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Thesis presented to the Postgraduate Program in Food Science of the Faculty of Pharmacy of the Federal University of Minas Gerais, as a partial requirement to obtain the Doctor Degree of Food Science.

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	UNIVERSIDADE FEDERAL DE MINAS GERAIS **PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DE ALIMENTOS**	**PPGCA**

FOLHA DE APROVAÇÃO

DETERMINAÇÃO DO PERFIL QUÍMICO DE CAGAITA (EUGENIA DYSENTERICA) E DO SEU SORVETE UTILIZANDO A ESPECTROMETRIA DE MASSAS COM IONIZAÇÃO POR PAPER SPRAY E A MICROEXTRAÇÃO EM FASE SÓLIDA NO MODO HEADSPACE COMBINADA COM A CROMATOGRAFIA GASOSA ACOPLADA A ESPECTROMETRIA DE MASSAS

MAURO RAMALHO SILVA

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Every good and perfec gift is from above, coming down from the Father of the heavenly lights, who does not change like shifting shadows.

James 1:17

ABSTRACT

The object of this study was to optimize the conditions to extract volatile organic compounds (VOCs), in order to determine the antioxidant activity and chemical profile of cagaita and cagaita ice cream. Initially, the optimization of the VOC extraction method involved the evaluation of parameters such as fiber type (PA, CAR/PMDS, DVB/CAR/PDMS, PDMS/DVB, and CW/DVB), the whipping speed (0, 50, and 100 rpm), the extraction time (20, 30, and 40 min), and the temperature (25, 45, and 65 °C). Then, VOCs were isolated and identified using headspace solid-phase microextraction combined with Gas Chromatography coupled to Mass Spectrometry (HS-SPME/GC-MS). The best condition for extracting VOCs from cagaita was obtained using Polyacrylate fiber, stirring at 50 rpm for 30 min at 45 °C. Most of the VOCs identified in the cagaita and ice cream were terpenes, followed by esters, carboxylic acids, and alcohols. Then the antioxidant activity of the extractable and non-extractable compounds from cagaita was determined. Finally, paper spray ionization mass spectrometry (PS-MS) was used to analyze cagaita and cagaita ice cream. With PS-MS, various compounds were identified including organic acids, sugars, amino acids, anthocyanins, hydroxycinnamic acids, hydrobenzoic acids, flavones, and flavonoids. Moreover, 78% of these compounds identified in the cagaita were also found in ice cream, demonstrating that most of the cagaita substances remained stable during the ice cream manufacturing process. Thus, HS-SPME/GC-MS and PS-MS proved to be suitable techniques to determine the chemical constituents of cagaita and cagaita ice cream samples, permitting identification of various volatile substances and bioactive compounds from various chemical classes.

Keywords: Cagaita. Ice cream. Volatile compounds. Antioxidant activity. Paper spray.

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Literature Review

Chapter I

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ACRONYMS

VOCs	Volatile organic compounds
HS-SPME	Headspace solid-phase microextraction
PS-MS	Paper spray ionization mass spectrometry
ABTS	2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazine
FRAP	Ferric reducing antioxidant power
ABIS	Brazilian Association of Ice Cream Industries
MS	Mass spectrometry
PA	Polyacrylate
CW/DVB	Carbowax/divinylbenzene
CAR/PDMS	Carboxen/Polydimethylsiloxane
PDMS	Polydimethylsiloxane
PDMS/DVB	Polydimethylsiloxane/Divinylbenzene
DVB/CAR/PDMS	Divinylbenzene/Carboxen/Polydimethylsiloxane
GAE	Gallic acid equivalents
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
ORAC	Oxygen Radical Absorption Capacity
TPTZ	2,4,6-tri (2-pyridyl)-s-triazine
CUPRAC	Cupric ion reducing antioxidant capacity
TROLOX	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
ESI	Electrospray ionization
DESI-MS	Desorption electrospray ionization mass spectrometry
ELDI	Electrospray assisted laser desorption/ionization
DART	Direct analysis in real time
EASI	Easy ambient sonic spray ionization
LESA	Liquid extraction surface analysis
PCA	Principal Components Analysis
AOAC	Association of Official Analytical Chemists
EDI/SIMS	Electrospray droplet impact/secondary ion mass spectrometry
HPLC	High-performance liquid chromatography
GC-MS	Gas chromatography-mass spectrometry
LC-MS	Liquid chromatography coupled to mass spectrometry

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1. INTRODUCTION

The Cerrado is considered the second largest biome in Latin America and covers around 22% of the Brazilian territory (BRASIL, 2019; MOREIRA-ARAÚJO *et al.*, 2019). As the richest savanna in the world, this biome has numerous species of medicinal plants and many edible fruits, which are consumed by the local population and are commercialized in urban centers fresh or incorporated into food products. One of these fruits is the cagaita, which is the fruit of the cagaiteira (*Eugenia dysenterica*), belonging to the Mirtaceae family. Cagaita has been widely used in products such as sweets, jams, juices, popsicles, and ice cream (DONADO-PESTANA *et al.,* 2018).

The quality of the fruit is related with various parameters including nutritional value, appearance, color, texture, taste, and aroma. The latter is one of the main parameters used by people to verify quality and is due to the presence of different volatile compounds in each fruit, corresponding to around 10^{-7} to 10^{-4} of its weight (JIANG; SONG, 2010). Among the techniques used to isolate volatile compounds, headspace solid-phase microextraction has been widely used due to its advantages over other methods that include low cost, simplicity, absence of solvents, and affinity for various analytes (UEKANE *et al.*, 2017; GARCIA *et al.*, 2019).

In addition to sensory aspects, studies have demonstrated that Cerrado fruits have bioactive compounds, with mostly antioxidant function. Different methods have been employed to evaluate this activity, in which ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-picrylhydrazine) and FRAP (Ferric Reducing Antioxidant Power) are the most widely employed (RUFINO *et al.*, 2010; FLOEGEL *et al.*, 2011; SILVA *et al.*, 2019).

This antioxidant activity is due to the action of various compounds such as vitamins, carotenoids, organic acids, and phenolic compounds, the latter being responsible for 90% of this property. Thus, studies were initially performed an organic aqueous extraction, and the antioxidant activity determined in the obtained supernatant. However, considerable quantities of phenolic compounds remained in the extraction residues. Thus, both the extractable and non-extractable polyphenols must be evaluated to correctly determine the antioxidant capacity of foods (HAMAUZU; SUWANNACHOT, 2019; LIU *et al.*, 2019).

Cagaita has been widely used in products such as sweets, jams, juices, popsicles, and ice cream. The incorporation of Cerrado fruits in food products such as ice cream has many advantages because, besides providing desirable characteristics, it represents a source of nutrients and bioactive compounds. According to ABIS (Brazilian Association

of Ice Cream Industries), Brazil is among the ten largest ice cream producers in the world (ABIS, 2019). This food is a creamy product obtained from heterogeneous mixtures of mainly milk, fats, sweeteners, emulsifiers, and flavors that are subjected to homogenization and pasteurization followed by whipping to incorporate air during freezing (YURKSEL, 2015; VITAL *et al.*, 2018; ÖZTÜRK, DEMIRCI, AKIN, 2018).

Given the demand for less laborious and less costly methods to analyze food, several ambient ionization mass spectrometry (MS) techniques have been proposed. An important method is paper spray ionization mass spectrometry (PS-MS). Compared to traditional methods, the advantages of PS-MS include short analysis time, absence or reduction of sample pretreatment steps, and low cost. In food analysis, this technique has already been employed to determine resveratrol in wine (DONNA *et al.*, 2017); quality control for teas (DENG; YANG, 2013), colorants (TAVERNA *et al.*, 2013), and alcoholic beverages (PEREIRA *et al.*, 2016; TEODORO *et al.*, 2017); caffeine in beverages (TAVERNA *et al.*, 2016; SNEHA; DULAY; ZARE, 2017); additive by-products (LI *et al.*, 2013); pesticides in fruits (EVARD *et al.*, 2015), corni fruits (GUO *et al.*, 2017), coffees (GARRETT *et al.*, 2013; ASSIS *et al.*, 2019), and cagaitas (SILVA *et al.*, 2019); and analysis of olive oils (MAZZOTTI *et al.*, 2013; BARTELLA *et al.*, 2019) and sorghum (CAMPELO *et al.*, 2019).

Thus, the scientific relevance and innovative character of this work are optimization of method for extracting volatile compounds from cagaita, determination of non-extractable total phenolic compounds, the evaluation of the antioxidant activity of the non-extractable fraction, and the determination of chemicals constituents in cagaita and cagaita ice cream through PS-MS and HS-SPME/GC-MS.

2. OBJETIVOS

2.1. Main Objective

The object was to develop and optimize volatile compound extraction methods and determine the chemical constituents of cagaita and cagaita ice cream using paper spray ionization mass spectrometry and headspace solid-phase extraction combined with gas chromatography coupled to mass spectrometry.

2.2. Specific Objectives

- Optimize a method for extracting volatile compounds from cagaita by assessing the effects of SPME fiber type, agitation, time, and temperature;
- Determine the extractable and non-extractable phenolic compounds of cagaita;
- Evaluate, in vitro, the antioxidant activity of cagaita;
- Identify the chemical constituents of cagaita using PS-MS;
- Determine the chemical profile of cagaita ice cream using PS-MS.

3. LITERATURE REVIEW

3.1. Cerrado Fruits

The second largest South American biome, Cerrado, corresponds to 22% of the Brazilian territory, covering an area of approximately 200 million hectares. Its extension covers several states such as Bahia, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Piaui, Rondônia, São Paulo, and Tocantins, as well as peripheral areas of Amapá, Amazonas and Roraima. This biome has an immense biodiversity, containing over 11627 cataloged native plants (BRASIL, 2019).

The Cerrado has several fruit species with peculiar characteristic flavors, colors, and aspects, which gives them economic importance. The consumption of these fruits occurs both in the fresh form and in products processed in the form of jams, popsicles, ice cream, juices, and others (DONADO-PESTANA *et al.,* 2018).

Fruit specimens of the Cerrado are characterized by their nutritional value represented by their content of fibers, minerals, high levels of vitamins, and bioactive compounds with antioxidant action such as carotenoids and phenolic compounds (MOREIRA-ARAÚJO *et al*., 2019). Among the various fruits of this biome, as those presented in Table 1.

Table 1 – Fruit species native to the cerrado

Fruits	Scientific name
Araçá	*Psidium* spp.
Araticum	*Annona crassiflora*
Buriti	*Mauritia flexuosa*
Cagaita	*Eugenia dysenterica*
Cajá	*Spondias mombin*
Cajuzinho	*Anacardium humile*
Jabuticaba	*Myrciaria cauliflora*
Jatobá	*Hymenaea stigonocarpa*
Jenipapo	*Genipa americana*
Macaúba	*Acrocomia aculeata*
Mangaba	*Hancornia speciosa*
Murici	*Byrsonima crassifolia*
Pequi	*Caryocar brasiliense*
Pitanga	*Eugenia uniflora*

Source: ROCHA *et al.* (2013); BAILÃO *et al.* (2015) MOREIRA-ARAÚJO *et al.* (2019)

Among typical Cerrado specimens, the cagaiteiras (*Eugenia dysenterica*) (Figure 1) belong to the Mirtaceae family, are usually between 4 and 8 m high and easily adaptable in nutrient-poor soils, which has allowed them to spread throughout the Cerrado. Its fruits called cagaitas are yellow, have a spherical shape of 2 to 3 cm diameter, length of 3 a 4 cm, and have a slightly acidic sweet flavor. Cagaiteiras produce fruit between October and December, with a 37-day cycle for physiological development of the cagaitas (GUEDES *et al.*, 2017; DONATO-PESTANO *et al.*, 2018; SANTANA, 2019).

Figure 1 – Illustrative image of a cagaiteira and its fruits in the Sete Lagoas microregion

Source: THE AUTHOR.

Studies about the physicochemical composition report that the cagaitas have low caloric value (32.56 a 35.37 kcal 100 g^{-1}) and high moisture content (91.72 a 92.06 100 g^{-1}) (SILVA *et al.*, 2019). Cagaitas also contain 0.63% protein, 0.30-0.57% lipids, 0.18-0.3% ashes, and 3.06-5.9% carbohydrates (GENOVESE *et al.*, 2008; GONÇALVES *et al.*, 2010; CARDOSO *et al.*, 2011; OLIVEIRA *et al.*, 2011). According to some works, these fruits have soluble solids between 7.75-10.65 (ºBrix), titratable acidity 9.66-12.69 (g citric acid 100 g^{-1}), and pH 2.94-3.40 (BUENO *et al.*, 2017).

Guedes *et al.* (2017) found that potassium is the most abundant mineral in cagaita fruits (767.27 mg 100 g^{-1}), followed by phosphorus (75.53 mg 100 g^{-1}), sulfur (40.06 mg 100 g^{-1}), calcium (27.16 mg 100 g^{-1}), magnesium (20.37 mg 100 g^{-1}). However, cagaita have low zinc (0.76 mg 100 g^{-1}), iron (0.91 mg 100 g^{-1}), maganese (0.41 mg 100 g^{-1}), copper (0.27 mg 100 g^{-1}) e boron (0.30 mg 100 g^{-1}) contents.

According to Cardoso *et al.* (2011), cagaita pulps are sources of vitamin C (34.11 mg 100 g^{-1}), and contain considerable folate (25.74 µg 100 g^{-1}). On the other hand, these fruits have low carotenoid values (0.77 mg 100 g^{-1}), which refer to *α*- and *β*-carotene. According to these authors, the 100 g of cagaita pulp may provide 71% of daily vitamin C requirements, 7.9% folate, and 7.5% vitamin A.

Of the polyunsaturated fatty acids, cagaita pulps have higher values of linoleic acid (10.5%) than palm oil and olive oil. They also contain more linolenic acid (11.86%) than peanut, corn, soybean, and sunflower oil (MARTINOTTO *et al.*, 2008; SILVA, 2016).

In addition, several phenolic compounds have been identified in mature cagaitas, including gallic acid (6.04 mg 100 g^{-1}), caffeic acid (0.28 mg 100 g^{-1}), vanillic acid (8.51 mg 100 g^{-1}), epicatechin (68.17 mg 100 g^{-1}), *p*-coumaric acid (2.79 mg 100 g^{-1}), syringing acid (1.66 mg 100 g^{-1}), ferulic acid (0.37 mg 100 g^{-1}), salicylic acid (18.37 mg 100 g^{-1}), quercetin (22.10 mg 100 g^{-1}) and rutin (4.50 mg 100 g^{-1}) (GUEDES *et al.*, 2017).

Investigating the physicochemical characteristics of these Cerrado fruits is important for both the local population and the food industry, with the goal of stimulating the consumption and development of new products (RIBEIRO *et al.*, 2013; SOUZA; NAVES; OLIVEIRA, 2013).

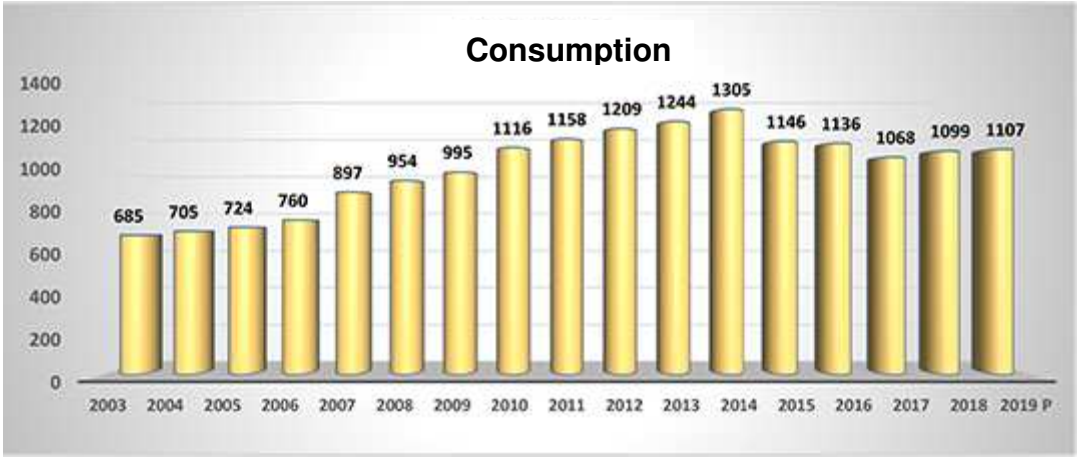
3.2. Ice creams

The development of food products is a way to add value to the fruits of the Cerrado. Under Brazilian law, ice cream is a food product in the category of frozen desserts, which is made from the emulsion of fats and proteins or the mixture of water and sugars. Other ingredients may be added as long as it does not change the basic characteristics of the product (BRASIL, 2005).

According to data from the Brazilian Association of Ice Cream Industries (ABIS), Brazil is among the ten largest producers of ice cream in the world. Considering the period from 2003 to 2016, Brazilian production increased from 686 million to 1 billion liters of ice cream (ABIS, 2019).

In Brazil, the consumption of ice cream in 2016 was 4.86 L/year per inhabitant, which represented revenues over 12 billion. Figure 2 and 3 represent the total consumption and per capita consumption, respectively, of ice cream in Brazil (ABIS, 2019).

Figure 2 – Consumption (million liters) of ice cream in Brazil



Source: ABIS (2019).

Figure 3 – Per capita consumption of ice cream in Brazil.

Source: ABIS (2019).

Ice creams are complex mixtures produced from the blending of various ingredients including milk, sugar, fat, stabilizer, flavoring, etc. The composition of these products may vary according to the laws of each country and the prevailing regional climate (RENHE *et al.*, 2015; GUO *et al.*, 2017; ÖZTÜRK *et al.*, 2018; YURKSEL, 2015).

The fat is responsible for the smooth texture and creaminess of ice cream, providing the structure (body) to the product. Fat content is known to influence the

sensation of cold, as low-fat ice cream increases the buccal sensation of cold, while high-lipid formulations decrease the sensation of cold due to fat globules concentrated on the surface of air bubbles (AKBARI; ESKANDARI; DAVOUDI, 2019).

Proteins contribute to the characteristic taste and structure. They also lower the freezing point and increase the viscosity of the remaining liquid, aeration, emulsification, and water retention capacity of ice creams as they coat the surface of fat globules and air bubbles (ORDÓÑEZ *et al*., 2005; SOUZA *et al*., 2010).

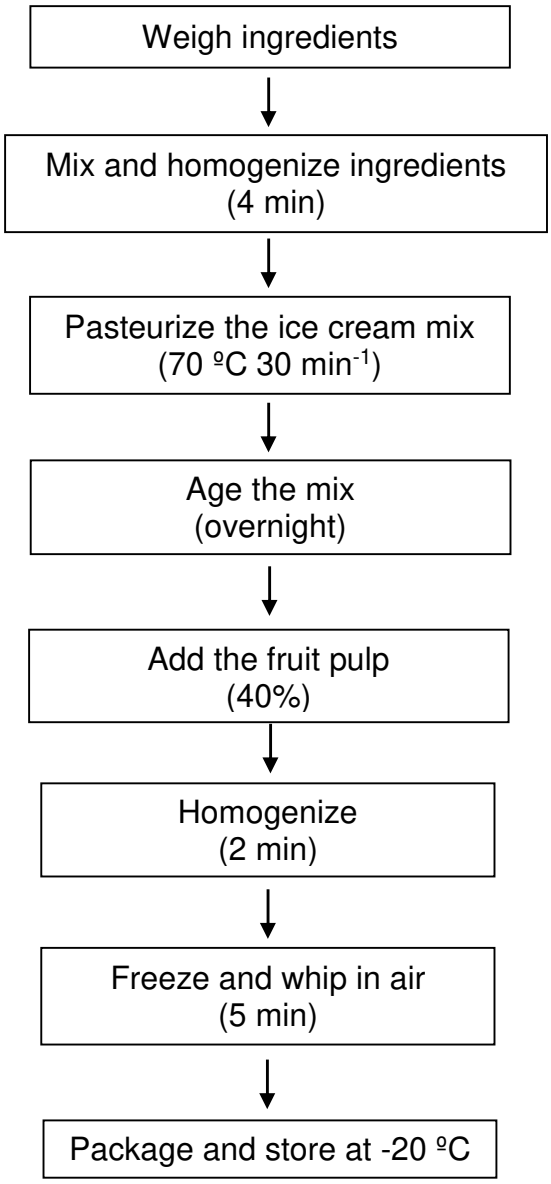
Carbohydrates in ice cream provide a pleasant taste, as well as increase the viscosity, which ensures greater creaminess and a lighter texture. In addition, they decrease the loss of volatile compounds, increase the viscosity of ice cream, and lower in the freezing point of the product (ORDÓÑEZ *et al*., 2005)

Stabilizers refer to compounds that can retain water by forming of hydrogen bridges between them. As a result, they reduce water mobility by structuring themselves in a three-dimensional network. Stabilizers prevent the appearance of lactose and ice crystals, in addition to the recrystallization of ice cream resulting from variations in exposure temperatures during storage (SILVA, 2012).

Ice crystals are vital to imparting the feeling of freshness and ensuring proper consistency. Air bubbles are essential for lighter ice cream, softness, and decreased of the sensation of intense cold. Other ingredients such as colorings, flavorings, and acidulants are employed to highlight desired aspects (ORDÓÑEZ *et al*., 2005).

Figure 4 is a simple flow chart of the ice cream manufacturing process.

Figure 4 – Flow chart of the ice cream manufacturing process

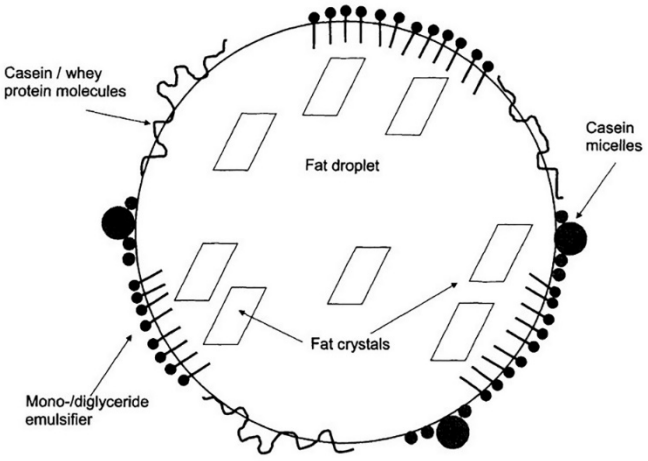


The production of ice cream initially involves weighing and mixing the ingredients, followed by the homogenization step, which consists of passing the ice cream mix through homogenizers that have narrow holes through which the mix flows under pressure. This reduces the diameter of fat globules, enlarging their surface area, promoting uniformity, and increasing product viscosity (SCHMIDT, 2004).

Subsequently, the mix is pasteurized, using temperatures of 80 °C/25 sec in continuous systems or 70 °C/30 min in batch processes. Then, the ice cream mix cools to temperatures of 4 °C or lower. Fruit pulp, juices, colorings, and flavorings may be added after the pasteurization step, provided that they have appropriate hygienic-sanitary conditions to avoid contamining the pasteurized mix (BRASIL, 2003).

The aging stage can be performed after cooling and involves subjecting the mix to refrigeration at temperatures of approximately 4 to 5 °C for a period of 2 to 24 h. In this period, the emulsifiers are adsorbed into the fat molecules, as shown in Figure 5. Thus, the aging step prepares the fat molecules to allow incorporation and stability of air bubbles during the step of freezing and whipping the ice cream mix (CLARKE, 2004).

Figure 5 – Fat droplet behavior during the setting period

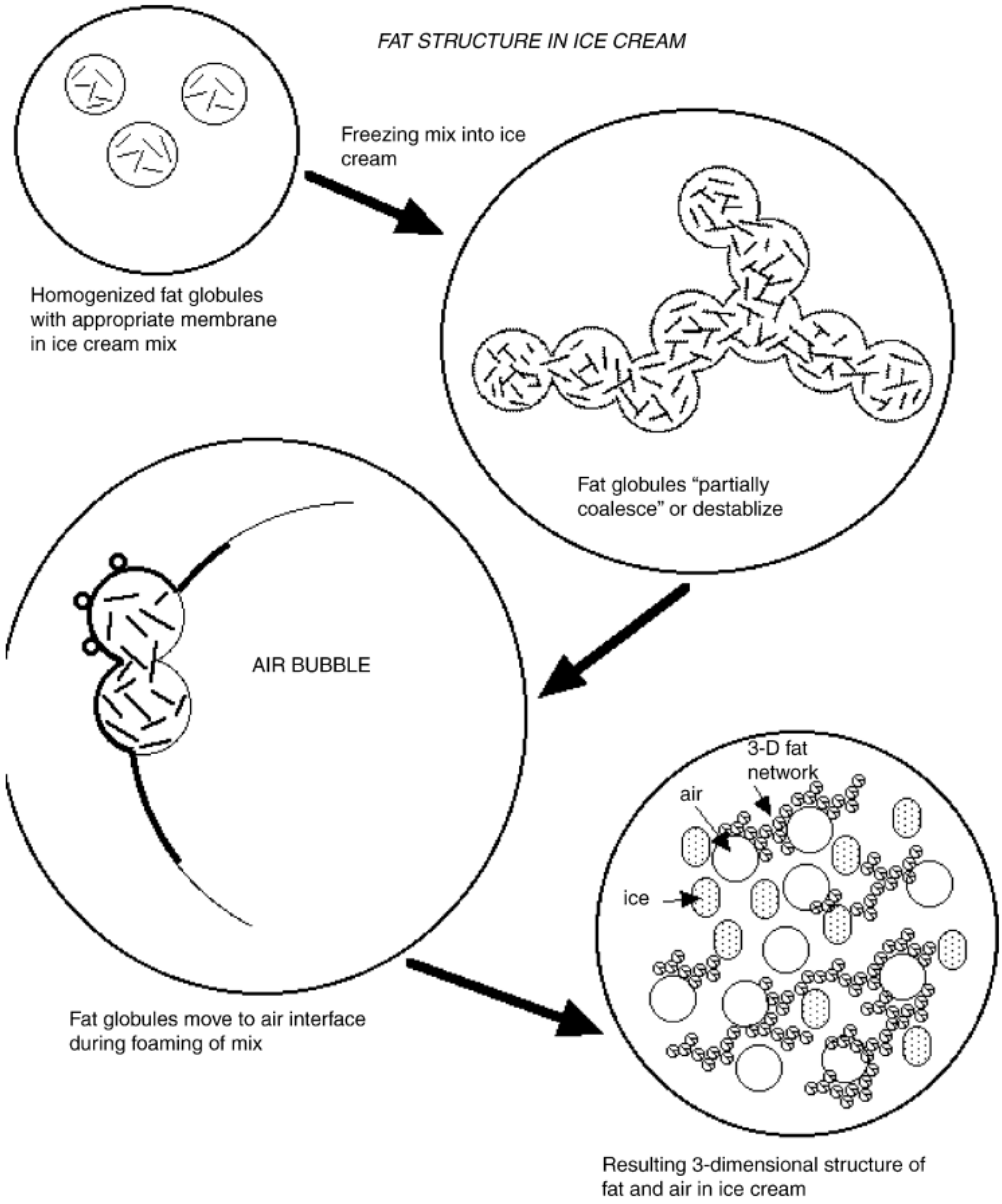


Source: CLARK (2004).

