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Analysis of the chemical profile of cerrado pear fixed compounds by mass spectrometry with paper spray and volatile ionization by SPME-HS CG-MS

Análise do perfil químico de compostos fixos da pera do cerrado por espectrometria de massas com ionização *paper spray* e voláteis por SPME-HS CG-MS

Análisis del perfil químico de compuestos fijados de pera cerrado por espectrometría de masas con paper spray e ionización volátil por SPME-HS CG-MS

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Abstract

Eugenia klotzschiana Berg is a native fruit of the cerrado, belonging to the Myrtaceae family, known as the cerrado pear and very little explored. The present study's objective was to obtain the profile of the fixed and volatile chemical constituents of the fruit. Mass spectrometry with ambient ionization paper spray (PS-MS) was used in positive and negative mode for fixed compounds. For volatiles, solid-phase microextraction by head space and gas chromatography was employed. The PS-MS identified 31 compounds, 21 in the negative and 10 in the positive mode, including fatty acids, flavonoids, phenolic acids, tannins and sugars. The solid-phase microextraction method was used in head space mode, using the polydimethylsiloxane-divinylbenzene fiber to extract volatile organic compounds (VOCs). The volatiles detected totaled 11 substances, belonging to the class of monoterpenes, sesquiterpenes, and esters. PS-MS was capable and efficient in determining the fixed compounds present in the cerrado pear pulp, providing the chemical profile of the fruit, which contains essential compounds with potential antioxidant bioactivity. The volatile profile was obtained with success, indicating menthol, fruity and vanilla notes in the pulp of the cerrado pear.

Keywords: Solid-phase microextraction; Paper spray; Cerrado pear; Volatile compounds.

Resumo

A Eugenia klotzschiana Berg é uma fruta nativa do cerrado, pertencente à família Myrtaceae, conhecida como pera do cerrado e muito pouco explorada. O objetivo do presente estudo é obter o perfil dos constituintes químicos fixos e voláteis da fruta. Para tal, utilizou-se a espectrometria de massas com ionização ambiente paper spray (PS-MS) no modo positivo e negativo para compostos fixos, e para os voláteis a microextração em fase sólida por head space e a cromatografia gasosa. Identificou-se pela PS-MS o total de 31 compostos, sendo 21 no modo negativo e 10 no positivo, dentre eles ácidos graxos, flavonoides, fenólicos, taninos e açúcares. Utilizou-se o método de microextração em fase sólida em modo head space, empregando a fibra polidimetilsiloxano-divinilbenzeno para a extração dos compostos orgânicos voláteis (COV's). Os voláteis detectados somaram-se em 11 substâncias, apresentadas na classe dos monoterpenos, sesquiterpenos e ésteres. A PS-MS foi eficiente na determinação dos compostos fixos presentes na polpa da pera do cerrado, fornecendo o perfil químico da fruta, que contêm importantes compostos com potencial bioatividade antioxidante. O perfil volátil foi obtido com êxodo, indicando a presença de notas mentoladas, frutadas e de baunilha na polpa da pera do cerrado.

Palavras-chave: Microextração em fase sólida; Paper spray; Pera do cerrado; Compostos voláteis.

Resumen

Eugenia klotzschiana Berg es una fruta autóctona del cerrado, perteneciente a la familia Myrtaceae, conocida como pera del cerado y muy poco explorada. El objetivo del presente estudio es obtener el perfil de los componentes químicos fijos y volátiles del fruto. Para ello, se utilizó espectrometría de masas con ionización ambiental paper spray (PS-MS) en modo positivo y negativo para compuestos fijos, y para volátiles, microextracción en fase sólida por head space y cromatografía de gases. Un total de 31 compuestos fueron identificados por el PS-MS, 21 en negativo y 10 en positivo, entre ácidos grasos, flavonoides, ácidos fenólicos, taninos y azúcares. Se utilizó el método de microextracción en fase sólida en modo head space, empleando la fibra de polidimetilsiloxano-divinilbenceno para la extracción de compuestos orgánicos volátiles (COVs). Los volátiles detectados se agregaron en 11 sustancias, presentadas en la clase de monoterpenos, sesquiterpenos y ésteres. PS-MS fue capaz y eficiente en la determinación de los compuestos fijos presentes en la pulpa del pera cerrado, proporcionando el perfil químico del fruto, que contiene importantes compuestos con potencial bioactividad antioxidante. El perfil volátil se obtuvo con éxodo, indicando la presencia de notas mentoladas, afrutadas y vainilladas en la pulpa de pera cerrado.

