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Otimização das condições de extração dos compostos voláteis da casca de pequi *(Caryocar brasiliense Camb.)* utilizando HS-SPME

Optimization of extraction conditions of volatile compounds from pequi peel *(Caryocar brasiliense Camb.)* using HS-SPME

Optimización de las condiciones para extraer compuestos volátiles de la corteza de pequi (*Caryocar brasiliense Camb*.) utilizando HS-SPME

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Resumo

O pequi é uma espécie nativa do cerrado brasileiro que possui grande importância econômica para a população da região. O pericarpo é considerado um resíduo agroindustrial, apesar de corresponder a aproximadamente 80% da massa total do fruto. Informações sobre a

composição química do pericarpo permitirão uma melhor utilização dessa porção da fruta. O objetivo deste estudo foi identificar as melhores condições de agitação, tempo de extração e temperatura de extração para maximizar a extração de compostos orgânicos voláteis presentes no pericarpo de pequi (Caryocar brasiliense Camb.), utilizando dois tipos de fibras de microextração em fase sólida. A extração dos compostos voláteis foi realizada pelo método de microextração em fase sólida do headspace com subsequente separação e identificação por CG-MS. A fibra de polidimetilsiloxano/divinilbenzeno (PDMS/DVB) foi estatisticamente mais eficiente do que a fibra de divinilbenzeno/carboxen/polidimetilsiloxano (DVB/CAR/PDMS). As duas fibras possibilitaram a extração e identificação de 35 compostos, principalmente terpenos (65,71%) e ésteres (14,29%). Um aumento na temperatura e tempo de extração permitiu maior extração dos compostos voláteis pelas duas fibras. Entretanto, em relação à agitação, a melhor condição para o uso da fibra DVB/CAR/PDMS foi a 100 rpm, enquanto a agitação não foi necessária para uma extração eficiente usando a fibra PDMS/DVB.

Palavras-chave: Pericarpo de pequi; Resíduo agroindustrial; Microextração em fase sólida em *headspace;* Compostos orgânicos voláteis.

Abstract

The pequi is a native specie from the Brazilian Cerrado that has great economic importance for the population of the region. The pericarp is considered an agroindustrial residue despite corresponding to approximately 80% of the total fruit mass. Informations about the chemical composition of pericarp would allow better utilization of this portion of the fruit. The objective of this study was to identify the best conditions of agitation, extraction time and extraction temperature to maximize the extraction of volatile organic compounds present in pequi pericarp (*Caryocar brasiliense* Camb.) using two types of solid phase microextraction fibers. The extraction of the volatile compounds was using the headspace solid-phase microextraction method with subsequent separation and identification by CG-MS. Polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was statistically more efficient than divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber. The both fibers enabled the extraction and identification of 35 compounds, mainly terpenes (65.71%) and esters (14.29%). An increase in the extraction temperature and time allowed greater extraction of volatile compounds by both fibers. However, in relation to agitation, the best condition for using DVB/CAR/PDMS was 100 rpm, while agitation was not necessary for an efficient extraction using the PDMS/DVB fiber.

Key words: Pequi pericarp; Agroindustrial residue; Headspace solid-phase microextraction; Volatile organic compounds.

Resumen

Pequi es una especie nativa del cerrado brasileño que tiene una gran importancia económica para la población de la región. El pericarpio se considera un residuo agroindustrial, aunque corresponde aproximadamente al 80% de la masa total de la fruta. La información sobre la composición química del pericarpio permitirá un mejor uso de esa porción de la fruta. El objetivo de este estudio fue identificar las mejores condiciones de agitación, tiempo de extracción y temperatura de extracción para maximizar la extracción de compuestos orgánicos volátiles presentes en el pericarpio de pequi (Caryocar brasiliense Camb.), Utilizando dos tipos de fibras de microextracción en fase sólida. La extracción de compuestos volátiles se llevó a cabo mediante el método de microextracción en fase sólida del espacio de cabeza con posterior separación e identificación por CG-MS. La fibra de polidimetilsiloxano / divinilbenceno (PDMS / DVB) fue estadísticamente más eficiente que la fibra de divinilbenceno / carboxeno / polidimetilsiloxano (DVB / CAR / PDMS). Las dos fibras permitieron extraer e identificar 35 compuestos, principalmente terpenos (65.71%) y ésteres (14.29%). Un aumento en la temperatura y el tiempo de extracción permitieron una mayor extracción de compuestos volátiles por las dos fibras. Sin embargo, en relación con la agitación, la mejor condición para usar la fibra DVB / CAR / PDMS fue a 100 rpm, mientras que la agitación no fue necesaria para una extracción eficiente usando la fibra PDMS / DVB.

