowing that this carbohydrate reach the colon, where it is degraded by β-fructanosidase enzymes which are prevalent in *Bifidobacterium* and *Lactobacilli*, and metabolized by these microorganisms, providing several health benefits (Arruda, Pereira, & Pastore, 2017; Gibson et al., 2017). Therefore, the inulin chemical structure directly affects the prebiotic effects of the food products. However, recent studies have demonstrated that several variables can affect the inulin chemical stability, including temperature, pH, degree of polymerization of inulin, food matrix, inulin origin, severity of the thermal treatment (time-temperature binomial) and thermal treatment method (batch or continuous) (Li, Ma, & Liu, 2019; Vega & Zuniga-Hansen, 2015; Wada, Sugatani, Terada, Ohguchi, & Miwa, 2005).

In order to ensure the microbiological safety of the final product, dairy beverages must be submitted to a thermal treatment after manufacturing (Guimarães et al., 2018). However, thermal processing can promote the functionality reduction of these products due to degradation of heat-sensitive nutritional compounds (*e.g.*, vitamins, antioxidants, proteins, carbohydrates and others) and loss of sensory characteristics (Monteiro et al., 2018). It is already known that inulin is capable of resisting the digestion and the absorption in the upper part of the gastrointestinal tract (Arruda, Pereira, & Pastore, 2017), but there is a lack of information about the effects of the industrial processes, mainly conventional food processing techniques such as thermal treatment, on the chemical stability of inulin. Although there are some studies reporting the thermal stability of inulin (Glibowski & Bukowska, 2011; Li et al., 2019; Wada et al., 2005), these studies are mostly performed in model systems (aqueous or buffer systems), and studies on complex systems such as food and beverages are scarce. Food and beverages as complex systems possess several other components that can interact with the inulin (*e.g.,* proteins and lipids in dairy beverages), increasing or reducing its thermal stability. However, the impact of these interactions on the thermal stability of inulin in complex systems

remains unknown. In this sense, this study aimed to evaluate the effect of different thermal treatments on the chemical stability of inulin in a complex food system, dairy beverage. To the best of our knowledge, this is the first research that reports the real impact of thermal treatments on the inulin stability in a dairy system and can be a reference material to support to the dairy beverage industry in the development of innovative prebiotic dairy beverages.

2. Material and methods

2.1. Chemicals and reagents

Authentic standards of 1-kestose (GF_2), nystose (GF_3) and 1-fructofuranosylnystose (GF_4) were obtained from Wako Pure Chemical Industries® (Osaka, Japan), whereas the authentic standards of glucose, fructose, galactose, sucrose and lactose were provided by Sigma-Aldrich Chemical Co.® (St. Louis, USA). Sodium hydroxide solution (50%) and sodium acetate for ionic cromatography were purchased from Sigma-Aldrich Chemical Co.® (St. Louis, USA). The water used was obtained from a Milli-Q water purification system (Millipore®, Bedford, USA). All other solvents and reagents used in this study were of analytical grade.

2.2. Plant material and sample preparation

The araticum fruits (*Annona crassiflora* Mart.) were collected in natural areas of the Cerrado Biome located in the municipality of Carmo do Paranaíba, Minas Gerais, Brazil. A voucher specimen (UEC 197249) has been deposited in the Herbarium of the Institute of Biology of the University of Campinas, Brazil (Herbarium UEC) (**Annex 1**). In addition, following Law n° 13.123/2015 and its regulations, the activity of access to genetic heritage was regimented by the Board of the Genetic Heritage Management under number AA9DDF7 (**Annex 2**).

The morphologically perfect and completely mature fruits were washed with distilled water, then manually peeled and pulped. The araticum pulp was freeze-dried (LIOTOP®, model L101, São Carlos, Brazil), ground using a knife grinder (Marconi®, model MA340, Piracicaba, Brazil) to obtain a powder and stored at -20°C until analysis.

2.3. Manufacture of prebiotic araticum whey beverage

The prebiotic araticum whey beverage was produced with whole pasteurized and homogenized milk of 3.4 g/100 g fat (30 g/100 g, Xandô®, São Paulo, Brazil), crystal sugar (8 g/100 g, União®, São Paulo, Brazil), whey powder (6 g/100 g, Alibra®, São Paulo, Brazil), araticum pulp freeze-dried (6 g/100 g), inulin GR (3 g/100 g, BENEO-Orafti®, São Paulo, Brazil) with average degree of polymerization (DP) = 10, and water (47 g/100 g). The inulin amount added to whey beverage was established from the consumption required to deliver health benefits. To be considered prebiotic, a beverage should contain from 3 to 8 g inulin per portion, considering a portion of 200 mL prebiotic beverage/day (Coussement, 1999).

For the beverage manufacturing, the whey powder and araticum pulp freeze-dried were dissolved in the milk. The inulin and sugar were dissolved together in hot distilled water (80°C) and incorporated to the other ingredients. Then, the beverage was submitted to homogenization at rotor-stator device (IKA T 25 digital ULTRA-TURRAX®, Staufen, Germany) for 3 min at 12,000 rpm.

2.4. Prebiotic araticum whey beverage processing

The prebiotic araticum whey beverage was subjected to different thermal treatments using a temperature-controlled oil bath (Marconi®, MA-159, Piracicaba, Brazil). The samples (25 mL) were treated in 50 mL plastic tubes, and the temperature in the setpoint (70 ± 1°C, 80 ± 1°C, 90 ± 1°C and 100 ± 1°C for 60 s) was monitored using a digital thermometer (Digital Thermometer Hanna®, Check temp, HI98501, Nusfalau, Romania). The samples were cooled to 10°C immediately after the treatments. The experiment was performed in duplicate.

2.5. Total soluble solids and pH analysis

The pH and total soluble solids (TSS) of the beverage submitted to different treatments were measured in duplicate immediately after manufacturing. TSS were determined by direct reading of a beverage aliquot using an Abbe refractometer (WAY-2S, Shanghai Yuguang Instrument Co. Ltd.®, PR China) and the results were expressed as °Brix. The pH of the beverage was determined by using a Mettler Toledo 320 pH meter (Mettler-Toledo AG®, CH-8603, Schwerzenbach, Switzerland).

2.6. ζ-potential

The surface charges of the prebiotic araticum whey beverage thermally processed were determined by measuring the ζ-potential using a chamber of microelectrophoresis (ZetaSizer Nano-ZS, Malvern Instruments Ltd.®, Worcestershire, UK). The samples were diluted in deionized water to 1% (v/v) for the measurements. The measurements were performed in triplicate at 25°C.

2.7. Fourier transform infrared spectroscopy (FTIR)

Freeze-dried previously samples of prebiotic araticum whey beverage were used in the preparation of KBr pellets. The mixture of 3 mg sample and 200 mg KBr powders were finely ground in an agate mortar. The mixture was compressed with a tablet press machine according to the manufacturer's instructions, forming pellets that used in a FTIR spectrometer (model IRPrestige-21, Shimadzu®, Kyoto, Japan). The measurements were performed at room temperature and the spectra were obtained in the range of wavenumber from 4000 to 400 cm^{-1} with total of 10 scans at a resolution of 4 cm^{-1}.

2.8. HPAEC-PAD sugars and fructo-oligosaccharides analysis

The analysis of sugars and fructo-oligosaccharides (FOS) in the prebiotic araticum whey beverage was performed by High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAEC-PAD) using an ion chromatographer Dionex ICS-5000 (Thermo Fisher Scientific®, Waltham, MA, USA) according to the method described by Pereira, Arruda, de Morais, Eberlin, and Pastore (2018), with some modifications. Sugars (glucose, galactose, fructose, sucrose and lactose) were separated using 0.2 M NaOH (eluent A) and ultrapure water (eluent B) on a Carbopac PA1 column (250 × 4 mm i.d., particle size 10 μm, Thermo Fisher Scientific®, Waltham, MA, USA). The elution gradient was performed as follows: 0-22 min, 4% A; 22-24 min, 4-50% A; 24-30 min, 50% A; 30-35 min, 100% A; and 35-40 min, 4% A. FOS were separated using three mobile phases: 0.2 M NaOH (eluent A), 1 M sodium acetate containing 0.2 M NaOH (eluent B) and ultrapure water (eluent C) on a Carbopac PA100 column (250 × 4 mm i.d., particle size 8.5 μm, Thermo Fisher Scientific®, Waltham, MA, USA). The elution gradient was performed as follows: 0-2 min, 48.5% A and 1.5% B; 2-44 min, 48.5-0% A and 1.5-50% B; 44-49 min, 50% B; and 49-54 min, 48.5% A and 1.5% B (the solution C was maintained at 50% along the chromatographic run).