After aging, the ice cream mix is transferred to the freezer, which is subjected to constant whipping to freeze and incorporate air (*overrun*) (CLARKE, 2004).

Ice creams are complex mixtures considered as emulsions and foams. Figure 6 shows the changes that occur in the structure of fat globules during the ice cream manufacturing steps. Small fat globules are formed due to the homogenization of the ingredients in the mix. During the step of simultaneous whipping, air incorporation, and freezing in the freezer, the fat globules flocculate or destabilize. Partially coalesced fat stabilizes the air bubbles formed during this phase (AKBARI; ESKANDARI; DAVOUDI, 2019).

Figure 6 – Change in fat globule structure during ice cream manufacturing



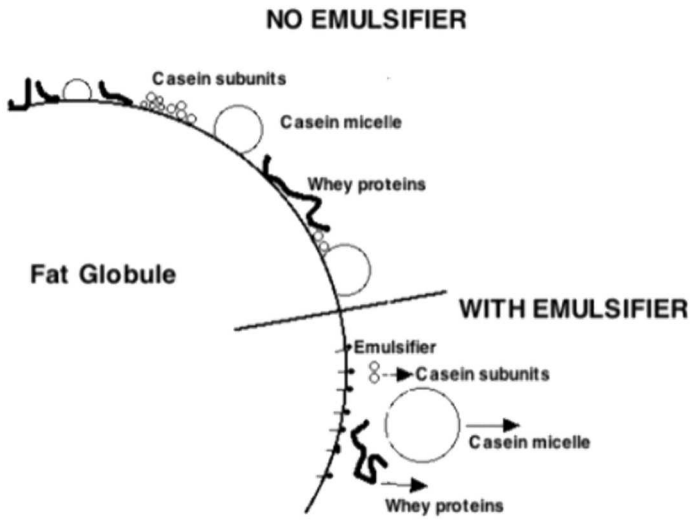
Source: GOFF (2019).

Many proteins present in the formulation act as emulsifiers providing stability required for fat emulsion. On the other hand, emulsifiers are used in the formulation of ice cream to replace proteins on the fat surface, thus reducing the stability of this fat emulsion (GOFF; HARTEL, 2013).

The effect of emulsifiers on proteins in the emulsion can be seen in Figure 7. Thinner and more coalescent membranes are formed during the constant whipping and freezing step in the ice cream machine. This agitation causes the fat emulsion to partially break, making the fat globules flocculate or destabilize. Partially coalesced fat stabilizes

air bubbles formed during whipping. Thus, emulsifiers generate a smoother texture ice cream. Ice creams without the addition of emulsifiers do not have this texture, as air bubbles do not have adequate stabilization due to the ability of fat globules to resist coalescence because proteins are adsorbed on their surface (GOFF, 2019). As a result of freezing, fat globules are destabilized, which coalesce and interact with the air bubbles surrounded by proteins, thus conferring the structural characteristics of ice cream such as texture, hardness, and softness (SILVA, 2012).

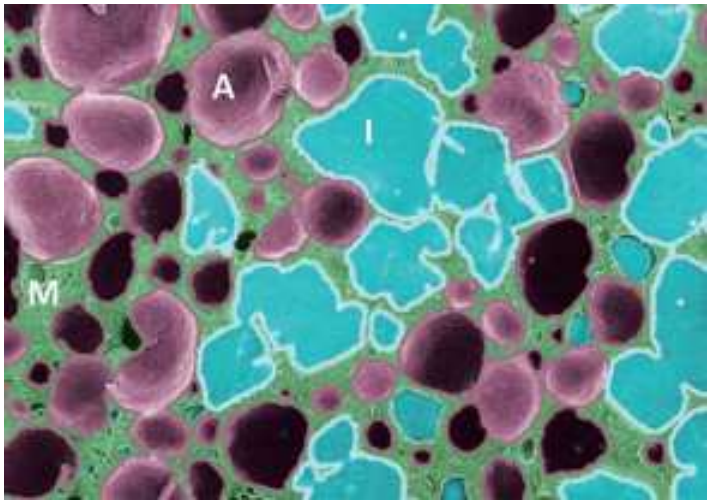
Figure 7 – Effect of emulsifier on ice cream structure



Source: GOFF; HARTEL (2013).

Figure 8 illustrates the structure of the ice cream. These products have air bubbles surrounded by fat globules, lactose crystals, and ice crystals.

Figure 8 – Schematic diagram of the ice cream microstructure highlighting the main components: Ice crystals (I), air bubbles (A), and unfrozen mix (M)



Source: GUO *et al.* (2017).

The last step of ice cream manufacturing involves putting these products into propylene cartons and storing them at temperatures of -18 °C. When displayed for sale, these products must be at or below -12 °C. According to the technical requirements established by legislation, the minimum apparent density of ice cream should be 475 g L^{-1} (BRASIL, 2003).

3.3. Volatile compounds

Food quality standards and acceptance by consumers involve various parameters such as appearance, color, texture, nutritional value, taste, and aroma. Sugars, acids, salts, and bitter compounds are responsible for the taste of the fruits, while volatile compounds give the characteristic aroma. Different volatile compounds are present in very low amounts in fruits, but due to the great sensitivity and complexity of the olfactory system, foods can be recognized by their aroma (JIANG; SONG, 2010; SANTOS, 2015).

Many factors influence the profile of volatile substances in fruits including species, variety, climate, and ripeness as well as handling pre- and post-harvest. During maturation and storage, these compounds are formed from precursors such as fatty acids, amino acids, carbohydrates, and carotenoids. The compounds formed belong to various chemical classes such as esters, acids, ketones, aldehydes, alcohols, lactones, and terpenoids (JIANG; SONG, 2010; BICAS *et al*., 2011). Table 2 lists volatile substances present in fruits.

Table 2 – Volatile substances present in fruits

Esters	Alcohols	Aldehydes	Ketones	Lactones	Terpenoids
Butyl acetate	Benzyl alcohol	Acetaldehyde	2,3-Butanedione	*γ*-Butyrolactone	*β*-Caryophyllene
Butyl butanoate	Butan-1-ol	Benzaldehyde	*β*-Damsenone	*γ*-Decalactone	1,8-Cineole
Butyl hexanoate	*(E)*-cinnamyl alcohol	*(E)*-Cinnamaldehyde	Eucalyptol	*δ*-Decalactone	Citral
Butyl-2-methyl butanoate	1-Hexanol	*(E,E)*-2,4-decadienal	Eugenol	*γ*-Dodecalactone	*β*-Damascenone
Butyl propanoate	*(E)*-2-hexenol	Hexanal	2-Heptanone	*δ*-Dodecalactone	Dihydroedulan
Ethyl acetate	*(Z)*-3-hexenol	*(E)*-2-hexenal	4-(*p*-Hydroxyphenyl)-2-butanone	*γ*-Jasmolactone	Farnesyl acetate
Ethyl butanoate	1-Octanol	*(Z)*-3-hexenal	3-Hydroxy-2-butanone	*γ*-Octalactone	Geraniol
Ethyl 9-decenoate	*(Z)*-6-nonenol	*(Z)*-3-hexenal	*β*-Ionone	*δ*-Octalactone	Hotrienol
Ethyl hexanoate	Hexan-1-ol	Nonanal	Linalool		*α*-Ionone
Ethyl 2-methylbutanoate	*(Z,Z)*-3,6-nonadienol	*(Z)*-6-nonenal	6-Methyl-5-heptene-2-one		*β*-Ionone
Ethyl 3-methylbutanoate	1-Phenylethanol	*(E,Z)*-2,6-nonadienal	Nerolidol		Limonene
Ethyl 2-methylpropanoate	2-Phenylethanol	*(E)*-2-nonenal	1-Octen-3-one		Linalool
Ethyl 2-methylbutanoate		Phenylacetaldehyde	2-Pentanone		Myrtenol
Ethyl propanoate			*(Z)*-1,5-octadien-3-one		Nerol
Ethyl 2-methylpropanoate			Terpenes		*α*-Phellandrene
Ethyl nonanoate					*α*-Pinene
(E)-2-hexenyl acetate					*β*-Pinene

Esters	Alcohols	Aldehydes	Ketones	Lactones	Terpenoids
(E)-3-hexenyl acetate					Terpinen-4-ol
Hexyl acetate					*α*-Terpineol
Hexyl butanoate					Terpinolene
Hexyl propanoate					*α*-Farensene
Hexyl-2-methyl butanoate					
Methyl acetate					
Methyl cinnamate					
Methyl butanoate					
Methyl hexanoate					
Methyl nonanoate					
Methyl octanoate					
Methyl-2-methylbutanoate					
Methyl-3-methylbutanoate					
2-Methylbutyl acetate					
3-Methylbutyl acetate					
2-Methylpropyl acetate					
(Z)-6-nonenyl acetate					
(Z,Z)-3,6-nonadienyl acetate					
Pentyl acetate					
Benzyl acetate					
Propyl acetate					
Propyl-2-methyl Butanoate					

Source: JIANG; SONG (2010).

3.4. Evaluation of volatile compounds

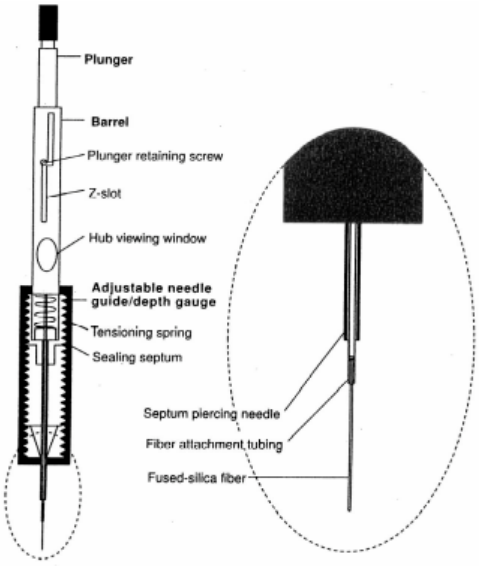
Technology to analyze food has advanced considerably. Efficient equipment and methods such as gas chromatography, high performance liquid chromatography, and the combination of both with mass spectrometry have emerged. However, these techniques are not able to perform direct sample analysis, requiring a preparation step to extract, concentrate, fractionate, and isolate the analytes (KATAOKA; LORD; PAWLISZYN, 2000).

Several sample preparation methods can perform this extraction including static headspace, dynamic headspace, supercritical fluid extraction, distillation, dialysis membranes, and extraction with solvent. However, these methods require extensive time and/or use a large amount of solvent (KATAOKA; LORD; PAWLISZYN, 2000; GUTIÉRREZ-ROSALES, 2010).

An alternative to these conventional methods involves headspace solid-phase microextraction (HS-SPME). This technique permits low cost, fast, and solvent-free extraction; can be used for numerous analytes; and is easily coupled to gas chromatography (KATAOKA; LORD; PAWLISZYN, 2000; GARCIA *et al.*, 2019).

HS-SPME is a simple technique that involves the extraction and concentration of volatile substances in a sheath fiber from a sample packed in a closed system. Fiber refers to an apparatus consisting of fused silica whose end is covered with a thin film which can be made of different types of polymer. This capillary filament is attached to a holder, which allows both exposure of the fiber cover during the analyte isolation and desorption steps and its retraction during penetration of containers or fiber storage (Figure 9) (ARTHUR PAWLISZYN, 1990).

Figure 9 – Device of Fiber SPME



Source: KATAOKA; LORD; PAWLISZYN (2000).

It is important to understand the types of stationary phases of SPME fibers commercially available for more efficient extraction because the number of extracted analytes depends on the polarity and thickness of the fiber coating. Table 3 lists the characteristics of different SPME fibers.

Table 3 – Fiber characteristics used in headspace solid-phase microextraction

Fiber	Film Thickness	Polarity	Temperature employed (°C)	Conditioning Time (h)	Physical State	Applications
PA	85 μm	Polar	220-300	1.0	Liquid	Semi-volatiles, polars
CW/DVB	65 μm	Polar	200-260	0.5	Solid Liquid	Polars, alcohols
CAR/PDMS	75 μm	Semipolar	250-310	1.0	Solid Liquid	Gases, compounds with low molecular weight
PDMS/DVB	65 μm	Semipolar	200-270	0.5	Solid Liquid	Volatiles, amines, and nitroaromatic compounds
DVB/CAR/ PDMS	50/30 μm	Semipolar	230-270	1.0	Solid Liquid	Polar compounds

Source: GARCIA (2016).

In addition to fiber type, an SPME method can evaluate other parameters such as agitation, temperature, and extraction time. The agitation employed during isolation of volatile compounds reduces equilibrium time, which promotes a faster extraction. Temperature and extraction time change the vapor pressure of volatile substances in the closed system, which can provide greater partition of the analytes in the sample, which results in a more efficient adsorption on the fiber coating (ABDULLAH; SULAIMAN, 2013).

The extraction time positively affects the concentration of the analytes until they reach equilibrium, but greatly increased levels may cause desorption (SANCHEZ-PALOMO; DÍAZ-MAROTO; PÉREZ-COELHO, 2005; MESQUITA *et al*., 2017).

Moreover, these parameters should always be analyzed to avoid the loss of volatile compounds, since they are thermosensitive and may be subject to hydrolysis, rearrangement, cyclization, and oxidation reactions (BICAS *et al*., 2011; ROCHA *et al*., 2017).

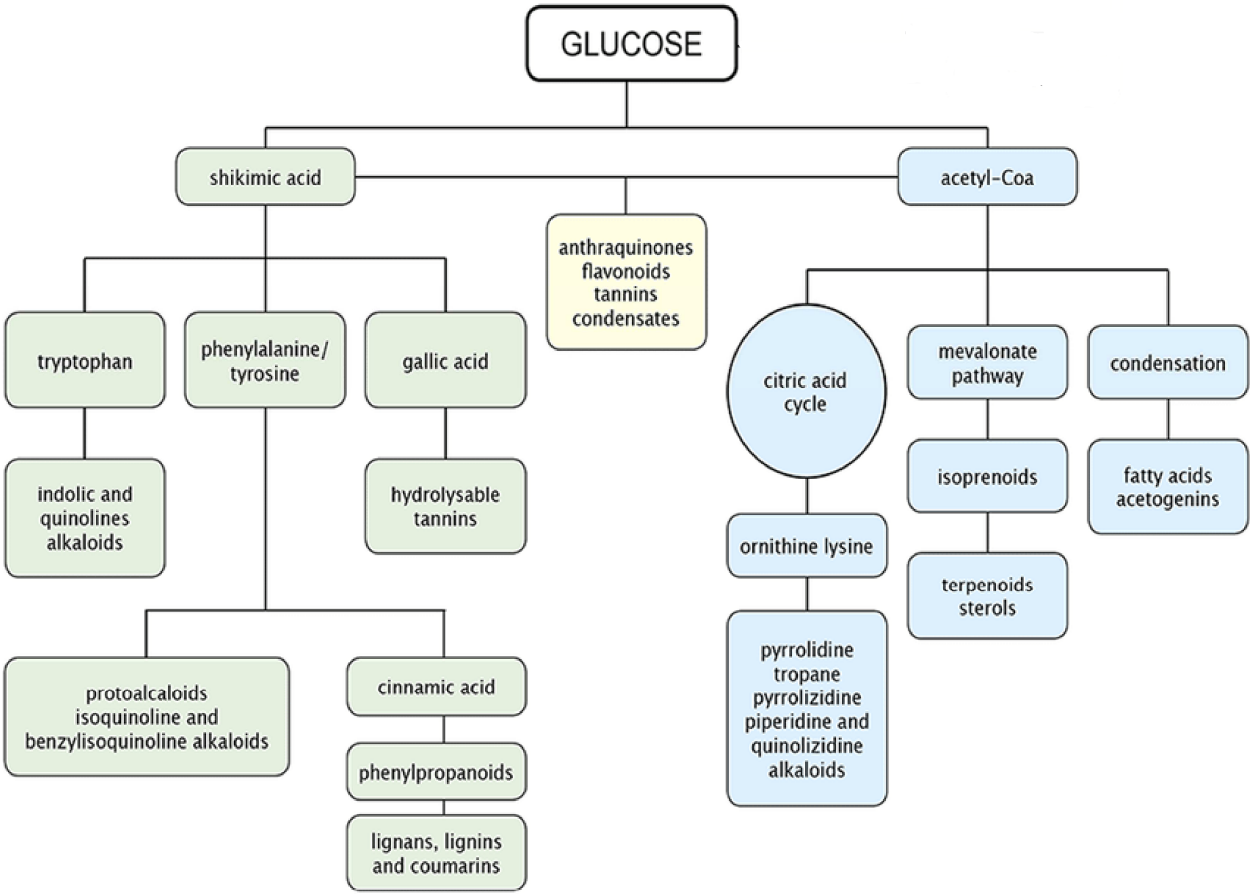
3.5. Evaluation of phenolic compounds

Due to the great concern about the impact of food on human health, the need for more consumption of fruits and vegetables in the diet is important, because in addition to their nutritional value, these foods are considered sources of bioactive compounds, including phenolic compounds (GUTIÉRREZ-GRIJALVA *et al*., 2016). Due to their antioxidant, anti-inflammatory, and modulation signal transduction, antimicrobial, and antiproliferation properties, regular consumption of fruits and vegetables rich in phenolic compounds has been associated with decreased risks of chronic non-communicable diseases, some cancers, type II diabetes, and osteoporosis (HAMINIUK *et al*., 2012; VELDERRAIN-RODRÍGUEZ *et al*., 2014). These compounds also influence the sensory quality of food, providing coloration, texture, bitterness, and astringency (ROCHA *et al*., 2011).

Phenolic compounds are secondary plant metabolites derived from glucose metabolism through acetate and shikimic acid (KABERA *et al*., 2014).

Figure 10 shows the biosynthetic cycle of secondary metabolites from glucose metabolism. In this figure, shikimic acid, and acetyl-Coa are the main intermediates of this metabolic pathway. Shikimic acid is the precursor of aromatic amino acids, which give rise to most aromatic secondary metabolites (TAVARES-DIAS, 2018).

Figure 10 – Biosynthesis cycle of the secondary metabolites



Source: TAVARES-DIAS (2018).

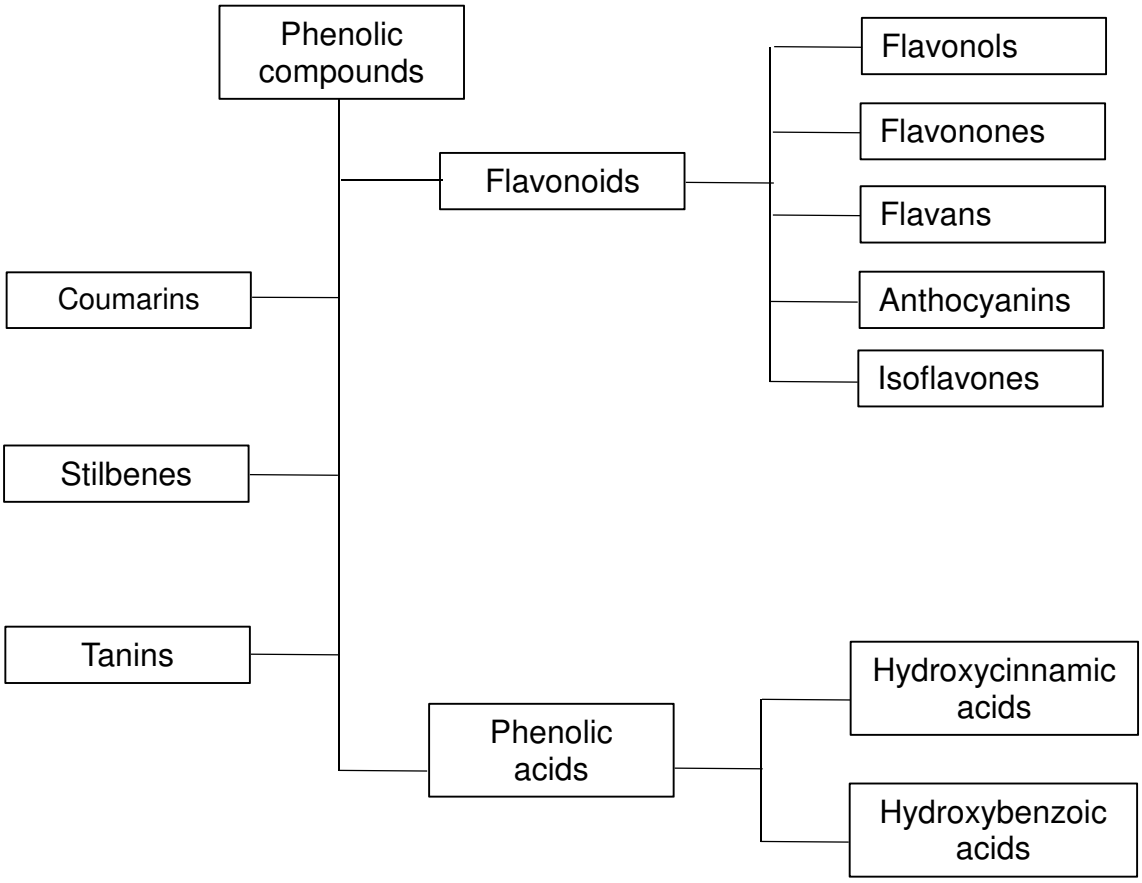
Secondary metabolites such as flavonoids, anthranquinones, and condensed tannins are from the combination of acetate or its derivatives with shikimic acid. On the other hand, acetyl-CoA is the precursor of several compounds, which are classified according to the metabolic pathway. Alkaloids (pyrrolidines, tropanes, pyrrolizidines, piperidines, and quinolizidines) are formed from the citric acid cycle pathway. Terpenoids and sterols originate from the mevalonate pathway. Fatty acids and acetogenins are formed by condensation of acetyl-CoA (SANTOS, 2010).

Among these secondary metabolites, phenolic compounds have an aromatic ring with one or more hydroxyl groups attached to it, forming structures that can range from a single molecule to highly polymerized tannins (VELDERRAIN-RODRÍGUEZ *et al.*, 2014; LIN *et al.*, 2016).

Production of these compounds occurs during normal plant development and in response to external agents, such as predators, UV radiation, and nutrient shortages. Their function is associated with the production of attractive substances to stimulate pollination, generate camouflage color, and protect against herbivores, as well as antibacterial and antifungal actions (HAMINIUK *et al*., 2012; LIN *et al*., 2016; SANTOS-SÁNCHEZ *et al*., 2019).

The quantity of these substances in each plant varies according to the maturity and variety of the fruit as well as climate type, soil composition, and geographic location. Phenolic compounds are classified mainly by their number of aromatic rings and can be divided into flavonoids, phenolic acids, coumarins, stilbenes, and tannins, as shown in Figure 11 (HAMINIUK *et al*., 2012).

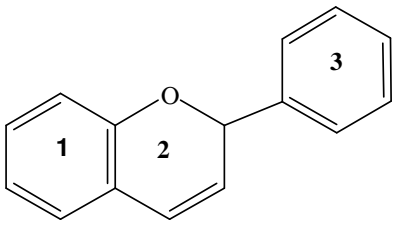
Figure 11 – Classification of the main classes of phenolic compounds



Source: RATNAM *et al*. (2006); GOODMAN *et al*. (2011); CAROCHO; FERREIRA (2013).

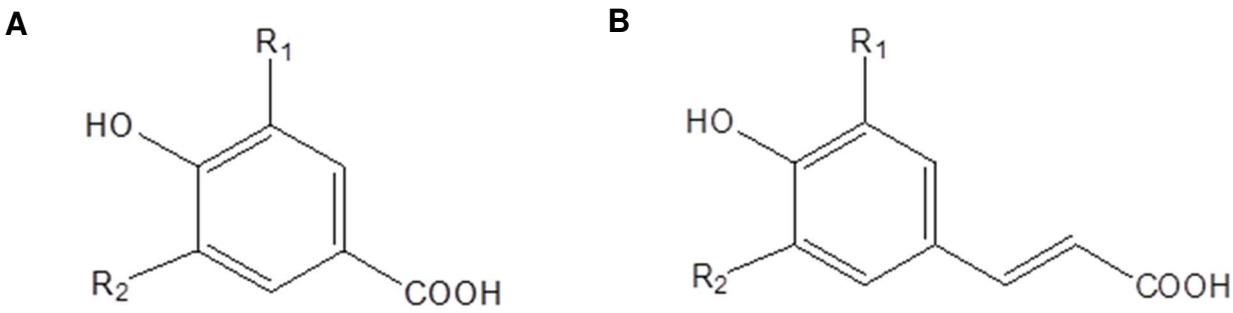
Among the secondary metabolites, flavonoids are one of the most abundant classes in nature, present in leaves, fruits, and seeds. They have 15 carbon atoms (C_6-C_3-C_6), in which the two aromatic rings (1 and 3) are joined by a heterocyclic ring (2) composed of 3 carbon atoms (Figure 12). Changes in ring 2 result in various flavonoid types such as flavonols, flavones, flavonones, flavanols, isoflavones, and anthocyanins (ANGELO; JORGE, 2007).

Figure 12 – Chemical structure of flavonoids



Phenolic acids include hydroxycinnamic and benzoic acids. The former is considered the simplest, consisting of 7 carbon atoms (C_6-C_1); while the latter has 9 carbon atoms (C_6-C_3). Both have a benzene ring with a carboxylic group and one or more hydroxyls and/or methoxyls in their structure, as illustrated in Figure 13 (ANGELO; JORGE, 2007).

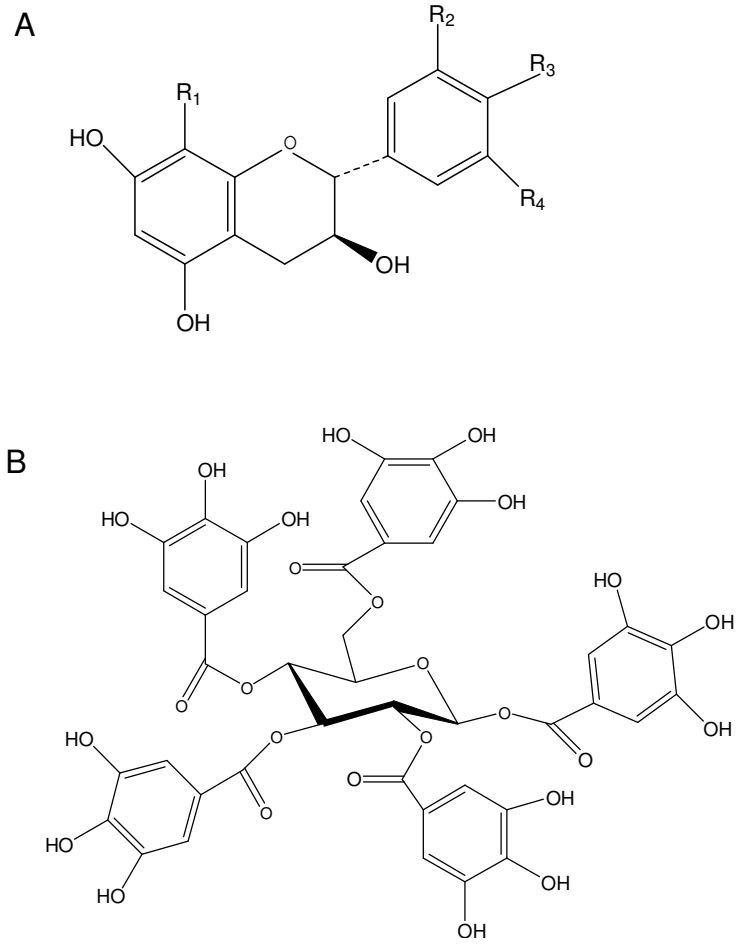
Figure 13 – Structure of hydroxybenzoic acids (A) and hydroxycinnamic acids (B)



Considered to be high molecular weight intermediate compounds, tannins have a molecular mass of 500 to 3000 Da. Their molecules have many hydroxyls and can combine with pectins, cellulose, and proteins, to form insoluble complexes responsible for astringency of many fruits and vegetables. Tannins can be classified into two groups: condensed tannins $(C_6\text{-}C_3\text{-}C_6)_n$ and hydrolysable tannins $(C_6\text{-}C_1)_n$ (Figure 14). The first, also called pro-

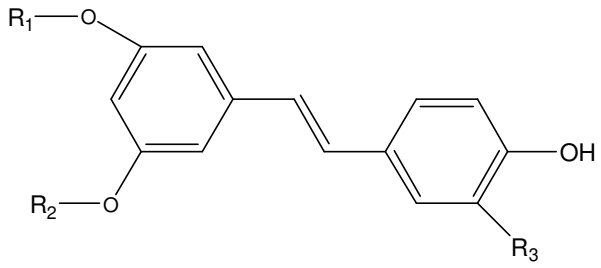
anthocyanidins, has a central polyol, usually *β-D*-glucose, whose hydroxyls are esterified with gallic acid, while the second are oligomers and polymers formed by the condensation of two or more molecules of flavan-3-ol and flavan-3,4-diol flavanols (BRAVO, 1998; SANTOS; MELO, 2010).

