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Optimization of extraction and identification of volatile compounds from *Myrciaria floribunda*[1]

Otimização da extração e identificação dos compostos voláteis de *Myrciaria floribunda*

Yesenia Mendoza García[2], Eurico Eduardo Pinto de Lemos[2], Rodinei Augusti[3] and Júlio Onésio Ferreira Melo[4]*

ABSTRACT – The composition of the volatile profile of rumberry fruits (*Myrciaria floribunda*) was determined using solid-phase microextraction in headspace mode and gas chromatography, coupled with mass spectrometry. The PA (*polyacrylate*) and DVB/CAR/PDMS (*divinylbenzene/carboxen/polydimethylsiloxane*) fibers were optimized for the extraction parameters (agitation, extraction time and temperature), in order to select the fiber with the highest number of isolated compounds. A total of 48 volatile compounds were identified using HS-SPME/GC-MS present in the ripe fruits of rumberry. The volatile compounds were classified into five chemical classes, the majority belonging to the sesquiterpenes class (71%). In addition, it was possible to verify that the fiber coated with *polyacrylate* (PA) had better performance, allowing for the extraction of a greater number of volatile compounds (n = 35). The extraction conditions that allowed the isolation of a greater number of volatile compounds corresponded to times greater than 26 minutes and temperatures above 85 °C, with agitation of 79 rpm for the PA fiber. Likewise, it was found that the hydrocarbon sesquiterpenes was the chemical class most present in the fruits, which is mainly related to the volatile profile of rumberry fruits.

Key words: Rumberry, Myrtaceae, Solid-phase microextraction.

RESUMO - Determinou-se a composição do perfil volátil de frutos de cambuizeiro (*Myrciaria floribunda*) utilizando-se microextração em fase sólida no modo headspace e cromatografia gasosa acoplada a espectrometria de massas. As fibras PA (*polyacrylate*) e DVB/CAR/PDMS (*divinylbenzene/carboxen/polydimethylsiloxane*) foram otimizadas quanto aos parâmetros de extração (agitação, tempo e temperatura de extração), a fim de selecionar a fibra com maior número de compostos isolados. Um total de 48 compostos voláteis foram identificados através do HS-SPME / GC-MS presentes nos frutos maduros de cambuí. Os compostos voláteis foram classificados em cinco classes químicas, sendo a maioria pertencente à classe dos sesquiterpenos (71 %). Além disso, foi possível verificar que a fibra revestida com poliacrilato (PA) teve melhor desempenho, permitindo a extração de um maior número de compostos voláteis (n = 35). As condições de extração que permitiram o isolamento de um maior número de compostos voláteis, corresponderam a tempos superiores de 26 min e temperaturas acima de 85 °C com agitação de 79 rpm, isto para a fibra PA. Da mesma forma, verificou-se que os sesquiterpenos hidrocarbonetos foi a classe química mais presente nos frutos, o que está relacionado principalmente ao perfil volátil dos frutos de cambuí.

Palavras-chave: Cambuí. Myrtaceae. Microextração em fase sólida.

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*Author for correspondence
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[1]Work extracted from the thesis of the first author, presented to the Graduate Program in Agronomy, Federal University of Alagoas/UFAL-AL
[2]Graduate Program in Agronomy-Plant Production, Center for Agricultural Sciences, Federal University of Alagoas, Maceió-AL, Brazil; jenny_thesiba@hotmail.com (ORCID ID 0000-0002-8833-259X), eurico@ceca.ufal. br (ORCID ID 0000-0002-0299-5676)
[3]Department of Chemistry, Federal University of Minas Gerais, Belo Horizonte-MG, Brazil; augusti.rodinei@gmail.com (ORCID ID 0000-0002-9448-9518)
[4]Department of Exact and Biological Sciences, Federal University of São João Del-Rei, Campus Sete Lagoas, Sete Lagoas-MG, Brazil; onesiomelo@gmail.com (ORCID ID 0000-0002-7483-0942)

INTRODUCTION

Among the many native species in Brazil, the Myrtaceae family stands out as one of the most important in neotropics; many phytosociological studies regard the family as having the highest species richness (SILVA; MAZINE, 2016).