Palabras clave: Pequi pericarpio; Residuos agroindustriales; Microextracción en fase sólida en el espacio superior; Compuestos orgánicos volátiles.

1. Introduction

The Brazilian Cerrado is one of the largest and most important biomes in South America, especially because of its wide variety of climates and soils and rich biodiversity of fauna and flora. This biome has various native species with peculiar characteristics, varied shapes, attractive colors, and uncommon flavors, which have great potential for agricultural and technological uses. The fruits are nutritious and commonly used in the popular diet, being generally consumed raw or in the form of juice, liqueur, ice cream, jam and other types of sweets (Ribeiro, et al., 2014; Silva & Fonseca, 2016).

A fruit called pequi (*Caryocar brasiliense* Camb.) stands among the several cerrado native fruits. Pequi is typically cultivated in the south-east, north-east and central regions of Brazil and constitutes an important source of food and income for the residents of the Cerrado region (Machado, et al., 2015; Monteiro, et al., 2015). Pequi fruit is composed of endocarp (edible portion of the fruit), pericarp (peel) and seed. The endocarp and the pericarp are rich in dietary fiber, carotenoids and polyphenols associated with high antioxidant capacity (Amorim, et al., 2016; Pinheiro, et al., 2018; Ribeiro, et al., 2014). Since the edible part of pequi is the endocarp, the pericarp, that corresponds to approximately 80% of the total fruit mass, becomes an agroindustrial residue and is usually discarded during pequi processing. Information on pericarp chemical composition is rather scarce and its proper characterization can provide valuable information to allow better utilization of this residue (Leão, et al., 2017).

The characteristic aroma and flavor are some of the most important quality criteria for fruit and its by-products, being decisive in the selection, acceptance and food intake (Ferreira, et al., 2016; Queiroga, et al., 2005). These characteristics are formed by a mixture of volatile substances, manly esters, aldehydes, alcohols, and terpenes (García, et al., 2019). Many extraction methods have been used to obtain volatile compounds such as the headspace solid-phase microextraction (HS-SPME). This technique is very simple, requires minimal sample treatment, provides high sensitivity, does not require solvent and it is low cost (Junior, et al., 2011; Setkova, et al., 2007; Silva, et al., 2019). HS-SPME is based on the extraction of analytes using a silica fiber coated with an sorbent layer, with subsequent thermal desorption of the analytes in the chromatographic system (Aguirre-Gonzalez, et al., 2011; Milhome, et al., 2011). The sensitivity of this technique depends on the type of fiber selected, agitation of the sample, extraction duration and temperature, among other factors (Aguirre-Gonzalez, et al., 2011; Silva, et al., 2019; Zacaroni, et al., 2017).

There are no studies in the literature about the optimization of extraction conditions of volatile compounds from pequi pericarp by the solid-phase microextraction (SPME) method, and only one study about the pequi endocarp. Belo et al. (2013) conducted a study to optimize a pequi endocarp volatile compound extraction method and evaluated the extraction capacity of polydimethylsiloxane/divinylbenzene (PDMS/DVB), polydimethylsiloxane (PDMS) and carboxen/polydimethylsiloxane (CAR/PDMS) fibers. According to the results, PDMS/DVB presented the best efficiency for volatile compound extraction.

In this context, the objective of the study was to determine the best conditions to maximize the extraction of volatile organic compounds (VOCs) of pequi pericarp using the fibers divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and

polydimethylsiloxane/divinylbenzene (PDMS/DVB) by analyzing the variables: agitation, temperature and extraction time.