Palabras clave: Microextracción en fase sólida; Paper spray; Cerrado pera; Compuestos volátiles.

1. Introduction

The cerrado is one of the largest Brazilian biomes. Its climatic characteristics differ from other savanna regions due to its precipitation conditions, of approximately 800-2000 mm, in the dry season (April-September), annual temperature ranging between 18 and 28°C and acid pH soil (Rufino et al., 2010). Due to these edaphoclimatic characteristics, different metabolic routes can be activated and interfere with the species compounds, such as polyphenols. They have their synthesis enhanced as a protection mechanism when plants are exposed to environments with low humidity, high exposure to light and solar radiation, and high temperatures, such as in the cerrado (Rufino et al., 2010; Silva, Freitas, et al., 2019). These substances and their potential bioactivity, such as the antioxidant action, make plants in the cerrado excellent candidates for bioprospecting for this capacity (Bailão, Devilla, da Conceição, & Borges, 2015).

Cerrado fruits are highlighted for their peculiarities of flavor, aroma, and functional properties (Arruda, Pereira, Morais, Eberlin, & Pastore, 2018; Flores et al., 2012; Silva, Freitas, et al., 2019; de Souza et al., 2012). One of the most frequent families in this biome is Myrtaceae, with approximately 102 genera and 3024 species, including araçá (*Psidium cattleianum*) (Ribeiro et al., 2018), gabiroba (*Campomanesia pubescens*) (Ribeiro et al., 2018), guava (*Psidium guajava*) (Ribeiro et al., 2018), and pitanga (*Eugenia uniflora*) (Ribeiro et al., 2018). Among the genera, Eugenia is distinguished by its economic and nutritional relevance. The fruits of the cagaiteira, *Eugênia dysenterica* (Silva, Freitas, et al., 2019), popularly known as cagaita, are an important source of minerals and phenolic compounds, such as gallic, vanillic, and salicylic acids and quercetin (Silva, Freitas, et al., 2019). Grumixama (*Eugenia brasiliensis*) (Ramos et al., 2020) has potential antioxidant capacity, with evidence for anthocyanins (Flores et al., 2012; Ramos et al., 2020).

Also noteworthy is *Eugenia klotzschiana* Berg, popularly known as pear of the cerrado. It is a fruit consumed by the regional population in the form of jams, sweets, jellies, and natural juices. In the past, its consumption was more frequent, however, currently, there is a shortage of its trees, as a result of the deforestation that has occurred in the cerrado regions for raising beef cattle or wood plantations, reducing access and consumption (Carneiro, Alves, Alves, Esperandim, & Miranda, 2017).

The cerrado pear is a fruit of variable size, thin-skinned, and greenish-yellow when ripe, with a soft brown pulp and an acidic,astringent flavor. On average, its diameter is 9 centimeters, with a weight of 102 grams (Carneiro, 2016). The tree flowering occurs in September to October and the fruiting between November to February (Carneiro, 2016; Ribeiro et al., 2018; Vallilo, Battello, Lamardo, & Lobanco, 2003). The pear chemical characteristics are high moisture and high of dietary fiber content, in addition to the presence of polyphenolic compounds and flavonoids such as quercetin (Carneiro, 2016). In the scientific literature, studies on the chemical characterization of the cerrado pear are limited. In this sense, research that provides data and information is of paramount importance in order to understand the chemical characteristics of the fruit and see that its consumption potential, use and preservation of the species are stimulated.

Among the analytical techniques for the characterization of these fruits, mass spectrometry stands out for being robust and having high sensitivity in detecting and subsequent identification of the fixed compounds present in the analyzed material, through the mass charge ratio of ions obtained by ionization of the aliquot. The technique can be used with different methods of ambient ionization, such as desorption electrospray ionization (DESI), electrospray ionization (ESI) (Hoffmann, Edmond de; Stroobant, 2007), and paper spray (PS) that was developed in the last decade (Liu, J et al., 2017). PS ionization is based on the formation of ions of a certain aliquot, arranged on a chromatographic paper in the form of a triangle moistened with solvent, through the application of a high potential originating from the mass spectrometer itself and conducted through a wire copper. The formed ions enter the spectrometer and are analyzed. PS is a fast technique, requiring little or no sample treatment, in addition to being sensitive and efficient for complex matrices, such as food, blood, and trace element analysis (Liu, J et al., 2017; Silva, Freitas, et al., 2019). Since its development, PS has been applied to different materials and made it possible to detect drugs of abuse, pesticides, food contaminants, and fraud (Pereira, Amador, Sena, Augusti, & Piccin, 2016). PS is also used on foods like cagaita (Silva, Freitas, et al., 2019) and the grumixama (Ramos et al., 2020), cereals like sorghum (Campelo et al., 2019), plus roots like ginger (C. T. Oliveira et al., 2020).