Figure 14 – Basic monomer of condensed tannin (A) and structure of hydrolyzable tannin (*β*-1,2,3,4,6-pentagaloyl-*D*-glucose) (B)



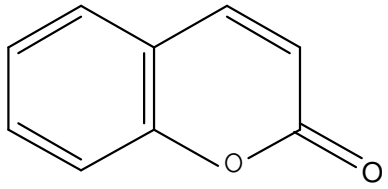
Stilbenes (C_6-C_2-C_6) (Figure 15) are present in low amounts in human food and are found in few plant families. Among them, resveratrol is the main representative, which is found mainly in red wine, grapes, and blueberries (ZHOU *et al.*, 2016).

Figure 15 – Basic structure of resveratrol stilbenes (R1 = R2 = R3 = H), pterostilbene (R1 = R2 = CH3, R3 = OH) and piceatannol (R1 = R2 = H, R3 = OH)



Coumarins (C_6-C_3) are lactones of o-hydroxy cinnamic acid (2H-1-benzopyran-2-ones), as illustrated in Figure 16. They are widely distributed in vegetables and are found mainly in angiosperms. Their strong odor warrents their use as flavorings in industrialized products (ROCHA; KUSTER, 2010).

Figure 16 – Chemical structure of coumarins



3.6. Methods to evaluate total phenolic compounds

Analysis of the content of phenolic compounds in foods begins with an extraction step. Garcia-Salas *et al*. (2010) discussed several methods for this including liquid-liquid, solid phase, supercritical fluid, pressurized liquid, microwave, and ultrasound extractions. Liquid-liquid extraction is the most employed, generally the aqueous-organic type in which methanol, acetone, and water are normally used. At the end of this step, extractable phenolic compounds will be present in the supernatant while non-extractable phenolics will remain in the extraction residue, requiring hydrolysis to break the bonds with the food matrix (SANZ-PINTOS *et al*., 2017).

Hence, most published works on the content of phenolic compounds present underestimated results, since they only analyze the supernatant obtained from aqueous-organic extraction and consider extractable polyphenols to be the total polyphenols.

However, significant amounts of non-extractable polyphenols remain in the extraction waste (SAURA-CALIXTO; 2012; PÉREZ-JIMÉNEZ; DÍAZ-RUBIO; SAURA-CALIXTO, 2013).

According to Saura-Calixto (2012), non-extractable phenolic compounds can be divided into two groups: pro-anthocyanidins, which are high molecular weight substances, and hydrolyzed polyphenols, which are low molecular weight compounds linked to macromolecules such as proteins and polysaccharides. According to Arranz *et al*. (2009), to quantify pro-anthocyanidins, the extraction residue must be hydrolyzed with butanol, hydrochloric acid, and ferric chloride, while evaluation of the hydrolyzed polyphenols involves hydrolysis with methanol and sulfuric acid.

These substances may be analyzed with chromatographic or spectrophotometric methods. The most commonly used spectrophotometric method involves the use of Folin-Ciocalteu Reagent, which contains phosphomolybdic/phosphotungstic acids. In this technique, reagents are reduced by the phenols in alkaline medium resulting in a blue complex that absorbs wavelengths at 765 nm. The color intensity obtained has a linear correlation with the phenol concentration of the samples. Thus, this method evaluates the reducing capacity of the sample extracts, expressing the results as the content of total phenolic compounds. Gallic acid is the most widely used standard for the calibration curve, and the results are expressed as milligrams of gallic acid equivalents (GAE) for 100 g^{-1} sample (SINGLETON; ORTHOFER; LAMUELA-RAVENTÓS, 1999; MARGRAF *et al*., 2016; HALDALGO; ALMAJANO, 2017).

The disadvantage of this method is related to the fact that the reagent may be reduced by other reducing substances such as organic acids, vitamins, proteins, and reducing sugars (HAMINIUK *et al*., 2012; HIDALGO; ALMAJANO, 2017).

3.7. Evaluation of antioxidant activity

Studies have reported that frequent consumption of fruits and vegetables are related to reduced risk of developing diseases such as cancer, cardiovascular, and cerebrovascular diseases. This protective effect has been associated with the presence of compounds with antioxidant capacity, such as vitamins, phenolic compounds, and carotenoids (ROCHA *et al*., 2011; ALVES *et al*., 2013; CÂNDIDO; SILVA; AGOSTINI-COSTA, 2015).

Antioxidant compounds are substances that can interact with free radicals and stop or slow the chain reaction that can cause cellular damage. Free radicals are formed when

electron transfer does not entirely occur, and an unpaired electron becomes free, which makes it unstable and very reactive with the molecules around it. Examples of these radicals include ROS (reactive oxygen species) and RNS (reactive nitrogen species). These radicals are naturally formed in metabolic pathways, but the body has endogenous defense mechanisms through endogenous antioxidants. However, excessive production of these radicals may occur in some cases, including inflammatory processes, improper diet, tobacco and alcohol use (GULÇIN, 2012).

This imbalance, called oxidative stress, is associated with the onset and aggravation of chronic diseases and is negatively related to aging. One way to prevent their deleterious effects involves the consumption of antioxidant-rich foods, especially fruits and vegetables, as they contain a large amount of phenolic compounds (HAMINIUK *et al*., 2012).

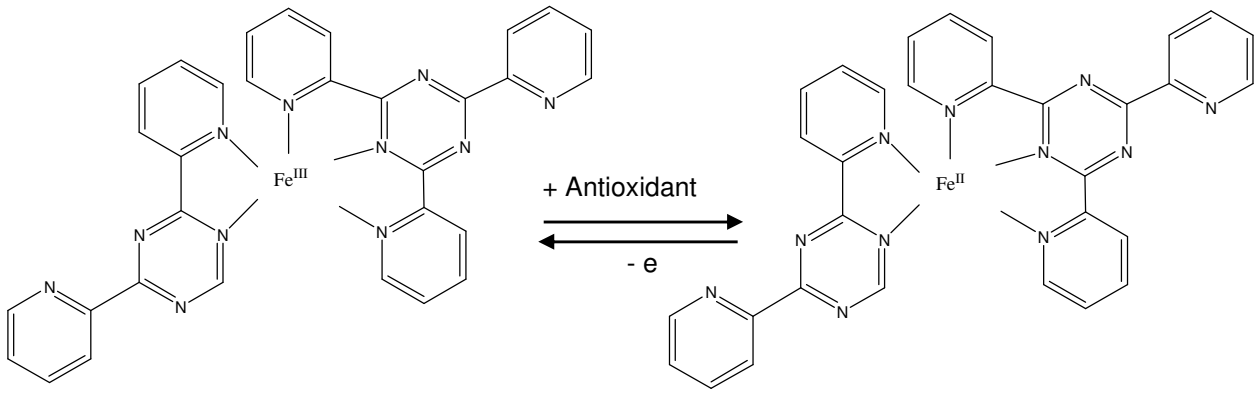
In recent years, many studies have been published that evaluate the antioxidant capacity of foods due to their beneficial health effects and their importance in food preservation by inhibiting or decreasing oxidative processes. Several methods with different mechanisms have been introduced to evaluate this activity in food, especially considering that they have a complex matrix and each technique is subject to some interference (SHAHIDI; ZHONG, 2015).

Some methods are based on peroxyl radical capture such as ORAC (Oxygen Radical Absorption Capacity) and TRAP (Total Reactive Antioxidant Potential), and others are related to metal reduction capacity such as FRAP (Ferric reducing antioxidant power) and CUPRAC (Cupric Ion Reducing Antioxidant Capacity). In addition, other principles of the methods include hydroxyl radical capture capability such as deoxyribose methods, organic radical scavenging capability such as ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picrylhydrazine), and the quantification of products formed during lipid peroxidation such as LDL oxidation, and β-carotene co-oxidation (RUFINO *et al*., 2010) .

A most reliable assessment employs various methods to determine antioxidant capacity. DPPH, FRAP, and ABTS have been the most used for in vitro evaluation of this bioactive property (RUFINO *et al*., 2010; OROIAN; ESCRICHE, 2015).

Originally the FRAP method was developed to measure reducing power in plasma, but its use has been extended to analysis of plants. This technique is based on the ability of antioxidants to reduce the TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) complex with light blue coloring to a ferrous (Fe^{+2}) complex with blue color, as shown in Figure 17 (HIDALGO; ALMAJANO, 2017).

Figure 17 – FRAP radical stabilization reaction by an antioxidante



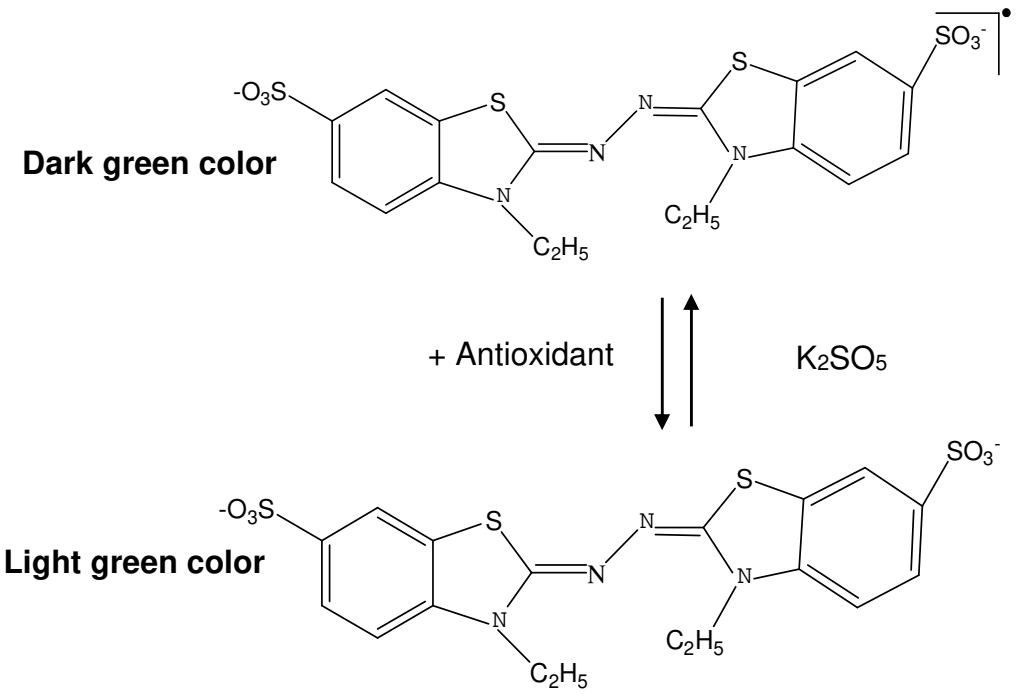
Light blue color **Dark blue color**

For this, the FRAP reagent is obtained by combining a solution of TPTZ with acetate buffer and ferric chloride. Ferrous sulphate is used as a standard for the calibration curve and results are obtained by increasing the absorbance at 595 nm and expressed as µM ferrous sulphate g^{-1} in the sample. FRAP is a simple, low cost, and fast method (RUFINO *et al*., 2006; SHAHIDI; ZHONG, 2015). The fact that some antioxidants do not have the ability to reduce Fe^{3+} and Fe^{3+} is reduced by other non-antioxidant substances works against this methodology (HIDALGO; ALMAJANO, 2017).

The ABTS method evaluates the ability of antioxidants to stabilize $ABTS^{+}$ cation (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)). This radical has a blue-green color and can be obtained from ABTS by the action of strong oxidizing agents such as potassium persulfate (RUFINO *et al*., 2007a; ALAM; BRISTI; RAFIQUZZAMAN, 2013). The addition of antioxidants to the reaction medium decreases color intensity, reducing $ABTS^{+}$ to ABTS, as shown in Figure 18.

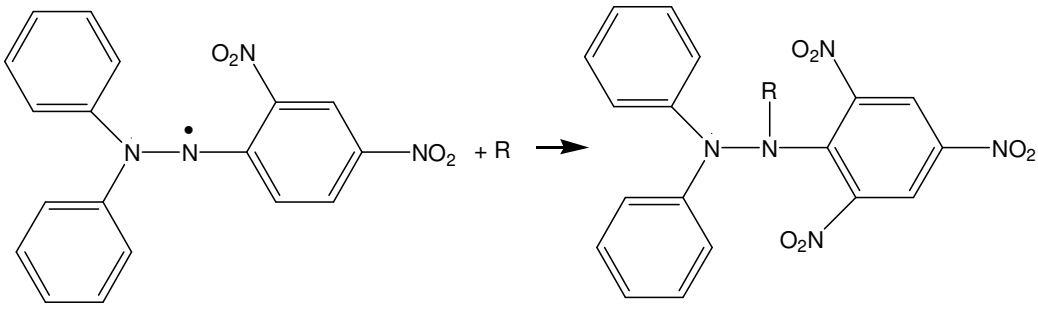
Depending on the pH of the medium or the structure of the antioxidant, this neutralization can occur either by donating electrons or by donating hydrogen atoms. In contrast to the FRAP method, the highest antioxidant activity in ABTS is obtained by decreasing the absorbance at 734 nm (PRIOR; WU; SCHAICH, 2005; SHAHIDI; ZHONG, 2015). Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a vitamin E analog, corresponds to the standard used and the results are expressed as equivalent trolox g^{-1} sample. Although this method is simple and easy, it relies upon use of a radical not found in food and biological systems (RUFINO *et al*., 2007a; SHAHIDI and ZHONG, 2015).

Figure 18 – $ABTS^{\bullet+}$ radical stabilization reaction by an antioxidant and its formation by potassium persulfate



Finally, the DPPH method evaluates the ability of the sample antioxidants to neutralize the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, which is commercially available and does not require any pre-preparation steps. This radical is stable due to the unpaired electron relocation in the molecule, which provides a dark violet coloration. When antioxidants are added to the reaction medium, 2,2-diphenyl-1-picrylhydrazyl is reduced to 2,2-diphenyl-1-picrylhydrazine altering the dark violet to light violet or pale yellow color depending on of the antioxidant power of the samples, as illustrated in Figure 19 (RUFINO *et al.*, 2007b; ALVES *et al.*, 2010; ALAM; BRISTI; RAFIQUZZAMAN, 2013).

Figure 19 – DPPH free radical stabilization reaction by an antioxidant R



Dark violet color **Light violet color**

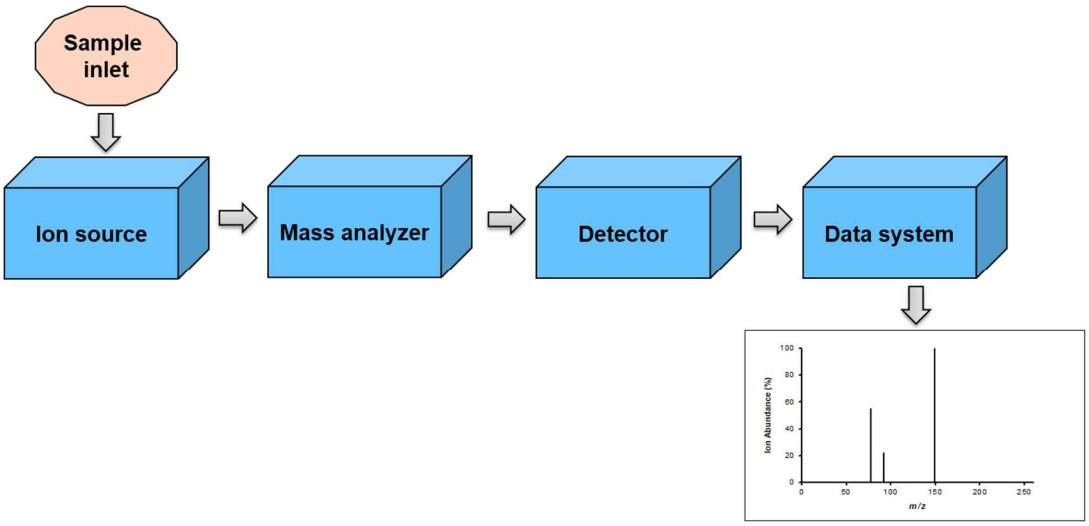
Trolox can also be used as a standard for the calibration curve. Reaction monitoring is performed by a reading at 517 nm; the results are expressed as both trolox g^{-1} sample and CE_{50} equivalents, which refer to the amount of antioxidant required to reduce 50% of DPPH radicals. The disadvantages of this methodology are related to the property of some compounds, such as anthocyanins, which absorb in the same wavelength range as DPPH (SHAHIDI; ZHONG, 2015). Nevertheless, the ease of execution, precision, and reproducibility make this method is one of the most used in the literature to evaluate the antioxidant activity of foods (ALVES *et al.*, 2010).

3.8. Mass spectrometry

Mass spectrometry (MS) has been widely used in several studies because it is a very versatile analytical technique that can analyze complex matrix samples with high sensitivity and selectivity and low detection limits. Its applications involve the discovery of new substances for drug development as well as studies of drug degradation, environmental monitoring, polymer sequencing, forensic research, and clinical diagnostics (PAVIA *et al.*, 2010; SHEN *et al.*, 2013).

The basic components of a mass spectrometer are shown in Figure 20.

Figure 20 – Scheme of the main components of a mass spectrometer



Source: THE AUTHOR.

After sample introduction, a flow of molecules is directed to the ionization source. Ion formation can occur by the electron ionization method or by chemical ionization. In the first, a heated filament emits high-energy electrons that collide with the flow of molecules from the sample input unit, which generates a cation if an electron is removed from the molecule. Anions can be formed by gaining electrons. In chemical ionization, a pre-ionized reagent gas collides with the sample molecules. This ionization process can occur by proton or electron transfer or by the formation of adducts. The ionization technique is chosen based on factors such as matrix and analyte characteristics (PAVIA *et al.*, 2010).

Subsequently, the ions are accelerated by an electromagnetic field generated by electrostatic lenses. The mass analyzer separates these ions according to their mass-charge ratio (*m/z*). Then the detector counts the ions formed and generates an electric current proportional to the ions that reach it. The detector signals are sent to the data acquisition system, producing the mass spectrum that is a graph of the abundance of ions relative to their *m/z* ratio (PAVIA *et al.*, 2010).

3.8.1 Environment Ionization

The compounds present in samples can only be analyzed on the mass spectrometer if their ionization occurs. Traditional ionization techniques such as electron ionization and chemical ionization require high vacuum, high ionization energy, and high temperatures. As

a consequence, until the 1980s, mass spectrometry was applied only to analyze thermally stable and volatile substances. Thus, the technique was very restricted, and it was not possible to study many analytes, especially those with high molecular weight (HOFFMAN; STROOBANT, 2007).

However, these difficulties were overcome by Fenn *et al*. (1989) who presented MS with electrospray ionization (ESI), in which ions are formed by displacing chemical equilibrium in a way that favors the generation of ions in the solution. Thus, this technique promotes the passage of ions from the solution to the gas phase, i.e. the ionization process occurs in solution. Moreover, this method does not require high vacuum and is classified as ionization at atmospheric pressure (CROTTI *et al*., 2006).

Ionization by ESI process occurs initially by injecting the solution containing the sample into the capillary. A high voltage field applied to the end of this capillary causes the Taylor cone to form. In addition, drops of the solution form microdrops due to the resulting effect of an inert gas parallel to the movement of the liquid. The employment of a gas flow perpendicular to the droplets further reduces its size and, due to the evaporation of the solvent, increases the ionic density. Thus, repulsion forces between charges lead to the expulsion of ionized analytes from these microdrops, and thus facilitates the transfer of ions to the gas phase (SILVA, 2014).

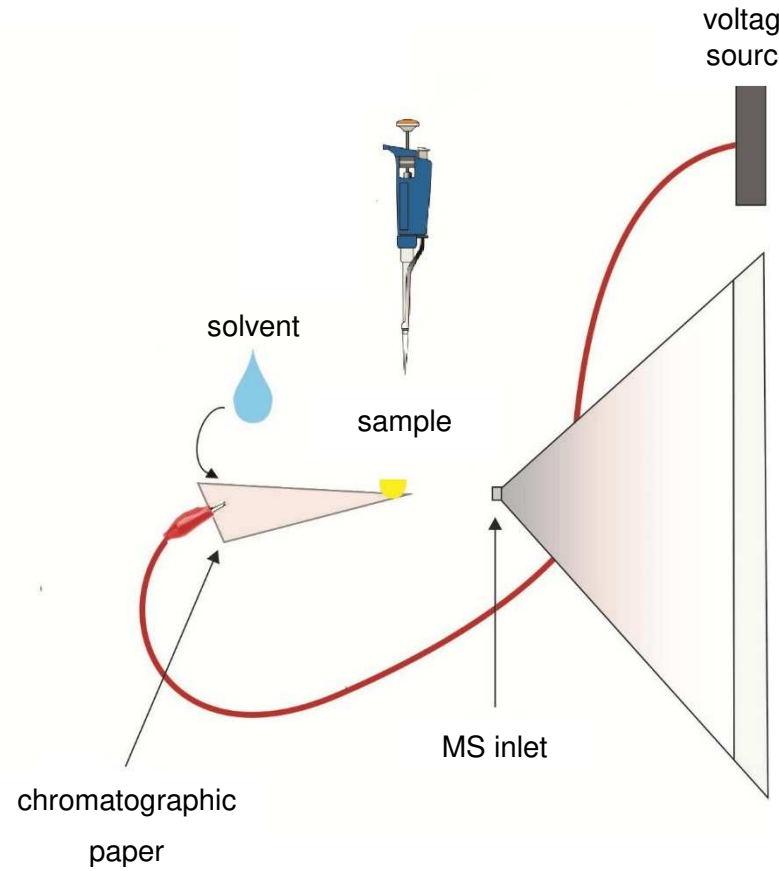
The traditional methods have some disadvantages associated mainly with the need for a sample pretreatment, high cost, and a longer analysis time. Thus, due to the demand for more advantageous techniques, ambient ionization mass spectrometry methods have achieved great progress because these tools enable ultra-fast analysis and high structural selectivity (ZHI-PING; XIAO-NING; YA-JUN, 2014; GUO *et al*., 2017). In the search for smoother ionization forms, other techniques were developed from ESI such as desorption electrospray ionization mass spectrometry (DESI-MS), electrospray assisted laser desorption/ionization (ELDI), and paper spray (SILVA, 2014).

3.8.1.1 Paper spray

Among the ambient ionization techniques, paper spray ionization mass spectrometry (PS-MS), proposed by Wang *et al*. (2010), has been widely used to analyze substances in complex matrices due to its simplicity, speed (seconds), low cost, sensitivity, selectivity, and minimal requirement for sample preparation.

The mechanism of PS-MS ionization is to place small amounts of sample, usually 1 to 5 µL, at the end of the triangle-shaped chromatographic paper, which acts as a porous medium for supporting and retaining the sample matrix. This paper is fixed to the front of the mass spectrometer input at a distance of usually 0.5 to 1 cm using an alligator clip connector, which is connected to a high voltage source. A volume of 40 µL of methanol is transferred to the chromatographic paper containing the sample drop. Then a potential difference is applied to the bottom of the paper, which generates a fine spray of electrically charged droplets containing the analytes inside. These analytes are charged by proton transfer or deprotonation. Due to the evaporation of the solvent, the repulsion between charges increases, generating desolvated ions in the gas phase entering the mass spectrometer (Figure 21) (WANG *et al*., 2010; CARVALHO, 2015; PEREIRA, 2016; SILVA *et al*., 2019).

Figure 21 – Schema of the paper spray ionization source



Source: THE AUTHOR.

The first study carried out with this technique aimed at the direct ionization of medications present in blood droplets. Due to its numerous advantages observed, research

was conducted with biological molecules (WANG *et al*., 2011) and used to differentiate bacteria without requiring sample preparation (HAMID *et al*., 2014).

Chen *et al*. (2017) used PS-MS for rapid analysis of bisphenol A and its analogues in food packaging. Several studies have also been performed with food matrices for quality control of products such as coffee (Garrett *et al*., 2013; Assis *et al*., 2019), tea (DENG; YANG, 2013) and corni fruits (GUO *et al*. , 2017); analysis of pesticides in fruits and vegetables (EVARD *et al*., 2015); evaluation of food additives and their by-products (LI *et al*., 2013); analysis of resveratrol in red wine (DONNA *et al*., 2017); discrimination of beer and whiskey forensics (PEREIRA *et al*., 2016; TEODORO *et al*., 2017), analysis of cagaitas (SILVA *et al*., 2019), caffeine in commercial beverages (TAVERNA *et al*., 2016; SNEHA; DULAY; ZARE, 2017), olive oils (MAZZOTTI *et al*., 2013; BARTELLA *et al*., 2019), and sorghum (CAMPELO *et al*., 2019).

PS-MS has been demonstrated suitable to evaluate various food matrices. Thus, the purpose of the present work was to employ PS-MS for the ultra-fast characterization of cagaitas and cagaitas ice cream for the first time.

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4. EXPERIMENTAL PART

The experimental part of this thesis involved obtaining a method to extract VOCs by HS-SPME and evaluate antioxidant activity, as well as the use of PS-MS to obtain fingerprints of samples of cagaita and its ice cream. The results were divided into three chapters.

The first chapter involved optimizing the conditions to extract volatile organic compounds of the cagaita through HS-SPME. For this, the pulps were obtained from a pool of cagaitas collected at Sete Lagoas, Minas Gerais, Brazil. An experimental design evaluated the parameters: fiber type (n=5), agitation speed (0, 50, and 100 rpm), temperature (25, 45, and 65 °C), and time (20, 30, and 40 min) of extraction.