The Myrtaceae family is found throughout the world, but is best adapted to tropical and subtropical climates (SOUZA; LORENZI, 2005). This family is most often found in Brazilian biomes, with 23 genera and 1,034 species, ranking eighth in diversity in the Northeast region (SOBRAL *et al*., 2015; SOBRAL; PROENÇA, 2006; STADNIK; OLIVEIRA; ROQUE, 2016).

Many of these species have a high economic value, as is the case with eucalyptus (*Eucalyptus* spp.), which is used in the production of wood and the production of aromas; and guava (*Psidium guajava*), appreciated mainly for the characteristics of its fruits, which can be consumed in nature or industrialized (CARVALHO *et al*., 2014).

Some other species in this family also produce fleshy fruits widely appreciated for being edible. However, few are exploited on a commercial scale; and, when exploited, production is very small and limited to certain regions, such as araçá (*Psidium cattleianum*), cagaita (*Eugenia dysenterica*), cambucá (*Plinia edulis* (Vell.), Cambuci (*Campomanesia phaea*), cambuí (*Myrciaria floribunda*), guabiroba (*Campomanesia xanthocarpa*), grumixama (*Eugenia brasiliensis* Lam.), Jabuticaba (*Myrciaria* spp. O. Berg), jambolão (*Syzygium cumini*), pitanga (*Eugenia uniflora* l. Cambess grape.) and the Cerrado pear (*Eugenia klotzchiana*) (BUENO *et al*., 2017; GUEDES *et al*., 2017; LORENZI *et al*., 2006).

Rumberry (*Myrciaria floribunda*), popularly known as "camboim," "jabuticabinha", "myrtle", "duke", "goiabarana" and "araçazeiro", is found across South America and Central America; in Brazil, it is found in an area ranging from Amazonia to the south of the country (LOZENZI *et al*., 2009; SOUZA; MORIM, 2008).

Rumberry is widely cultivated for its edible fruits, which have unique organoleptic characteristics, due to their high content of vitamin C and antioxidant action (PINHEIRO; ALMEIDA; SILVA, 2011; SILVA *et al*., 2012). They are relevant in the chemical composition of the essential oils of leaves, flowers and stems, in addition to being rich in phenolic compounds and having excellent biological functions, including antimicrobial, anticholinesterase, antioxidant, antitumor and insecticide (APEL *et al*., 2006; RAMOS *et al*., 2010; TIETBOHL *et al*., 2014).

Fruits are interesting for the fruit market, not only because of the technological potential they may have, but also because they can contribute to the diversification of the local cultivation of fruits, introducing new options for aromas and flavors to the market (MÜLLER *et al*., 2012).

The aroma, one of the most appreciated characteristics of fruits, consists of a complex mixture of volatile compounds belonging to various chemical classes (esters, acids, ketones, aldehydes, alcohols and terpenes), present in different concentrations and intensities, and are generally specific both for each species and for each variety (SINESIO *et al*., 2010; UEKANE; ROCHA-LEÃO; REZENDE, 2013).

The taste of fruits depends mainly on the perception of the mouth (sweetness, acidity or bitterness), as well as the smell produced by several of these volatile compounds, which determine the perception and acceptability of the products by consumers (EL HADI, 2013).

Given the above, there is a need to explore the chemical composition of rumberry fruits, so our objective was to optimize a method to determine the volatile profile of rumberry fruits, evaluating different extraction parameters (time, temperature and agitation) by solid-phase microextraction in mode headspace.

