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Comparison of *Spondias tuberosa* Arruda accessions by fruit volatile compounds using multivariate analysis

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Abstract

In this study, the volatile organic compounds from *Spondias tuberosa* fruits of 16 accessions with different origins were extracted by solid-phase microextraction in headspace mode (HS-SPME) and analyzed by gas-chromatography coupled to mass spectrometry (GC-MS). The volatile compounds present in the fruit from the 16 accessions were identified and submitted to multivariate analysis, with the main volatile compounds identified (from a total of 25) being: ethyl butanoate, α-pinene, myrcene/ethyl caproate, limonene, ocimene isomers, linalool/nonanal and p-menth-1-en-4-ol. The major chemical classes were terpenes (about 7-72%) and esters (about 14-72%). The relative chromatographic areas of the volatile compounds were used in Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), which accumulated 67.69% of the explained variance. The compounds classified as esters and terpenes were the main compounds responsible for forming distinct groups. The PC1 and PC2 enabled distinguishing the percentage of compounds responsible for grouping in the PCA quadrants and the HCA shows the grouping of these compounds.

Keywords: umbu; headspace extraction; VOCs; aromatic fruit; caatinga fruits.

Practical Application: Provide information for future understanding of flavor and organisms' interaction.

1 Introduction

The Caatinga is an exclusive biome in Brazil which covers approximately 10% of Brazil's territorial area and presents typical biodiversity of fruit trees that exert an important social role as a source of income for the local population (Alves et al., 2009; Filizola & Sampaio, 2015). The *Spondias tuberosa* tree, known as *umbuzeiro* is a great example of this as part of the population depends on its fruit as a source of income or food (Matta et al., 2019; Santos et al., 2005; Saturnino & Souza, 2019).

In this sense, the EPAMIG Norte created an accession collection of *S. tuberosa* trees from the north region of Minas Gerais state on the institution's experimental farm (Moreira et al., 2007). To date, few studies have been carried out comparing fruits from this or other *S. tuberosa* collections or considering the species variability (Dutra et al., 2017; Nascimento et al., 1999, 2002; Oliveira et al., 2015). These studies evaluated physical attributes and chemical aspects such as titratable acidity, soluble solids and pH (Dutra et al., 2017; Nascimento et al., 1999, 2002; Oliveira et al., 2015). Thus, there is a lack of studies involving characterization of the volatile organic compounds of several accessions of this species. Although some previous studies have already characterized the volatile compounds of *S. tuberosa* fruits, none of them have considered more than one variety of the species (Thomazini et al., 1998 apud Franco & Janzantti, 2005; Galvão et al., 2010, 2011). Furthermore, no study so far has involved solid-phase microextraction in headspace mode (HS-SPME), which is an extraction method that does not use solvents and high temperatures (Rahman et al., 2022; Yang et al., 2020; Zhang et al., 1994).

Volatile organic compounds contribute to the fruit aroma and some of them may be important secondary metabolites for plant communication (Aragüez & Fernández, 2013; Taylor, 1998). Therefore, a comparison of the volatile compound profiles from different fruits of this species may provide relevant chemical information to foster future studies in order to select and protect accessions, as well develop phytosanitary strategies. Multivariate analysis tools have generally been used to characterize the profile of volatile organic compounds from different accession.

Therefore, the main aim of this study was to determine the volatile composition of *S. tuberosa* fruits of 16 accessions from the EPAMIG NORTE collection in Nova Porteirinha city in Minas Gerais State, Brazil, and use multivariate analysis to characterize the profile of volatile organic compounds in *S. tuberosa* fruit.

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2 Materials and methods

2.1 Fruit

Fruits from 16 accessions with different geographic origins were harvested in the experimental accession collection from EPAMIG NORTE in Nova Porteirinha city, Minas Gerais State, Brazil (SisGen registration number: A6AA0F7). The codes of the 16 accessions are EPAMIG-C01, EPAMIG-C02, EPAMIG-C03, EPAMIG-C04, EPAMIG-C05, EPAMIG-C06, EPAMIG-C07, EPAMIG-C08, EPAMIG-C09, EPAMIG-C10, EPAMIG-C11, EPAMIG-C12, EPAMIG-C13, EPAMIG-C15, EPAMIG-C18 and EPAMIG-C19. The fruits were harvested in January and February of 2019.