In both analyses, the samples were diluted in ultrapure water, filtered through a 0.22 μm PTFE filter and injected into the column using an auto-sampler. The column temperature was maintained at 30°C, the flow-rate was 1.0 mL/min, and the injection volume of the samples was 25 μL. Data were acquired and processed using Chromeleon software version 7.0. The sugars (glucose, galactose, fructose, sucrose and lactose) and FOS (GF_2, GF_3 and GF_4) were identified in samples by comparing the retention times of authentic standards and the samples. Calibration curves were constructed with commercial standards (0.25-12.50 μg/mL) to quantify the sugars/FOS in the prebiotic araticum whey beverage. The content of individual sugars and FOS was expressed as mg/mL beverage.

2.9. Statistical analysis

A randomized factorial design of experiment was carried out and conducted in duplicate. The results were expressed as the mean ± standard deviation. The data were analysed using Minitab software version 18.0 (Minitab Inc.®, State College, PA, USA). The temperature effects were assessed by linear regression analysis at 5% of significance level (p-value ≤ 0.05).

3. Results and discussion

3.1. Physicochemical properties

Thermal treatments are the main processing used by the food industry to control pathogenic and spoilage microorganisms as well as undesirable enzymes in foods. However, several reactions can take place during thermal processing (*e.g.*, hydrolysis, degradation, oxidation, Maillard and caramelization reactions, and others), affecting the physicochemical and nutritional properties of the food product and, consequently, its final quality (Ling, Tang, Kong, Mitcham, & Wang, 2015). The effects of thermal treatment on the physicochemical properties of the prebiotic araticum whey beverage were evaluated and the results are presented in **Table 1**. According to results, thermal treatments did not modify the pH (p-value = 0.941), TSS (p-value = 0.166) and ζ-potential (p-value = 0.266) values after processing, as also observed in other studies. No changes in pH and TSS after thermal treatment were also reported by Barba, Cortés, Esteve, and Frígola (2012) and Zulueta, Barba, Esteve, and Frígola (2013) in orange juice-milk beverage (90 and 98°C/15 and 21 s, and 90°C/15 s, respectively), and by Amaral et al. (2018) in whey-grape juice drink (72 °C/15 s). Our results suggest that the thermal

treatments did not cause significant reactions in the samples, possibly due to the reduced processing time (60 s).

Table 1 Physicochemical characterization of the prebiotic araticum whey beverage.

Treatment	pH	TSS (°Brix)	ζ-potential (mV)
Untreated	5.59±0.01	24.63±0.32	-32.77±1.28
70°C	5.59±0.01	25.06±0.38	-33.10±0.49
80°C	5.59±0.01	25.19±0.47	-29.05±0.79
90°C	5.59±0.01	24.88±0.43	-31.12±0.65
100°C	5.59±0.00	24.75±0.29	-31.28±1.06
p-value	0.941	0.166	0.266

The ζ-potential describes the magnitude of the surface charge of the molecules that make up the evaluated system. Thus, the modification in ζ-potential can represent some type of structural change caused by hydrolysis, degradation and oxidation reactions of the molecules present in the whey beverage. As can be seen in **Table 1**, the ζ-potential values of the prebiotic araticum whey beverage processed by thermal treatment maintained almost stable (p-value = 0.266). Guimarães et al. (2018) also reported no change in ζ-potential values of the prebiotic soursop whey beverage after thermal treatment (72°C/15 s). This result suggests that the thermal treatments maintain the chemical characteristics of the whey beverage processed. To prove this hypothesis, the prebiotic araticum whey beverage was analysed by HPAEC-PAD to evaluate in detail the qualitative and quantitative molecular profile of inulin before and after the thermal processing. These results will be discussed in the **Section 3.4**.

3.2. FTIR analysis

FTIR is a useful technique to identify the presence of new functional groups in food samples, which can be formed and/or intensified during thermal treatment. The FTIR spectra of the inulin and prebiotic araticum whey beverage before and after thermal treatments can be observed in **Fig. 1**. Pure inulin powder presented a broad strong band centred at 3385 cm^{-1} which correspond to the stretching of hydroxyl groups (O-H) of associated glucose and fructose units in the carbohydrate backbone (frequently detected in the region of 3500 to 3200 cm^{-1}). Two bands around 2933 cm^{-1}/2891 cm^{-1} correspond to C-H stretch of the methyl group and peak at 1637 cm^{-1} can be assigned to the hydroxyl bending mode. In addition, it was evidenced two bands at 1030 cm^{-1} and 933 cm^{-1} corresponding to the C-O-C stretching, respectively

(Rahul, Jha, Sen, & Mishra, 2014). In the case of prebiotic araticum whey beverage, apart from these bands and peaks, there was the appearance and shift and/or intensification of some bands/peaks of characteristic bands of the inulin. These alterations are due to the presence of other macromolecules (mainly proteins and lipids) present in the system from the other ingredients added to the whey beverage formulation.

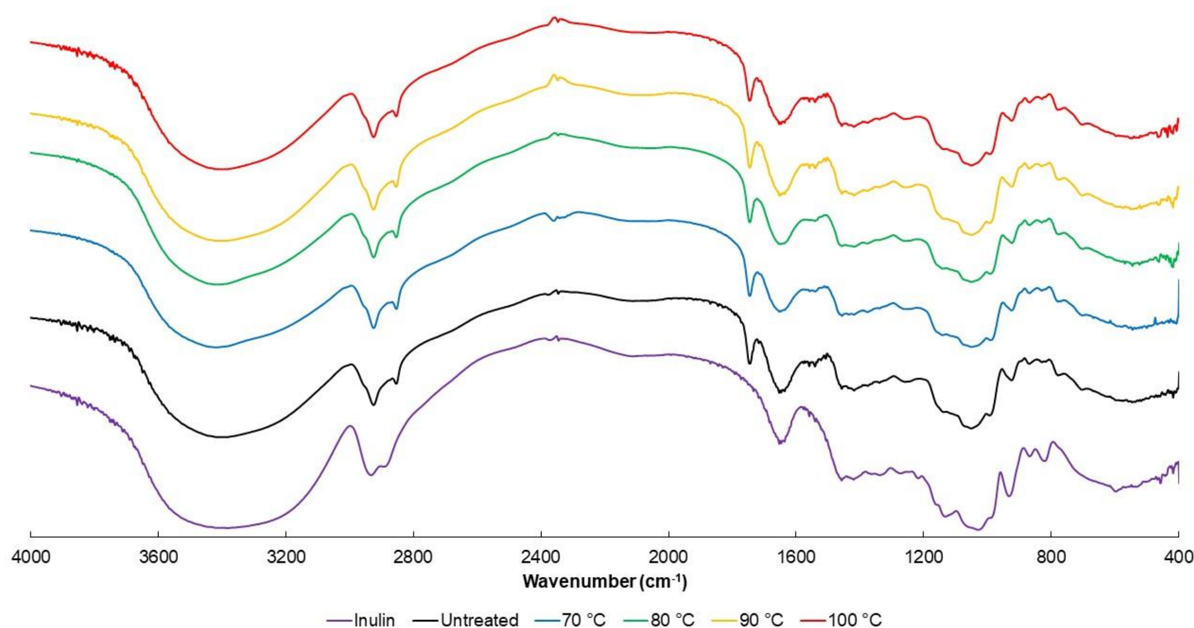

Fig. 1. FTIR spectra of the inulin and prebiotic araticum whey beverage.