MATERIAL AND METHODS

Vegetal material

The rumberry fruits of a single access (AC132) of those available in the Active Germplasm Bank of Rumberry (BAG – Rumberry) were collected manually, stored in polyethylene bags and transported to the Laboratory of Plant Biotechnology in the Center for Agricultural Sciences at the Federal University of Alagoas (CECA/UFAL), located in the municipality of Rio Largo – AL (09º 28 '42 "S and 35º 51 '12" W) with an altitude of 127 m.

Approximately 250 g of rumberry fruits were washed and disinfected with 20 mL of sodium hypochlorite solution (200 ppm) for 5 minutes with water movement, followed by a second rinse with running water for 2 minutes. Then, the fruits were crushed with the help of a mixer, discarding only the seeds, and the pulp obtained was stored in a freezer at -60 °C until the time of analysis.

Extraction of volatile compounds

The frozen samples were transported to the Mass Spectrometry Laboratory of the Department of Chemistry at the Federal University of Minas Gerais (UFMG). For the extraction of volatile compounds, the *polyacrylate* (PA) polar fiber (85 μm) and the *divinylbenzene/carboxyne/polydimethylsiloxane* (DVB/CAR/PDMS) semi-polar fiber (50/30 μm) were used, using the solid-phase-headspace microextraction method (SPME-HS).

We weighed 0.5 g of pulp from rumberry and put it in bottles with a capacity of 20 mL, which were closed with an aluminum sell and a rubber septum (GARCÍA *et al.*, 2019), to optimize the conditions of extraction of volatile organic compounds (VOC). The matrices were subjected to the experimental conditions established in the experimental design.

Experimental planning

The central composite design (CCD) was used, consisting of a factorial system 2^3 (three factors on two levels), with 5 central points and 6 axial points, totaling 19 tests. The independent variables were extraction time, adsorption temperature and agitation, as shown in Table 1. Statistical analyses were performed using the software Statistica v.10 (Stat-Soft Inc., Tulsa, USA) (STATSOFT, 2011).

Gas chromatography coupled to mass spectrometry

The analysis of VOCs was carried out by means of a gas chromatograph (Trace GC Ultra) coupled to mass spectrometry (Polaris Q) (GC-MS), with an "ion trap" analyzer (Thermo Scientific, San José, CAUSE.). The separation was carried out on an HP-5 MS capillary column (5% phenyl and 95% methylpolysiloxane), 30 m wide, 0.25 mm internal diameter, 0.25 μm film thickness and helium as a carrier gas, with a constant flow of 1 mL per minute. 1 (Agilent Techonolgies Inc, Germany). The injector temperature was 250 °C in splitless mode, with a time of 5 minutes; the temperature of the ion source was 200 ºC and the temperature of the interface was 270 ºC. If you use the next hourly schedule: start at 40 ºC, stay 5 minutes at a heating speed of 2.5 ºC min-1 to 125 ºC and temperature from 10 ºC min-1 to 245 ºC; for the temperature thereafter, keep the isotherm for 3 minutes (GARCÍA *et al.*, 2019).

For the identification of VOCs, the retention indices of each peak of the chromatogram were compared with the mass spectra obtained by electron impact ionization (EI) at 70 eV, and the fullscan scanning range from 50 to 300 m/z. The peaks of the chromatogram that had an area greater than 0.15% and a similarity level (RSI) greater than 500 were considered, which were compared with the data obtained by the NIST library (National Institute of Standards and Technology). Peak areas were obtained from the Xcalibur 1.4 program from Thermo Electron Corporation (Thermo Electron, San Jose, CA, USA.), and transferred to Microsoft Excel 2013 (GARCÍA *et al.*, 2016, 2019).

Table 1 – Variables used in factorial design 2^3 with central component for the optimization of the HS-SPME conditions of the volatile substances from rumberry

Variables	Levels of variation		
	-1	0	+1
Extraction time (min)	10	20	30
Adsorption temperature (°C)	30	65	100
Agitation (rpm)	0	50	100

RESULTS AND DISCUSSION

To determine the ideal conditions for the extraction of VOCs for HS-SPME, the optimization of the factorial system 2^3 was carried out, thus evaluating the effect of agitation, temperature, and extraction time for each of the tested fibers.