Physiologically ripe fruits on the tree with smooth surfaces and uniform characteristics were chosen, visually identified in a maturity stage regionally called "swollen". These accessions are planted in the same land (delimited by the following geographic coordinates: 15° 48' 8.932" S, 43° 17' 49.445" W; 15° 48' 3.060" S, 43° 17' 43.811" W; 15° 48' 4.075" S, 43° 17' 42.648" W; 15° 48' 10.159" S, 43° 17' 48.188" W) which minimizes differences in fruit caused by environmental and climatic factors. These fruits are shown in Figure 1. The differences in some chemical and physical characteristics have been previously reported (Saturnino & Gonçalves, 2011 apud Donato et al., 2019).

2.2 Sample preparation

The samples were prepared on the day after harvest using a similar method to a previous study (Ferreira et al., 2022). First, the seeds and peels from 9 fruits from each accession were removed, and the pulps were homogenized using a domestic mixer for 30 s. Next, 0.20 g of NaCl salt was added per gram of fruit pulp to increase the polarity of the matrix and reduce the solubility of volatile compounds in the matrix and again mixed for 60 s. Finally, 4.8 g of fruit pulp was added into 20 mL vials and sealed for volatile compound extraction.

2.3 Extraction method

The volatile compound extraction was performed by solid-phase microextraction in headspace mode (HS-SPME) according to a previous study (Ferreira et al., 2022). A manual holder and SPME-fiber divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco - São Paulo, Brazil) were used in the procedure. Each vial with 4.8 g from samples were incubated in a thermostatic bath at 40 °C for 10 min and then the SPME-fiber was inserted in the vials for 20 min. All procedures were performed in duplicate.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

After the extraction, the SPME-fiber was inserted into the GC-MS injector for compound desorption and it was kept in the equipment until the end of the chromatographic analysis. Static headspace analysis was performed using a PAL Syr HS 2.5 mL for combi-PAL. GC-MS analysis was carried out using a gas-chromatograph from Agilent Technologies (GC 7890A model) coupled with a mass spectrometer (MS 5975C model). Analyses were performed using a DB-5 MS capillary column of 30 m x 0.32 mm x 0.25 µm. All samples were injected in splitless mode at 220 °C. The chromatograph oven temperature-programmed started at 60 °C, then increased by 3 °C min^{-1} to 240 °C. Helium (99.9999% purity) was used as carrier gas at a flow rate of 1 mL min^{-1}. The interface temperature was 280 °C, and the acquisition mass range was 45-600 m/z. A standard solution from a series of saturated alkanes (C7-C40) purchased from Sigma-Aldrich was injected under the same conditions to calculate the Van den Dool and Kratz retention index (LRI) of the sample compounds. The volatile compounds' identification was performed by comparing the mass spectra (NIST 2.0 library) and the retention index (LRI).

2.5 Multivariate analysis

The mean values of volatile compounds in the pulp of the 16 populations were examined by multivariate analysis.

The data in the multivariate analysis were arranged into a matrix consisting of variables (columns) which constituted the values of relative chromatographic peak area from detected compounds and the objects (rows) which were the 16 different accessions. The matrix was self-scaled and then the PCA and HCA were performed, with the latter using the algorithm of means (Correia & Ferreira, 2007). Data were analyzed using the MATLAB Chemometrics Software program (5.3 version) and the PLS_Toolbox package (2.0 version) (Wise & Gallagher, 1999).