All spectra of whey beverage showed a band at 1745 cm^{-1} that is associated with the vibration of axial deformation of C=O bonds of the ester carbonyl groups present in the triacylglycerol molecules (Rohman & Man, 2010). Peaks similar, but more intense and slightly shift, to the characterised in the inulin were observed in the FTIR spectra of whey beverage at 2924 cm^{-1} and 2854 cm^{-1}. The small changes observed in peaks were attributed to symmetric and asymmetric stretching of methyl and methylene groups in the membrane phospholipids (frequently detected in the region of 3050 to 2800 cm^{-1}) (Stafussa et al., 2016). In addition, some peaks were detected in the region between 1700 and 1500 cm^{-1}, indicating the presence of amide groups, mainly from protein peptide bonds (C=O stretching and N-H bending) (Stafussa et al., 2016).

As can be seen in **Fig. 1**, the untreated whey beverage showed very similar FTIR spectrum to those of thermally treated whey beverages by bare eyes. No distinct band was formed, intensified or shift after the thermal treatments. The bands observed from 1200 to 900 cm^{-1} are mainly related to complex vibrations from carbohydrates skeleton (vibration of the glycosidic bonds, stretching band of C-O-C and C-O) (Rahul et al., 2014). Thus, this region was used as reference to analyse whether there was any type of modification of the native inulin added to whey beverage after the thermal treatments. The absence of FTIR spectra changes in this region confirmed that the backbone of the inulin remained intact in the whey beverage after thermal treatments.

3.3. Sugars content

The increase in the monosaccharides content together with the reduction of the disaccharides content may indicate that the thermal processing is causing hydrolysis of disaccharides and/or polysaccharides, whereas the reduction in both mono- and disaccharides content may suggest that the thermal processing is degrading these sugars through chemical reactions, such as the Maillard and oxidation reactions. These reactions may cause changes in the physicochemical, sensory, nutritional and functional characteristics of thermally processed foods (Hardy, Parmentier, & Fanni, 1999; Peñaflor et al., 2018). Thus, sugar content analysis can provide relevant information on the effect of thermal processing on the carbohydrates chemical stability present in food products.

The mono- and disaccharides content of the prebiotic araticum whey beverage before and after thermal treatments was analysed by HPAEC-PAD and the results are presented in **Table 2**. Glucose, fructose, sucrose and lactose were identified in the whey beverage before and after thermal treatments. The results demonstrated that the thermal treatments did not alter the mono- and disaccharides content in the prebiotic araticum whey beverage (p-values $\geq$ 0.319). These findings are in accordance with the TSS data (**Table 1**), where the thermal treatments also did not affect the TSS values in the whey beverage. Vervoort et al. (2011) and Cappelletti et al. (2015) also reported no effect of the thermal processing on glucose, fructose and sucrose contents in orange juice (72°C/20 s) and coconut water (90°C/60 s). On the other hand, Andrés, Villanueva, and Tenorio (2016) observed limited effect of the thermal treatment (80°C/3 min) on sugar contents in milk-smoothies. The authors verified that the thermal processing significantly affected only the lactose content, keeping the glucose and fructose contents. Therefore, our findings demonstrated that the thermal treatments used were not

enough to modify the chemical structure of the soluble sugars from prebiotic araticum whey beverage.

Table 2 Sugars content in the prebiotic araticum whey beverage.

Treatment	Sugars content (mg/mL beverage)				
	Glucose	**Fructose**	**Sucrose**	**Lactose**	**Total sugars**
Untreated	17.23±0.60	18.91±1.05	92.40±3.67	63.11±0.51	191.65±4.84
70°C	16.54±0.07	17.75±0.48	89.52±1.30	61.82±2.68	185.64±4.17
80°C	17.97±0.82	19.49±1.43	94.14±1.40	67.19±4.13	198.79±6.95
90°C	16.11±0.82	18.74±1.31	89.00±1.13	61.59±0.53	185.44±3.10
100°C	17.25±0.36	18.87±0.60	93.37±2.87	66.52±4.38	196.02±8.04
p-value	0.902	0.319	0.332	0.357	0.346

3.4. Inulin stability

Inulin possesses a number of health benefit properties (for more details, see Arruda et al. (2017)) and is, therefore, used in different foods and beverages as functional ingredients. However, to achieve these properties, considerable amounts of inulin have to be added to food and/or beverage systems, frequently, from 1 to 6% by weight, leading to approximately 3 to 8 g per portion (Coussement, 1999). In addition, its stability in the final product must be guaranteed, which does not always happen when foods/beverages are processed and/or stored under severe conditions (*e.g.*, high-temperatures, low or high pH and long-time). Therefore, it is extremely important to study the effects of the industrial processes, mainly conventional food processing techniques such as thermal treatment, on the chemical stability of inulin, in order to find process conditions that besides guaranteeing the food safety, also preserve the prebiotic substances added to the foods/beverages.

Although all the results obtained from the previous analyses have supported the hypothesis that inulin structure was not affected by the thermal treatments applied to the whey beverage, HPAEC-PAD analysis was performed to prove this hypothesis. The inulin chromatographic profiles obtained by HPAEC-PAD in the prebiotic araticum whey beverage before and after thermal treatments are shown in **Fig. 2**.

Inulin is a fructan made up (2→1) β-*D*-fructofuranosyl units (F*n*) ($2 \leq n \leq 60$), usually with a (1↔2) α-*D*-glucopyranose (GF*n*) terminal group. Thus, the general formula of carbohydrate polymer is GF*n*, where G = glucose terminal unit, F = fructose residue and n = number of fructose units in the fructan chain (Gupta, Jangid, Pooja, & Kulhari, 2019). During

the thermal treatments, there can be the hydrolysis of inulin chain leading to an increase in the quantities of low molecular weight GF*n* while reducing the high molecular weight ones. In addition, inulin thermal hydrolysis could lead to the formation of F*n*. As can be seen in **Fig. 2**, the untreated whey beverage showed very similar inulin chromatographic profile to those of thermally treated whey beverages. No distinct peak was formed after the thermal treatments, demonstrating that there was no formation of any F*n*. Although the qualitative profile of inulin has provided sufficient evidence to prove that the inulin chain was not degraded by the applied thermal treatments, quantitative analysis of the FOS that make up the inulin structure were carried out based on analytical curves from authentic standards (GF_2-GF_4) and in the peak areas (GF_5-GF_{20}) and the results are shown in **Tables 3** and **4**, respectively. As can be seen from the statistical analysis, thermal treatments did not modify the FOS content (GF_2-GF_{20}) in the prebiotic araticum whey beverage (p-values $\geq$ 0.141).

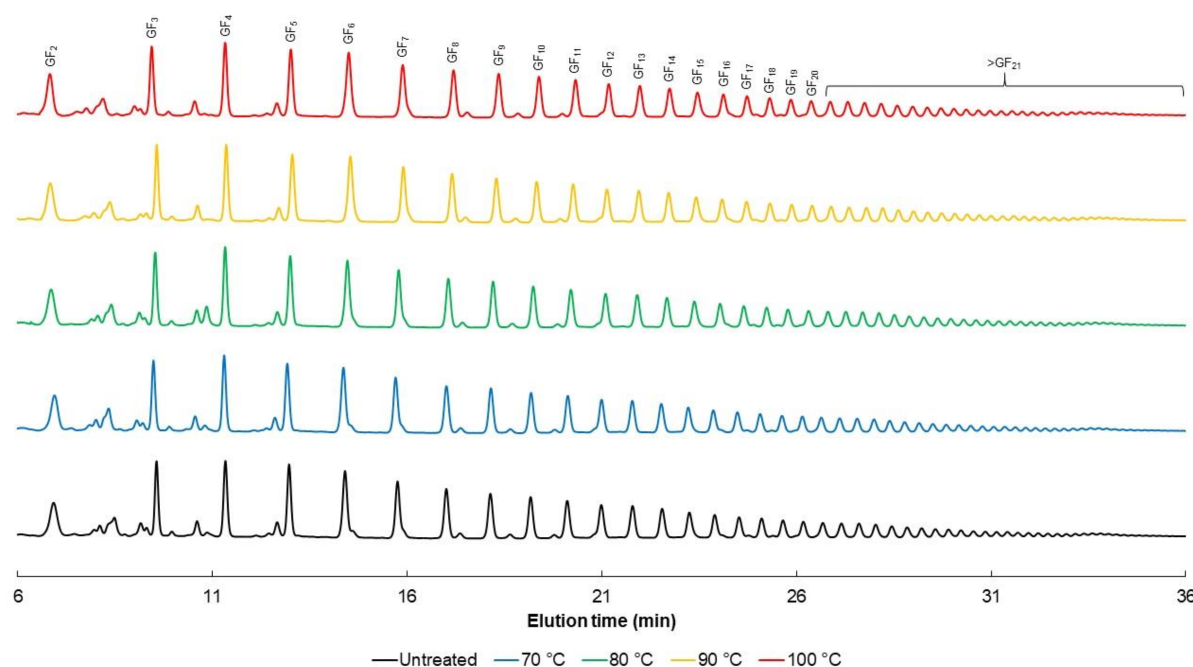

Fig. 2. Inulin chromatographic profile of the prebiotic araticum whey beverage.