The solid-phase microextraction fibers (SPME), PA (polar) and DVB/CAR/PDMS (semipolar) were evaluated and compared individually according to the sum of the peak areas obtained in the chromatograms of the 19 tests, in such a way that a central composite design (CCD) was applied.

Table 2 shows the relative areas (%) of the volatile compounds isolated for each of the SPME fibers. It is observed that the fiber with polyacrylate (PA) coating had better efficiency having the largest chromatographic area when the rumberry samples were submitted to 85 °C and 79 rpm of agitation for 26 minutes.

Studies by Silva *et al.* (2019), in which they evaluated five types of SPME fibers (PA, CAR/PDMS, PDMS/DVB, DVB/CAR/PDMS and CW/DVB) showed that the PA fiber was the most effective when submitting the cagaita samples to 45 °C, with agitation of 50 rpm for 30 minutes. On the other hand, in García *et al*. (2019), where they optimized a methodology by HS-SPME-GC-MS for the study of VOCs from Acerola, reported better results when exposing the PA fiber to temperatures above 65 °C for 20 minutes.

With the above, the efficiency of SPME fibers for the extraction of volatiles in fruits belonging to the Myrtaceae family is demonstrated. However, none of these studies reported the extraction and identification of volatile substances in fruits of rumberry.

Thus, Pareto plots (Figure 1) were generated with a 95% confidence limit, which show the influence of independent variables on the response variable, as well as their respective interactions.

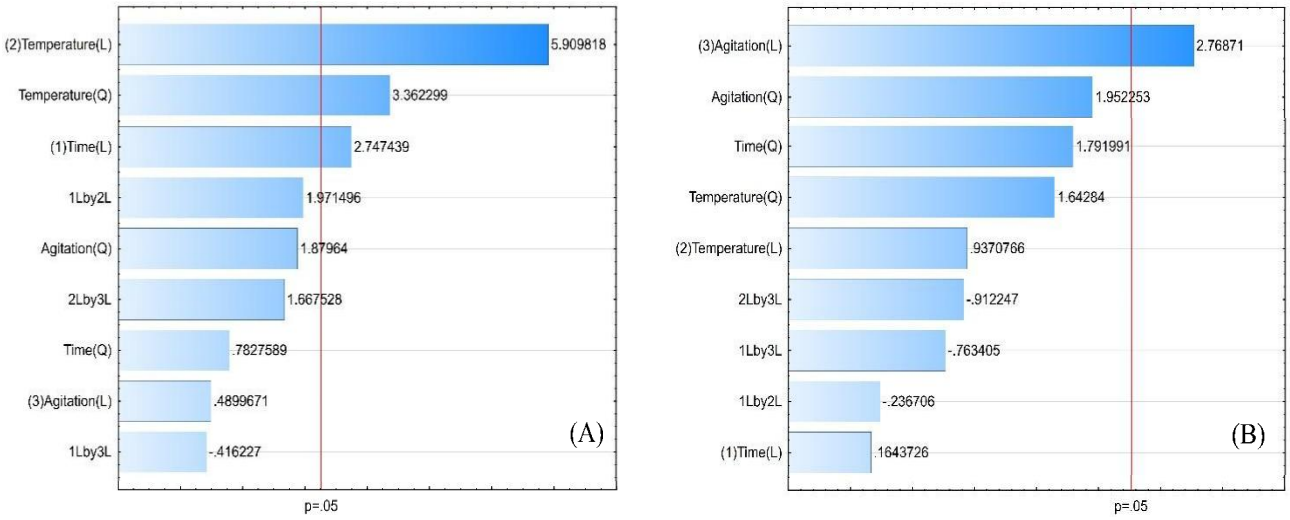
Note that the independent variables, extraction time and temperature, showed a significant effect only for the PA fiber. An increase in these two variables allowed for greater extraction and, consequently, the identification of a greater number of volatile compounds.