In the second chapter, pool of cagaita fruits from each microregion (Sete Lagoas, Paraopeba, and Prudente de Morais, Minas Gerais, Brazil) was used to determine the content of total phenolic compounds and antioxidant activity (extractable and non-extractable fraction) employing three methods (ABTS, DPPH, and FRAP). In addition, PS-MS was used for the first time to characterize the chemical profile of cagaitas from the three microregions (fruit pulps from 9 cagaiteiras from each of the 3 microregions, n = 27 samples). Principal component analysis was also used to differentiate the fingerprints obtained from the fruits.

The third chapter involved the extraction of the volatile compounds from the cagaitas (pool of cagaitas collected in Sete Lagoas, Minas Gerais, Brazil) and cagaita ice cream using SPME PDMS/DVB and PA fibers. Total phenolic compounds were also quantified. The chemical profile of the ice cream and cagaita pulp was determined using PS-MS and HS/SPME/GC-MS.

5. CHAPTER I

Evaluation of the Influence of Extraction Conditions on the Isolation and Identification of Volatile Compounds from Cagaita (*Eugenia dysenterica*) Using HS□SPME/GC□MS

SCIENTIFIC PRODUCTION

SILVA, M. R.; BUENO, H. B.; ARAÚJO, R. L. B.; LACERDA, I. C. A.; FREITAS, L. G.; MORAIS, H. A.; AUGUSTI, R.; MELO, J. O. F. Evaluation of the influence of extraction conditions on the isolation and identification of volatile compounds from cagaita (*Eugenia dysenterica*) using HS□SPME/GC□MS. **Journal of the Brazilian Chemical Society**, v. 30, n. 2, p. 379-387, 2019.

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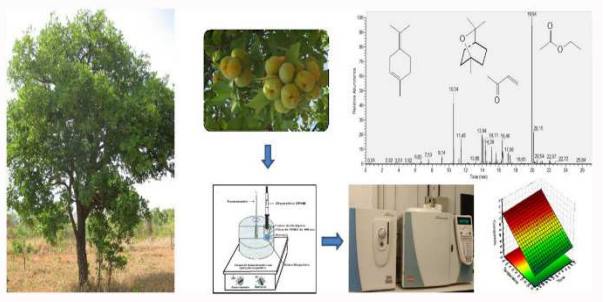
Graphical Abstract (GA)



Conditions optimization of extraction of volatile compounds from fruit of *Eugenia dysenterica* using different SPME fibers.

Evaluation of the Influence of Extraction Conditions on the Isolation and Identification of Volatile Compounds from Cagaita (*Eugenia dysenterica*) using HS-SPME/GC-MS

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Abstract

The objective of this study was to explore the extraction and identification of volatile organic compounds (VOCs) present in cagaita fruit (*Eugenia dysenterica*). Parameters such as type of extraction fiber, agitation, extraction time and extraction temperature were investigated. Initially, the VOCs were extracted using headspace solid-phase microextraction. Then, the compounds were identified using gas chromatography coupled to mass spectrometry. Results revealed the presence of at least 26 different compounds and the polyacrylate fiber (PA) promoted the extraction of a larger number of VOCs. Regarding the PA fiber, the most efficiency extraction was achieved using a 50 rpm agitation at 45 ºC for 30 minutes. The majority were monoterpenes (34.64%) and esters (36.28%). An increase in the extraction temperature promoted the isolation of more VOCs when using carboxen/polydimethylsiloxane (CAR/PDMS) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers. However, these fibers allowed the isolation of a smaller number of VOCs. The extraction time and agitation had no significant influence.

Keywords: Cagaita, HS-SPME, GC-MS, volatile organic compounds.

Introduction

The Cerrado, which covers 22% of Brazil's territory, is the second largest biome in Latin America, only behind the Amazonian forest, and it is a source of many plant species that play an important role in folk medicine and in food. In this biome, there are different types of fruits, with unique sensory and nutritional characteristics, which gives them economic importance, through the commercialization of derived products, and nutritional, by their consumption.[1-3]

One of these fruits is the *Eugenia dysenterica* DC, an exotic fruit of the Myrtaceae family, which is popularly known as cagaita. This fruit has received significant attention because it has features that make it ideal for use in its fresh form or in preparations such as jams, fruit jellies, juices, liqueurs and ice cream.[3] These fruit-bearing plant species have peculiar characteristics, including their scents, flavours and colours, and highly contents of phenolic compounds, flavonoids and tannins, besides the presence of saponins and terpenes. The characteristic aroma of fruits is determined by the kind of volatile substances they produce. These substances are typically carboxylic acids, alcohols, aldehydes, ketones, esters, phenylpropanoids and terpenes.[4] Therefore, several studies have been conducted to identify volatile organic compounds (VOCs) present in fruits such as pitanga (*Eugenia uniflora* L.),[5] cherry (*Prunus avium*),[6] pineapple (*Ananas comosus*),[7] strawberry and lemon guavas (*Psidium cattleianum* Sabine),[8] araça-boi (*Eugenia stipitata*),[9] mango (*Mangifera indica* L.),[10] jaboticaba (*Myrciaria jaboticaba*)[11] and apple (*Malus domestica* Borkh).[12]

To better characterize these fruits, various techniques have been used to isolate VOCs, such as headspace solid-phase microextraction (HS-SPME),[13-15] the headspace static,[16] the dynamic headspace,[16] supercritical fluid extraction,[16] purge and vapour, solid-phase extraction, and liquid-liquid extraction.[16] Among these techniques, HS-SPME stands out due to its concentration of the volatile compounds, absence of solvent, ease of incorporating into gas chromatography and applicability to a wide variety of analytes.[16]

The development of VOCs extraction method by HS-SPME involves the assessment of many parameters like fiber type, agitation, time and the temperature of the extraction. The fiber features are critical since each fiber has different polarities, polymeric coating thicknesses, all of which are important variables for extracting the largest amount of a broad range of VOCs with distinctive features. Use of agitation may influence the equilibrium time and number of VOCs extracted. [13,15,17-19]

Thus, the present study aimed to optimize the best conditions for the identification of maximum VOCs of cagaita pulp by analysing various parameters, such as type of extraction fiber, agitation, temperature and time. Finally, the research will provide relevant information about the fresh fruit in order to establish identity and quality standards.

Experimental

Materials

Five types of HS-SPME fibers with distinct characteristics were used to optimize the extraction of VOCs from cagaita samples. The fibers were manufactured by Sigma-Aldrich (St. Louis, MO, USA). Therefore, the semi-polar fibers Carboxen/polydimethylsiloxane (CAR/PMDS, 75 μm), Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) and Polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm) and the polar fibers Carbowax/divinylbenzene (CW/DVB, 65 μm) and Polyacrylate (PA, 85 μm) were evaluated.

Methods

Preparation of samples of cagaita

The ripe fruit of cagaita were collected at Sete Lagoas, Minas Gerais, Brazil (latitude 19° 28' 36" and longitude 44° 11' 43"), in 2016. Then, the fruit were transported to the laboratory of the Federal University of São João Del-Rey, Sete Lagoas/MG. The sample was washed in running water and then sanitized using 500 mL of sodium hypochlorite solution (200 ppm) for 15 min followed by a subsequent rinse under running water for 2 min. After that, the seeds were discarded, and the pulp was homogenized using a mixer. The homogenized pulp was stored at -18 °C until further use.

Extraction of volatile compounds

The extraction of VOCs was performed using SPME fibers (CAR/PMDS, DVB/CAR/PDMS, PDMS/DVB, CW/DVB and PA). For this process, 2.0 g samples of cagaita pulp were transferred to 20 mL vials, which were sealed with aluminium and rubber septa. Then, the vials were placed in an aluminium heating block (8.5 cm x 10 cm) with a hotplate, and the same fibers for adsorption of volatile substances were inserted into each of them. The optimization of extraction conditions (sample stirring time, temperature, and extraction time) was conducted based on the procedure described by Rodrigues and Iemma.[20] To this end, a 2^3 full factorial design with triplicates at the central point was used, and the dependent variables were the extraction time (20, 30 and 40 min), the extraction temperature (25, 45 and 65 °C) and agitation (0, 50 and 100 rpm). The number of volatile substances isolated for each test was used as response for the parameters evaluated.

After the adsorption of VOCs by the HS-SPME fibers, the fibers were directly introduced into the gas chromatograph injector, occurring desorption and separation of compounds in the chromatographic column, ionized by EI and finally identified by the mass analyzer.

Identification of volatile compounds

VOCs were identified in the Laboratory of Mass Spectrometry of the Department of Chemistry - UFMG, using a gas chromatograph (Trace GC Ultra) coupled to a mass spectrometer (Polaris Q, Thermo Scientific, San Jose, CA, USA), with an ion trap, using a split/splitless capillary injector. The chromatographic analysis was performed as described by Belo *et al.*[21] Therefore, the following conditions were used: injector temperature of 250 °C, 5 min desorption, ion source temperature of 200 °C and 275 °C interface. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}, and the VOCs were separated using a HP-5ms (5% phenyl and 95% methylpolysiloxane) capillary column (30 m x 0.25 mm x 0.25 μm; Agilent Technologies Inc., Munich, Germany). Initially, the column was held at 40 °C for 5 min, and then, the temperature was increased at a rate of 2.5 °C min^{-1} up to 125 °C followed by an increase of 10 °C min^{-1} up to 245 °C and held for 3 min.

The acquisition of the data occurred in full scan mode with a range of 50 to 350 *m/z*, ionization by electron impact (EI), and a power of 70 eV.

The VOCs were identified based on the comparison between mass spectrum obtained and the database available in library of the National Institute of Standards and Technology (NIST). In addition, the data were confirmed through comparison with compounds previously reported in the literature.

Results and Discussion

Optimization of extraction conditions using HS-SPME

The optimization of VOCs extraction conditions by HS-SPME were performed trough multivariate analysis seeking to evaluate the effect of the agitation, time and temperature of extraction

on each type of SPME fiber. The number of VOCs identified was considered as response, since this study aimed to optimize a method to determine the largest amount of volatile compounds in cagaita.

In Table 1 is presented the relative area (%) of the volatile compounds isolated using the fiber PA in the best response assay. When using the PA fiber, the best experimental condition was obtained by subjecting cagaita samples to 50 rpm agitation at 45 ºC for 30 min. The greatest part of VOCs identified was monoterpene (34.64%) and esters (36.28%).

Table 1. Relative composition (%) of VOCs identified in the cagaita using the PA fiber.

VOCs	Class	PA Area (%)
3-buten-2-one	ketone	2.04
Oleic alcohol	alcohol	9.83
Ethyl acetate	ester	5.53
Dibutylphthalate	ester	25.05
(*Z*)-9-Methyl octadecenoate	ester	5.70
Eucalyptol	monoterpene	20.56
α-Terpinene	monoterpene	14.08
Propanoic acid	carboxylic acid	1.16
Nonanoic acid	carboxylic acid	4.49
Tetradecanoic acid	carboxylic acid	1.77
1-Methyl-4-(1-methylethyl)-1,3-cyclohexadiene	hydrocarbon	1.65
2-Methyl-1,3-butadiene	hydrocarbon	2.55
Estragole	ether	5.59

Garcia *et al.*[22] has found a different result when Barbados Cherry 'BRS-366 Jaburú' samples were subjected to temperature and time of 65 ºC for 20 min, using the PA fiber. Under these conditions, predominant extracted compounds were terpenes and carboxylic acids. These different results are associated with VOCs profile, which is characteristic for each matrix evaluated.

Effect of some parameters on the number of VOCs extracted

To investigate the influence of some parameters (agitation, time and temperature) on the extraction and subsequent identification of VOCs using each fiber type, Pareto diagrams were constructed with a 95% confidence limit, as shown in Figure 1.

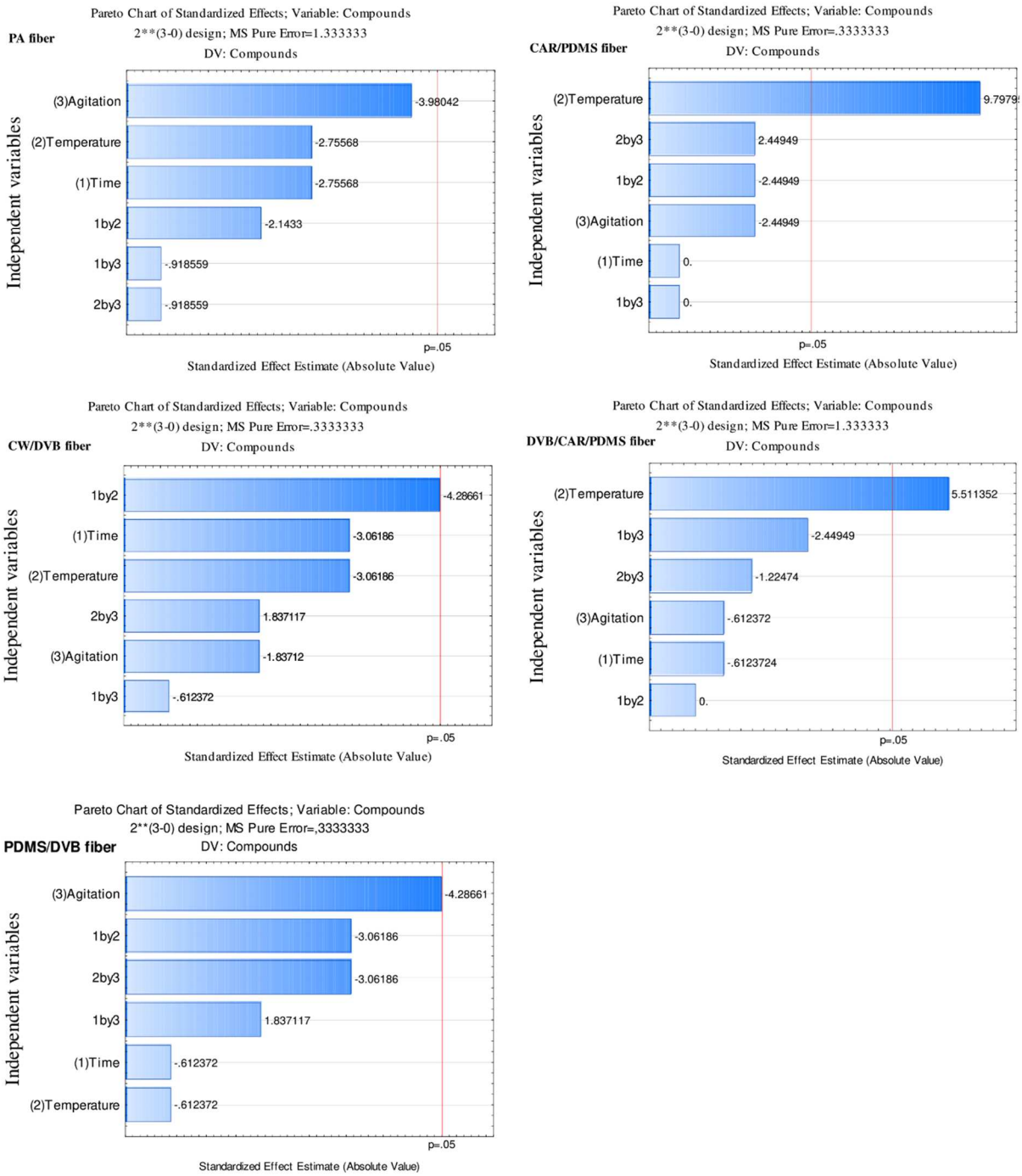

Figure 1. Effects of extraction parameters (agitation, time and temperature) on the isolation of VOCs from cagaita using different types of fibers.

As seen in Figure 1, the extraction behaviour varied between the parameters tested for each type of fiber. Extraction temperature showed a significant effect only with CAR/PDMS and DVB/CAR/PDMS fibers. An increase in temperature with these two fibers allowed greater extraction and consequently the identification of a larger number of compounds. Each type of coating extracts

different groups of VOCs. In that way, polydimethylsiloxane (PDMS) coating has affinity with non-polar compounds such as esters and monoterpenes, which are predominant in the samples of this study. These results are in agreement with those observed by Belo *et al.*,[21] who observed predominance of non-polar VOCs (esters and terpenoids) in pequi samples.

Agitation and time of extraction did not have a significant effect with any of the evaluated fibers. It was not found previous studies evaluating chemometric tools to optimize the extraction and identification of VOCs in cagaita using different fiber coatings.

Effect of using different types of HS-SPME fiber

The influence of fiber type on the number of VOCs extracted from cagaita fruit is shown in Table 2. This table show the total number of compounds found in the 11 assays of factorial planning for each fiber.

Based on the number of isolated compounds from each set of experimental conditions, PA fiber was the most efficient (n=17), followed by CAR/PDMS (n=16), PDMS/DVB (n=15) and DVB/CAR/PDMS (n=14). The CW/DVB fiber was the least effective, allowing isolation of only 12 compounds. It was observed that the PA polar fiber promoted the extraction of several compounds like alcohol, esters, ether, quinones, being efficient even for isolation of some nonpolar substances such as hydrocarbons shown in Table 2. These results highlight the importance of the use of different fibers and adsorption conditions in HS-SPME to achieve a more complete identification of all volatile substances present in an extract.

No previous reports have evaluated the use of different types of fibers and HS-SPME to identify VOCs in cagaita. However, this type of study has previously been carried out for other types of fruits.

Sanchez-Palomo, Díaz-Maroto and Pérez-Coello[17] evaluated three fibers with samples of grapes, and showed that the most effective fibers were a PDMS/DVB fiber, followed by a CAR/DVB/PDMS fiber and, finally, a CW/DVB fiber.

Table 2. Volatile organic compounds (VOCs) identified from cagaita using different fibers HS-SPME.

N°	VOCs	Class	SPME fibers				
			CAR/ PDMS	CW/ DVB	DVB/CAR/ PDMS	PA	PDMS/ DVB
1	3-buten-2-one	ketone	x	x	x	x	
2	oleyl alcohol	alcohol	x	x	x	x	x
3	2-propyn-1-ol	alcohol				x	
4	ethyl acetate	ester	x	x	x	x	x
5	isopropyl myristate	ester	x				
6	dibutyl phthalate	ester	x	x	x	x	x
7	(*z*)-9-methyl octadecenoate	ester	x			x	x
8	3-methyl acetate-1-butanol	ester	x		x		x
9	ethyl hexenoate	ester		x			
10	eucalyptol	monoterpene	x	x	x	x	x
11	terpinolene	monoterpene					x
12	*α*-terpinene	monoterpene	x	x	x	x	x
13	propanoic acid	carboxylic acid	x	x	x	x	
14	heptanoic acid	carboxylic acid					x
15	nonanoic acid	carboxylic acid	x	x	x		x
16	undecylenic acid	carboxylic acid	x	x			
17	dodecanoic acid	carboxylic acid	x		x	x	x
18	tridecanoic acid	carboxylic acid					x
19	tetradecanoic acid	carboxylic acid	x	x	x	x	x
20	1,3-butadiene, 2-methyl	hydrocarbon			x		
21	1,3-diethyl-benzene	hydrocarbon				x	
22	2-methyl-1,3-butadiene	hydrocarbon	x	x	x	x	x
23	estragole	ether	x		x	x	
24	2,3-di-*tert*-butyl-p-benzoquinone	quinone				x	x
25	2,4-bis-(1,1-dimethylethyl)-phenol	phenol				x	
26	2-propen-1-one, 3-(4-methylphenyl)-1-phenyl-chalcone, 4-methyl	phenol				x	

HS-SPME: headspace solid-phase microextraction; CAR: carboxen; PDMS: polydimethylsiloxane; CW: carbowax; DVB: divinylbenzene; PA: polyacrylate.

In the work of Pino and Quijano,[23] the influence of three different fibers on the extraction of VOCs from plums was assessed, and the most efficient fiber was found to be a PDMS fiber, followed by a CAR/PDMS fiber, and a PDMS/DVB fiber being the least efficient. The consistency of these results in relation to the present study is worth noting; the CAR/PDMS fiber was more advantageous than the PDMS/DVB fiber.

In a third study, five types of fibers were employed by Garcia *et al.*[24] for the adsorption of volatile substances present in acerola that was obtained using a more efficient extraction process with a PA fiber, which allowed the identification of 37 compounds. In this work, the author noted that the least effective fiber was a CW/DVB fiber. Thus, these results are in agreement with the results obtained in the present study.

Effect of temperature and extraction time

The effects of temperature and extraction time on the number of VOCs extracted are presented in Figure 2.

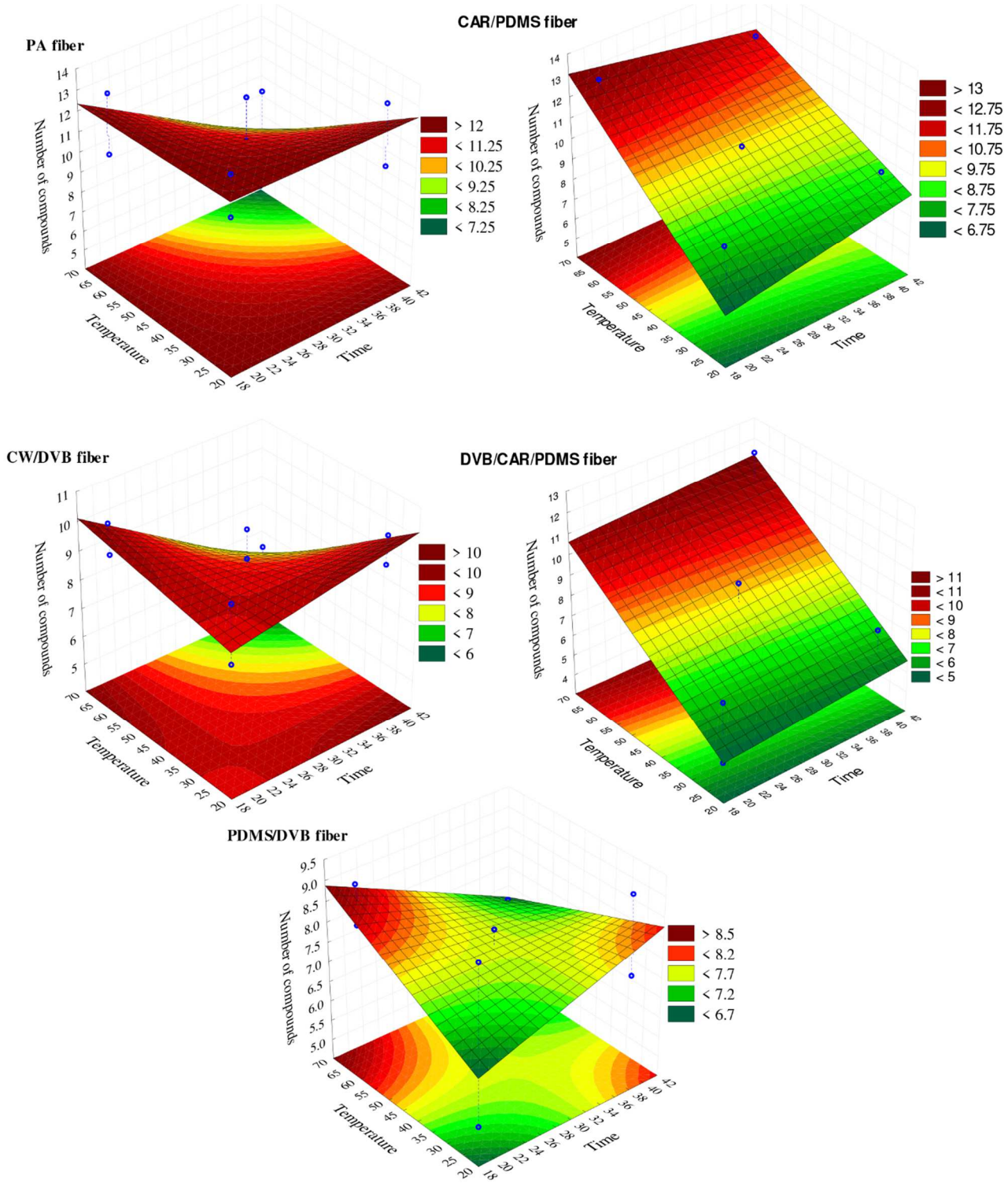

Figure 2. Effects of extraction temperature and time on the isolation of VOCs from cagaita using different types of fibers.

It appears that the shorter the extraction time and higher the extraction temperature, the greater the number of VOCs identified. The shortest time (20 min) and the highest temperature (65 ºC) were the best conditions for the adsorption of volatile substances when using the CAR/PDMS and DVB/CAR/PDMS fibers. This result shows that the use of longer time do not always enable greater detection of volatile substances depending on the fiber type. This may be related to the increased partition of volatile substances in the headspace, which increased the adsorption by the fibers.[18,25]

Considering that no previous reports have evaluated the effects of temperature and HS-SPME extraction time on VOCs of fruits grown in the cerrado, the results obtained here were compared with previously reported results for grape samples, which were used in the only previous study that assessed this parameter. Sanchez-Palomo[17] who evaluated the optimal extraction conditions when employing a CAR/DVB/PDMS fiber, temperatures of 40 to 70 °C, and times ranging from 10 to 50 min, observed that the best results based on the sum of the peaks of aromatic compounds identified were obtained at 70 °C for 20 min.

Effects of agitation and extraction time

Figure 3 shows the binomial agitation and time on the extraction of VOCs. As observed in the Pareto diagrams, agitation and time had no significant effect on extraction. In some fibers such as DVB/CAR/PDMS, the longer time and minimal or no agitation allowed the identification of the largest number of VOCs. It should be noted that no previous studies in the literature have addressed these two variables.