In addition to fixed compounds, fruit stands out in volatile organic compounds (VOCs), which are substances that impart the fruity aroma and characterize its olfactory sensory perception. In fruits, VOCs are important in defense against pests and consumer preferences (Gao, Li, Xu, Zeng, & Guan, 2018). These constitute the volatile profile in different maturation periods until senescence (Nagarajan & Chandiramouli, 2018), comprising the class of alcohols, esters, and terpenes (sesquiterpenes and monoterpenes). However, VOCs must be extracted from the sample matrix to be identified and quantified to identify the fruit volatile profile. To this end, techniques were developed from the beginning, when they still included the use of solvents and very laborious execution (Bianchin, 2015). Currently, some methodologies do not require the use of solvents, such as the sorting of magnetic bar (SBSE) and the solid-phase microextraction (SPME) (Bianchin et al., 2012). SPME in head space mode has stood out for its efficiency in complex arrays (Canuto, Garruti, & Magalhães, 2011; Santos, Augusti, Melo, Takahashi, & Araújo, 2020) like food, including beer (Pereira et al., 2016), acerola (Garcia et al., 2016; García et al., 2019), cagaita (Silva, Bueno, et al., 2019) and grumixama (Ramos et al., 2020), and for not requiring the use of sophisticated instruments. Also, it is easy to handle, requires less extraction time, has low cost, and allows the application of different extraction fibers (Silva, Bueno, et al., 2019).

The fibers most used for food samples are PA (Polyacrylate), PDMS/DVB (Polidimethylsiloxane/Divinylbenzene), and CAR/PDMS/DVB (CarboxPolydimethylsiloxane/Divinylbenzene semipolar), with emphasis on PDMS/DVB for volatiles in fruits. The difference between them is, especially, their polarity, which will define the type of compound extracted. That is, semipolar fibers such as PDMS/DVB and CAR/DVB/PDMS adsorb volatiles with medium polarity, whereas PA, those that are highly polar (Silva, Bueno, et al., 2019). The volatiles isolated by SPME HS are separated, detected, and quantified by gas chromatography coupled with mass spectrometry (Silva, Bueno, et al., 2019).

Given this, the present study proposes the characterization of the chemical profile of fixed constituents present in the cerrado pear using mass spectrometry with PS ionization and their identification, and characterization of the volatile chemical compounds profile in the fruit pulp.

2. Materials and Methods

Obtaining the fruit

The cerrado pear fruit samples were obtained through donation, from trees located in Turmalina, Minas Gerais – Brazil (Lat -17.287410, Long -42.718210). The fully ripened fruits (greenish-yellow peel color) were harvested directly from the trees, from January to February 2019, obtaining about 3 kg.

After collection, the fruits were washed with running water to remove dirt and sanitized with a chlorinated solution (200 ppm) for 15 minutes, followed by rinsing under running water. Subsequently, they were packed entirely in polyethylene bags and stored at of -18ºC until analyzes. The samples were transported in a secondary package containing ice to the food chemistry laboratory of the Faculty of Pharmacy of the Federal University of Minas Gerais to be analyzed.

The analyzes were performed using the fruit pulp, obtained by manual pulping, followed by homogenization with a mixer (Mixer Mondial Versatile Black M-08).

Extraction of cerrado pear pulp

The extraction of cerrado pear pulp was carried out according to the methodology described by (Rufino et al., 2010). 0.5 g of pulp was weighed in falcon-type tubes lined with aluminum foil. Subsequently, 1 ml of methanol/water solution (50:50, v/v) was added, then incubated for 1 hour. Tthey were then centrifuged for 15 min at 25,406 xg. The supernatant was collected and stored in a 5mL volumetric flask. One (1) ml of acetone/water solution (70:30, v/v) was added to the residue, followed by incubation and centrifugation under the same conditions previously mentioned. The supernatant was transferred to the 5 ml flask, manually shaken and divided into Eppendorfs in the dark, for subsequent analysis.