Table 2 – 2^3 factorial design matrix and CCD in response to the sum of the chromatographic peaks obtained for each fiber by the HS-SPME-GC-MS method

Assay	Factors			Response Variables	
	Time (min)	Temperature (ºC)	Agitation (rpm)	PA (%)	DVB/CAR/PDMS (%)
1	14	45	21	130660658	5712883,925
2	26	45	21	197421957	7884439,133
3	14	85	21	171669590	18991038
4	26	85	21	473628360	14316985,9
5	14	45	79	72949751	23487624,8
6	26	45	79	137765497	14074138,76
7	14	85	79	318966807	23841402,9
8	26	85	79	540193684	17013588,8
9	10	65	50	85949619	2763265,76
10	30	65	50	124736784	15844343
11	20	30	50	76694399	8766278,08
12	20	100	50	415749747	8365294,01
13	20	65	0	155937458	165076,265
14	20	65	100	174558957	20028526,6
15*	20	65	50	130723212	2107432,83
16*	20	65	50	116735828	9760817,99
17*	20	65	50	91195137	1145328,51
18*	20	65	50	106424019	3496468,34
19*	20	65	50	105421098	3797181,45

SPME fibers: *divinylbenzene/carboxen/polydimethylsiloxane* (DVB/CAR/PDMS) and *polyacrylate* (PA). * Central points

Figure 1 – Pareto graph of the PA (a) and DVB/CAR/PDMS (b) fibers, in relation to the partial area of the analyzed chromatograms of the fruits of rumberry



However, only one independent variable (agitation) showed an influence on the extraction of volatile compounds directly with a positive effect for the DVB/CAR/PDMS fiber, which presented a linear model (indicated by the letter L). The other factors had no significant effect, for a level of 5% of significance.

The effects of the significant variables for both fibers are shown in Figure 2. It is noted in Figures 2 (a) and 2 (c) that the longer the extraction time and temperature (time > 20 minutes and temperature > 90 °C), the greater the number of identified VOCs. Thus, the best extraction combination is obtained at 26 minutes at 85 °C and 79 rpm, which refers to assay no. 8, since it was the one that obtained the highest value in the sum of the areas of the chromatographic peaks (Table 2).

Figures 2 (b) and 2 (d) show that, at higher revolutions per minute (agitation > 60 ℃) and high temperature values, better results are obtained in the extraction of volatile compounds, which refers to assay no. 7 (Table 2).

Thus, comparing both fibers, it was found that the extraction time was an important factor in the extraction of VOCs from the fruits of rumberry, as shown in assays 7 and 8 of Table 2; and the fiber DVB/CAR/PDMS, when exposed for shorter times (< 14 minutes) extracted fewer VOCs compared to PA fiber, which required longer times (> 25 minutes) to isolate a greater number of VOCs.

This result shows that the use of higher temperatures, times or agitations will not always allow the detection of a greater number of volatile substances, as these parameters are influenced according to the type of fiber used.

Once the extraction conditions were determined, the analysis of the volatile compounds present in the rumberry samples was carried out. We identified 48 compounds belonging to different chemical classes, with sesquiterpene hydrocarbons being the most abundant (54%), followed by oxygenated monoterpenes (19%), oxygenated sesquiterpenes (17%) and monoterpene hydrocarbons (10%) (Table 3).