Previous studies have shown that thermal treatments are not feasible for the manufacture of prebiotic foods/beverages, since FOS are highly susceptible to hydrolysis/degradation during thermal processing (Courtin, Swennen, Verjans, & Delcour, 2009; Forgo, Kiss, Korózs, & Rapi, 2013; Klewicki, 2007; Vega & Zuniga-Hansen, 2015). Wada et al. (2005) studied the effect of thermal treatment of inulin enzymatically synthesized

from sucrose and they verified that the residual ratio of inulin was more than 96% after thermal treatment (100°C/15 min) at pH 4-7, while that at pH 3 was drastically decreased to 12%. Glibowski and Bukowska (2011) reported that inulin chemical stability at pH ≤ 4 decreased as the heating time and temperature increased, whereas in a neutral and basic environment inulin was chemically stable regardless of heating time (up to 55 min) and temperature (up to 100°C). In another study, Li et al. (2019) reported that inulin with different DP had strong ability to resist heating degradation (only less than 2% reducing sugar occurred under 20-100°C/1 h). Moreover, the authors observed that inulin with higher DP was more thermally stable. Indeed, Glibowski and Wasko (2008) demonstrated that high polymerised inulin (DP ≥ 23) was highly stable even at 80°C for 30 min in neutral and slightly acidic conditions (pH 5), but in acidic conditions (pH 3) its thermal stability was significantly decreased as the heating time and temperature increased. Klewicki (2007) and Vega and Zuniga-Hansen (2015) studied the FOS stability during thermal processing of juices and fruit drinks (beverages with low pH) and concluded that the FOS added to fruit juices and drinks were highly susceptible to hydrolysis in pasteurization conditions. In addition to temperature, pH and degree of polymerization of the FOS that make up the inulin structure, food matrix, FOS/inulin origin, severity of the thermal treatment (time-temperature binomial) and thermal treatment method (batch or continuous) can also affect the inulin chemical stability (Vega & Zuniga-Hansen, 2015; Wada et al., 2005). As can be observed above, most studies regarding inulin thermal stability have been conducted on model systems (aqueous and buffer systems). Despite presenting relevant results, further studies such as ours are needed to evaluate the real effect of thermal treatments on inulin stability in complex food systems.

Table 3 FOS content (GF_2-GF_4) in the prebiotic araticum whey beverage.

Treatment	FOS content (mg/mL beverage)			
	GF_2	**GF_3**	**GF_4**	**$\sum GF_2$-GF_4**
Untreated	0.89±0.01	0.98±0.01	1.22±0.01	3.09±0.02
70°C	0.92±0.03	1.00±0.04	1.22±0.04	3.14±0.11
80°C	0.96±0.03	1.04±0.04	1.28±0.07	3.28±0.14
90°C	0.92±0.04	0.95±0.02	1.17±0.03	3.04±0.08
100°C	0.96±0.02	1.01±0.01	1.22±0.01	3.18±0.03
p-value	0.250	0.549	0.300	0.711

Thus, understand the behaviour of prebiotic carbohydrates in a food matrix during processing is extremely necessary for their food applications once the chemical structures of

these carbohydrates must be kept after processing to claim the food as prebiotic (Fonteles & Rodrigues, 2018). According to results from **Tables 3** and **4**, the FOS (GF_2-GF_{20}) that make up the inulin chain added to the prebiotic araticum whey beverage were not hydrolysed/degraded by the thermal treatments (70-100°C/60 s). These results are supported by the studies abovementioned since the pH of the whey beverage developed in this work was close to neutrality (pH = 5.59, **Table 1**). Therefore, our findings evidence that the inulin chain was not susceptible to hydrolysis/degradation under high-temperature conditions in araticum whey beverage, maintaining its prebiotic properties. However, applications involving foods/beverages thermally processed with low pH (*e.g.*, fruit juices) can decompose the FOS/inulin structures, resulting in a loss of physicochemical and functional properties.

Our findings demonstrate, for the first time, that the inulin (average DP = 10) had a strong ability to resist thermal degradation in a dairy system and thus could maintain the prebiotic properties in the final product. This way, these results can be used by the food industry to support the development of innovative prebiotic dairy beverages.

Table 4 Effect of temperature on FOS (GF_5-GF_{20}) in the prebiotic araticum whey beverage.

Treatment	FOS (peak area)							
	GF_5	**GF_6**	**GF_7**	**GF_8**	**GF_9**	**GF_{10}**	**GF_{11}**	**GF_{12}**
Untreated	11.77±0.09	13.44±0.35	11.67±0.13	9.62±0.05	9.14±0.08	8.47±0.06	7.62±0.03	7.51±0.05
70°C	11.93±0.44	14.12±0.82	11.88±0.39	9.70±0.32	9.24±0.30	8.55±0.29	7.69±0.27	7.52±0.22
80°C	12.49±0.53	15.04±0.72	12.33±0.65	10.12±0.42	9.61±0.41	8.89±0.38	7.99±0.33	7.83±0.33
90°C	11.57±0.35	14.12±0.32	11.53±0.28	9.36±0.19	8.96±0.20	8.21±0.24	7.32±0.30	7.20±0.23
100°C	12.09±0.03	14.74±0.08	11.98±0.09	9.68±0.06	9.29±0.06	8.60±0.07	7.57±0.04	7.38±0.08
p-value	0.701	0.538	0.659	0.342	0.537	0.499	0.189	0.141

Table 4. (Continued)

Treatment	FOS (peak area)							
	GF_{13}	**GF_{14}**	**GF_{15}**	**GF_{16}**	**GF_{17}**	**GF_{18}**	**GF_{19}**	**GF_{20}**
Untreated	6.66±0.07	6.18±0.05	5.58±0.09	5.05±0.04	3.96±0.02	3.70±0.02	3.31±0.03	3.46±0.02
70°C	6.77±0.25	6.24±0.21	5.68±0.16	5.10±0.20	3.99±0.14	3.74±0.14	3.34±0.11	3.47±0.10
80°C	7.11±0.29	6.49±0.29	5.89±0.28	5.37±0.20	4.15±0.17	3.91±0.17	3.50±0.16	3.63±0.16
90°C	6.59±0.17	6.01±0.13	5.45±0.12	4.98±0.12	3.82±0.10	3.58±0.09	3.22±0.11	3.33±0.09
100°C	6.87±0.05	6.24±0.04	5.68±0.05	5.20±0.05	3.91±0.02	3.68±0.03	3.31±0.01	3.43±0.03
p-value	0.732	0.381	0.395	0.878	0.134	0.170	0.283	0.212

4. Conclusion

For the first time, the effect of thermal treatments on the chemical stability of inulin in a dairy system was evaluated. When compared with corresponding untreated whey beverage, we found that the thermally treated whey beverage under different temperatures (70-100°C/60 s) did not exhibit any changes in the physicochemical properties (pH, TSS and ζ-potential), FTIR spectra and soluble sugars content (glucose, fructose, sucrose and lactose). In addition, the HPAEC-PAD analysis proved that the thermal treatments had no effect on the qualitative and quantitative profiles from inulin added in the whey beverage. Our findings showed that the inulin backbone remained intact in the dairy system after thermal treatments, providing evidences that the prebiotic properties of the araticum whey beverage thermally treated are preserved. Thus, inulin can be successfully used to enrich thermally treated whey beverages without prejudice their functional properties. Moreover, our study can support the food industry in the development of innovative prebiotic dairy beverages.