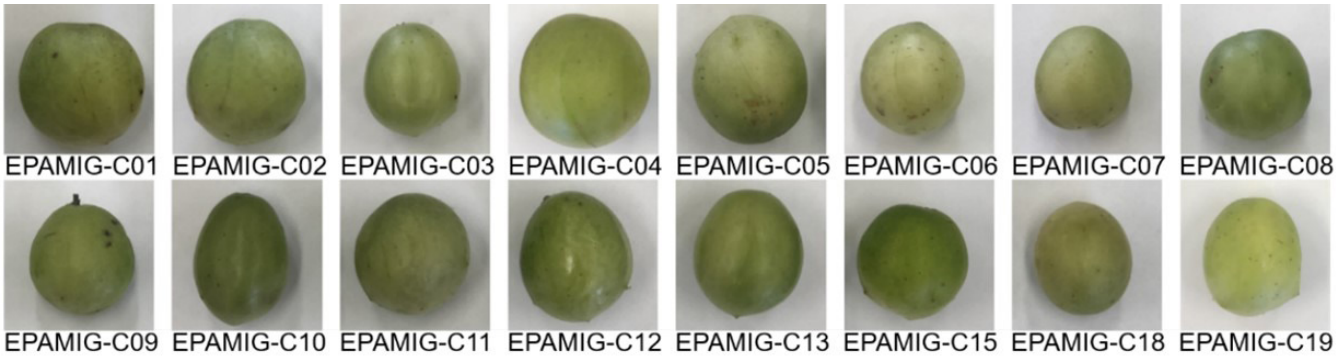

Figure 1. *S. tuberosa* fruit harvested from each of the studied accessions.

3 Results and discussion

The 16 chromatograms of the volatile compounds of the *S. tuberosa* fruits from the accessions are shown in Figure 2 and the identified compounds are summarized in Table 1.

It was observed that eight signals were detected and seven identified in all accessions. These signals are attributed to ethyl butanoate, α-pinene, a mixture of myrcene and ethyl caproate, limonene, ocimene isomers mixed, a mixture of linalool and nonanal and p-menth-1-en-4-ol. The relative chromatographic areas of the main chemical classes identified in the volatile compounds from each *S. tuberosa* fruit accession may be seen in Figure 3.

It is observed that the predominant chemical classes in most fruits are terpenes and esters, about 6.7-72.3% and 13.7-72.1%, respectively. Aldehydes and alcohols were also detected in small amounts for all fruits, which presented the areas 0.1-4.6 and 0.6-7.7%, respectively. Other relevant information is the high sesquiterpene content identified in the EPAMIG-C02 (about 45%), while it is always less than 5% for the other accessions. A previous study already indicated that swollen fruits from EPAMIG-C02 could be different from others, presenting the lowest pH and the highest °Brix among them (Saturnino & Gonçalves, 2011 apud Donato et al., 2019).

3.1 Classification by principal component analysis

The exploratory analysis initially showed that from the 25 compounds detected (among those identified and unidentified), 14 main components accumulated 100% of total variance (Figure S1). The differentiation of each accession (Figure 4) was explained by the first two principal components (PC1 and PC2) which accumulated 67.69% of the variance explained by the data, with PC1 showing 54.09% and PC2 with 13.60% of variance. In turn, the compounds responsible for the differentiation are shown in Figure 5.

In Figure 4, it was observed that the accessions EPAMIG-C03, EPAMIG-C06, EPAMIG-C07, EPAMIG-C08, EPAMIG-C15 and EPAMIG-C19 were grouped into negative PC1 and PC2. In Figure 5, it was observed that the compounds responsible for this grouping were the α-pinene, limonene and ocimene isomers, numbered respectively as 3, 5 and 6 in Figure 2 and Table 1. Interpreting the negative PC1 and positive PC2 quadrants, also in Figures 4 and 5, the grouping of EPAMIG-C01, EPAMIG-C04, EPAMIG-C05, EPAMIG-C09, EPAMIG-C10, EPAMIG-C11, EPAMIG-C12, EPAMIG-C13, and EPAMIG-C18 accessions was influenced by an unidentified compound with retention time equal to 2.2 min, ethyl butanoate, myrcene/ethyl caproate mixture, ethyl caprylate, dec-2-enal and methyl geranate, respectively numbered 1, 2, 4, 10, 11 and 12 in Figure 2 and Table 1. It is also observed that PC2 separated the two groups described above, as well as the EPAMIG-C02 accession, which together with the second group was positive in PC2 positive and the first group in PC2 negative. The EPAMIG-C02 accession differed from the others due to the greater relative abundance of the isoledene, copaene, caryophyllene, γ-elemene, and aromadendrene compounds, an unidentified compound (retention time = 26.7 min), viridiflorene, as well as a mixture of α-selinene, epizonarene and