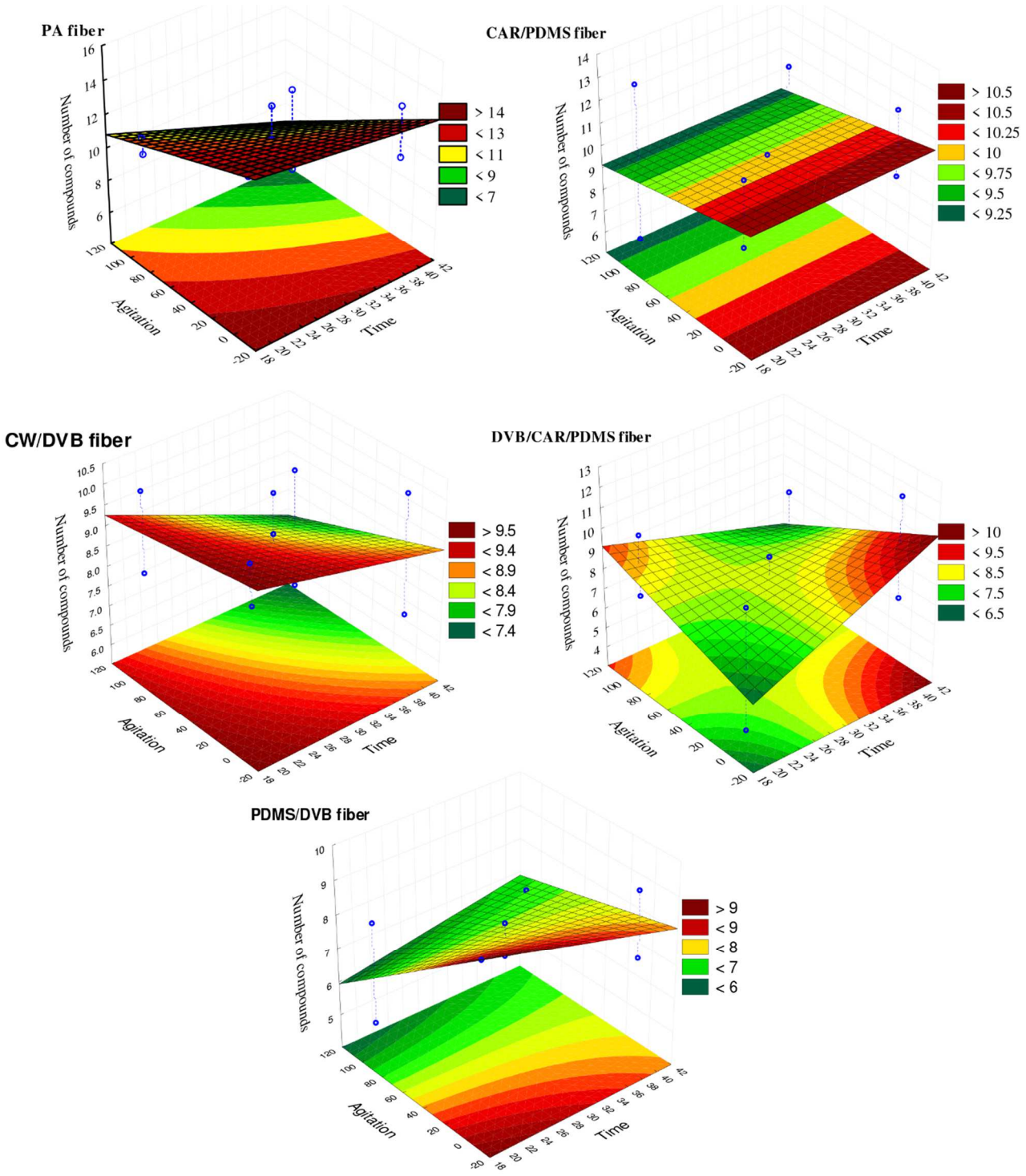

Figure 3. Effects of agitation and extraction time on the isolation of VOCs from cagaita using different types of fibers.

Effects of agitation and extraction temperature

The effects of minimal agitation and higher extraction temperatures are shown in Figure 4. Note that these parameters had an influence on the number of VOCs only when CAR/PDMS and DVB/CAR/PDMS fibers were used and that minimal or no agitation and higher temperatures promoted the identification of a larger number of volatile substances. As observed previously, higher values of temperature are associated to a more efficient VOCs extraction.

It should be noted that this is the first report to address the dual effects of agitation and extraction temperature.

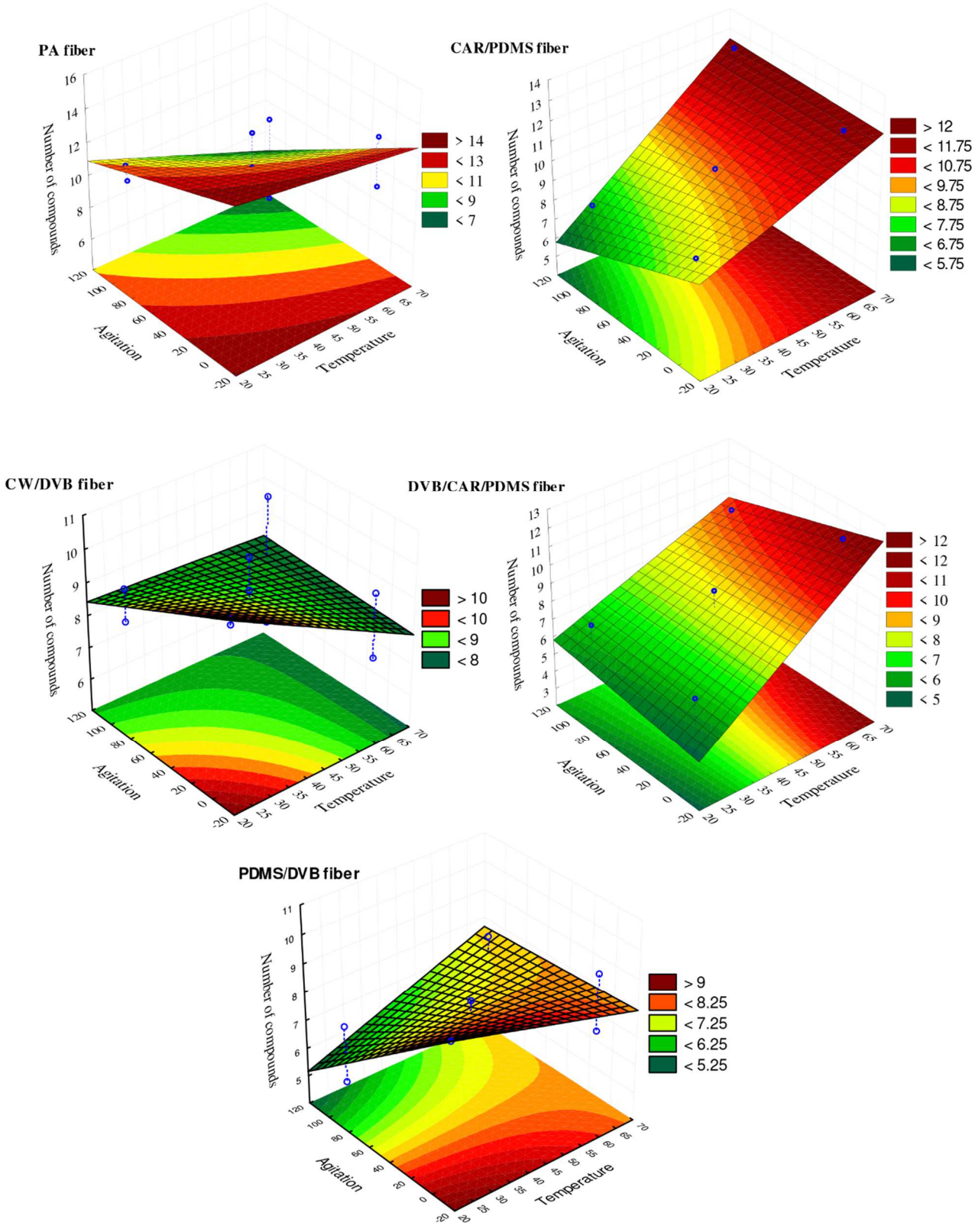

Figure 4. Effects of agitation and extraction temperature on the isolation of VOCs from cagaita using different types of fibers.

Conclusion

HS-SPME coupled to GC/MS has proven to be an efficient technique for the extraction and identification of the VOCs present in cagaita, allowing the identification of 26 compounds. The predominant substances were monoterpenes (34.64%) and esters (36.28%). The effects of the parameters studied were varied, and in terms of the number of compounds identified in all trials, PA fibers proved to be the most efficient. The use of higher temperatures allowed the identification of a larger number of VOCs, especially with CAR/PDMS and DVB/CAR/PDMS fibers. No significant effect of agitation and extraction time was observed.

Supplementary Information

Supplementary Information (chromatograms and table of experimental design results) is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgements

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Supplementary Information

Evaluation of the Influence of Extraction Conditions on the Isolation and Identification of Volatile Compounds from Cagaita (*Eugenia dysenterica*) using HS-SPME/GC-MS

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Table S1. Summary of the results obtained in the optimization of VOCs by the HS-SPME/GC-MS from cagaita by experimental design.

Fiber	Equation	R^2	Lack of fit
PA	Parameters were not significant		
CAR/PDMS	Y = 5.32 +0.1t	0.7686	$p = 0.22$
CW/DVB	Y = 10.68 - 0.0014Tt	0.5202	$p = 0.19$
DVB/CAR/PDMS	Y = 3.03 + 0.1125t	0.7376	$p = 0.51$
PDMS/DVB	Y = 8.51 - 0.018A	0.4211	$p = 0.25$

R^2: determination coefficient; PA: polyacrylate; CAR: carboxen; PDMS: polydimethylsiloxane; CW: carbowax; DVB: divinylbenzene; T = time; t = temperature; A = agitation.

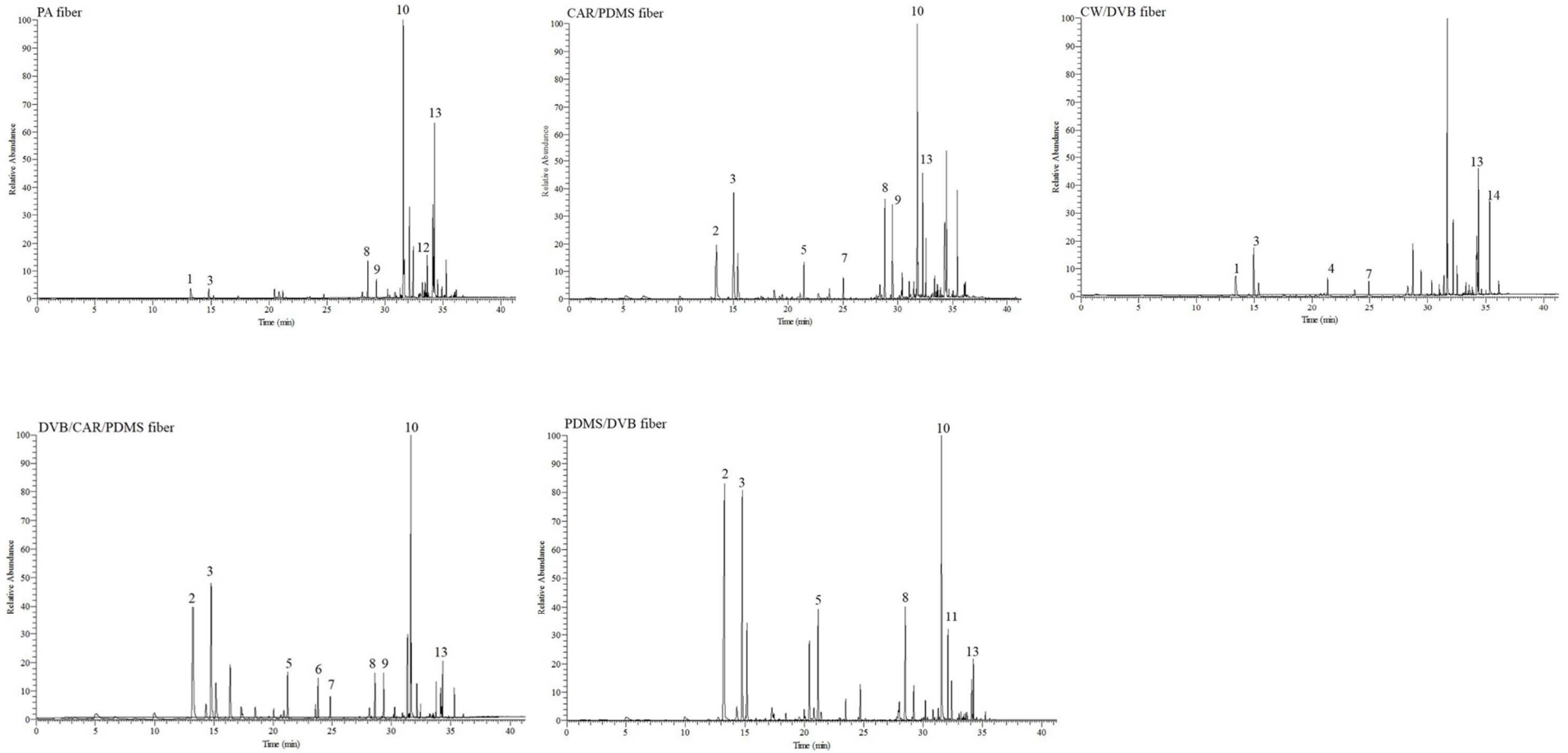


Figure S1. HS-SPME/GC–MS chromatograms from cagaita. Peaks: (1) Ethyl acetate; (2) 3-Methyl acetate-1-butanol; (3) α-terpinene; (4) 3-buten-2-one; (5) nonanoic acid; (6) tetradecanoic acid; (7) 2-methyl-1,3-butadiene; (8) dodecanoic acid; (9) estragole; (10) eucalyptol; (11) (z)-9-methyl octadecenoate; (12) 2-propen-1-one, 3-(4-methylphenyl)-1-phenyl-chalcone, 4-methyl; (13) Oleyl alcohol; (14) dibutyl phthalate.

6. CHAPTER II

Antioxidant Activity and Metabolomic Analysis of Cagaitas (*Eugenia dysenterica*) using Paper Spray Mass Spectrometry

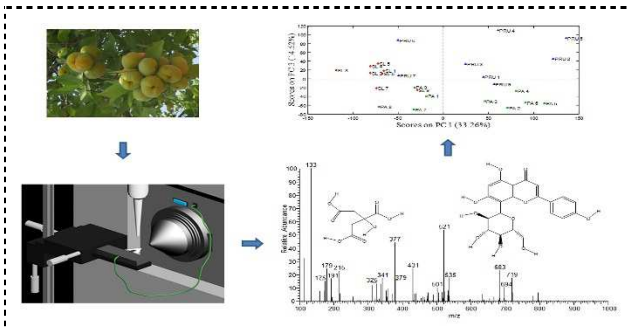
SCIENTIFIC PRODUCTION

SILVA, M. R.; FREITAS, L. G.; SOUZA, A. G.; ARAÚJO, R. L. B.; LACERDA, I. C. A.; PEREIRA, H. V.; AUGUSTI, R.; MELO, J. O. F. Antioxidant activity and metabolomic analysis of cagaitas (*Eugenia dysenterica*) using paper spray mass spectrometry **Journal of the Brazilian Chemical Society**, v. 30, n. 5, p. 1034-1044, 2019.

GUEDES, M. N.; SILVA, M. R.; ARAÚJO, R. L. B.; LACERDA, I. C. A.; MELO, J. O. F. Análise metabolômica da cagaita pela espectrometria de massas com ionização por *paper spray*. XXVI CONGRESSO BRASILEIRO DE FRUTICULTURA, 2019, Juazeiro. **Anais do XXVI Congresso Brasileiro De Fruticultura.** Juazeiro: Editora do Evento, 2018, v. 1, p. 1-4.

REINA, L. C. B.; SOUZA, A. G.; SILVA, M. R.; FREITAS, L. G. ARAÚJO, R. L. B.; LACERDA, I. C. A.; AUGUSTO, R.; MELO, J. O. F. Caracterização dos constituintes da cagaita empregando-se a PS-MS. In: 41º REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE QUÍMICA, 2018, Foz do Iguaçu. **Anais do 41º Reunião Anual da Sociedade Brasileira de Química.** Foz do Iguaçu: Editora do Evento, 2018. v. 1. p. 1-1.

Graphical Abstract (GA)



Discrimination of *Eugenia dysenterica* using paper spray mass spectrometry.

Antioxidant Activity and Metabolomic Analysis of Cagaitas (*Eugenia dysenterica*) using Paper Spray Mass Spectrometry

Mauro R. Silva,[a] Lucas G. Freitas,[a] Amauri G. Souza,[b] Raquel L. B. Araújo,[a] Inayara C. A. Lacerda,[a] Hebert V. Pereira,[c] Rodinei Augusti[c] and Júlio O. F. Melo[b]*

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Abstract

Cagaita is a fruit from Brazilian Cerrado, belongs to Myrtaceae family, and has important economic value. This work aimed to determine the total antioxidant capacity (extractable and non-extractable fractions) by different methods and to evaluate the use of paper spray mass spectrometry to obtain fingerprints of cagaita from different regions with the aid of principal components analysis. Cagaitas had higher antioxidant activity than those found in other fruits mentioned in literature, and the non-extractable fraction was 18.90 to 21.05% of the antioxidant capacity. The analysis of paper spray mass spectrometry in positive and negative ionization modes identified several substances, including organic acids, sugars, amino acids, and several other classes of phenolic compounds. Analysis of the main components of cagaita samples permitted discrimination of the major constituents such as sugars and different kinds of phenolic compounds. Thus, this study demonstrated that paper spray mass spectrometry is a simple and ultrafast method with minimum sample preparation that allows the analysis of the chemical profile of cagaita.

Keywords: Paper spray, principal components analysis, ABTS, FRAP, DPPH.

Introduction

Cerrado, also known as Brazilian Savanna, is a South American biome of around 200 million hectares and covers about 22% of Brazil's territory. It is recognized for its biodiversity of fauna and flora, as well as the abundance of natural resources. For the sustainable exploitation of these resources, the native species of this region need to be characterized.[1,2] A typical specie of this biome is cagaiteira (*Eugenia dysenterica),* an angiosperm belonging to Mirtaceae family. Its fruit, called cagaita, is widely used by local residents.[1,3,4]

Scientific studies report that this fruit is a source of bioactive substances with antioxidant activity, which have been associated with prevention of diseases such as cancer, cardiovascular, and cerebrovascular diseases. The antioxidant activity of foods needs to be determined with different methods that use other mechanisms to reliably quantify foods with a complex matrix. Among the most used methods are the DPPH (2,2-diphenyl-1-picrylhydrazil), FRAP (Ferric reducing antioxidant power), and ABTS (2,20-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid).[5]

Studies about the components of cagaitas have focused on determining extractable phenolic compounds, in other words, components isolated in solution from solid-liquid extraction of fruit pulp. However, significant levels of these substances remain present in the sample, which can lead to hasty conclusions. Therefore, it is important to quantify both the extractable and non-extractable polyphenols.[6]

For broader characterization of cagaita, instrumental analytical techniques can be used, such as, High Performance Liquid Chromatography with UV Detection and/or mass spectrometry, Capillary Electrophoresis, and Gas Chromatography Mass Spectrometry. In general, they provide accurate and precise qualitative and quantitative analyses, despite disadvantages as requiring laborious sample preparation, extensive time, and expense. Recent developments in mass spectrometry techniques with direct analysis have overcome such limitations and provide ultrafast analyses of complex matrices at

low cost. They minimize or eliminate sample preparation and promote ionization of analytes under gentle experimental conditions.[7,8]

Among the ambient ionization techniques, several methods have been developed from Electrospray ionization (ESI) such as desorption electrospray ionization mass spectrometry (DESI-MS), paper spray, and electrospray assisted laser desorption/ionization (ELDI). Paper spray mass spectrometry (PS-MS), developed by Wang *et al.*,[9] has been widely used to analyze substances in complex matrices. A great advantage of PS-MS is the possibility of rapidly obtaining fingerprints in wide ranges of masses. Thus, the PS-MS has been used in studies involving resveratrol in red wine,[10] olive oil analysis,[11] chemical composition of whisky and beer fraud verification,[12,13] coffee classification,[14] caffeine analysis in commercial beverages[15,16] and medicines, pesticide analysis in fruits and vegetables,[17] quality control of tea,[18] *Corni fructus*,[8] dyes,[19] and food additives and their byproducts.[20] Other ambient ionization techniques, such as desorption electrospray ionization (DESI),[21-24] direct analysis in real time (DART),[21,23,24] easy ambient sonic spray ionization (EASI),[21,23] and liquid extraction surface analysis (LESA)[24] have been also successfully explored for fingerprint.

The present study aimed to determine the total antioxidant activity of cagaitas (extractable and non-extractable fraction) using DPPH, ABTS, and FRAP methods. We also identified other chemical constituents using PS-MS. In addition, fingerprints of cagaitas collected in three distinct regions were differentiated with aid of Principal Components Analysis (PCA).

Experimental

Cagaita sample and material

During the 2016 harvest, ripe fruit were collected separately from 27 matrices of cagaiteira in different places within three microregions of Minas Gerais state (Brazil): Paraopeba (Latitude 19º 16'

23" and Longitude 44º 24' 52"), Prudente de Morais (Latitude 19º 28' 54" and Longitude 44º 08' 37"), and Sete Lagoas (Latitude 19° 28' 36" and Longitude 44° 11' 43"). Samples were transported to the Research Laboratory – Food Chemistry Unit of the Federal University of Minas Gerais.

Then, ripe fruit were washed in running water and sanitized using a solution of 200 mg L^{-1} sodium hypochlorite for 15 min followed by running water rinse. Later, samples were stored in a freezer at -18 °C. Before each analysis, the fruit pulp was obtained by thawing 3 fruit from each sample, seeds discarded, and homogenized using a mixer.

The physical-chemical properties and antioxidant activity were determined from a pool made of 3 fruit from 9 cagaiteiras from each microregion. The analysis by PS-MS considered the crude analysis of fruit pulps from 9 cagaiteiras from each of the 3 microregions (27 samples).

All the standards for Folin & Ciocalteu's phenol reagent, 2,2-di-phenyl-1-picrylhydrazil (DPPH•), 2,20-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS•), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were acquired from Sigma Aldric (São Paulo, SP, Brazil). Methanol HPLC grade was acquired from J. T. Baker (Phillipsburg, NJ, USA) and chromatography paper 1 CHR from Whatman (Little Chalfont, Buckinghamshire, UK).

Physical-chemical analyses

Titratable acidity, pH, soluble solids (°Brix), moisture, protein, and ashes were determined, in triplicate, following the methods described by the Association of Official Analytical Chemists (AOAC).[25] Lipids were analyzed according to the Bligh and Dyer[26] extraction method, using methanol, chloroform, and water. Carbohydrate content was calculated by the difference between 100 and the sum of percentages of moisture, protein, lipids, and ashes. The calculation of energy content was performed using conversion factors of 4 kcal g^{-1} of protein and carbohydrate and 9 kcal g^{-1} of lipids.[27]

Samples treatment

Extractable polyphenols

Extraction was done according to the procedure described by Rufino *et al.*[5] For this, 0.5 g of sample and 1 mL of methanol/water (50:50, v/v) were added inside a 2 mL eppendorf tube. After 1 h at room temperature, the tubes were centrifuged at 25,406 *x g* for 15 min and the supernatant retrieved. Afterward, 1.0 mL of acetone/water (70:30, v/v) was added to the residue, with a new incubation and centrifuging at the same conditions above. Both supernatants were mixed, and distilled water added until 5.0 mL was reached.

Hydrolysable polyphenols and non-extractable proanthocyanidin

The hydrolysable polyphenols and the non-extractable proanthocyanidin were obtained according to the methods described by Hartzfeld *et al.*[28] and Arranz *et al.*[29]

Total phenolic compounds and antioxidant activity

The obtained extracts were used to determine the content of phenolic compounds and to evaluate the antioxidant activity. Thus, the phenolic compounds of cagaita were determined following the procedure of Singleton *et al.*[30] The antioxidant activity were evaluated by FRAP, ABTS, and DPPH methods; the first two were carried out according to Rufino *et al.*[5] and the last one according to the AOAC[25] protocol.

PS-MS fingerprints

The chemical profile analysis of the samples was done using a mass spectrometer LCQ Fleet (Thermo Scientific, San Jose, CA, USA) equipped with a paper spray ionization source. The 27 samples were analyzed in positive and negative ionization modes.

Figure 1 is the diagram of the ionization source for paper spray. To carry out the analyses, chromatographic paper was cut in an equilateral triangle shape (1.5 cm) and positioned in front of the mass spectrometer entrance. This paper was supported by a metal connector and positioned 0.5 cm away with the aid of a movable platform (XYZ). This apparatus was connected to a high-voltage source of the spectrometer through a copper wire. Ultimately, 2.0 µL of pulp was applied on the edge of the triangles, 40.0 µL of methanol was transferred to the chromatographic paper and the voltage source was connected for data acquisition. The non-extractable part of the pulp remains retained in the paper after PS-MS analysis. It is important to mention that this part probably contains antioxidant species which, however, could not be detected by the PS-MS approach. The analyses of each individual pulp were done in triplicate for both ionization modes (positive and negative).

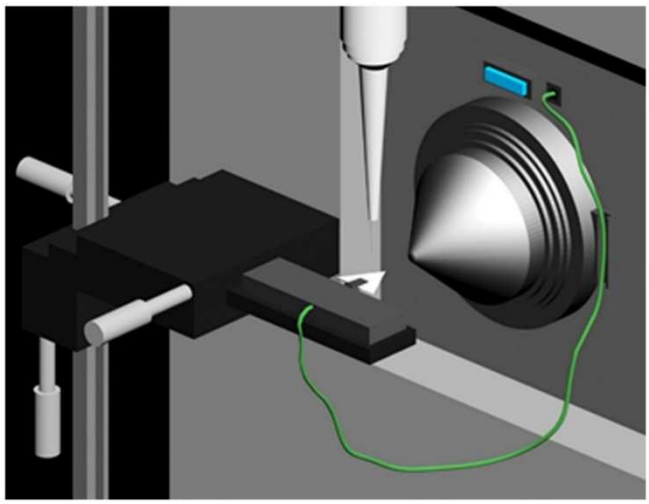

Figure 1. Diagram of ionization source for paper spray.

For the analyses, the instrumental was operated at: voltage of the PS-MS source equal to + 4.0 kV (positive ionization mode) and – 3.0 kV (negative ionization mode); capillary voltage of 40 V; transfer tube temperature of 275 °C; tube lenses voltage of 120 V; and mass range from 50 to 600 *m/z* (positive ionization mode) and from 50 to 1000 *m/z* (negative ionization mode). The ions and their fragments obtained in this analysis were identified based on the data described in literature. Collision energies used to fragmentize the compounds ranged from 15 to 30 eV.

Statistical analysis

Results of physical-chemical analysis and antioxidant activity were subjected to variance analysis by ANOVA one way and Tukey Test ($p < 0.05$) to evaluate the means. Determination of evaluated factor correlation was executed by Pearson test ($p < 0.05$), using software Statistica.[31]

The mass spectra obtained were processed with the software Xcalibur.[32] Mean PS-MS spectra in positive and negative ionization mode for each sample were determined using a spreadsheet of Excel.[33]

Fingerprints of the samples in positive and negative ionization mode were disposed, respectively, in X (25 × 551) and Y (25 × 901) matrices. Data were centered on the mean, and the principal components analysis were carried out using the software MatLab,[34] with aid of PLS Toolbox.[35]

To analyze the differentiation capacity of PCA model, a data fusion model was proposed.[36] This is possible because the detected compounds in both ionization modes were distinct; thus, different information pattern are present in PS(+)-MS and PS(-)-MS results. Therefore, X and Y matrices were concentrated to obtain a Z (25 × 1452) matrix. Resulting matrix data were treated as the previous ones.

Results and Discussion

Physical-chemical assay

In general, cagaitas have high moisture content and low energy content (Table 1). The chemical composition data of cagaitas evaluated in this work corroborates values in other studies on this fruit, including moisture (91.1%),[37,38] protein (0.63%),[39] lipids (0.20 to 0.57%),[1,39] ashes (0.18 to 0.33%),[1,39] carbohydrate (5.54 to 8.73%),[1,39] and energy content (29.83 kcal 100 g^{-1}).[39] Moreover, agreement with literature data was observed for the following parameters: soluble solids (ºBrix) (8.3 to 9.64),[40,41] titratable acidity (13.78 g 100g^{-1}),[1] and pH (2.79 to 3.31).[40,41]

Table 1. Physical-chemical characteristics of cagaitas from Paraopeba, Sete Lagoas, and Prudente de Morais.