2 Material and methods

2.1 Material

Mature pequi fruits (30 kg) were purchased from a single producer in Paraopeba – MG, at the Minas Gerais Supply Center (CEASA), in Contagem - MG, Brazil, during the 2018 harvest. The fruits were transported in styrofoam boxes to the Food Chemistry Laboratory of Federal University of Minas Gerais, Belo Horizonte – MG, where the healthy fruits were selected, sanitized and then rinsed. The parts corresponding to the pericarp were removed from fruits, cut into smaller pieces with a stainless steel knife and homogenized using a food processor. The homogenized sample was stored in glass amber jars at -18 °C until the moment of analysis.

The fibers DVB/CAR/PDMS (50 μm) and PDMS/DVB (65 μm) (Sigma-Aldrich, St. Louis, United States of America) were used to optimize the extraction of VOCs from pequi pericarp.

2.2 Experimental design

The influence of the dependent variables (extraction temperature, extraction time and agitation) was evaluated by a factorial planning 2^3 with triplicates at the central point (Rodrigues & Iemma, 2009) (Table 1). The sum of the peak areas of all the compounds captured in each test was used as response for optimization of the evaluated parameters.

Table 1 - Factorial planning (2^3) with triplicates at the central point

Experiment	Conditions		
	T (ºC)	t (min)	A (rpm)
1	25	20	0
2	25	40	0
3	65	40	100
4	25	40	100
5	65	40	0

6	25	20	100
7	65	20	0
8	65	20	100
9	45	30	50
10	45	30	50
11	45	30	50

T: temperature; ºC: degrees Celsius; t: time; min: minutes; A: agitation; rpm: rotations per minute.

2.3 Extraction of VOCs

For the extraction, 2.0 g of processed pequi pericarp samples were weighed in headspace flasks, which were sealed with aluminium seal and rubber septa. The SPME fibers were inserted into the flasks and exposed to the headspace of the samples in order to adsorb the volatile substances extracted under the different conditions. After the extraction period, the fibers were inserted into the gas chromatograph injector for 5 minutes for desorption of the volatile compounds (Belo, et al., 2013).

2.4 GC-MS analysis and identification of volatile compounds

The VOCs of pequi pericarp samples were analyzed using a gas chromatograph (Trace GC Ultra) coupled to a mass spectrometer (MS, Polaris Q, Thermo Scientific, San Jose, CA, USA), with an ion trap, using a split/splitless capillary injector, in splitless mode (Belo, et al., 2013).

It was used helium as the carrier gas at a flow rate of 1 mL min^{-1}. Chromatographic analysis conditions were: injector temperature of 250 °C, desorption time 5 minutes, ion source temperature 200 °C, and interface temperature 275 °C. An HP-5ms capillary column (5% phenyl and 95% methylpolysiloxane) (30 m × 0.25 mm × 0.25 µm) (Agilent Technologies Inc., Munich, Germany) was used to separate the VOCs. Column heating was programmed starting at 40 °C, remaining for 5 minutes, heating from 2,5 °C min^{-1} to 125 °C then an increase of 10 °C min^{-1} to 245 °C, at which temperature the isotherm was maintained for 3 minutes (Belo, et al., 2013).

The identification of the volatile constituents of pequi pericarp was performed through the comparison between mass spectrum obtained by electron impact ionization (EI) at 70 eV, in a

full scan mode with a range of *m/z* 30 to 400 and the database of the National Institute of Standards and Technology (NIST). For confirmation of the volatile compounds, a comparison was carried out with compounds identified by other authors (Belo, et al., 2013).

3 Results and discussion

A multivariate analysis was performed in order to assess the influence of temperature, time and agitation employed in the extraction of VOCs present in pequi pericarp samples using the fibers DVB /CAR/PDMS and PDMS/DVB. The sum of the relative area of the peaks of all compounds present in the sample was considered as response. Table 2 presents the identification of all VOCs extracted from pequi pericarp samples using both SPME fibers.