Determination of the chemical constituents of the cerrado pear pulp

To carry out the analyzes, the standardized process by (Silva, Freitas, et al., 2019) using a mass spectrometer with an IQ-trap analyzer model LCQ Fleet from Thermo Scientific (San Jose, CA, USA) and a paper spray ionization source. A chromatographic paper cut in the shape of an equilateral triangle and measuring 1.5 cm was inserted into a metal claw at a distance of 0.5 cm from the mass spectrometer entrance. The copper wire connected to the equipment and the clamp allows the application of a high voltage current. A 2 µL aliquot of the obtained extract was added to the end of the triangular paper, followed by 40 µL of methanol. After that, the high voltage source was turned on to promote ionization and obtain the data.

The experiments were carried out in the negative ionization mode using -3.2 kV and positive with +5.0 kV. The established mass range was 100 to 1000 m/z, and the transfer tube temperature was 275°C with a capillary voltage of 40 V and lens voltage of 120 V. In addition to the full scan, fragmentation was carried out in both ionization modes by applying collision energy ranging from 14 to 40 eV.

The Xcalibur software version 2.2 SP1 was used to extract and analyze the data from the spectra obtained (Thermo Scientific, San Jose, CA, USA).

Extraction and identification of volatile compounds

For the extraction of volatile compounds, the SPME-HS method was used, according to García et al. (2019). Two (2) g of the pulp, homogenized by a mixer, were weighed and placed in a 20 mL glass bottle. The 20 mL head space bottle was coupled to an aluminum block and heated to 30°C on a hot plate. After 5 minutes of heating, the semipolar fiber, polydimethylsiloxane-divinylbenzene (PDMS/DVB) mounted in a holder, was exposed to the pulp sample for 20 minutes in head space mode. The holder containing the polymeric film was then removed and manually inserted into the gas chromatograph injector coupled to the mass spectrometer, exposing the polymeric film for 5 minutes for the desorption of the extracted volatile organic compounds. The VOCs were separated using a capillary column (HP-5 ms) 5% phenyl and 95% methyl polysiloxane (30 mx 0.25 mm x 0.25 µm; Agilent

Technologies Inc., Germany) with helium carrier gas at a flow rate of 1 mL.min-1. The column was maintained for 1 minute at 40°C at the beginning of the process, increasing 12°C per minute until reaching 120°C and remaining stable for 2 minutes. Then, the temperature rose to 15ºC per minute until the 150ºC mark. After two minutes at that temperature, 245°C was gradually reached at 20°C every minute.

The chromatograms were obtained in full scan mode, and the identification of VOCs was based on the m/z ratio of the fragments obtained in the mass range of 50 to 300 m/z. The data were compared using the Xcalibur software version 2.1 (Thermo Scientific, San Jose, CA, USA) and the NIST library (National Institute of Standards and Technology) considering the similarity level (reverse search index, RSI) greater than 600.

3. Results and Discussion

Chemical constituents of the cerrado pear pulp

In the cerrado pear samples, 31 substances were identified, 21 compounds in the negative mode, and 10 in the positive mode. The compounds are shown in Table 1.

Table 1. Proposed classification for the compounds identified in the cerrado pear pulp (-) PS-MS