Parameters	Place of sample collection		
	Paraopeba	Sete Lagoas	Prudente de Morais
Moisture (%)	92.06 ^{a} ± 0.40	91.72 ^{a} ± 0.64	92.05 ^{a} ± 0.25
Protein (g 100 g^{-1})	0.76 ^{ab} ± 0.08	0.71^{b} ± 0.09	0.81^{a} ± 0.05
Lipids (g 100 g^{-1})	0.34 ^{a} ± 0.04	0.36 ^{a} ± 0.02	0.27 ^{b} ± 0.01
Ash (g 100 g^{-1})	0.25 ^{ab} ± 0.04	0.22 ^{b} ± 0.02	0.28 ^{a} ± 0.04
Carbohydrate (g 100 g^{-1})	6.59 ^{a} ± 1.18	6.99 ^{a} ± 0.58	6.59 ^{a} ± 0.19
Total energy value (kcal 100 g^{-1})	35.09 ^{a} ± 0.06	35.37 ^{a} ± 0.34	32.56 ^{b} ± 0.01
Soluble solids (°Brix)	9.56 ^{a} ± 1.02	8.69 ^{ab} ± 0.99	8.23 ^{b} ± 0.89
pH	3.18 ^{a} ± 0.15	3.06 ^{a} ± 0.16	3.24 ^{a} ± 0.18
Titratable acidity (g citric acid 100 g^{-1})	9.66 ^{a} ± 0.22	11.89 ^{ab} ± 0.22	12.69 ^{b} ± 0.22

Means indicated by the same letters on the same line do not differ at 5% significance compared to different regions.

Phenolic compounds and antioxidant activity

Table 2 presents the total phenolic compounds content and antioxidant activity of the sample pool from each microregion. Cagaitas from Paraopeba region exhibited significantly higher amounts of phenolic compounds than those from the other two regions. Phenolic compounds in non-extractable fraction corresponded to between 14.8% and 17.4% of total content of cagaitas according the region, which demonstrates the importance of evaluating this fraction that is underestimated by other authors (141.95 to 150 mg GAE $100g^{-1}$).[2,37] Considering other fruits of the Mirtaceae family, cagaitas contained higher values than cambui (*Campomanesia phaea* Berg.) (246 mg GAE 100 g^{-1}), araçá-boi (*Eugenia stipitata* Mc. Vaugh) (87 mg GAE 100 g^{-1}), and araçá (*Psidium guineensis* Sw.) (129 mg GAE 100 g^{-1}).[37]

Considering the total antioxidant activity obtained by DPPH, FRAP, and ABTS methods (Table 2), cagaitas from Paraopeba presented statistically higher results than the other regions. The importance must be emphasized in non-extractable fraction determination, as it represented 18.90% to 21.05% of total antioxidant capacity obtained by FRAP method.

Table 2. Total phenolic compounds and antioxidant activity of cagaitas of Paraopeba, Sete Lagoas, and Prudente de Morais.

Parameters	Fraction	PA	SL	PRU
Phenolic compounds (mg GAE 100 g^{-1} sample)	EPP	$367.67^{a} \pm 17.40$	$276.31^{b} \pm 13.62$	$277.02^{b} \pm 19.59$
	NEPA	$39.90^{a} \pm 1.82$	$36.62^{a} \pm 0.54$	$37.00^{a} \pm 1.52$
	HPP	$24.12^{a} \pm 0.16$	$18.34^{b} \pm 0.33$	$21.22^{c} \pm 0.22$
	TOTAL	$431.69^{a} \pm 15.80$	$331.27^{b} \pm 13.26$	$335.24^{b} \pm 17.66$
FRAP (µM ferrous sulphate g^{-1} sample)	EPP	$17.29^{a} \pm 0.34$	$12.45^{b} \pm 0.14$	$12.83^{b} \pm 0.32$
	NEPA	$3.61^{a} \pm 0.35$	$3.09^{a} \pm 0.07$	$2.21^{b} \pm 0.21$
	HPP	$0.42^{a} \pm 0.01$	$0.23^{b} \pm 0.01$	$0.33^{c} \pm 0.01$
	TOTAL	$21.33^{a} \pm 0.39$	$15.77^{b} \pm 0.07$	$15.38^{b} \pm 0.28$
ABTS (µM trolox g^{-1} sample)	EPP	$9.34^{a} \pm 0.34$	$6.44^{b} \pm 0.26$	$6.80^{b} \pm 0.19$
	NEPA	nd	nd	nd
	HPP	nd	nd	nd
	TOTAL	$9.34^{a} \pm 0.34$	$6.44^{b} \pm 0.26$	$6.80^{b} \pm 0.19$
DPPH (µM TE g^{-1} sample)	TOTAL	$11.47^{a} \pm 0.87$	$7.09^{b} \pm 0.47$	$7.64^{b} \pm 0.50$

PA = Paraopeba; SL = Sete Lagoas; PRU = Prudente de Morais; GAE = gallic acid equivalentes; FRAP: ferric reducing antioxidant power; ABTS: 2,20-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazil; EPP = extractable polyphenols; NEPA = non-extractable proanthocyanidins; HPP = hydrolysable polyphenols; nd = not detected. Means indicated by equal letters on the same line do not differ at 5% significance in the comparison with different regions.

Many studies have reported the significant contribution of phenolic compounds to the antioxidant property of fruits.[2,5,38,42] The present work corroborates with these data, since a positive correlation with strong intensity ($r > 0.7$) and significant ($p < 0.05$) between total content of phenolic compounds and total antioxidant activity was observed for ABTS ($r = 0.910$), FRAP ($r = 0.937$), and DPPH ($r = 0.999$) methods; and between FRAP and ABTS ($r = 0.963$), FRAP and DPPH ($r = 0.985$), and ABTS and DPPH ($r = 1.00$).

PS-MS Fingerprints

Examples of PS-MS spectra of cagaitas in positive and negative ionization modes are illustrated in Figure 2. The proposed molecules from the obtained ions in the negative and positive ionization modes were amino acids, sugars, delphinidins, coumarins, organic acids, sugars, and phenolic compounds.

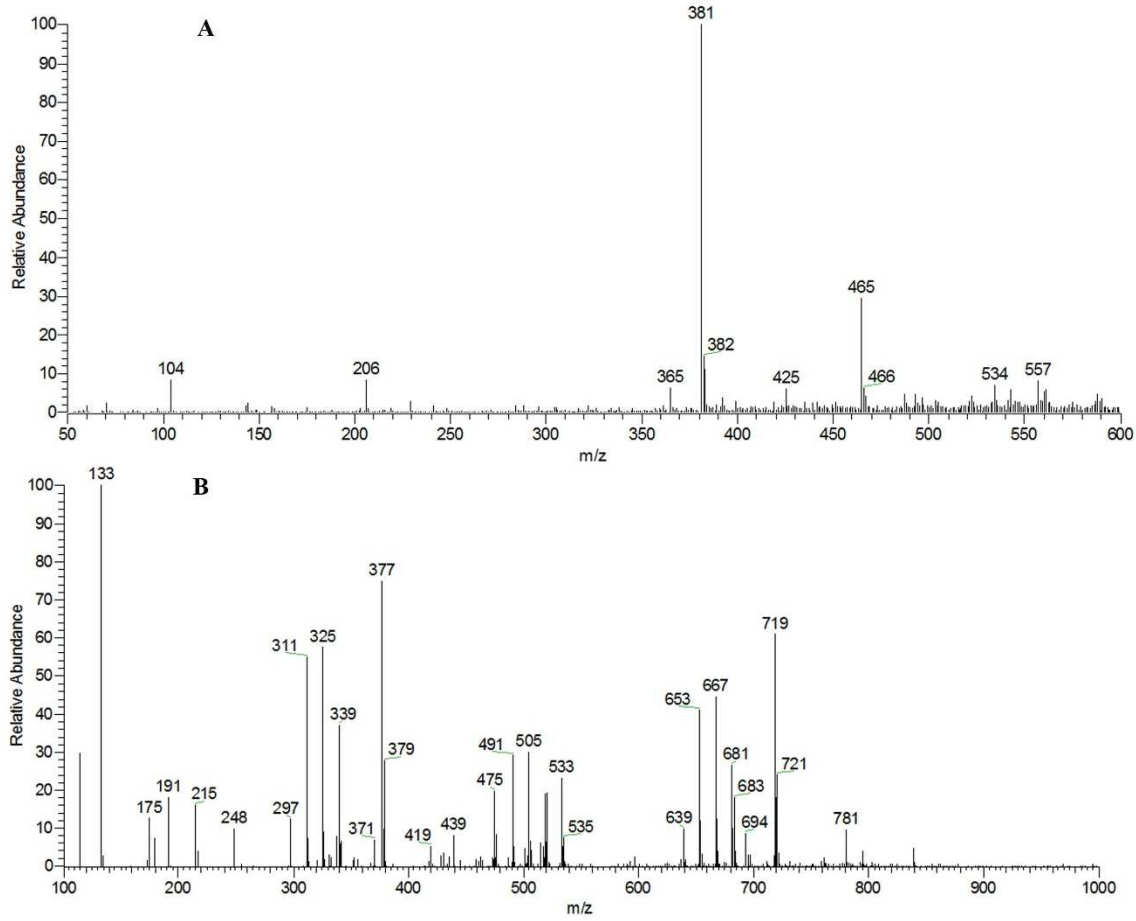

Figure 2. Representation of (a) (+)PS-MS and (b) (-)PS-MS of a cagaita sample.

PS(+)-MS Fingerprints

Table 3 shows the possible compounds detected in the fingerprints (positive ionization mode) of cagaitas. The analysis with the positive ionization mode were performed specifying the ratio mass

charge from 50 up to 600, since in previous assays, we observed the sample has not presented any ion above this range.

The signal *m/z* 175 probably refers to protonated *L*-arginine. This amino acid had a different fragmentation pattern than commonly observed in other amino acids, not being characterized by the loss of NH_3. Its classification was confirmed by distinguishing ions obtained after the fragmentation reactions (*m/z* 70 and 129).

Table 3. Compounds identified in cagaitas by PS(+)-MS.

Tentatively identification	*m/z*	ID	MS/MS	Reference
L-arginine	175	$[M+H]^+$	70, 129	Gogichaeva *et al.*,[43] Ozcan and Senyuva[44]
Citropten	206	$[M]^+$	121	Ledesma-Escobar *et al.*[45]
Sucrose	381	$[2Hex + K - H_2O]^+$	201, 219	Yuan *et al.*,[46] Asakawa and Hiraoka[47]
Delphinidin-3-glucoside	465	$[M]^+$	303	Flores *et al.*,[48] Silva *et al.*[49]

The signal with *m/z* 381 was proposed as being sucrose $[2Hex + K - H_2O]^+$. Such characterization was performed in works by Asakawa and Hiraoka[47] as well and Chen *et al.*[50] when they investigated the presence of oligosaccharides in fruits using mass spectrometry. The existence of this sugar in pulp of unpeeled cagaitas has already been related by Ribeiro *et al.*,[1] when they found 0.59% of sucrose.

The ion with *m/z* 206 can be recognized as citropten, based on its transition MS/MS 206→121, which was also observed by Ledesma-Escobar *et al.*[45] when evaluating the identification parameters of coumarins in lemon (*Citrus limon*) by LC-MS. The ion with *m/z* 465 was assigned as delphinidin-3-glucoside, and the confirmation was based on its ion MS^2 (*m/z* 303, -162 amu), resulting from the loss of a hexose unit. Previous studies with cagaitas evaluated only total anthocyanins as reported by Siqueira *et al.*,[51] who found 0.38 mg 100 g^{-1}.

PS(-)-MS Fingerprints

Fingerprints of cagaitas obtained using PS-MS in negative ionization mode are in Table 4. This method helped identify several compounds including organic acids, sugars, and phenolic compounds.

Organic acids

The signals with *m/z* 115 and 133 showed fragmented ions with *m/z* 71 [$C_3H_3O_2$] and 89 $[M - H - CO_2]^-$; thus, they were proposed as malic acid. Ion with *m/z* 191 was recognized as citric acid based on obtained ions after the fragmentation reaction with *m/z* 85 and 111 $[M - H_2O - COOH - OH]^-$. Ramos *et al.*[52] also founded these two organic acids when investigating contents of araçá-pera (*Psidium acutangulum*) by HPLC-MS.

Table 4. Ions identified in cagaitas by PS(-)-MS.

Tentatively identification	*m/z*	ID	MS/MS	Reference
Malic acid	115	$[M - H_2O - H]^-$	71	Wang *et al.*[54]
Malic acid	133	$[M - H]^-$	89, 115	Roesler *et al.*[53]
Hexose	179	$[M - H]^-$	71, 89	Roesler *et al.*,[53] Wang *et al.*[54]
Citric acid	191	$[M - H]^-$	85, 111	Wang *et al.*[54]
Hexose	215	$[Hex + 2H_2O - H]^-$	71, 89, 179	Guo *et al.*,[8] Wang *et al.*[54]
Caftaric acid	311	$[M - H]^-$	133	Abu-Reidah *et al.*[55]
p-Coumaric acid hexoside	325	$[M - H]^-$	119, 145	Aaby *et al.*,[56] Kajdžanoska *et al.*[57]
Cafeoil-D-glucose	339	$[M - H]^-$	159	-
Syringic acid hexoside	359	$[M - H]^-$	153, 197	Abu-Reidah *et al.*,[55] Barros *et al.*[58]
Hexose or sucrose	377	$[2Hex + H_2O - H]^-$ or $[Suc + 2\ H_2O - H]^-$	341	Chen *et al.*[50]
Vitexin	431	$[M - H]^-$	341	Wang *et al.*,[54] Koolen *et al.*[59]
dimethylellagic acid hexoside	491	$[M - H]^-$	454	Gordon *et al.*[60]
Hexose	521	$[3Hex - H_2O - H]^-$	341	-
Galloylated caffeic acid hexoside	681	$[M - H]^-$	511	-
Caffeic acid hexoside dimer	683	$[M - H]^-$	341	Spínola *et al.*[61]

Sugars

Based on the fragmentation profile shown in Table 4, signals with *m/z* 179, 215, 377, and 521 were recognized as sugars. Ribeiro *et al.*[1] found by HPLC presence of glucose and fructose (1.75 and 2.54 g 100 g^{-1}, respectively) in pulp of unpeeled cagaitas. Results obtained in this present study agree with Chen *et al.*,[50] who determined the oligosaccharides present in fruits through electrospray droplet impact/secondary ion mass spectrometry (EDI/SIMS), and Roesler *et al.*[53] who investigated the main contents of araticum (*Annona crassiflora*) by ESI-MS.

Hydroxycinnamic acids

The signal with *m/z* 311 showed fragmentation ion with *m/z* 133, recognized as caftaric acid, which corresponds to a non-flavonoid phenolic compound originating from the esterification of caffeic acid with tartaric acid. Previous studies have already reported its presence in a Chinese medical plant (*Taraxacum formosanum*) and in wines.[62,63]

The ion with *m/z* 325 probably corresponded to a hydroxycinnamic acid conjugated to a hexose called *p*-coumaric acid hexoside. Classification was proposed by mass spectra MS^2 with *m/z* 119 and 145. This substance was reported previously by Kajdžanoska *et al.*[57] in strawberries and by Mikulic-Petkovsek *et al.*[64] in blueberries (*Vaccinium myrtillus* L.) through LC/DAD/ESI-MS.

The signal with *m/z* 339 was assigned to caffeic acid in the conjugated form with a hexose (cafeoil-*D*-glucose). The obtained fragmented ion (*m/z* 159) resulted from the loss of caffeic acid (-170 amu). Caffeic acid has already been described in cagaitas by Guedes *et al.*,[65] employing HPLC. This substance in this conjugated form was reported by Chen *et al.*[63] in Chinese medical herbs through LC-MS/MS.

The ion with *m/z* 681 may be defined as galloylated caffeic acid hexoside, since it presented characteristic fragmentation profile from the loss of gallic acid (ion *m/z* 511, -170 amu). The

substance with *m/z* 683 may be recognized as caffeic acid hexoside dimer, as it had transition MS/MS 683→341, probably resulting from loss of hexose (-342 amu).

Hydroxybenzoic acids

The signal with *m/z* 359 may be recognized as syringic acid hexoside, because it presented fragmented ions with *m/z* 153 ([M - H - CO_2]) and 197, which resulted from elimination of a hexosyl group (-162 amu). Guedes *et al.*[65] already described syringic acid in cagaitas. The signal with *m/z* 491 presented a fragmented ion with *m/z* 454, identified as dimethylellagic acid hexoside.

Flavones

A substance with *m/z* 431 with fragmentation profile with *m/z* 341 ($[C_{18}H_{13}O_7]^-$) was recognized as vitexin. This flavone, belonging to the flavonoids class, has already been reported by Silva *et al.*[49] in jussara fruit (*Euterpe edulis* Mart.), using HPLC-DAD-MS/MS. Koolen *et al.*[59] also identified this flavonoid when investigating phenolic compounds of buriti (*Mauritia flexuosa* L. f.) by UPLC-ESI-MS/MS.

Principal Components Analysis

Principal components analysis was executed for data matrices X (25 × 551) and Y (25 × 901) made from PS(+)-MS and PS(-)-MS spectra. Results of these models showed the differentiation of some samples, mainly due to compounds *L*-arginine, sucrose, delphinidin-3-glucoside, and citropten in positive ionization mode and to the substances citric acid, caftaric acid, *p*-coumaric acid hexoside, hexoses, and dimethylellagic acid hexoside in negative ionization mode. Both

models were constructed by selecting 3 principal components, which explained, respectively, 92.23% (positive ionization mode) and 70.79% (negative ionization mode) of total data variability.

As the information patterns obtained by both ionization methods were different, classification models were also constructed from the approach of data fusion. Thus, distinct information could be correlated from this data set to enhance the classification capacity of this model.

Data fusion was carried out from the simple concatenation of the data matrices X and Y, what resulted in a Z matrix (25 x 1452). Resulting PCA model consisted in selection of three principal variables, which explain a total of 65.32% of total data variability.

Figure 3 illustrates the scores of PC 1, PC 2, and PC 3 of the PCA model after data fusion.

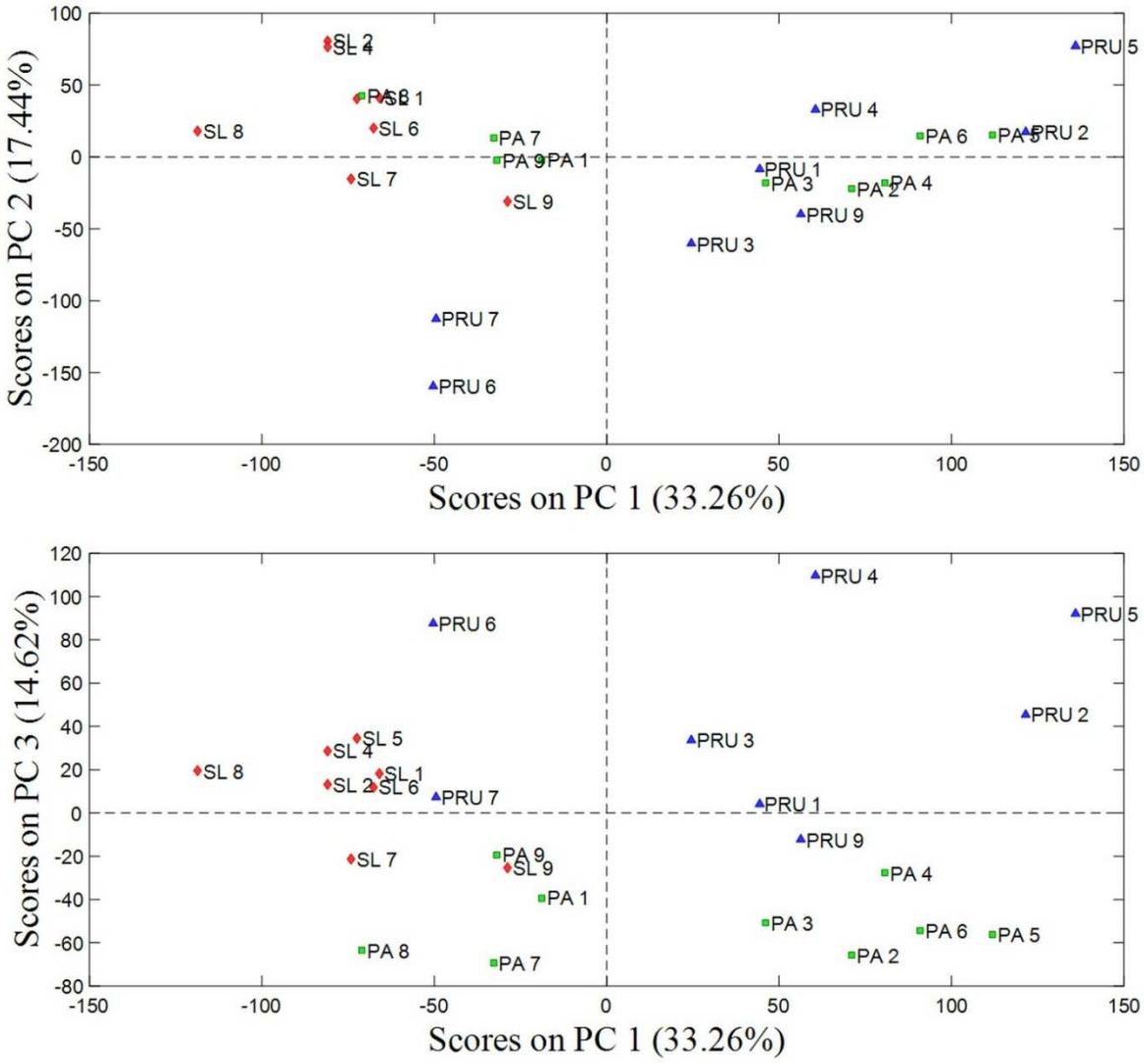

Figure 3. PC 1, PC 2, and PC 3 scores. PA = Paraopeba, SL = Sete Lagoas, PRU = Prudente de Morais.

The principal component 1 (33.26% of data variability) allowed most of the samples from the microregions Prudente de Morais and Paraopeba (positive scores) to be separated from Sete Lagoas samples (negative scores). Loading analysis of PC 1 shows that samples with positive values presented ions with *m/z* 381 (positive ionization mode) and *m/z* 179, 191 215, 311, 325, 340, 371, 491, 505, and 519 (negative ionization mode). Samples with negative score values presented the variables *m/z* 377, 431, 521, 683, and 719 (negative ionization mode) and *m/z* 206, 392, and 465 (positive ionization mode) with higher loading values.

This indicates that factors that may differentiate samples from these regions differ mainly in the kind of phenolic substance that come from the conditions of metabolic stress and genetic variability. This hypothesis is plausible, since some samples from Paraopeba (PA 1, 7, 8, and 9) are grouped together to the Sete Lagoas samples.

Principal component 2 (17.44% of the variability) differentiated some of the samples from Prudente de Morais (PRU 6 and PRU 7) in relation to the other samples. Loadings analysis of this component indicates that sample PRU 6 and PRU 7 presented signal with *m/z* 133, 191, 333, and 339 (negative ionization mode) and *m/z* 70, 104, 143, 175, 206, 392, and 465 (positive ionization mode).

As for the principal component 3 (14.62% of the variability), it allowed the discrimination of cagaitas from Paraopeba (negative scores). Loadings analysis of this PC showed that the differentiation of these samples occurred mainly due to signals with *m/z* 133, 175, 191, 371, 431, and 521 (negative ionization mode) and *m/z* 381 (positive ionization mode).

Figure 4 shows the loadings of variables of PC 1 (Figure 4a), PC 2 (Figure 4b), and PC 3 (Figure 4c) after data fusion. Signals of some sugars with *m/z* 377 ($[2Hex + H_2O - H]^-$ or $[Suc + 2H_2O - H]^-$) and the signal with *m/z* 719 contributed with stronger negative signals for the formation of grouping 1 and 2 in PC 1. On the other hand, grouping 3 was a function of the positive signal from potassium adduct of sucrose ($[2Hex + K - H_2O]^+$, *m/z* 381) and from the signals of hexose (*m/z* 179), citric acid (*m/z* 191), hexose (*m/z* 215), caftaric acid (*m/z* 311), *p*-coumaric acid hexoside (*m/z* 325), and dimethylellagic acid hexoside (*m/z* 491).

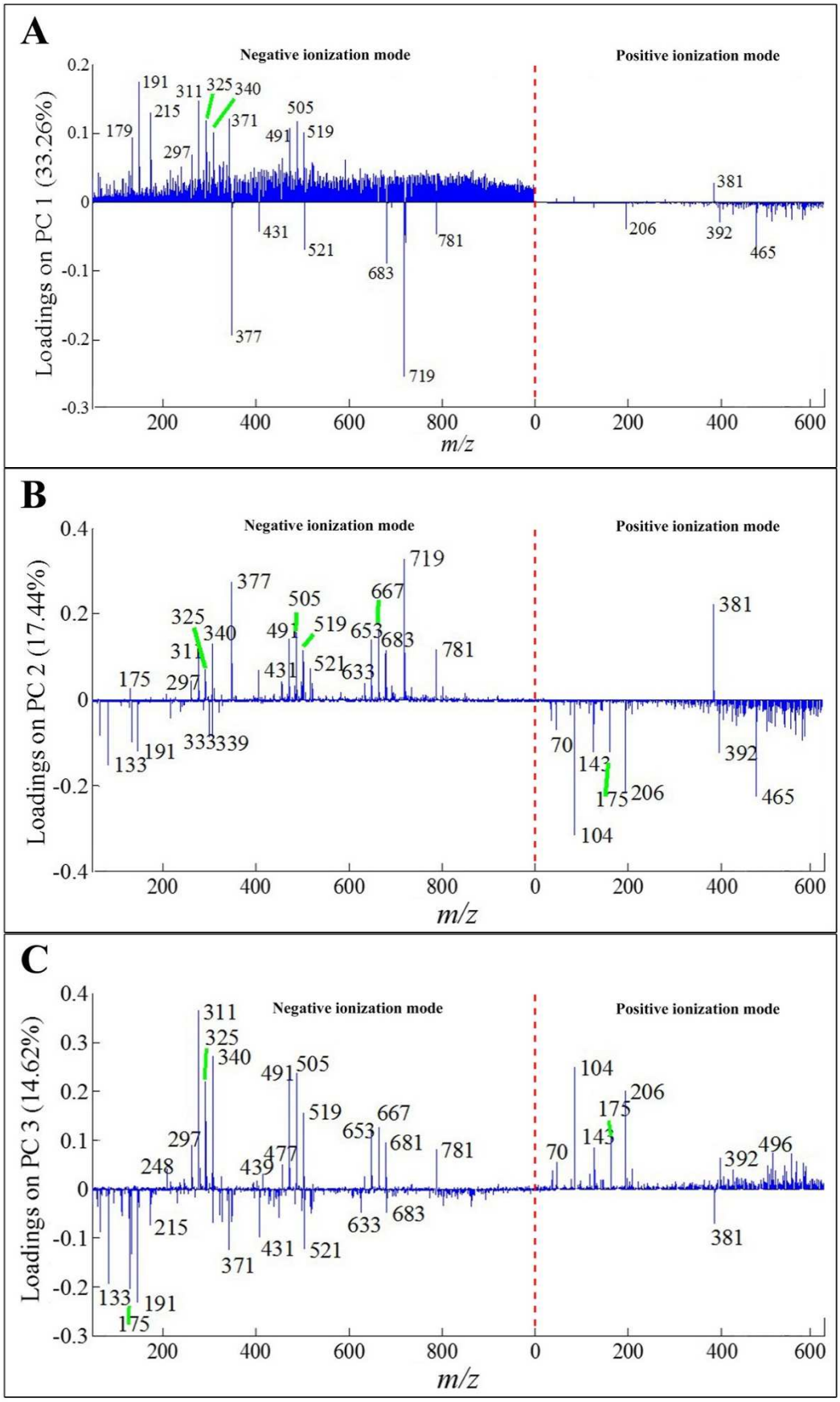

Figure 4. Representation of the loadings responsible for the discrimination of the samples scores in (a) PC 1, (b) PC 2 and (c) PC 3.