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Conflict of interest

The authors report no conflict of interest.

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GENERAL DISCUSSION

Knowledge of the potential from Brazilian flora, mainly of the endemic species, is essential to stimulate researches related to its use as new sources of nutrients and value-added compounds for the development of innovative products and valuation of the national biodiversity. Araticum (*Annona crassiflora* Mart.) is an endemic fruitful plant from Brazilian Cerrado with high potential nutritional, functional and economic but still little explored. In this sense, this research was developed in order to obtain a more in-depth phytochemical characterization this fruit, as well as to purpose ways of exploiting the edible fruit part and its by-products.

Initially a literature extensive review was made presenting the araticum chemical composition, bioactive compounds identified so far, biological activities confirmed *in vitro* and *in vivo*, and processed pulp-based food products (**Chapter I**). It was possible to verify the presence of several bioactive compounds (*e.g.*, phenolics, alkaloids, annonaceous acetogenins, tocols, phytosterols, vitamins, minerals and dietary fiber) and biological properties (*e.g.*, antioxidant, hepatoprotective, anti-inflammatory, antitumoral, analgesic, antidiabetic, skin healing, antidiarrhoeic, antimicrobial, antiparasitic, insecticide and herbicide) from different botanical parts of araticum. Although it presents high nutritional and functional potential, araticum fruit and based products are available only in some niche and local markets and its by-products are totally discarded. In addition, its chemical composition is not yet fully understood, especially as concerns phytochemicals present in the fruit. Therefore, there is a demand for studies that focus on the phytochemical characterization, techniques for extraction and purification of value-added compounds, and the development of commercial pulp-based food products.

Aiming to fill the gaps noted from the literature review, **Chapter II** intended to obtain the profile and content of phenolic compounds from the different parts of araticum fruit (pulp, peel and seeds); on the other hand, **Chapter III** aimed to study an emerging technique (high-intensity ultrasound technology) for extracting phenolic compounds from araticum peel in a simpler, faster, safer and less environmental impact way; whereas **Chapter IV** had as objective to develop a prebiotic araticum whey beverage enriched with inulin and evaluate the effect of industrial conservation process (thermal treatments) on the chemical stability of inulin added to whey beverage.

Previous studies have reported high total phenolic compounds values in araticum pulp (Arruda et al., 2017), peel (Justino et al., 2016; Roesler, Catharino, Malta, Eberlin, & Pastore, 2007) and seeds (Roesler et al., 2007). However, little information is available related to the profile and contents of individual phenolics in the araticum fruit. To help close this major gap, the phenolic compounds of the araticum fruit (pulp, peel and seeds) was fractionated and, then they were characterized and quantified by high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) (**Chapter II**). Phenolic compounds were predominantly present in the insoluble-bound and esterified forms in all of the araticum fruit parts. Moreover, soluble phenolics (sum of free, esterified and glycosylated fractions) was higher than of the insoluble-bound fraction in all araticum fruit parts. Although phenolics in araticum fruit parts would have been found mainly in soluble form, the phenolics extracted from the insoluble-bound fraction accounted for 23.84, 39.96 and 47.38% of total phenolics in araticum peel, pulp and seed, respectively. Insoluble-bound phenolics can be released into the gastrointestinal system forms by the action of intestinal enzymes or colonic microbiota and then be absorbed, contributing to the overall biological effects of the food consumed (Wang et al., 2016). In addition, their release during extraction process can also increase the extraction yields, reducing industrial costs. These findings demonstrate the importance of evaluating the insoluble-bound phenolics in food plants, since they comprise a significant portion of their phenolic compounds. Ignoring this fraction in analytical procedures would therefore inevitably lead to substantial underestimation of their functional and industrial potentials.

Flavonoids made the highest contribution for the araticum pulp (80.74%) and peel (90.49%), whereas phenolic acids comprised the most phenolics from araticum seed (62.21%). Quantification of phenolic compounds using HPLC-ESI-MS/MS revealed that catechin and epicatechin were the major compounds from araticum pulp and peel (catechin: 768.42 and 3526.78 µg/g fdw; and epicatechin: 661.81 and 1632.98 µg/g fdw, respectively), whereas seed displayed caffeic acid, catechin and epicatechin (45.36, 33.60 and 25.48 µg/g fdw) as its main phenolics. Individual phenolics were identified and quantified here for the first time in araticum pulp. In addition, protocatechuic, *p*-coumaric and gentisic acids; and catechin, epicatechin, and protocatechuic, chlorogenic and gentisic acids were identified in araticum peel and seeds, respectively, for the first time in this study.

The localization of a phenolic compound within a tissue reflects its physiological role for the plant. The plants often store the phenolic compounds at strategically important sites

where they act as a signalling or in plant defence mechanisms (Ewané, Lepoivre, Lapeyre, de Bellaire, & Lassois, 2012). Araticum peel showed the highest contents of total phenolics (31.65 mg GAE/g fdw), total flavonoids (22.05 mg CE/g fdw), condensed tannins (17.96 mg CE/g fdw), antioxidant activities (189.32, 292.28 and 448.29 μmol TE/g fdw for the results from total DPPH, TEAC and $ORAC_{FL}$, respectively) and total phenolics by HPLC-ESI-MS/MS (5735.52 μg/g fdw), followed by pulp (20.49 mg GAE/g fdw; 13.51 mg CE/g fdw; 13.67 mg CE/g fdw; 132.73, 214.38 and 259.26 μmol TE/g fdw ; and 1792.58 μg/g fdw for the results from total phenolics, total flavonoids, condensed tannins, total DPPH, TEAC and $ORAC_{FL}$, and total phenolics by HPLC-ESI-MS/MS, respectively) and seeds (12.68 mg GAE/g fdw; 7.33 mg CE/g fdw; 3.08 mg CE/g fdw; 71.66, 94.81 and 260.22 μmol TE/g fdw; and 164.66 μg/g fdw for the results total phenolics, total flavonoids, condensed tannins, total DPPH, TEAC and $ORAC_{FL}$, and total phenolics by HPLC-ESI-MS/MS, respectively). These results demonstrate the role of phenolics in fruit defence against adverse biotic (*e.g.*, pathogens, insects and herbivores attack) and abiotic (*e.g.*, UV radiation and temperature) factors, since they are localized primarily in its peel.

Food and agricultural industries generate a large amount of residues and by-products which can cause and/or contribute to immense environmental problems. However, wastes and by-products derived from food processing are rich sources of potentially valuable bioactive compounds and their sustainable use for the production of value-added products could contribute to mitigate environmental problems, improve the economic growth and promote human health benefits through foods enriched with bioactive compounds (Sagar, Pareek, Sharma, Yahia, & Lobo, 2018; Sepúlveda, Romaní, Aguilar, & Teixeira, 2018). Araticum peel is the main by-product derived from araticum fruit processing, accounting for 44 to 49% of the total weight of the ripe fruit (Braga Filho, Naves, Chaves, Pires, & Mazon, 2014). Moreover, araticum peel showed the highest contents of phenolic antioxidants as previously mentioned and studied (**Chapter II**). Thereby, araticum peel can emerge as potential source for obtaining phenolic compounds. Therefore, a more detailed study on the extraction process and the phytochemical profile was conducted with araticum peel (**Chapter III**).