Nº	**Identification**	**Formula**	**m/z [ ]$^-$**	**MS/MS**	**Reference**
Organic acid					
1	Malic acid	C_4-H_6-O_5	115	71	Silva et al. 2019
2	Malic acid	C_4-H_6-O_5	133	115	Silva et al. 2019
3	Traumatic Acid	C_{12}-H_{20}-O_4	227	165, 183	Wang et al. 2017
Phenolics					
4	Isocitric acid	C_6-H_8-O_7	213	85, 111	El-Sayed et al. 2017
5	1-O-Dihydrocaffeoylglycerol	-	255	181, 179, 135	Kang et al. 2016
6	Tryhydroxyflavone	C_{15}-H_{10}-O_5	269	225, 241	Mena et al. 2012
7	Tinosposide A	$C_{27}H_{35}O_{11}$	535	373, 517	Jiao et al.2018
Fatty acids					
8	Oleic acid	$C_{18}H_{34}O_2$	281	237	Wang et al. 2017
9	Stearic acid	$C_{18}H_{36}O_2$	283	265	Wang et al. 2017
10	Eicosanoic acid	$C_{20}H_{40}O_2$	311	293	Wang et al. 2017
11	Oxo-dihydroxy-octadecenoic acid isomer	$C_{18}H_{32}O_5$	327	183, 291, 309	Ağalar et. al 2018
Flavonoids					
12	Gallocatechin	C_{15}-H_{14}-O_7	305	--	Said et al. 2017
13	Scoparin	C_{22}-H_{22}-O_{11}	461	341	El-Sayed et al. 2017
14	Delphinidin-3-caffeoyl glucosid	$C_{30}H_{26}O_{15}$	625	463	Wang et al. 2017
Hydroxinamic Acids					
15	Caffeic acid	C_9-H_8-O_4	179	135, 179	Roesler et al. 2007
16	Caffeoyl hexose	-	341	179	Kajdžanoska et al. 2010
Sugar					
17	Glucose	$C_6H1_2O_6$	179	71, 89	Wang et al. 2017
18	Sucrose	C_{12}-H_{22}-O_{11}	377	215, 341	Chen et al. 2011
Lignans					
19	Cyclo lariciresinol hexoside	-	521	359	Mena et al. 2012
20	Conidendrin	C_{20}-H_{20}-O_6	355	337	Sanz et al. 2012
Tannin					
21	HHDP-galloyl-glucose	-----	633	301	Kajdžanoska et al. 2010

Source: Author (2020).

The compounds ionized in the positive mode with the identification suggestion are shown in Table 2.

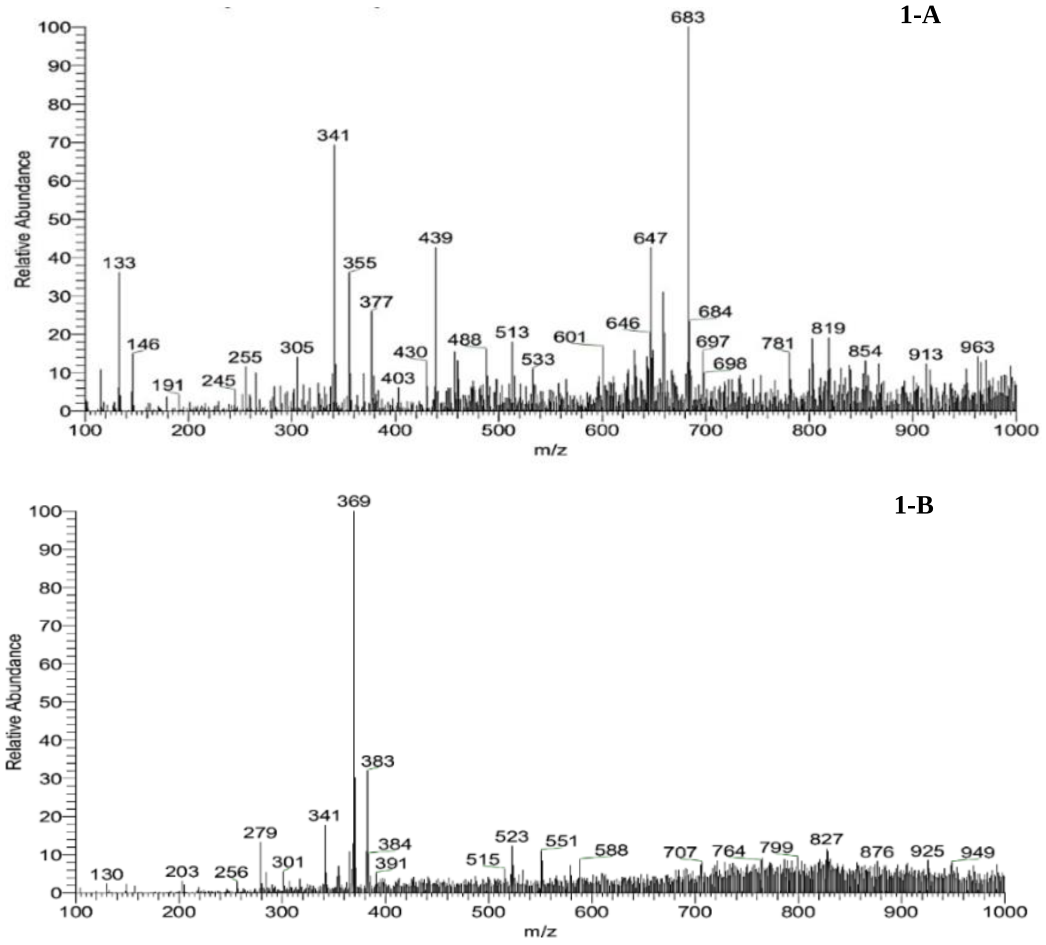
Table 2. Proposed classification for the compounds identified in the cerrado pear pulp (+) PS-MS.