Table 2 - VOCs identified from pequi pericarp using different fibers

No.	Volatile organic compounds	SPME fiber	
		DVB/CAR/PDMS	PDMS/DVB
Ketone			
1	1-(2,8,8-trimethyl-4,5,6,7-tetrahydrocyclohepta[b]furan-5-yl)ethanone	X	-
2	(*E*)-3-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptan-2-yl)but-3-en-2-one	-	X
Alcohol			
3	1-decenol	X	-
4	2,3,5,5,8a-pentamethyl-6,7,8,8a-tetrahydro-5H-benzo[b]pyran-8-ol	X	-
Ester			
5	pentyl octanoate	X	-
6	ethyl hexanoate	X	X
7	2-cyclohexenyl-3-[1-(2-oxopropyl)ethenyl]-2,4,4-trimethyl acetate	-	X

8	2-ethylhexyl salicylate	X	-
9	hexyl salicylic acid	-	X
Aldehyde			
10	(*E*)-5-(4,5-dimethyl-7*a*-prop-1-en-2-yl-2,3,3*a*,5,6,7-hexahydro-1*H*-inden-4-yl)-3-methylpent-2-enal	X	X
11	Benzeneacetaldehyde	-	X
Terpenes			
12	6-camphenol	X	-
13	*p*-cymene	X	X
14	*γ*-terpinene	X	X
15	*cis*-sabinene hydrate	X	X
16	3-carene	X	-
17	2-cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)	X	-
18	*trans*-sabinene hydrate acetate	X	X
19	*trans*-sabinene hydrate	X	-
20	7,8-dehydro-8a-hydroxy-isolongifolene	X	-
21	Khusimol	X	-
22	1-epi-cubenol	X	-
23	*α*-cadinol	X	-
24	5-neo-cedranol	X	-
25	3,7-dimethyl-1-octanol	X	-
26	1,8-dimethyl-8,9-epoxy-4-isopropyl- spiro[4,5]decan-7-one	X	-
27	Ocimene	-	X
28	terpinen-4-ol	-	X
29	Azulene	-	X
30	4-ethenyl-4,8,8-trimethyl-2-methylidenebicyclo [5.2.0]nonane	-	X
31	7-methoxy-2,2,4,8-	-	X

	tetramethyltricyclo [5.3.1.0(4,11) undecane		
32	Cubenol	-	X
33	α-muurolol	-	X
34	2,2,8,8-tetramethyl-octahydro-1H-2,4a-methanonapthalen-9-ol	-	X
Hydrocarbon			
35	1-decene	X	-

SPME: solid phase microextraction; DVB/CAR/PDMS: divinylbenzene/carboxen/polydimethylsiloxane; PDMS/DVB: polydimethylsiloxane/divinylbenzene. Source: Authors.

When using DVB/CAR/PDMS and PDMS/DVB fibers, the best experimental condition was obtained when pequi pericarp was submitted to 65 °C for 40 minutes. In relation to agitation, there were different results according to the fiber used, since the best condition for extraction with DVB/CAR/PDMS was achieved using 100 rpm while PDMS/DVB worked better in the absence of agitation.

The semipolar fibers used promoted the extraction of several polar and nonpolar compounds such as ketones, alcohols, esters, hydrocarbons, aldehydes and terpenes. The most significant part of VOCs identified were terpenes (65.71%) and esters (14.29%). A total of thirty-five VOCs were identified in pequi pericarp using the DVB/CAR/PDMS and PDMS/DVB fibers. Only four of these compounds (ocimene, α-muurolol, α-cadinol, ethyl hexanoate) were previously identified in pequi pulp, seed and leaves using different extraction techniques (Belo, et al., 2013; Cordeiro et al., 2003; Damiani et al., 2009; Maia, et al., 2008; Passos et al., 2003).