The analysis of full factorial experimental design showed that the ultrasonic parameters (nominal ultrasonic power and process time) significantly affected the phenolics recovery and antioxidant activity from araticum peel. Total phenolic content, individual phenolic contents, and antioxidant activities by the DPPH, TEAC and ORACFL assays increased as the applied nominal ultrasonic power and extraction time increased, demonstrating

that the ultrasonication process intensification had a positive effect on the phenolic compounds recovery and antioxidant activities values from araticum peel. This effect can be attributed to the increase in energy density, since the intensity of the acoustic cavitation is increased as energy density increases. Acoustic cavitation phenomenon caused by the propagation of ultrasound pressure waves promotes the formation and implosion of microbubbles on the matrix surface resulting in micro-jetting which generates diverse effects such as surface peeling, fragmentation, erosion, sonocapillary effect, sonoporation, local shear stress and destruction/detexturation of plant structures. In addition, the implosion of cavitation microbubbles in a liquid media leads to macro-turbulences and micro-mixing. These combined effects result in the collapse of the plant matrix, enabling solvent penetration into the plant material and improving the release of intracellular substances into the solvent, consequently increasing extraction rates (Goula, Ververi, Adamopoulou, & Kaderides, 2017; Chemat, Rombaut, Sicaire, Meullemiestre, Fabiano-Tixier, & Abert-Vian, 2017).

Due to synergistic effect between nominal ultrasonic power and process time, it was found that the recovery of a high phenolic antioxidants content could be achieved at short extraction times (≤ 5 min) when operating with higher nominal ultrasonic powers, whereas the previous studies took from 40 min to 6 days to obtain the phenolic-rich extracts (Arruda et al., 2018; Justino et al., 2016; Roesler et al., 2007). Thus, our findings demonstrated that the use of high-intensity ultrasound was able to recover high phenolic contents significantly reducing extraction time.

HPLC-ESI-QTOF-MS/MS revealed the presence of a total of 142 phytochemicals belonging to different classes in the hydroethanolic extract from araticum peel, including 5 organic acids, 3 jasmonates, 33 phenolic acids, 73 flavonoids, 6 other phenolics, 21 annonaceous acetogenins and 1 other compound. To the best of our knowledge, the most part of these compounds (123 of them) was reported here for the first time in araticum peel. The presence of a large number of phenolic compounds (112 of the 142 identified phytochemicals) in araticum peel extract may explain the high antioxidant activity demonstrated for this extract.

Araticum extracts studied in this research exhibited high phenolic contents as can be seen in **Chapters II** and **III**. The biological and technological properties of phenolic compounds are mainly due to their antioxidant capacity. Phenolic compounds can act as antioxidants in chemical or biochemical systems by several ways, such as donating hydrogen atoms, chelating transition metals, scavenging reactive oxygen and/or nitrogen species (ROS/RNS), inhibiting enzymes involved in oxidative stress, and upregulating and/or

protecting endogenous defence systems (Arruda, Pereira, de Morais, Eberlin, & Pastore, 2018). Antioxidant activities (DPPH, TEAC and $ORAC_{FL}$) from araticum extracts were high and positively correlated with their phenolic contents ($p < 0.01$), indicating that the phenolic compounds are the main responsible for the antioxidant activity in araticum extracts.

Araticum fruits have unique sensory features such as attractive colour, intense flavour and exotic aroma, as well as high content of nutrients and bioactive compounds (Bezerra et al., 2018; Cardoso et al., 2013). However, this fruit present a very short production cycle and is highly perishable, making its consumption possible only at certain times of the year and in limited regions (Botrel, Rodrigues, Souza, & Fernandes, 2016). One way to increase availability and add even more value to this fruit is to process its pulp and/or create new products. In this sense, one of the approaches of this research was to develop a prebiotic inulin-enriched araticum whey beverage and to evaluate the impact of thermal treatments (70 to 100°C for 60 s) on chemical stability of the inulin (**Chapter IV**).

It was verified that thermal treatments did not modify the physicochemical properties (pH, TSS and ζ-potential), FTIR spectra and soluble sugars content (glucose, fructose, sucrose and lactose) of whey beverages after processing ($p \geq 0.05$), suggesting that the thermal treatments did not cause significant reactions and/or structural modifications in the samples, possibly due to the reduced processing time (60 s). To prove this hypothesis, the prebiotic araticum whey beverages were analysed by HPAEC-PAD to evaluate in detail the qualitative and quantitative molecular profile of inulin before and after the thermal processing and the results revealed no significant change ($p \geq 0.05$).

Previous studies have shown that thermal treatments are not feasible for the manufacture of prebiotic foods/beverages, since FOS are highly susceptible to hydrolysis/degradation during thermal processing (Courtin, Swennen, Verjans, & Delcour, 2009; Forgo, Kiss, Korózs, & Rapi, 2013; Klewicki, 2007; Vega & Zuniga-Hansen, 2015). However, the medium pH plays a key role in the thermal stability of inulin/FOS. Wada, Sugatani, Terada, Ohguchi, and Miwa (2005), Glibowski and Bukowska (2011), Klewicki (2007) and Vega and Zuniga-Hansen (2015) reported that the thermal stability of inulin/FOS is decreased in low pH (≤ 4). FOS (GF_2-GF_{20}) that make up the inulin chain added to the prebiotic araticum whey beverages were not hydrolysed/degraded by the thermal treatments (70-100°C/60 s). These results are supported by the studies abovementioned since the pH of the araticum whey beverages developed in this work was close to neutrality (pH = 5.59). Therefore, these findings evidence that the inulin chain is not susceptible to hydrolysis/degradation under

high-temperature conditions in non-acid beverages, and thus it can be successfully used to enrich araticum whey beverages thermally treated without prejudice to their functional properties.

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GENERAL CONCLUSION

The bibliographic research carried out in **Chapter I** was the basis for understanding the sensory, nutritional and functional aspects of araticum as well as its potential for use in the development of innovative food and non-food products. The review revealed that different botanical parts of araticum (fruit pulp, fruit peel, seeds, leaves, stem and root) contain a variety of bioactive compounds such as phenolics, alkaloids, annonaceous acetogenins, carotenoids, tocols and phytosterols, as well as several biological activities including antioxidant, hepatoprotective, anti-inflammatory, antitumoral, analgesic, antidiabetic, skin healing, antidiarrhoeic, antimicrobial, antiparasitic, insecticide and herbicide activities. In addition, this review was also useful in identifying some scientific gaps, such as the need for more in-depth studies on: (1) chemical composition of the fruit, mainly related to secondary metabolites; (2) techniques for extracting and obtaining its phytochemicals; (3) development of commercial pulp-based food products; and (4) use of fruit by-products (peel and seeds).

Chapter II revealed, for the first time, the profiles and contents of free, esterified, glycosylated and insoluble-bound phenolics from araticum fruit parts (pulp, peel and seed). This study showed that: (1) araticum peel had the highest phenolics content and antioxidant activity, followed by pulp and seeds, respectively; (2) flavonoids made the highest contribution for the araticum pulp and peel, whereas phenolic acids comprised the most phenolics from araticum seed; (3) catechin and epicatechin were the major compounds from araticum pulp and peel, whereas seed displayed caffeic acid, catechin and epicatechin as its main phenolics; and (4) phenolics were predominantly present in the insoluble-bound and esterified forms in all of the araticum fruit parts. Moreover, this study also confirmed the need to explore the fraction of insoluble-bound phenolics from araticum fruit, since this fraction had an important contribution for total phenolics, especially for araticum pulp (39.96%) and seed (47.38%), demonstrating that simple extraction of soluble phenolics might lead to underestimation of phenolic contents and their actual contribution on the biological activities.

Chapter III was developed for investigating the effects of high-intensity ultrasound parameters on phenolic antioxidants recovery from araticum peel, looking the exploitation of this food industry by-product as a source of phenolics antioxidants. The evaluation of ultrasound conditions, ultrasonic power (W) and process time (min), led to obtaining extracts rich in phenolic antioxidants from araticum peel. Ultrasonication process intensification significantly increased the phenolics antioxidants recovery, demonstrating that

high phenolic yields and antioxidant activities values can be obtained from araticum peel at short extraction times, while operating in high nominal ultrasonic powers due to synergic effect between these process parameters. Therefore, it can be concluded that high-intensity ultrasound technology is a simple, fast and of low environmental impact process to recover high-added value compounds like phenolics antioxidants from araticum peel for further applications in the food, nutraceutical, cosmetic and pharmaceutical industries.