Nº	Identification	Formula	m/z $[\ ]^+$	MS/MS	Reference
Flavonoids					
1	Galloylpyrogallol	$C_{13}H_{10}O_7$	279	153	Abu Reidah et al. 2015
2	Peonidin 3-O-glucoside	C_{16}-H_{13}-O_6.C_l	301	---	Gordon et al. 2012
3	Petunidin	C_{16}-H_{13}-O_7.C_l	317	---	Sharma et al. 2015
4	Stigmasterol	C_{29}-H_{48}-O	413	---	Sharma et al, 2015
5	Quercetin arabinoside	$C_{20}H_{18}O_{11}$	435	301	Abu Reidah et al. 2015
6	Myricetin-3-glucoside	C_{21}-H_{20}-O_{13}	481	318	Sharma et al, 2015
7	Apigenin neohesperidoside I	C27H30O$_{14}$	579	433	Abu Reidah et al. 2015
8	Quercetin-3-O- (6’’-3-hydroxy-3-methyl) glutaroyl-â-galactoside	$C_{27}H_{28}O_{15}$	593	301	Abu Reidah et al. 2015
Sugars					
9	Glucose	C_6-H_{12}-O_6	203	---	Guo et al. 2017
10	Sucrose	C_{12}-H_{22}-O_{11}	365	185, 203	Guo et al. 2017

Source: Author (2020).

The mass spectra obtained by the PS-MS methodology, using negative ionization mode is shown in Figure 1-A and the positive in Figure 1-B.

Figure 1. Mass spectra obtained in full scan mode with negative and positive ionization.



Source: Author (2020).

The compounds detected in the negative ionization mode are distributed in phenolics, flavonoids, organic acids, fatty acids, sugar, and lignans. Most of them are concentrated in the classes of flavonoids and phenolics.

The main flavonoids identified negatively in the cerrado pear pulp using spectral evidence and by comparing the data obtained with those in the literature were gallocatechin, scoparin, delphinidin-3-caffeoylglucosid, pelargonidin-succinyl-arabinoside, and delphinidin-3- caffeoylglucosid, with molecular ion characteristics of m/z 305, m/z 461, m/z 625, respectively. In the positive ionization mode, 7 of the ten compounds identified are flavonoids and simple sugars, mono and disaccharides, which are often found in fruits and provide the sweet taste. The identification of these constituents corroborates and reaffirms data from existing studies that the fruits of the Myrtaceae family, such as the cerrado pear, *Eugenia brasilienses Lam*. (Ramos et al., 2020; Teixeira, Bertoldi, Lajolo, & Hassimotto, 2015), *Myrciaria floribunda* (L. M. de Oliveira et al., 2018; Tietbohl et al., 2017) and *Syzygium* *alternifolium* (Babu et al., 2016) are an important source of bioactives, such as flavonoids (Teixeira et al., 2015).

Flavonoids have been investigated for their potential biological activities, including antitumor (Chailungka, Junpirom, Pompimon, Nuntasaen, & Meepowpan, 2017) with a toxic effect on cancer cells, antioxidant (Dong, Huang, Wang, & He, 2019), maintaining the oxidative balance by neutralizing and eradicating oxygen-reactive species, anti-inflammatory (Yang et al., 2020) and gastroprotective action against carcinogenic cells, being a potential natural alternative, with greater efficacy and less negative impact of side effects caused by synthetic drugs (Babu et al., 2016).

Phenolic acids represented (19%) of the chemical compounds identified in the present study, surpassed only by flavonoids (70%). Phenols are frequently detected in Eugenias, and these are highlighted by the content and profile of these substances. This chemical marker is an important factor in the economic value added to cagaitas (*Eugenia dysenterica*). Upon obtaining the fingerprints of these fruits, a range of polyphenols was detected, including delphinidin-3-glucoside m/z 465 and malic acid m/z 115, frequently cerrado pear. In addition to these, hydroxycinnamic acids, organic acids, flavonones, derivatives of caffeic acid, and sugars constituted the phytochemical profile of cagaita (Silva, Freitas, et al., 2019).