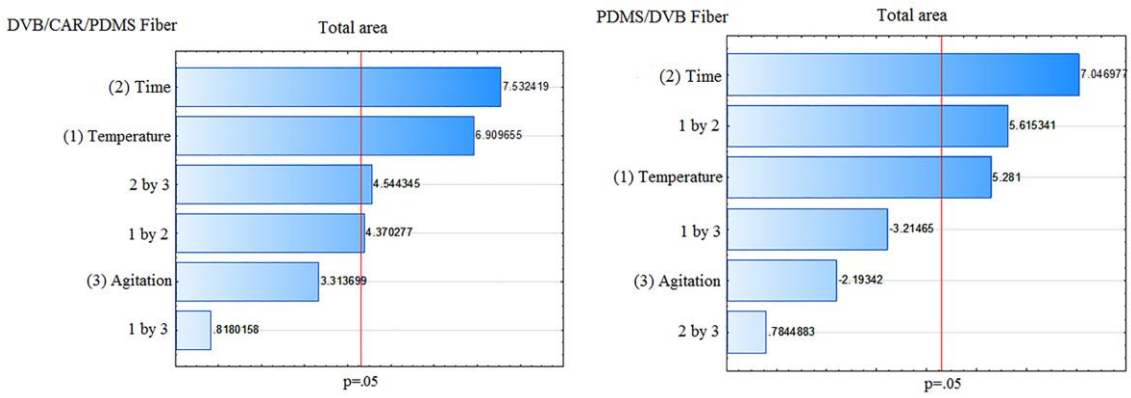
The data obtained indicated that penthyl octanoate, 2-ethylhexyl salicylate and 1,8-dimethyl-8,9-epoxy-4-isopropyl-spiro[4,5]decan-7-one were the main volatile compounds extracted from pequi pericarp by DVB/CAR/PDMS while using PDMS/DVB the main volatile compounds were hexyl salicylic acid and (*E*)-3-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptan-2-yl)but-3-en-2-one.

In order to evaluate which parameters had significant effect on the extraction of volatile compounds using the two different types of fiber a Pareto graph was constructed with a 95% confidence level (Figure 1). The result shows that extraction time and temperature had significant effect for both fibers evaluated. The increase in the levels of these two parameters

enabled greater extraction of VOCs. In addition, the interaction between these two variables also generated significant effect for both fibers while the interaction between the parameters extraction time and agitation generated significant effect only for the fiber DVB/CAR/PDMS. No studies were found using chemometric tools to upgrade the extraction of VOCs from pericarp of pequi using these different fiber types.

Pellati et al. (2005) used the fiber DVB/CAR/PDMS to extract VOCs from fruits of *Evodia* species and also observed better response with increasing temperature, as shown in the present study. A similar result was reported by Silva et al. (2019) who observed significant temperature effect on increasing the extraction of VOCs from the pulp of *Eugenia dysenterica* fruit using the fiber DVB/CAR/PDMS. However, according to the authors, agitation and the extraction time showed no significant effect.

Figure 1 - Influence of parameters on the extraction of VOCs from the pequi pericarp



Source: Authors.

Based on the sum of the area of all peaks identified in 11 tests performed for each fiber, PDMS/DVB was statistically more efficient than DVB/CAR/PDMS. Corroborating the present study, Sánchez-Palomo et al. (2005) observed greater efficiency in extraction of VOCs from grapes utilizing PDMS/DVB fiber in comparison to DVB/CAR/PDMS fiber.

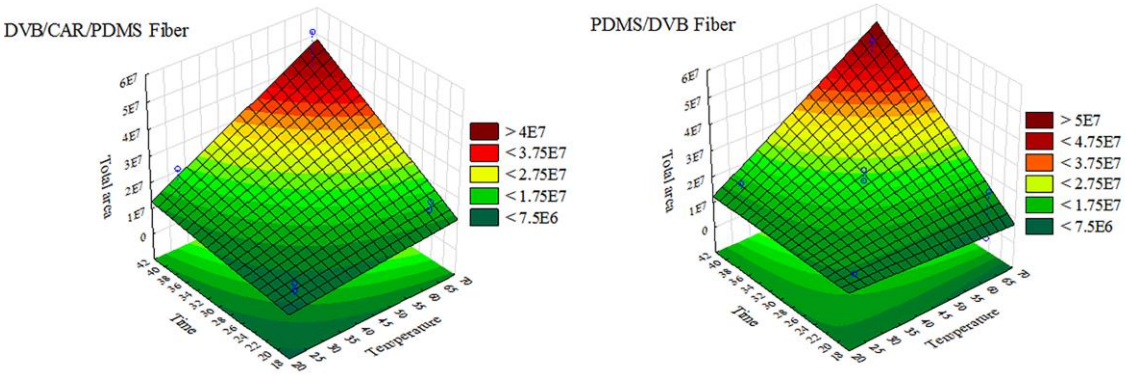
The impact of HS-SPME fiber coating on the extraction of volatile compounds has been studied by Bicchi et al. (2000) that showed that the selection of the most appropriate fiber type depends on the target and compound matrices targeted. The authors observed that the fibers composed of liquid (PDMS) and solid (DVB and/or CAR) components had higher efficiency in extraction of VOCs from tropical fruits. According to Sánchez-Palomo et al. (2005), the PDMS/DVB fiber has the highest sensitivity for aromatics of medium and low volatility and, for this reason has been widely used for analysis of aromatic compounds from fruits.