The variations in cagaita composition may be associated with some factors like geographic location of the fruit, genetic variability, stress factors, sun exposure, and temperature.[1]

Therefore, PS-MS method may be a more adequate analysis tool to determine content of phenolic substances, because it permits fast, simple, and low cost analysis of each individual fruit.

Conclusion

Cagaitas contained high levels of phenolic compounds and intermediate values of antioxidant activity found with the three assessed methods (ABTS, FRAP, and DPPH), and these values were higher for fruit from Paraopeba when compared with other regions. Compounds in the non-extractable fraction such as pro-anthocyanidin and hydrolysable polyphenols contained 14.8% to 17.4% of phenolic compounds and 16 to 21% of antioxidant activity in relation to the total. The levels of total phenolic compounds showed good positive correlation with the three methods used to evaluate the antioxidant activity. PS-MS was demonstrated to be a simple and fast technique to obtain the fingerprint of cagaita content, identifying several compounds such as organic acids, sugars, amino acids, and phenolic compounds.

Supplementary Information

Supplementary Information are available free of charge at http://jbcs.sbq.org.br as PDF file.

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Supplementary Information

Antioxidant Activity and Metabolomic Analysis of Cagaitas (*Eugenia dysenterica*) using Paper Spray Mass Spectrometry

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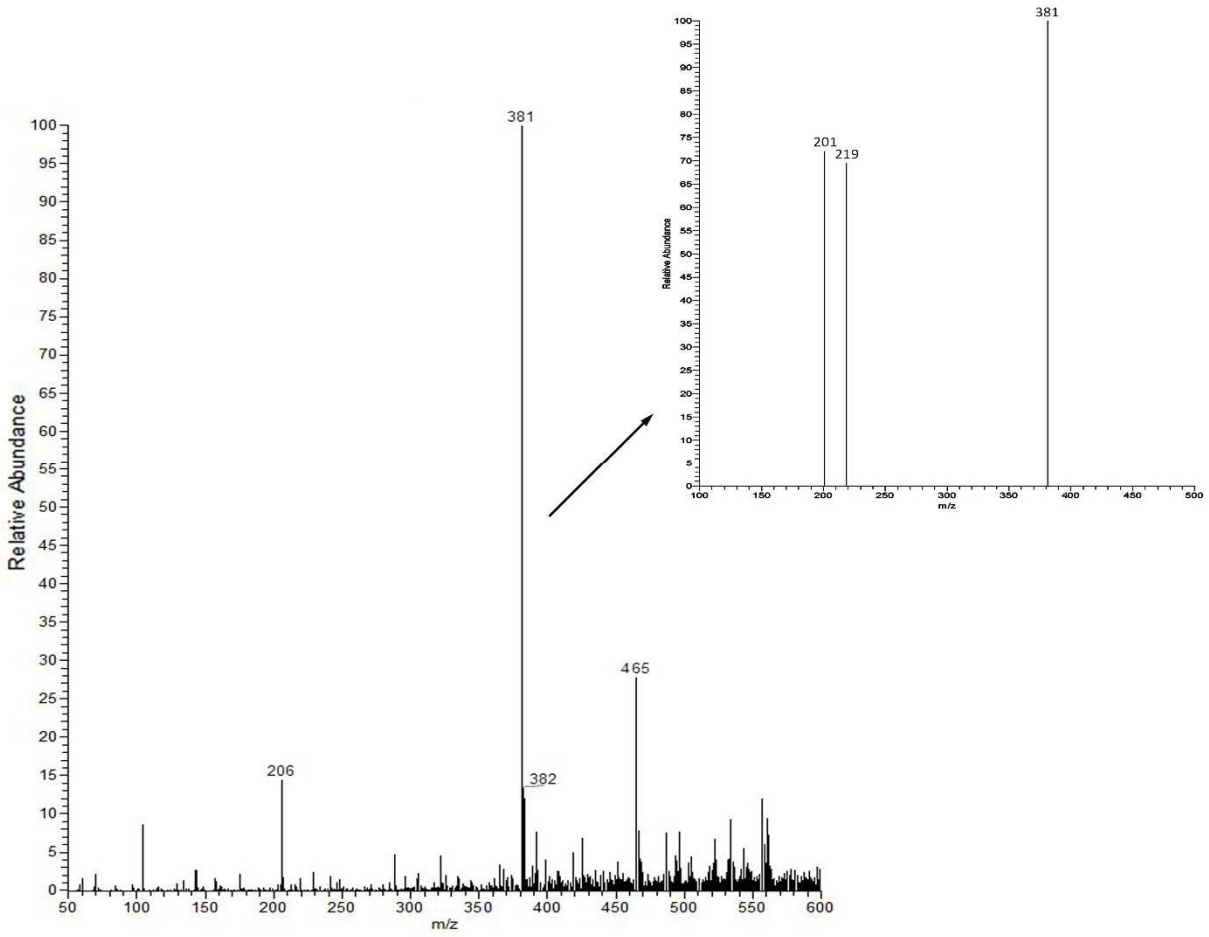


Figure S1. Fragmentation spectrum of the sucrose found in cagaita.

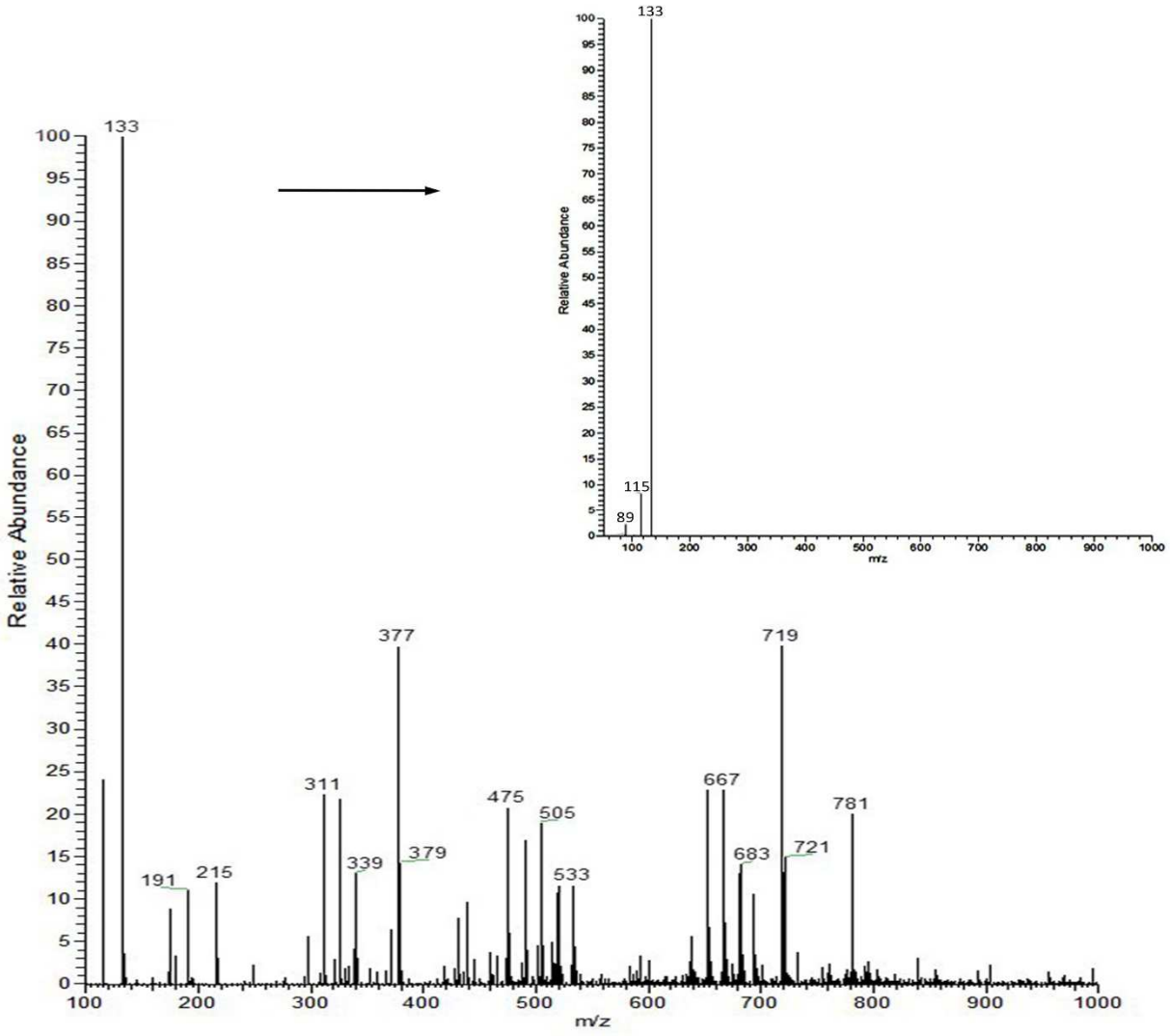


Figure S2. Fragmentation spectrum of the malic acid found in cagaita.

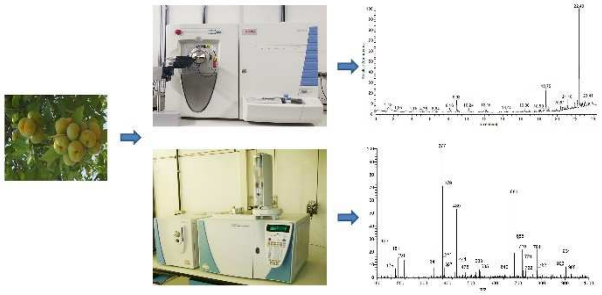
7. CHAPTER III

Determination of chemical profile of *Eugenia dysenterica* ice cream using PS-MS and HS/SPME/CG-MS

SCIENTIFIC PRODUCTION

SILVA, M. R.; FREITAS, L. G.; SOUZA, A. G.; ARAÚJO, R. L. B.; LACERDA, I. C. A.; PEREIRA, H. V.; AUGUSTI, R.; MELO, J. O. F. Determination of chemical profile of Eugenia dysenterica ice cream using PS-MS and HS/SPME/CG-MS. **Journal of the Brazilian Chemical Society**. (Submitted article)

Graphical Abstract (GA)



Volatile profile and use of paper spray ionization mass spectrometry for rapid analysis of cagaita (*Eugenia dysenterica)* ice cream

Determination of chemical profile of *Eugenia dysenterica* ice cream using PS-MS and HS-/SPME/CG-MS

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Abstract

The paper spray ionization mass spectrometry (PS-MS) technique was applied for the first time to characterize cagaita ice cream rapidly. The profile of volatile compounds was determined by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry. Fingerprints obtained through PS-MS identified various classes of compounds, such as flavones, anthocyanins, sugars, organic acids, hydroxybenzoic acids, fatty acids, hydroxycinnamic acids, lignin, and phenylpropanoid. The two SPME fibers used found 21 volatile compounds in cagaita, and 19 of them were also identified in cagaita ice cream. The most common volatile compound found in both cagaita and ice cream was 3-carene monoterpene. Considering the effect of processing on cagaita constituents, 90% of the volatile compounds and total phenolics remained in the ice cream. Also, 78% of the fruit chemical compounds analyzed by PS-MS were found in the product, which mainly belonged to the flavonoid class. Thus, the results indicate that most of the fruit compounds remained in the ice cream after processing.

Keywords: Cerrado, cagaita, ice cream, paper spray, chemical profile, volatile compounds.

Introduction

The Cerrado, the richest savanna on earth, encompasses 22% of the Brazilian territory and is home to approximately 11,627 native plant species. Among the edible fruits found in this biome, cagaiteira trees (*Eugenia dysenterica*) produce fruit with low caloric value and high moisture content that are an excellent source of vitamin C containing several phenolic compounds.[1] Cagaita are consumed fresh or used to make jellies, jams, liqueurs, and juices.[2] However, despite their social importance to inhabitants who obtain income from this natural resource, many species are at risk of extinction due to deforestation caused by the expansion of various agriculture and livestock sectors.[3]

Development of food products is a way to add value to the Cerrado fruits. According to ABIS (Brazilian Association of Industries and the Ice Cream Industry), Brazil is among the ten largest producers of ice cream in the world. In the period from 2003 to 2016, Brazilian production increased from 686 million to 1 billion liters of ice cream.[4] Ice cream is made of milk, sugars, stabilizers, emulsifiers, flavors, among others. These ingredients provide a stable emulsion when this mixture is subjected to agitation, freezing, and incorporation of air.[5-8]

The literature describes many techniques for chemical analysis of ice cream, such as spectrophotometric assay,[8,9] high-performance liquid chromatography (HPLC),[7,10] gas chromatography-mass spectrometry (GC-MS),[11] liquid chromatography coupled to mass spectrometry (LC-MS).[12] However, some of these techniques require longer analysis time with multiple sample preparation steps and generate chemical waste.

Several techniques of ambient ionization mass spectrometry have overcome these disadvantages, allowing fingerprints to be obtained through ultrafast analysis and with minimal sample preparation. Among them, paper spray ionization mass spectrometry (PS-MS) has been employed to analyze various food matrices, such as corni fructus,[13] cagaita,[2] colorings,[14] red wine,[15] olive oils[16] as well as coffee,[17] acoholic beverages,[18,19] and teas.[20]

This work aimed to characterize the chemical constituents of cagaita ice cream through paper spray ionization mass spectrometry and volatile compounds using headspace solid phase microextraction combined with gas chromatography coupled to mass spectrometry.

Experimental

Material

Solid phase microextraction fibers Polyacrylate (PA, 85 μm) and Polydimethylsiloxane/Divinylbenzene (PDMS/DVB, 65 μm) and Folin-Ciocalteu reagent were purchased from Sigma Aldrich (São Paulo, SP, Brazil). Chromatography paper 1 CHR was from Whatman (Little Chalfont, Buckinghamshire, UK) and HPLC grade methanol was supplied by J. T. Baker (Phillipsburg, NJ, USA). The other reagents were analytical grade.

Sample preparation and ice cream processing

Ripe cagaita fruits were collected in the municipality of Sete Lagoas, MG (Latitude 19° 28' 35.8" e Longitude 44° 11' 42.4") in December 2018. The cagaitas were transported to the Chemistry and Analytical Research Laboratory of the Universidade Federal de Minas Gerais. The fruits were washed in running water, sanitized for 15 min using sodium hypochlorite (200 mg L^{-1}), rinsed in running water, and stored in a freezer at -20 °C. The pulps were produced from thawed fruit. The peels and seeds were removed, and the pulp homogenized in a mixer.

The production of cagaita ice cream was performed as described by Goff and Hartel[21] with modifications. The formulation consisted of the following ingredients: cagaita pulp (40%), nonfat powdered milk (13.62%), sugar (5%), inulin (4.92%), maltitol (3.59%), sorbitol (3.15%), palm

kernel oil (2.36%), glucose (0.61%), and emulsifier (0.4%). All ingredients were weighed and then mixed in a household blender. Next, the mix was pasteurized at 70 °C for 30 min and cooled to 4 °C. The cagaita pulp was added and the mixture homogenized again. Subsequently, the mixture was placed in a domestic freezer and kept under constant agitation to incorporate air during freezing. Finally, the ice cream was packed in polypropylene jars and stored in a freezer at -20 °C.

Sample Extraction

Cagaita and ice cream samples were extracted according to the method of Rufino *et al.*[22] In a 2 mL Eppendorf tube, 0.5 g of sample and 1 mL of methanol/water (50:50, v/v) were added. After incubation at room temperature for 1 h, the tubes were centrifuged at 25,406 × g for 15 min and supernatants were collected. Subsequently, 1 mL of acetone/water (70:30, v/v) was added to the tubes and a new incubation and centrifugation step was performed in the same conditions. The two supernatants obtained after the centrifugation steps were placed in a 5 mL volumetric flask and the volume completed with distilled water. The extracts were used to analyze total phenolic compounds and chemical constituents.

Total phenolic compounds

Total phenolic compounds were found using the method proposed by Singleton *et al.*[23] For this, a 150 µL volume of the sample extract, 3,850 mL distilled water and 250 µL Folin-Ciocalteu were mixed in 15.0 mL falcon tube coated with aluminum foil and incubated at room temperature for 8 min. Then 750 µl of 20% sodium carbonate was added. After 2 h incubation, the samples were read at 765 nm and the data expressed as mg of gallic acid equivalent (AGE) 100 g^{-1} sample.

Chemical Profile of the Cagaita and Cagaita Ice Cream by paper spray ionization mass spectrometry (PS-MS)

The chemical constituents of the samples were identified as described in Silva *et al.*[2] by using an LCQ Fleet ion trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) with paper spray ionization (Figure 1). For this, the following experimental conditions were employed: mass range: 100 to 1000 *m/z*; PS-MS voltage: + 5.0 kV (positive ionization mode) and -3.0 kV (negative ionization mode); and capillary voltage of 40 V; tube lens voltage: 120 V.

Aliquots with 2 µL of the sample extracts were placed on the tip of a triangular-shaped chromatographic paper (1.5 cm dimensions) positioned 0.5 cm from the mass spectrometer inlet using a clamp attached to an XYZ platform. This clamp was connected to a high voltage source of the spectrometer by a copper wire. Subsequently, 40 µL of HPLC grade methanol was applied to the base of the triangular paper and the voltage source was switched on to obtain the mass spectra.

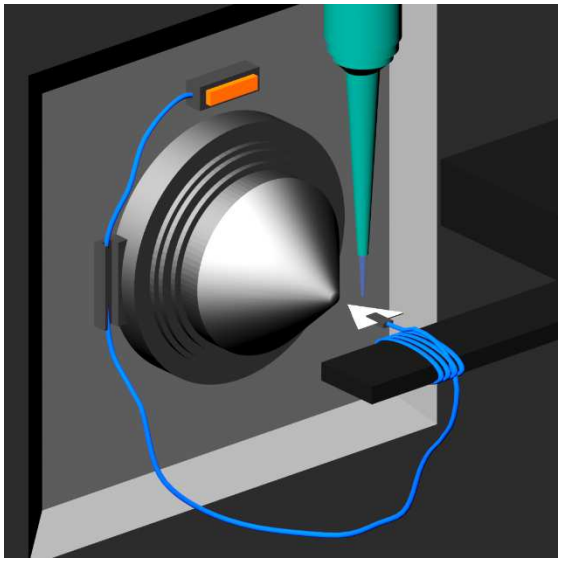

Figure 1. Ilustration of ionization source for paper spray.

Extraction and identification of volatile compounds

Headspace solid phase microextraction (HS-SPME) and volatile compound identification were performed as described by Silva *et al.*[24] For this, 0.5 g of the samples (cagaita and ice cream) were transferred to 20 mL vials, which were closed and placed inside aluminum blocks (8.5 × 10 cm). After a 5 min preheating step, the fibers were inserted into these vials and kept at 60 °C for 10 min and then taken to the GC/MS injector, remaining in that equipment for 5 min.

A gas chromatograph (Trace GC Ultra) equipped with a Polaris Q mass spectrometer from Thermo Scientific (San Jose, CA, USA) with an ion-trap analyzer equipped with a split/splitless injector was used in splitless mode. Helium gas (1 mL min^{-1} flow) and an HP-5 MS capillary column; 30 m × 0.25 mm × 0.25 μm (Agilent Techonolgies INC, Munich, Germany) were used. Oven temperatures were: 40 °C (1 min), subsequent heating to 110 °C (10 °C min^{-1}), then to 180 °C (15 °C min^{-1}). The conditions used in the mass spectrometer were: 35 to 300 *m/z* mass range, 70 eV electron impact ionization mode, 275 °C transfer line temperature, and 200 °C ion source temperature. The National Institute of Standards and Technology Research Library was used to identify detected volatile substances. The results obtained were also compared with the articles available in the literature.

Statistics

All experiments were performed in triplicate. The results of the total phenolic compounds content were evaluated by one-way ANOVA and Tukey test ($p < 0.05$) used to evaluate the means. Chromatograms for the analysis of volatile compounds were analyzed using the programs Xcalibur version 1.4 (Thermo Scientific, San Jose, CA, USA)[25] and Excel version 2013 (Microsoft, Redmond, WA, USA).[26]

Mass spectra were evaluated using Xcalibur version 2.1 software (Thermo Scientific, San Jose, CA, USA).[27] The Principal Component Analysis (PCA) model was built with data centered mean using MatLab version 7.9.0.529 software (Mathworks, Natick, MA, USA)[28] with the aid of PLS Toolbox version 5.2.2 (Eigenvectors Research, Manson, WA, USA).[29]

Results and Discussion

Volatile compounds profile

Table 1 shows the volatile compounds identified in samples of cagaita pulp and cagaita ice cream.

Twenty-one volatile compounds were identified in cagaita, including 19 using PA fibers and 20 using PDMS/DVB fiber. Of these, 19 compounds were also found in cagaita ice cream (PA: n=14; PDMS/DVB: n=18). The 3-carene monoterpene was the most abundant in both cagaita (PA = 14.47%, PDMS/DVB=32.74%) and cagaita ice cream (PA = 9.51%, PDMS/DVB = 23.49%). The higher percentage of 3-carene found when using the PDMS/DVB fiber may be because that this fiber has a semi-polar characteristic, which promoted a higher adsorption of this monoterpene in relation to the PA polar fiber. An increase of 4-penten-2-ol in ice cream over cagaita pulp was also observed when using PA fiber. This may be related to some effect by the ice cream mixture that provided a higher fiber adsorption.

Table 1. Relative composition (%) of volatile substances found in cagaita and cagaita ice cream using PDMS/DVB and PA fibers by SPME/GC-MS.

Nº	Volatile compounds	Class	PA fiber		PDMS/DVB fiber	
			Cagaita	Cagaita ice cream	Cagaita	Cagaita ice cream
1	4-Penten-2-ol	Alcohol	+	+	+	+
2	Ethyl butanoate	Ester	nd	nd	+	+
3	2-Buten-1-ol, 3-methyl-, acetate	Ester	nd	nd	+	+
4	4-Heptenoic acid, methyl ester, (E)-	Ester	+	nd	nd	nd
5	Pinene	Monoterpene	+	nd	+	+
6	Hexanoic acid ethyl ester	Ester	+	+	+	+
7	Eucalyptol	Monoterpene	+	nd	+	+
8	3-carene	Monoterpene	+	+	+	+
9	Ocimene	Monoterpene	+	+	+	+
10	Linalyl acetate	Monoterpene	+	+	+	+
11	Decanoic acid	Carboxylic acid	+	+	+	+
12	Hexanoic acid, 4-pentenyl ester	Ester	+	nd	+	+
13	Hexanoic acid, 3-methyl-2-butenyl ester	Ester	+	+	+	+
14	Caryophyllene	Sesquiterpene	+	+	+	nd
15	Dodecanoic acid, propyl ester	Ester	+	+	+	+
16	Humulene	Sesquiterpene	+	+	+	+
17	Muurolene	Sesquiterpene	+	+	+	+
18	Guaiene	Sesquiterpene	+	+	+	+
19	Cadinene	Sesquiterpene	+	+	+	+
20	Copaene	Sesquiterpene	+	+	+	+
21	Linalyl isobutanoate	Monoterpene	+	nd	+	nd

Table 1 shows that most of the compounds identified in cagaita were also found in cagaita ice cream, thus the manufacturing process did not cause large losses of these volatile substances in the product. The relative area of volatile compounds in ice cream generally decreased compared to cagaita pulp, which was expected because the manufacturing processing involves several steps such as homogenization of ingredients in a blender, agitation, and freezing. No articles were found

in the literature that determined volatile compounds in fruit ice cream or evaluated the effect of processing on these volatile substances.

The relative area of the chemical classes of volatile compounds found in the samples evaluated as a function of the fibers used are in Figure 2.

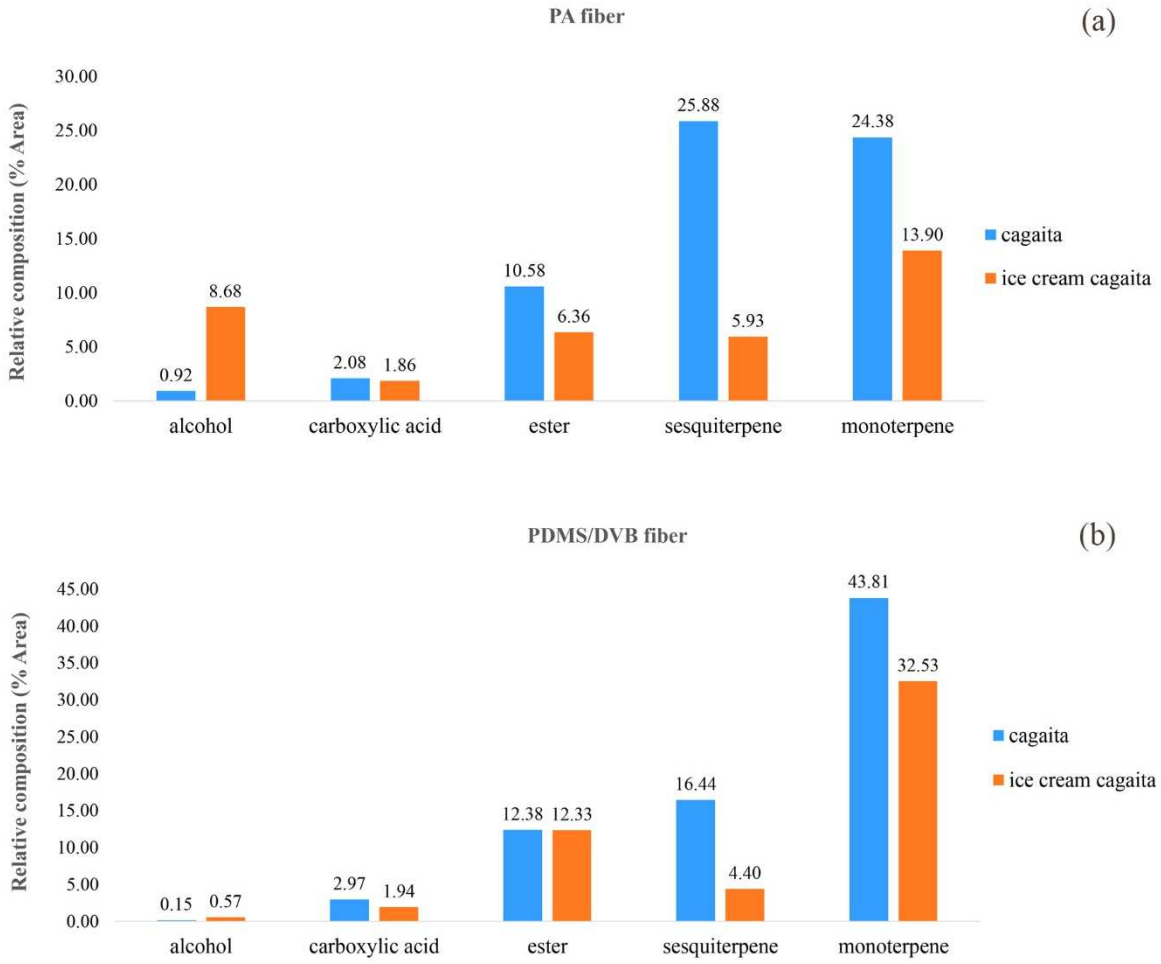

Figure 2. Relative area (%) of chemical classes of volatile compounds identified in cagaita and cagaita ice cream using (a) PA and (b) PDMS/DVB fibers by SPME/GC-MS.