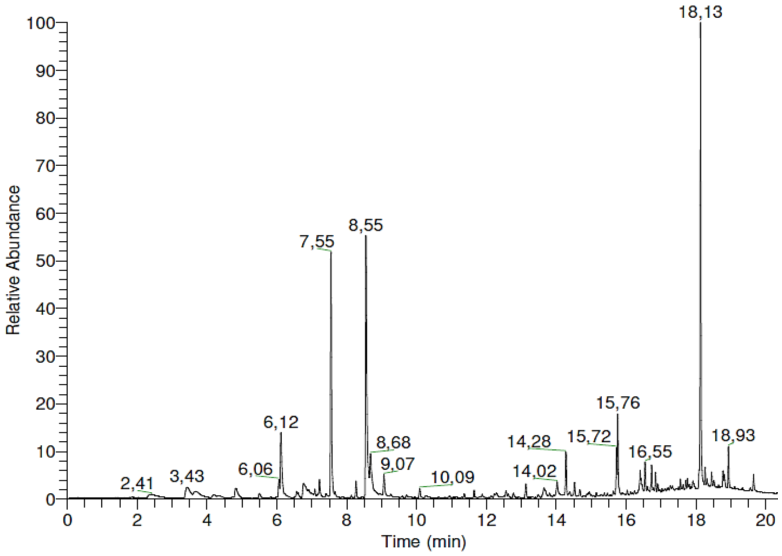
The grumixama (*Eugenia brasiliensis Lamarck*) presented higher content of phenolic compounds than the cerrado pear, (26.67%) of the total of phytochemicals screened in the fruit pulp, being tinosposid A (m/z 535) common to both chemical profiles (Ramos et al., 2020). The consumption of this class of secondary metabolites is of paramount importance to human health, due to its bioactive potential It has an anti-inflammatory action (Grace, Esposito, Dunlap, & Lila, 2014) in doses that can be obtained through food, an antioxidant effect due to its anti-oxidation capacity that also enables important activity in the pathophysiology of obesity, being able to inhibit adipogenesis, configuring an anti-adipogenic effect (Krongyut & Sutthanut, 2019). Although obesity does not only have organic factors as individual determinants, it constitutes a multifactorial pathology that involves the food environment (Carlos, Ii, & Foreyt, 1999). Pathophysiological mechanisms are intrinsic to disease, especially inflammation. Nevertheless, phenolics have a potential anticarcinoma effect (Barros et al., 2015) and hepatoprotective action (Sobeh et al., 2018).

Fatty acids are nutrients that make up the chemical profile of the cerrado pear and have been identified negatively as the molecular ions m/z 281, 283, 311, and 327, which correspond, respectively, to oleic acid, stearic acid, and eicosanoic acid oxo -dihydroxy-octadecenoic acid isomer. They are constituents that differentiate the composition of the chemical profile of the cerrado pear. It is observed that they do not occur in fruits of the same family as in cagaita (Silva, Freitas, et al., 2019) and grumixama (Ramos et al., 2020). However, fruit seeds such as from a pine (*Rollinia sylvatica*) and pitanga (*Eugenia uniflora*) presented an important lipid profile (Andrade et al., 2012), since they contained essential fatty acids that is, they are necessary for our organism and obtained exogenously, through food (Mahan, Escott-stump, & Raymond, 2013). In common with cerrado pear, stands out the stearic and oleic acid. Oleic acid is essential to human nutrition and has a protective effect on beta cells that are insulin producers. The mechanisms involved in the action are based on the anti-inflammatory and anti-oxidation capacity of unsaturated fatty acids, such as oleic (Liu et al., 2019). In addition to pancreatic B cells, anti-inflammatory activity extends to other tissues, such as the epithelial (Pegoraro et al., 2019) and intestinal (Reddy & Naidu, 2016). Emphasizing lipid bioactivity, it is reiterated that these compounds provide energy, being an essential nutrient for the functioning and maintenance of physiological functions. (Mahan et al., 2013).

Volatile Organic Compounds present in the cerrado pear pulp

The chromatogram obtained with the separation of the substances present in the cerrado pear pulp is shown in Figure 2.

Figure 2. Chromatogram with volatile substances from the cerrado pear pulp obtained after extraction by SPME and analysis by CG-MS.



Source: Author (2020).

Altogether, it was possible to identify eleven volatile compounds in the pulp of the cerrado pear. The substances are shown in Table 3.

Table 3. Volatile organic compounds identified in the cerrado pear pulp by CG-MS.