Figure 2 – Response surfaces and contour curves obtained from the CCD for the variables of temperature and time of extraction through Fiber PA (a, c) and temperature of extraction and agitation through Fiber DVB/CAR/PDMS (b, d)

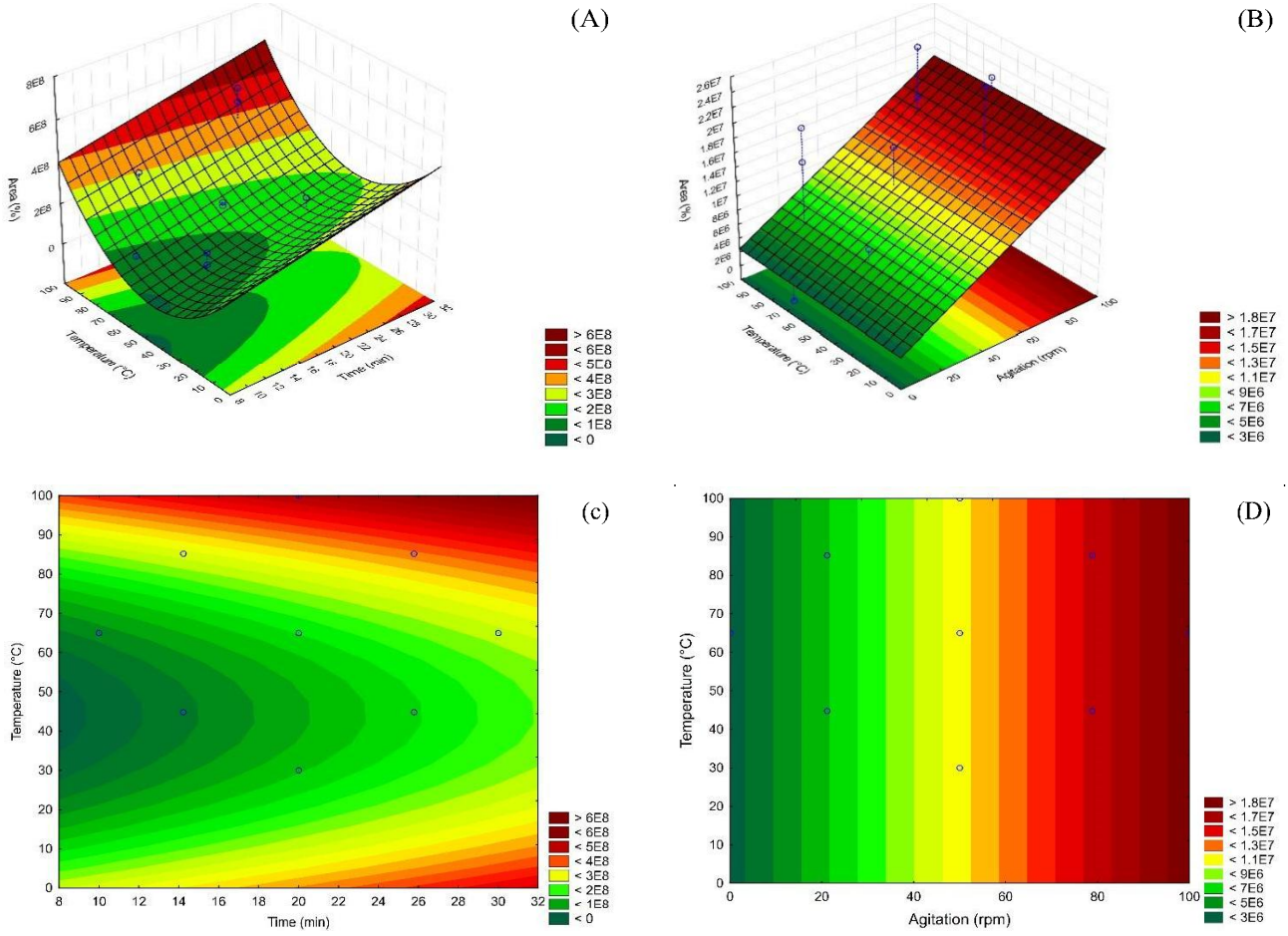


Table 3 – Volatile compounds detected in rumberry fruits, using HS-SPME/GC-MS

Nº.	Classification	COVsa	Formula	CAS	SPME fibers	
					PA	DVB/CAR/PDMS
1.	Oxygenated monoterpenes	Sabinene hydrate	$C_{10}H_{18}O$	15537-55-0		X
2.		endo-Keto Borneol	$C_{10}H_{16}O_2$	114529-11-2	x	
3.		6-Camphenol	$C_{10}H_{16}O$	55925-49-0		X
4.		cis-Crisantenol	$C_{10}H_{16}O$	55722-60-6		X
5.		Dihydrocarbyl acetate	$C_{12}H_{20}O_2$	20777-49-5	x	
6.		Eucalyptus	$C_{10}H_{18}O$	470-82-6		X
7.		cis-p-Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	22771-44-4		X
8.		Linalyl propionate	$C_{13}H_{22}O_2$	144-39-8	x	
9.		α-campholenaldehyde	$C_{10}H_{16}O$	4501-58-0	x	
10.	Monoterpene hydrocarbons	Carene	$C_{10}H_{16}$	13466-78-9	x	X
11.		Sylvestrene	$C_{10}H_{16}$	1461-27-4	x	
12.		α-Fenchene	$C_{10}H_{16}$	7378-37-2	x	
13.		α-Pinene[1,3]	$C_{10}H_{16}$	2437-95-8	x	X
14.		β-Terpinene[2]	$C_{10}H_{16}$	99-84-3	x	
15.	Oxygenated sesquiterpenes	Agarospirol	$C_{15}H_{26}O$	23811-08-7	x	