The type of the fiber coating influences the extracted chemical compounds. According to Silva et al. (2019) the PDMS coating has higher affinity with non-polar compounds such as terpenes and esters. In the present study, these chemical groups were also predominant using the two types of fibers (Table 2). Belo et al. (2013) found similar results when extracting the VOCs from pequi pulp at 60 ºC for 10 minutes without agitation, using the PDMS/DVB fiber. In their work, seventy-seven VOCs of pequi pulp were isolated, predominantly esters, hydrocarbons and terpenoids.

Sánchez-Palomo et al. (2005) observed that the use of PDMS/DVB fiber to extract VOCs from grapes allowed higher extraction of alcohols, aldehydes, esters and benzene compounds. In another study, Pellati et al. (2005) reported that DVB/CAR/PDMS extracted mainly monoterpenes from fruits of *Evodia* species, while the PDMS/DVB fiber presented greater ability to extract sesquiterpenes.

The effects of temperature and extraction time in the extraction of volatile compounds from pequi pericarp are presented in Figure 2. The analysis of the response surface graphic shows that the use of high temperature (65 °C) associated with the longest extraction time (40 minutes) allowed better extraction of VOCs regardless of the fiber used). This result is justified by the influence of temperature on the partition coefficients of compounds and the increased concentration of VOCs in the headspace allowing higher adsorption by the fibers (Pellati, et al., 2005; Pellati, et al., 2013).

Figure 2 - Influence of extraction time and temperature on the extraction of VOCs from pequi pericarp



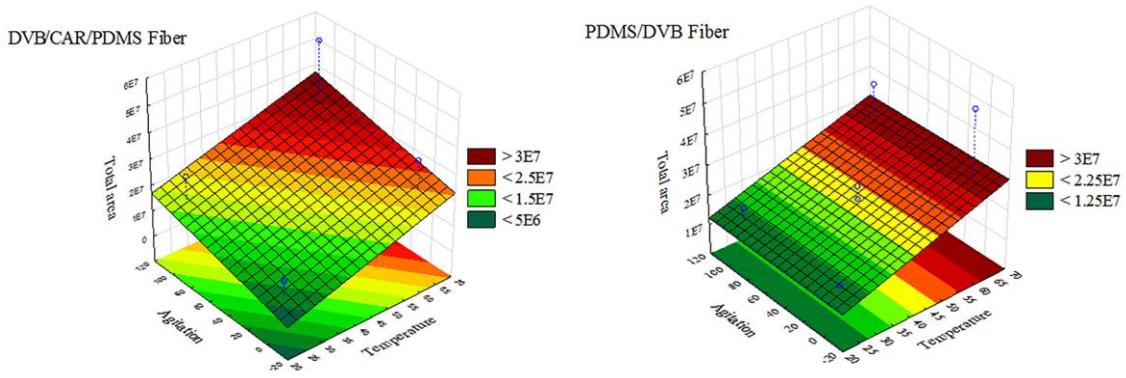
Source: Authors.