Obtaining an araticum whey beverage with functional potential through enrichment with a prebiotic carbohydrate (inulin) as well as the effect of thermal treatments on the chemical stability of inulin added were evaluated in **Chapter IV**. Physicochemical properties (pH, TSS and ζ-potential), FTIR spectra, soluble sugars content (glucose, fructose, sucrose and lactose) and qualitative and quantitative profile from inulin of the inulin-enriched araticum whey beverages were not affected by the thermal treatments applied. This study demonstrated that the backbone of the inulin added remained intact in the araticum whey beverages after thermal treatments, providing evidences that the prebiotic properties of the araticum whey beverages thermally treated are preserved. Thus, inulin-enriched araticum whey beverages can be successfully manufactured industrially without prejudice to their functional properties. Moreover, this study opens the way for the commercial exploitation of araticum fruit in the development of functional food products.

Overall, from the present research, it can be concluded that the araticum fruit has a high potential for use in the development of commercial functional food products due to its high phenolic content and antioxidant activity, whereas its by-products (peel and seeds) offer promising sources of natural antioxidants, especially phenolics, for further development of nutraceuticals, functional ingredients, foods, cosmetics, drugs or value-added products.

FUTURE PERSPECTIVES

The use of exotic fruits for the development of novel food products and the agroindustrial by-products utilization to obtain value-added ingredients are a worldwide trend. In this context, araticum fruit and its by-products represent an interesting alternative for the development fruit pulp-based products and obtaining value-added compounds, especially phenolics, for food, feed, cosmetic and pharmaceutical applications. However, there are still several gaps to be filled so that araticum can become a sustainable cash crop, requiring more comprehensive and in-depth studies. Thus, from the results obtained with the development of the present work, the following lines of action for future researches were identified:

- Study agronomic and genetic aspects in order to develop technical plantations to increase productivity and fruit availability at different times of the year;
- Study and develop suitable fruit pulp conservation methods;
- Develop and evaluate sensorial, physicochemical and functionally novel food fruit pulp-based products for their implementation in the food products market;
- Evaluate the digestibility, bioavailability and bioaccessibility of the nutrients and bioactive compounds present in araticum fruit;
- Assess the association between araticum fruit consumption and health benefits;
- Study phenolics concentration and purification methods from araticum peel;
- Investigate the effect of water activity, pH, temperature and oxygen on the stability of phenolic extract or purified phenolics from araticum peel to determine the critical limits of shelf life;
- Study the toxicity of phenolic extract or phenolics purified from araticum peel both *in vitro* and *in vivo* models;
- Study the use of phenolic extract or phenolics purified from araticum peel for several technological and therapeutic purposes;
- Study the obtaining annonaceous acetogenins from araticum peel and their potential bioactivities and applications.

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ANNEXS

Annex 1: Voucher specimen deposited to Herbarium of the Institute of Biology of the University of Campinas

Annex 2: 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 AA9DDF7

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:	**AA9DDF7**
Usuário:	**UNICAMP**
CPF/CNPJ:	**46.068.425/0001-33**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Annona crassiflora Mart.

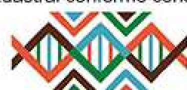
Título da Atividade:	**Avaliação do potencial funcional e tecnológico do fruto do Cerrado araticum (Annona crassiflora Mart.)**

Equipe

Henrique Silvano Arruda	**UNICAMP**
Damila Rodrigues de Moraes	**Unicamp**
Célio Fernando Figueiredo Angolini	**Unicamp**
Eric Keven Silva	**Unicamp**
Maria Angela de Almeida Meireles	**Unicamp**
Marcos Nogueira Eberlin	**Unicamp**
Glaucia Maria Pastore	**Unicamp**
Gustavo Araujo Pereira	**Unicamp**

Data do Cadastro:	**16/03/2018 11:43:13**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **11:44** de **16/03/2018**.



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Annex 3: Article published to Food Research International

Food Research International 123 (2019) 450–480

Contents lists available at ScienceDirect

Food Research International

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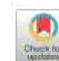
Review

Araticum (*Annona crassiflora* Mart.) as a source of nutrients and bioactive compounds for food and non-food purposes: A comprehensive review



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ARTICLE INFO

Keywords:
Biological activity
Health benefits
Nutritional composition
Chemical constituents
Phytochemicals
Nutraceuticals
Marolo
Cerrado fruit

ABSTRACT

Araticum (*Annona crassiflora* Mart.) is a fruitful tree native to the Brazilian Cerrado biome that holds high nutritional, functional and economic potential. This plant has been used since ancient times by folk medicine for the treatment of several pathological conditions. There has been increasing interest in the development of pulp-based food products as well as the by-products utilization to obtain value-added ingredients. Understanding the chemical composition and biological activities of different botanical parts of *Annona crassiflora* Mart. provides a basis to support future researches and applications. In this context, this paper carries out an exhaustive review of the scientific literature, on the main phytochemicals of different botanical parts of *Annona crassiflora* Mart. (fruit, leaves, stem and root) and their biological activities, assessing their potential uses for several industrial segments. *Annona crassiflora* Mart. fruits and especially their by-products (peel and seeds) and leaves have been shown a wide range of bioactive compounds such as phenolic compounds, alkaloids, annonaceous acetogenins, tocols, carotenoids, phytosterols, dietary fiber, vitamins, minerals and essential oils. These compounds contribute to various biological activities, including antioxidant, hepatoprotective, anti-inflammatory, antitumoral, analgesic, antidiabetic, skin healing, antidiarrhoeic, antimicrobial, antiparasitic, insecticide and herbicide activities of *Annona crassiflora* Mart. extracts. Therefore, these findings demonstrate that *Annona crassiflora* Mart. fruit, by-products and leaves can be excellent candidates to be used as functional foods and/or sources for obtaining bioactive compounds for the food, cosmetics and pharmaceutical applications.

1. Introduction

Brazil is home to one of the greatest biodiversity in the world (20–22% of the total), housing a large number of plant species with medicinal potential (Silva et al., 2018). For example, in the Brazilian Cerrado biome (second largest biome in Brazil, accounting for approximately 25% of the Brazilian territory) there are > 600 medicinal plants, among which we can highlight the *Annona crassiflora* Mart. (Arruda, Fernandes, Botrel, & Almeida, 2015; da Costa Oliveira et al., 2018).

Annona crassiflora Mart., popularly known as "araticum-do-cerrado", "ariticum", "articum", "marolo", "bruto", "cabeça-de-negro", "cascudo", "panã", "pinha-do cerrado" and "pasmada" (Arruda et al., 2015; Arruda, Pereira, & Pastore, 2018), is a tree native to the Brazilian Cerrado, widely spread throughout the biome spanning across the states of Minas Gerais, São Paulo, Bahia, Mato Grosso do Sul, Mato Grosso, Tocantins, Goiás, Maranhão, Piauí, Pará and Federal District (Cota et al., 2011). This species has been used since ancient times as a traditional medicine in preparations for the treatment of several pathological conditions (Arruda & Almeida, 2015). Besides being used by folk medicine, *Annona crassiflora* Mart. tree produces fruits with acceptable sensory characteristics as well as significant nutritional and functional potential (Arruda, Botrel, Fernandes, & Almeida, 2016). Its fruits are highly appreciated by the local population and are among the 20 most used species in the regional foodstuffs (Cavalcante, Naves, Seraphin, & Carvalho, 2008), being consumed either fresh or processed (ice cream, popsicles, jellies, jams and juices). *Annona crassiflora* Mart. fruits present unique sensory features such as attractive colour, intense flavour and exotic aroma, as well as a high content of nutrients and bioactive compounds (Bezerra, Pereira, Prado, Barros Vilas Boas, & Resende, 2018; de Cardoso, Oliveira, de Bedetti, Martino, & Pinheiro-Sant'Ana, 2013). These attributes make this fruit a promising ingredient for the development of innovative and healthy products in the food industry. In addition, fruit by-products and other plant parts have been shown to be potential sources of value-added compounds. However, *Annona crassiflora* Mart. fruit and based products are available only in

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Title: Araticum (Annona crassiflora Mart.) as a source of nutrients and bioactive compounds for food and non-food purposes: A comprehensive review

Author: Henrique Silvano Arruda,Glaucia Maria Pastore

Publication: Food Research International

Publisher: Elsevier

Date: September 2019

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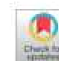
Annex 5: Article published to Food Chemistry