16.		Carotol	$C_{15}H_{26}O$	465-28-1	x	
17.		Cubebol	$C_{15}H_{26}O$	23445-02-5	x	X
18.		Globulol[2]	$C_{15}H_{26}O$	489-41-8		X
19.		Guaiol	$C_{15}H_{26}O$	489-86-1	x	
20.		Terpinen-4-ol acetate	$C_{12}H_{20}O_2$	4821-04-9	x	
21.		Viridiflorol	$C_{15}H_{24}O$	21747-46-6	x	X
22.		γ-Eudesmol[2]	$C_{15}H_{26}O$	1209-71-8	x	
23.	Sesquiterpene hydrocarbons	Aromadendene	$C_{15}H_{24}$	109119-91-7		X
24.		Allo-aromadendene[2]	$C_{15}H_{24}$	25246-27-9		X
25.		Cadina-1 (10), 4-diene	$C_{15}H_{24}$	16729-01-4	x	
26.		Caryophyllene	$C_{15}H_{24}$	13877-93-5	x	X
27.		Cyperene	$C_{15}H_{24}$	2387-78-2	x	
28.		Epizonarene	$C_{15}H_{24}$	5975-30-4	x	
29.		Germacrene D	$C_{15}H_{24}$	37839-63-7		X
30.		Longifolene	$C_{15}H_{24}$	475-20-7	x	
31.		Patchoulene	$C_{15}H_{24}$	1405-16-9	x	
32.		Presilphiperfol-7-ene	$C_{15}H_{24}$	80931-09-5	x	
33.		Sativene	$C_{15}H_{24}$	3650-28-0	x	
34.		Valencene[1]	$C_{15}H_{24}$	4630-07-3	x	
35.		α-Cubebene	$C_{15}H_{24}$	17699-14-8	x	X
36.		α-Guaiene[3]	$C_{15}H_{24}$	53863-54-0	x	X
37.		α-Gurjunene[2]	$C_{15}H_{24}$	489-40-7		X
38.		α-Muurolene	$C_{15}H_{24}$	10208-80-7		X
39.		α-Copaene	$C_{15}H_{24}$	3856-25-5		X
40.		Calarene	$C_{15}H_{24}$	17334-55-3	x	
41.		β-Selinene[1,2,3]	$C_{15}H_{24}$	17066-67-0	x	X