The most evident change, when using PA fiber (Figure 2a) and PDMS/DVB fiber (Figure 2b), was the increase of alcohols and the reduction of monoterpenes and sesquiterpenes in the ice cream in relation to the cagaita pulp. These results are related to the interaction between the volatile compounds of cagaita and the ice cream mixture. In addition to the effect of processing,

the reduced percentage of terpene after product processing may have occurred due to greater interaction with the lipids in the ice cream mixture.

The cagaita pulp contained a predominance of terpenes in this study. These results are in agreement with the work done by Silva *et al.*[24] with cagaitas collected in the 2016 harvest in the municipality of Sete Lagoas, MG, which also observed a high proportion of monoterpenes (34.64%). The profile of volatile fruit compounds is known to be related to factors such as degree of ripeness, climate, and pre- and post-harvest handling.[30,31] No studies were found in the literature that evaluate changes in the relative composition of chemical classes of volatile compounds ice cream and other cold deserts.

The contents of total phenolic compounds obtained from two types of extractions investigated are presented in Table 2. The content of total phenolic compounds found in cagaita in the present study are in agreement with those reported in the literature, which ranged from 171.76 to 367.67 mg 100 g^{-1}.[2,32] The total phenolic content of the produced ice cream is also within the range described in the literature for ice creams made with various types of fruits as reported in the works of Vital *et al.*[7] (46 to 117 mg 100 g^{-1} GAE), Goraya and Bajwa[33] (81 to 257 mg 100 g^{-1} GAE), and Öztürk *et al.*[6] (7.5 to 65 mg 100 g^{-1} GAE).

Table 2. Average results of the content of total phenolic compounds of cagaita and cagaita ice cream.

Sample	Total phenolic compounds (mg GAE 100^{-1} g sample)
Cagaita	241.26 $\pm$ 4.27
cagaita ice cream	79.97 $\pm$ 1.44

GAE = Gallic acid equivalent

Considering that cagaita ice cream was produced with 40% fruit, a 17.13% loss occurred from the theoretical value expected in ice cream (96.50 mg GAE 100 g^{-1}). In previous works,

Goraya and Bajwa[33] evaluated the influence of the added alma (Indian gooseberry) pulp on the functional properties of ice cream. With the total phenolic values found in alma pulp (1.48 g 100 g^{-1} GAE) and in ice creams produced with 20% pulp (0.257 g 100 g^{-1} GAE), they observed a reduction of 13.18% in relation to the expected theoretical value (0.296 g 100 g^{-1} GAE). In another study, Vital *et al.*[7] when incorporating grape juice residue (2.5 to 10%) into ice cream, obtained losses of 38.36 to 54.20%.

Paper spray ionization mass spectrometry (PS-MS) fingerprints

Figure 3 shows the fingerprints (positive and negative ionization modes) of cagaita ice cream as well as the fragmentation spectra of some characteristic ions.

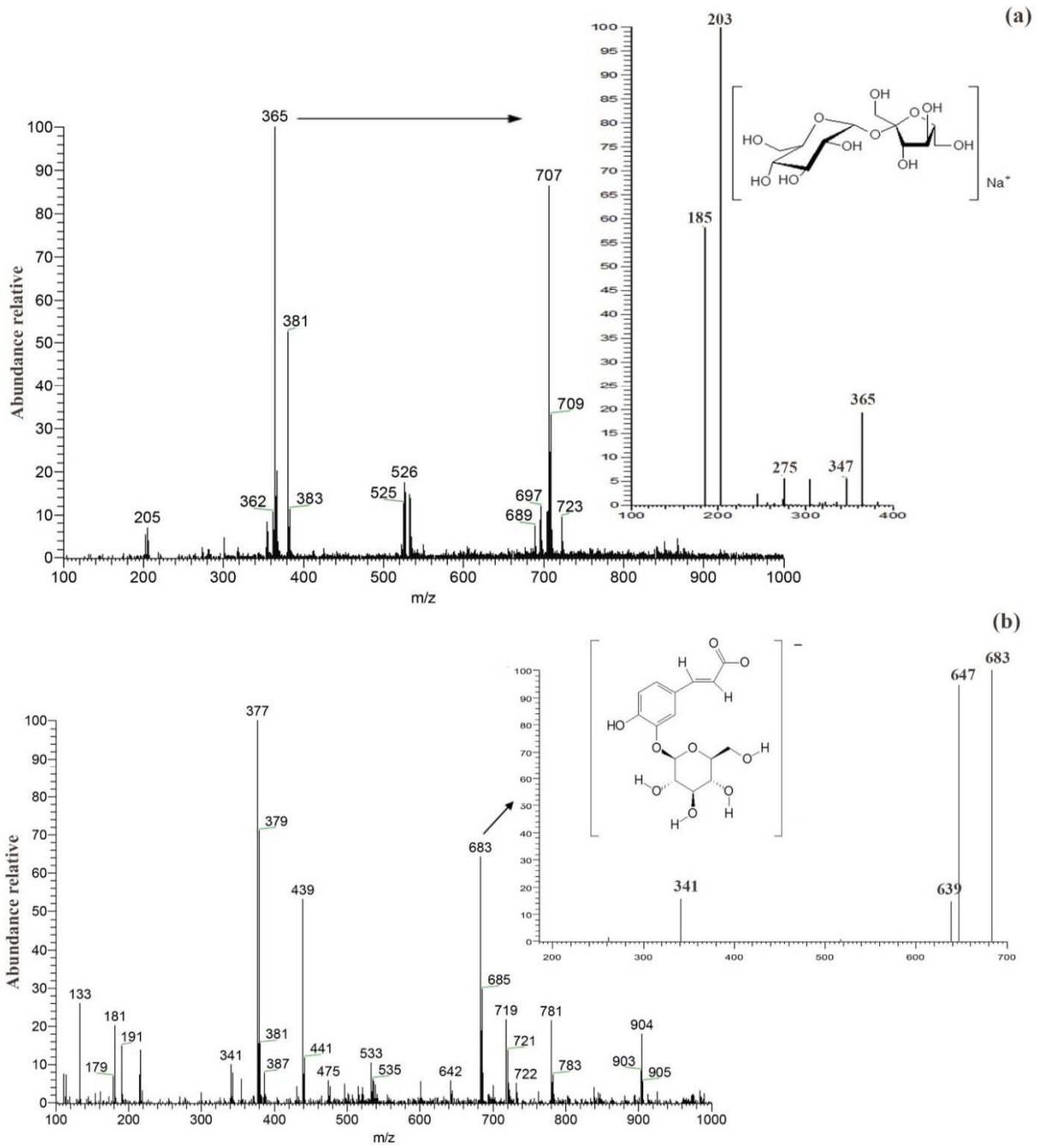

Figure 3. Representation of (a) PS(+)-MS and (b) PS(-)-MS of cagaita ice cream sample and MS/MS spectra of characteristic ions.

The PS(+)-MS identified 5 compounds of the flavone, anthocyanins, and sugars classes as in the form of sodium and potassium adducts (Table 3), which were also reported by Silva *et al.*[2] when employing PS-MS in cagaita collected in the city of Sete Lagoas, MG, Brazil.

Chrysoeriol (n = 1; 20% of the compounds) was the only compound found in cagaita that was not in the cagaita ice cream. Thus, 80% (n = 3) of the substances identified in the fruit remained in the product after manufacturing.

Table 3. Proposed assignments for ions detected in cagaita and cagaita ice cream by PS(+)-MS.

Nº	Tentative identification	*m/z*	MS/MS	Reference	Class	Cagaita	Cagaita ice cream
1	Chrysoeriol	301	258	Abu-Reidah *et al.*[34]	Flavone	+	nd
2	Sucrose	365	185, 203	Guo *et al.*[13]	Sugar	+	+
3	Sucrose	381	201, 219	Silva *et al.*,[2] Yuan *et al.*,[35] Asakawa and Hiraoka[36]	Sugar	+	+
4	Pelargonidin 3-rutinoside	579	271, 519	Silva *et al.*[37] Oliveira *et al.*[38]	Anthocyanin	+	+
5	[2 Sucrose + Na]$^+$	707	365	Furlan *et al.*[39]	Sugar	+	+

nd = not detected

The proposed classification for ions identified in PS(-)-MS is presented in Table 4. The 31 compounds found included organic acids, hydroxybenzoic acids, sugars, hydroxycinnamic acids, flavone, anthocyanins, and carboxylic acids. Among them, 13 (Compounds 1, 2, 5, 7, 8, 9, 10, 12, 15, 16, 17, 22, and 26) were also described by Silva *et al.*,[2] when evaluating the chemical profile of cagaitas in different microregion using the PCA and PS(-)-MS. The observed differentiation occurred due to 15 compounds.

For the compounds identified in PS(-)-MS, the processing resulted in a 22.58% loss (n = 7; pimelic acid, shikimic acid, galloyl glucose, chlorogenic acid, syringic acid hexoside, delphinidin 3-*O*-arabinoside, and delphinidin 3-*O*-glucoside).

Table 4. Assignments for the cagaita and cagaita ice cream ions detected by PS(-)-MS.

Nº	Tentative identification	*m/z*	MS/MS	Reference	Class	Cagaita	Cagaita ice cream
1	Malic acid	115	71	Wang *et al.*,[40] Silva *et al.*[2]	Organic acid	+	+
2	Malic acid	133	89, 115	Silva *et al.*[2]	Organic acid	+	+
3	Pimelic acid	159	97, 115, 141	Wang *et al.*[40]	Organic acid	+	nd
4	Shikimic acid	173	73, 111, 155	Wang *et al.*[40]	Hydroxybenzoic acids	+	nd
5	[Hexose + Cl]$^-$	179	71, 89	Wang *et al.*,[40] Silva *et al.*[2]	Sugar	+	+
6	[Hexose + Cl]$^-$	181	-	-	Sugar	+	+
7	Citric acid	191	85, 111	Wang *et al.*,[40] Silva *et al.*[2]	Organic acid	+	+
8	Hexose	215	71, 89, 179	Guo *et al.*,[13] Wang *et al.*,[40] Silva *et al.*[2]	Sugar	+	+
9	Palmitic acid	255	237	Wang *et al.*[40]	Fatty acid	+	+
10	Caftaric acid	311	133	Abu-Reidah *et al.*,[34] Silva *et al.*[2]	Hydroxycinnamic acids	+	+
11	*p*-Coumaric acid hexoside	325	119, 145	Aaby *et al.*,[41] Kajdžanoska *et al.*,[42] Silva *et al.*[2]	Hydroxycinnamic acids	+	+
12	Galloyl glucose	331	169	Ramirez *et al.*[43]	Hydroxybenzoic acids	+	nd
13	Conidendrin	355	337	Sanz *et al.*[44]	Lignin	+	+
14	Cafeoil-*D*-glucose	339	159	Silva *et al.*[2]	Hydroxycinnamic acids	+	+
15	Caffeoyl-glucose	341	179	Ramirez *et al.*[43]	Hydroxycinnamic acids	+	+
16	Chlorogenic acid	353	173, 179, 191	Koolen *et al.*,[45] Wang et *al.*[40]	Hydroxycinnamic acids	+	nd
17	Syringic acid hexoside	359	153, 197	Abu-Reidah *et al.*,[34] Silva *et al.*[2]	Hydroxybenzoic acids	+	nd
18	Hexose or sucrose	377	215, 341	Chen *et al.*,[46] Silva *et al.*[2]	Sugar	+	+
19	Vitexin	431	341	Wang *et al.*,[40] Silva *et al.*[2]	Flavones	+	+
20	Delphinidin 3-O-arabinoside	435	303	Junqueira-Gonçalves *et al.*[46]	Anthocyanin	+	nd
21	Icariside D1	439	403, 421	Jiao *et al.*[48]	Phenylpropanoid	+	+
22	Delphinidin 3-*O*-glucoside	465	303	Junqueira-Gonçalves *et al.*[47]	Anthocyanidin	+	nd
23	5-pyranopelargonidin-3-glucoside	501	339	Aaby *et al.*[41]	Anthocyanidin	+	+
24	Dicaffeoylquinic acid	515	173	Catarino *et al.*[49]	Hydroxycinnamic acids	+	+
25	Hexose	521	341	Silva *et al.*[2]	Sugar	+	+
26	5′-Methoxy-demethylpiperitol-4-*O*-glucoside	533	371	Simirgiotis *et al.*[50]	Other Phenolic Compounds	+	+
27	Coumaroyl iridoid isomer 1	535	311, 491	Mikulic-Petkovsek *et al.*[51]	Hydroxycinnamic acid	+	+
28	Lithospermic acid	537	493	Wang *et al.*[52]	Carboxylic acid	+	+
29	Caffeic acid hexoside dimer	683	341	Spínola *et al.*,[53] Silva *et al.*[2]	Hydroxycinnamic acids	+	+
30	Sinapic acid dihexoside hydroxy benzoyl	685	667	Silva *et al.*[37]	Phenylpropanoid	+	+
31	[Tetraose + Cl]$^-$	719	-	-	Sugar	+	+

nd = not detected

Thus, the overall calculation (positive and negative ionization mode) found that 77.78%, which corresponds to 23 of the compounds identified in the cagaita, remained in the processed ice cream. This loss of only 22.22% (n = 8) may be related to the steps of homogenizing the cagaita pulp with other ice cream ingredients as well as agitating and freezing in the ice cream maker during manufacturing.

No articles were found that evaluated changes in the profile of chemical constituents (organic acids, phenolic compounds, and other secondary metabolites) of fruits during ice cream manufacturing.

Principal Component Analysis (PCA)

Effect of processing on the chemical profile of samples

Figures 4 and 5 exhibit the PCA that demonstrate the effect of ice cream processing on the chemical profile of the cagaita pulp used. The resulting PCA model was able to explain 84.24% [(+)PS-MS] and 77.76% [(-)PS-MS] of total data variability.

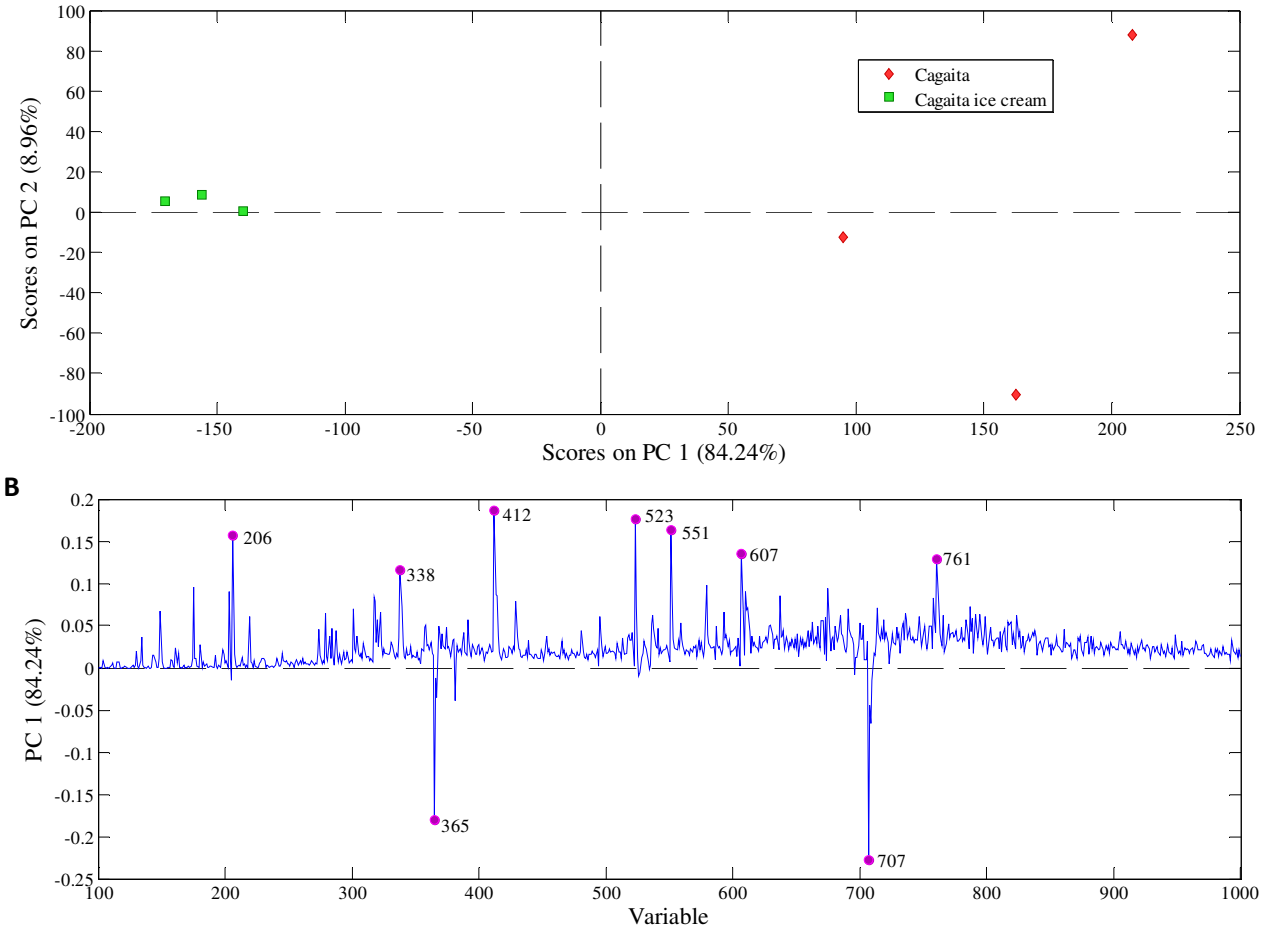

Figure 4. PC 1 and PC 2 scores (positive ionization mode).

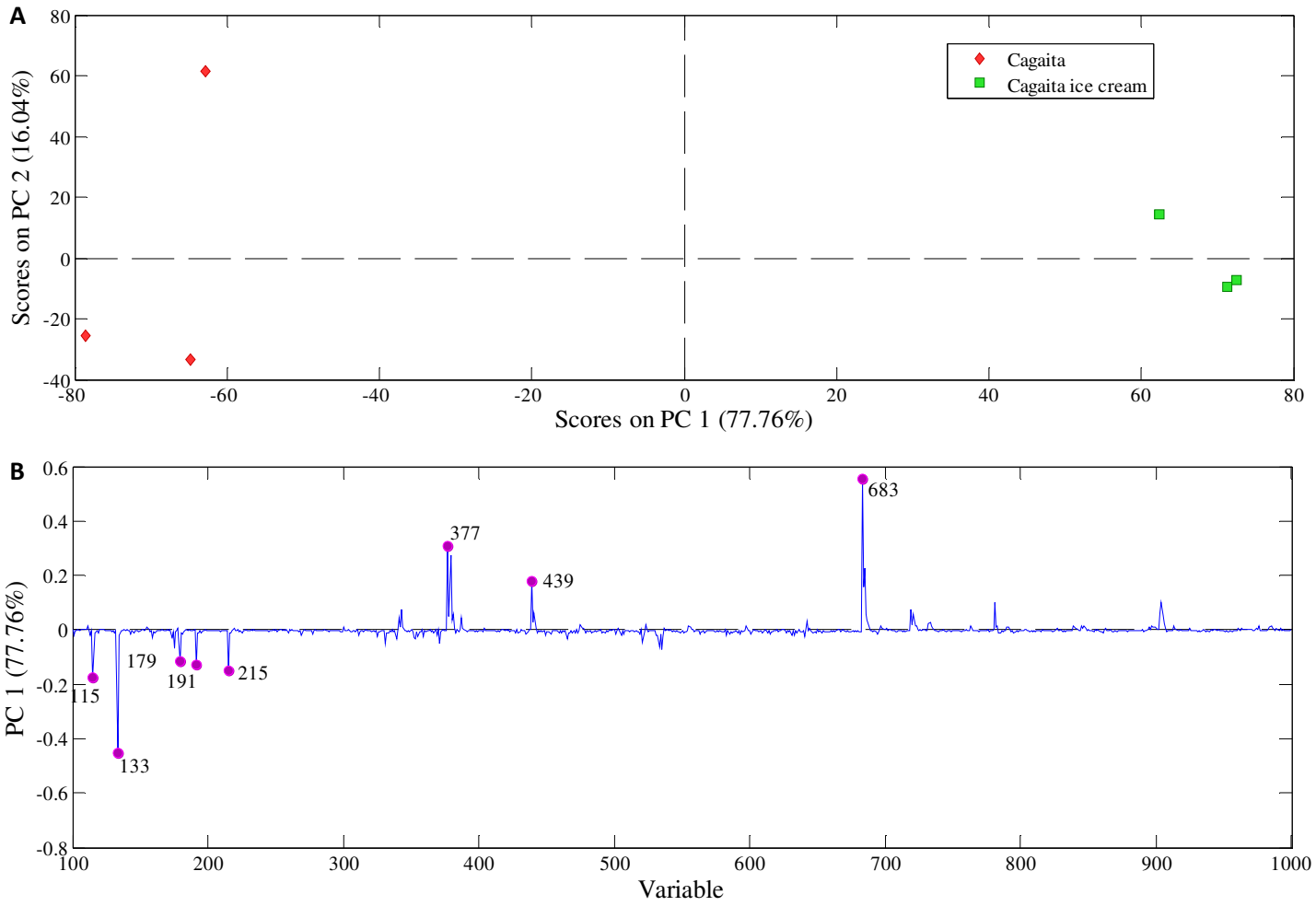

Figure 5. PC 1 and PC 2 scores (negative ionization mode).

PC 1 in the positive ionization mode (Figure 4a) recognized differences between the fruit (positive scores) and the cagaita ice cream (negative scores). Analysis of the weights of this component (Figure 4b) found that this differentiation of ice cream occurred due to the signals with *m/z* 365 and 707 related to sugars, while the cagaita differed as a function of the signals with *m/z* 206, 412, 523, and 551.

In the PCA, generated from the spectra obtained by PS(-)-MS presented in Figure 5, the composition of the cagaita ice cream (positive scores) differed from the cagaita pulp due to the ions with *m/z* 377, 439, and 683, while the pulp showed more intense signs with *m/z* 115, 179, 191, and 215.

No other articles were found that evaluated changes in the profile of compounds (organic acids, phenolic compounds, and other secondary metabolites) of fruits during ice cream manufacturing.

Conclusion

The SPME PA and PDMS/DVB fibers efficiently revealed the volatile compounds present in cagaita and cagaita ice cream. Most of the volatile compounds (90%) present in cagaita pulp were also found in ice cream, although in a smaller proportion, most of them belonging to the monoterpenes class. After processing the ice cream, a reduction of 10% in the content of total phenolic compounds was observed in relation to cagaita pulp. Fingerprints obtained from the evaluated samples found of 36 compounds, 28 of which were also present in the ice cream produced. Thus, PS-MS proved to be an adequate, simple, and fast technique to the determine the chemical profile of these food matrices by identifying the bioactive compounds of different chemical classes.

Supplementary Information

Supplementary Information is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Supplementary Information

Determination of chemical profile of *Eugenia dysenterica* ice cream using PS-MS and HS-/SPME/CG-MS

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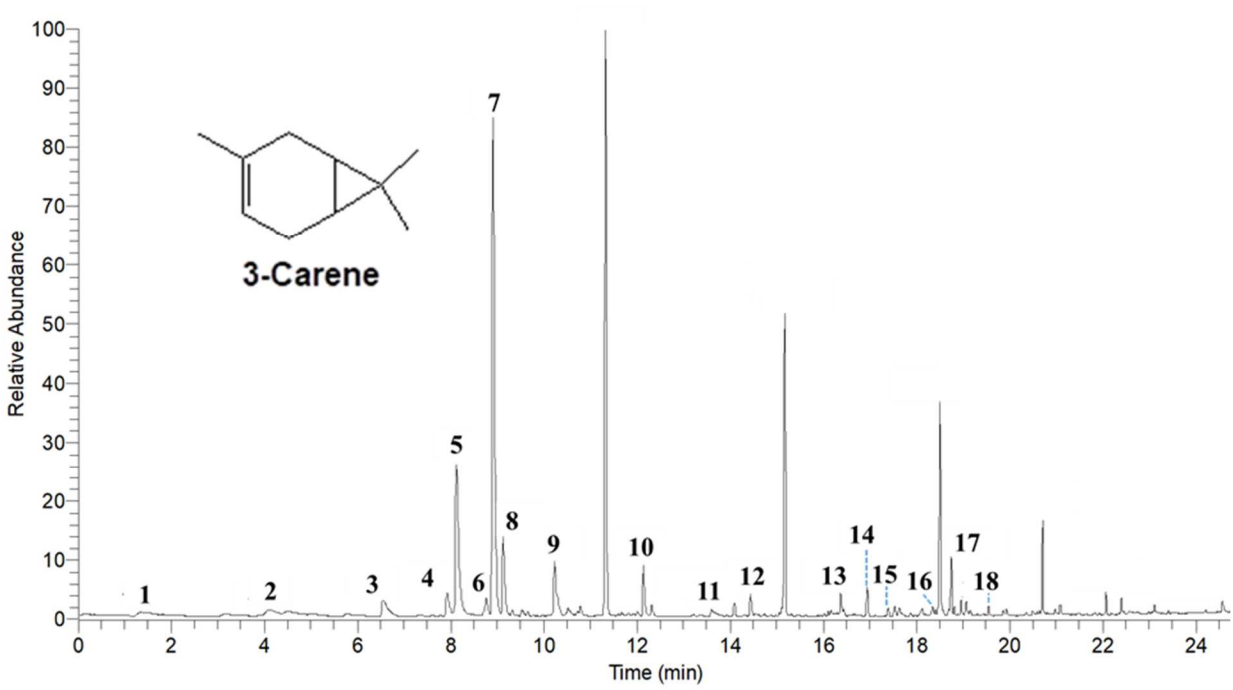

Figure S1. HS-SPME/GC-MS chromatograms from cagaita ice cream. Peaks: (1) 4-penten-2-ol; (2) ethyl butanoate; (3) 2-buten-1-ol, 3-methyl-, acetate; (4) pinene; (5) hexanoic acid ethyl ester; (6) Eucalyptol; (7) 3-carene; (8) ocimene; (9) linalyl acetate; (10) decanoic acid; (11) hexanoic acid, 4-pentenyl ester; (12) hexanoic acid, 3-methyl-2-butenyl ester; (13) dodecanoic acid, propyl ester; (14) humulene; (15) muurolene; (16) guaiene; (17) cadinene; (18) copaene.