Food Chemistry 245 (2018) 738–749

Determination of free, esterified, glycosylated and insoluble-bound phenolics composition in the edible part of araticum fruit (*Annona crassiflora* Mart.) and its by-products by HPLC-ESI-MS/MS



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A R T I C L E I N F O

Chemical compounds studied in this article:
Catechin (PubChem CID: 9064)
Epicatechin (PubChem CID: 72276)
Rutin (PubChem CID: 5280805)
Quercetin (PubChem CID: 5280343)
Protocatechuic acid (PubChem CID: 72)
Gentisic acid (PubChem CID: 3469)
Chlorogenic acid (PubChem CID: 1794427)
Caffeic acid (PubChem CID: 689043)
p-Coumaric acid (PubChem CID: 637542)
Ferulic acid (PubChem CID: 445858)

Keywords:
Brazilian Cerrado fruit
Antioxidant activity
Bioactive compounds
Natural phenolic antioxidants
Extraction procedure

A B S T R A C T

Phenolics present in the free, esterified, glycosylated and insoluble-bound forms of araticum pulp, peel and seed were for the first time characterized and quantified using HPLC-ESI-MS/MS. Levels of total phenolics, flavonoids, condensed tannins and antioxidant activities from araticum fruit followed the order peel > pulp > seed. Overall, insoluble-bound and esterified phenolics were the dominant forms of phenolics from araticum fruit parts and the highest contributors to their antioxidant activities. Extracts were found to contain contrasting levels of phenolics that were specific to each fruit part. From 10 phenolics quantified in araticum fruit, catechin and epicatechin were the major ones from pulp and peel, whereas seed displayed caffeic acid, catechin and epicatechin as its main phenolics. Araticum fruit was found to provide a good source of phenolics, and the full exploitation of this fruit may find applications in the food, cosmetic and pharmaceutical industries.

1. Introduction

Araticum or marolo (*Annona crassiflora* Mart.) is an exotic fruit of Brazilian Cerrado belonging to the *Annonaceae* family. This fruit has oval or rounded shapes, tomentose surfaces of green colour when developing and brown when ripe with dimensions ranging from 9 to 15 cm in length per 10 to 15 cm diameter, and weighing from 0.5 to 4.5 kg. Its seeds (70 to 190 per fruit) are dark-brown with obovate-flattened shape, measuring from 10 to 13 mm per 20 to 27 mm, whereas its pulp is lightly sweet with a pleasant aroma, and colour ranging from white to yellow (Arruda & de Almeida, 2015). Araticum is one the most consumed fruits in the Cerrado region (within the top 20) (Arruda, Pereira, & Pastore, 2017a). Apart from its unique sensory features such as colour, flavour and aroma, the araticum fruit holds high nutritional value (1.52 g proteins, 3.50 g lipids, 14.39 g carbohydrates, 0.47 g ash, 6.80 g dietary fibre and 95.12 kcal per 100 g of pulp) and a great diversity of phytochemicals such as carotenoids, tocopherols, folates, some vitamins (Cardoso, Oliveira, Bedetti, Martino, & Pinheiro-Sant'Ana, 2013), and mainly phenolic constituents (Arruda, Pereira, & Pastore, 2017b; Roesler, Catharino, Malta, Eberlin, & Pastore, 2007). The rich profile of nutrients and phytochemicals in the araticum fruit has attracted growing interest of researchers, consumers and food, cosmetic and pharmaceutical industries.

Recent studies have demonstrated diverse biological properties of extracts from different parts of the araticum fruit, such as the ability to inhibit digestive enzymes and formation glycation products (Justino et al., 2016; Pereira et al., 2017), antioxidant (Roesler, 2011), antibacterial (Silva et al., 2014) and hepatoprotective (Justino et al., 2017) activities of fruit peel; antiproliferative, anticholinesterase (Formagio et al., 2015), insecticidal (Krinski & Massaroli, 2014), larvicidal

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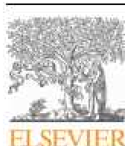
Annex 7: Article published to Ultrasonics Sonochemistry

Ultrasonics - Sonochemistry 50 (2019) 82–95



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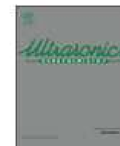

Effects of high-intensity ultrasound process parameters on the phenolic compounds recovery from araticum peel

Henrique S. Arruda[a], Eric Keven Silva[b,*], Gustavo A. Pereira[a], Célio Fernando F. Angolini[a], Marcos N. Eberlin[c], M. Angela A. Meireles[b], Glaucia M. Pastore[a]

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ARTICLE INFO

Keywords:
Emerging technology
Ultrasonic power
Bioactive compounds
By-products
HPLC-ESI-MS/MS
Annona crassiflora Mart

ABSTRACT

In this work, we investigated the effects of the nominal ultrasonic power (160–640 W) and process time (0.5–5.0 min) on the phenolic compounds recovery and antioxidant activity from araticum peel. The individual and synergistic effects of the process variables on the phenolic recovery were estimated using a full factorial experimental design. Operating at high nominal ultrasonic powers was possible to obtain high phenolic yields and antioxidant activities at short process times (≤5 min). The HPLC-ESI-QTOF-MS/MS analysis revealed that the araticum peel sample possessed 142 phytochemicals, 123 of which had not been reported in the literature for this raw material yet. The most abundant phenolic compounds recovered were epicatechin, rutin, chlorogenic acid, catechin and ferulic acid. Thus, high-intensity ultrasound technology proved to be a simple, efficient, fast and low environmental impact method for obtaining phenolic compounds from araticum peel. In addition, araticum peel showed to be a promising source bioactive natural phenolics for further applications in the food, nutraceutical, cosmetic and pharmaceutical industries.

1. Introduction

Phenolic compounds are secondary metabolites ubiquitously distributed in the plant kingdom. These plant metabolites are characterized by possessing at least one aromatic ring containing one or more hydroxyl groups substituents, called phenol groups, which are derived from L-phenylalanine or tyrosine. In addition, their structures can be associated with carbohydrates, organic acids, lipids, amines and cell wall components [1]. Currently, more than 8000 phenolic structures have been described and isolated from natural sources, and among them, over 4000 are flavonoids [2]. Phenolic compounds from plant materials present a wide variety of applications in several industry sectors, such as food and beverage, pharmaceutical and cosmetic industries. These compounds have been effectively used as dietary supplements, ingredients in functional foods and cosmetics, and replacers for synthetic antioxidants [3]. Moreover, numerous studies have shown that the consumption of natural phenolic compounds can prevent, slow or reverse the development of chronic diseases, such as cardiovascular diseases [4,5], metabolic syndrome [6], cancer [7,8], and type 2 diabetes [9].

Annona crassiflora Mart., a fruitful plant native from Brazilian Cerrado belonging to the *Annonaceae* family, produces fruits (popularly known as "araticum" or "marolo") that are very much appreciated in this region (within the top 20 more consumed) [10]. Since the peel accounts for about 40% of the whole fruit weight [11], very large amounts of by-products are formed after its consumption or processing. Araticum peel extracts have demonstrated to exhibit several biological properties, such as potent antioxidant capacity [10,12,13], ability to inhibit digestive enzymes and formation of glycation products [14,15], antibacterial [16] and hepatoprotective [12,13] activities. These beneficial effects have been associated with the bioactive compounds present in araticum peel like phenolics [10,14] and alkaloids [15]. The araticum peel has been recently shown by our group as a source of phenolics [10]. Therefore, araticum peel can be a potential material to recover highly valuable biomolecules, mainly phenolics, for further applications in the food, nutraceutical and pharmaceutical industries, and consequently add more value to the Cerrado fruits industry.

The choice of extraction procedure is one the most important steps in the recovery and purification of phenolic compounds from plant matrices and it should be based on simplicity, versatility, cost and the capacity to both extract and preserve these compounds [3]. Thus, several emerging extraction methods, mainly those based on high-

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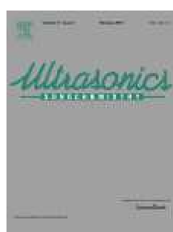

Title: Effects of high-intensity ultrasound process parameters on the phenolic compounds recovery from araticum peel

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