Continuation table 3

42.	γ-Cadinene[2]	$C_{15}H_{24}$	5957-55-1	x	X
43.	γ-Elemene	$C_{15}H_{24}$	370572-92-2	x	
44.	γ-Gurjunene	$C_{15}H_{24}$	22567-17-5	x	X
45.	γ-Muurolene[2]	$C_{15}H_{24}$	24268-39-1	x	X
46.	γ-Himachalene[1]	$C_{15}H_{24}$	53111-25-4	x	X
47.	δ-Cadinene[2]	$C_{15}H_{24}$	483-76-1	x	X
48.	δ-Selinene	$C_{15}H_{24}$	28624-23-9		X

[a] Compounds identified by comparing their mass spectra and retention indices according to the NIST library (2007); SPME fibers: *divinylbenzene/carboxen/polydimethylsiloxane* (DVB/CAR/PDMS) and *polyacrylate* (PA); compounds identified by other authors in different parts of the *M. floribunda* plant: [1] Oliveira *et al*. (2018), (lyophilized fruits); [2] Ramos *et al*. (2010) (fresh leaves); [3] Tietbohl *et al*. (2012), (leaves, stems and flowers); CAS: unique numerical identifier for chemical compounds

These results are similar to those obtained in fruits such as *Campomanesia adamantium* (Gabiroba), *Eugenia dysenterica* (cagaita) and *Myrciaria dubia* (camu-camu), in which the volatile composition is mainly represented by the chemical group of sesquiterpenes (OLIVEIRA *et al*., 2016; SILVA *et al*., 2019).

Some volatile compounds found in the present study have already been previously identified by other authors, among which are α-pinene, β-terpinene, globulol, γ-eudesmol, Valencian, α-guaiene, α-gurjunen, β-selinen, cadinene and himachalene (OLIVEIRA *et al*., 2018; RAMOS *et al*., 2010; TIETBOHL *et al*., 2012). However, a large part of these compounds had not yet been identified in rumberry pulp.

Of the compounds detected, 13 were similar between both fibers, the most representative being carene, caryophyllene, cubebol, viridiflorene, α-cubebene, and γ-gurjunene because there are no reports in the literature on these compounds.

Most of the volatile substances identified in the present study corresponded to the class of hydrocarbon sesquiterpenes. These results corroborate studies carried out on the chemical composition of the volatile oil of four species of Mirtaceae, in which the presence of the groups cariolifeno, aromadendreno and germacreno were detected (APEL *et al*., 2006).

On the contrary, studies by Mehta *et al*. (2018), where they determined the VOCs of jambolan (*Syzygium cumini* L.) fruits in three maturation stages, concluded that the main compounds that grant the aromatic characteristics to the fruit were karyophylene, δ-cadinene and α-fenchene.

Ramos *et al*. (2010) and Tietbohl *et al*. (2012, 2014), when isolating the essential oil from different parts of the *M. floribunda* plant (leaves, stems and flowers), mentioned the class of monoterpenes and sesquiterpenes as the most important components. Similarly, the group of hydrocarbon sesquiterpenes (40.6%) was found in greater amounts in lyophilized fruits of rumberry, of which (through the CAR/DVB/PDMS fiber) α-pinene, longicycline and α-humulene were also reported in the present study (OLIVEIRA *et al*., 2018).

CONCLUSIONS

1. The application of the HS-SPME-GC-MS method proved to be satisfactory for the extraction of VOCs from rumberry fruits;
2. The methodology used to evaluate the efficiency of the fibers showed that the PA fiber is the most suitable for the extraction of VOCs from rumberry fruits, since it extracted a greater number of compounds;
3. It was also found that the technique is effective and adequate at temperatures above 85 °C for 26 minutes and 79 rpm, thus extracting compounds belonging mainly to the class of sesquiterpenic hydrocarbons, such as δ-cadinene, γ-himachalene, γ-muurolene, γ-gurjunen, γ-cadinene, β-selene, α-guaiene, α-cubebene and caryophyllene, which were detected by both fibers.

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