Since studies evaluating the influence of extraction time and temperature on the volatile compounds of pequi fruit by HS-SPME were not found, the results herein obtained were compared to data reported for other fruits. Silva et al. (2019), using the DVB/CAR/PDMS fiber also observed greater extraction of VOCs from the pulp of *Eugenia dysenterica* fruit at a temperature of 65 °C for 20 minutes. Zacaroni et al. (2017) evaluated the extraction of VOCs from *cachaça* (a typical Brazilian spirit) and obtained similar results when using the DVB/CAR/PDMS fiber at 45 °C for 50 minutes.

In another study, García et al. (2019) observed that elevation of temperature to 65 °C ensured greater extraction of VOCs from *Malpighia emarginata* fruit using the PDMS/DVB fiber, as found in the present study. However, a contrary result was obtained by the authors using the DVB/CAR/PDMS fiber.

The effects of agitation and extraction temperature on the number of VOCs extracted from the pericarp of pequi are presented in Figure 3. The response surface graph analysis indicates that agitation and extraction temperature were parameters that did not significantly impact the extraction of VOCs by the SPME fibers utilized. García et al. (2019) also found no significant influence of agitation during the extraction of VOCs *Malpighia emarginata* fruit using different SPME fibers.

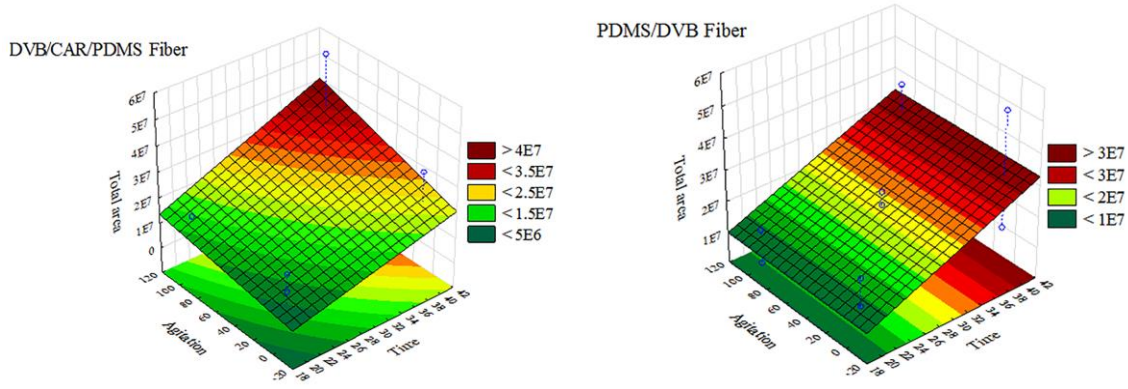
Figure 3 - Influence of agitation and extraction temperature on the extraction of VOCs from pequi pericarp



Source: Authors.

As observed in the Pareto graph, the interaction between agitation and extraction time showed no significant effect on the extraction of VOCs using the fiber PDMS/DVB. When the fiber DVB/CAR/PDMS was employed, minimum use of agitation (50 rpm) and the longest extraction period (40 minutes) allowed better extraction of VOCs (Figure 4). Silva et al. (2019) found similar results in the extraction of VOCs from the fruit of *Eugenia dysenterica*, i.e, longer extraction period and the absence or minimal use of agitation provided greater extraction of VOCs using the DVB/CAR/PDMS fiber.

Figure 4 - Influence of agitation and extraction time on the extraction of VOCs from pequi pericarp



Source: Authors.

4 Conclusions

The use of HS-SPME analysis allowed the identification of thirty-five volatile compounds present in the pequi pericarp, mainly terpenes and esters. Comparing the fibers, PDMS/DVB was statistically more efficient than DVB/CAR/PDMS. The use of high temperature during a long period allowed greater extraction of volatile compounds by both fibers. In relation to agitation, the best condition for using DVB/CAR/PDMS was 100 rpm, while agitation was not necessary for an efficient extraction using the fiber PDMS/DVB.

For future works regarding the composition of volatile compounds from pequi pericarp, it is suggested the use of HS-SPME fibers with other types of coating to allow the extraction and identification of new compounds and thus contribute even more to the characterization of this agro-industrial residue.

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