Nº	**Name**	**Formula**	**CAS**	**Reference**
Esters				
1	Ethyl ester Hexanoic acid	$C_8H_{16}O_2$	123660	(Vârva et al. 2015)
2	Benzyl alcohol	C_7H_8O	100516	(Yang et al. 2019)
3	Benzoic acid	$C_9H_{10}O_2$	93890	(Gao et al. 2018)
Monoterpenes				
4	p-Mentha-1,8-dien-7-ol	$C_{10}H_{16}O$	536594	(Thai et al.2018)
5	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	$C_{10}H_{16}$	586629	(Somkuwar et al. 2019)
6	Linalyl acetate	$C_{12}H_{20}O_2$	115957	(Salvo et al. 2018)
7	Menthofuran	$C_{10}H_{14}O$	494906	(Shigeto et al. 2019)
8	1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	514954	(Gecibesler et al. 2017)
9	Octane, 2,6-dimethyl-, hexadehydro deriv.	$C_{10}H_{16}$	29714872	(Frias et al. 2016)
Sesquiterpenes				
10	γ-Muurolene	$C_{15}H_{24}$	30021740	(Corrêa et al. 2017)
11	Bicyclogermacrene	$C_{15}H_{24}$	24703353	(Govindarajan et al. 2016)
12	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,7α,7aβ,7bα)]-	$C_{15}H_{24}$	21747466	---------------

Source: Author (2020).

The volatile compounds identified are divided into esters, monoterpenes, and sesquiterpenes. When considering the relative area of the chromatogram occupied by each peak, monoterpenes have the highest, 66.54% of occupation, followed by esters with 26.40% and sesquiterpenes 7.05%.

However, when analyzed from the number of substances, different results are obtained. Esters represent 27.27% and sesquiterpenes (27.27%) of the number of constituents in the volatile profile of the cerrado pear. Therefore, the most considerable fraction of compounds (45.45%) corresponds to the class of monoterpenes.

Monoterpenes contributed most significantly to the cerrado pear pulp aromatic content, based on the chromatogram area and the number of compounds. Among these, menthofuran and linalyl acetate cooperate with the largest relative area. Menthofuran is the primary precursor of p-menthane lactones, which are mint, isomintlactone, and mentofurolactone. The presence of these lactones provides volatile aromatic sensory characteristics of mint, coconut, and vanilla. In the complexity of the aroma, they contribute to the perception of notes of freshness, mint, and menthol nuances, which are identified in the presence of menthofuran (Picard, de Revel, & Marchand, 2017). Linalyl acetate is an elemental constituent in the aroma of lavender essential oil (25.46%) (Wińska et al., 2019), it has a medium volatile intensity, also contributing to the odor of orange juice and is established as an important chemical compound for consumer choice (Mastello, Janzantti, Bisconsin-Júnior, & Monteiro, 2018). Linalyl acetate is responsible for characterizing the floral, fruity, and lavender notes (Xiao et al., 2017).

The results obtained corroborate those found in acerola pulp (*Malpighia emarginata* DC), in which the highest concentration was terpenoids, that is, monoterpenes and sesquiterpenes, with emphasis on ethyl acetate (Garcia et al., 2016; García et al., 2019). In cagaita, the largest number of volatile compounds were monoterpenes (34.64%) and esters (36.28%) (Silva, Bueno, et al., 2019). Esters were also significant in the cerrado pear, but with a lower percentage (27.27%) and monoterpenes with a higher percentage (45.45%). Although the samples are from the same family and the predominant aroma compound classes are the same, the chemical constituents' profile is different, which implies the uniqueness of each fruit's aroma. In the aromatic profile of the grumixama, not only was there a higher concentration of terpenoids, all volatile chemical constituents are mono and sesquiterpenes, which reiterates their influence on the genus Eugenia (Ramos et al., 2020). However, the cerrado pear has a more diverse aroma, including esters, and although one of the terpenoids is the majority as in grumixama, the highest concentration is monoterpenes.

4. Final Considerations

The paper spray ionization technique with mass spectrometry was able to identify the chemical profile of the cerrado pear pulp, consisting mainly of phenolics, flavonoids, fatty acids, organic acids, and sugars. The results demonstrate that the fruit has a complex chemical composition and has the potential to be explored, providing an important benefit to human food.

The volatile profile was successfully identified and tracked by SPME HS and CG, with monoterpenes and sesquiterpenes being the substances that mostly cooperate to form the cerrado pear.

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