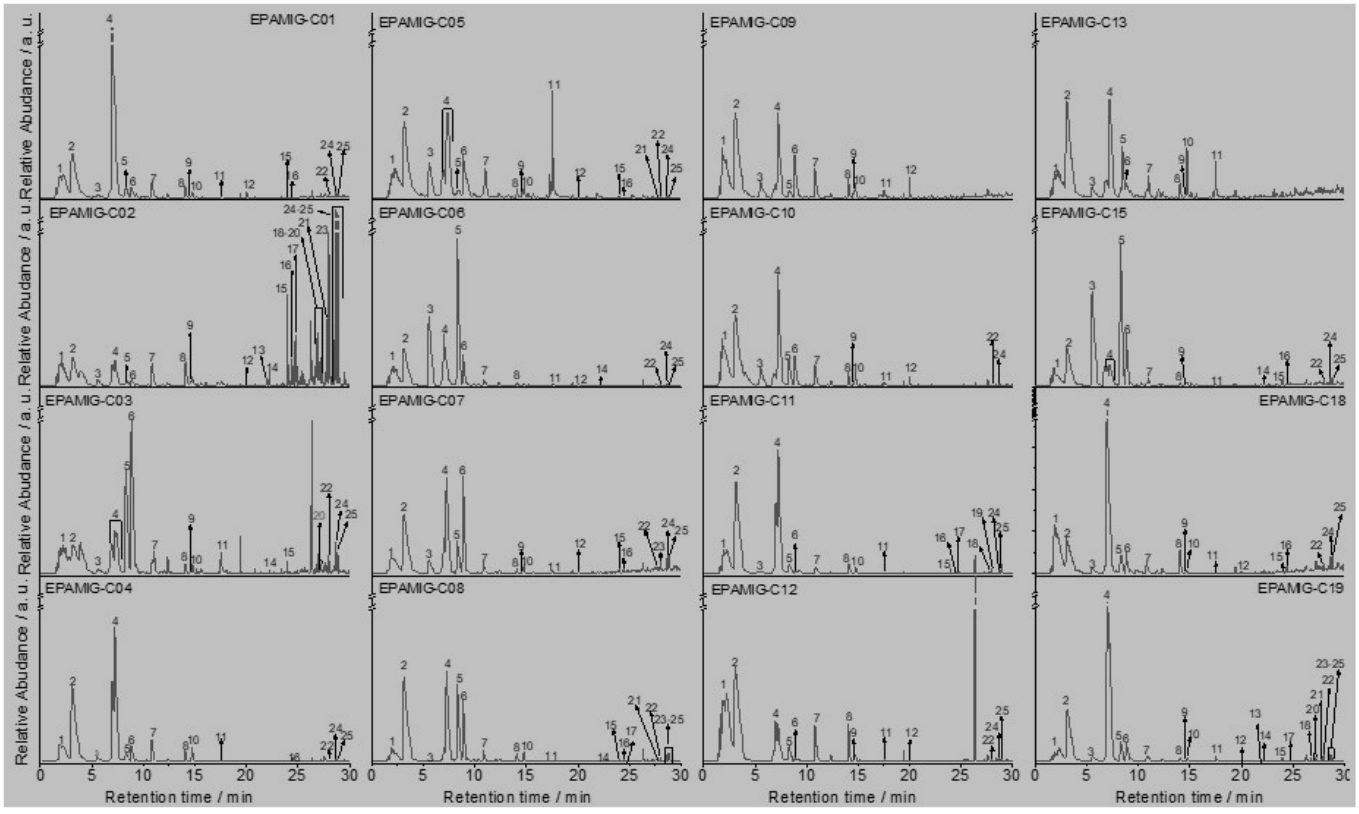

Figure 2. Total ion chromatograms of the volatile compounds of the *S. tuberosa* fruits from the 16 accessions.

Table 1. Volatile compounds of *S. tuberosa* fruits from the analyzed accessions.

Peak[a]	Compound	RT	LRI[b]	LRI[c]	Accession name (EPAMIG-C)															
					01	02	03	04	05	06	07	08	09	10	11	12	13	15	18	19
1	Unidentified compound	2.2	< 800		d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
2	Ethyl Butanoate	3.1	815	808	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
3	α-pinene	5.5	937	942	d	d	d	d	d	d	d	d	d	d	d		d	d	d	d
4	Myrcene/Ethyl Caproate	7.0/7.2	992/1001	992/999	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
5	Limonene	8.3	1031	1035	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
6	Ocimene isomers	8.8	1046	1046	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
7	Linalool/Nonanal	10.8/11.0	1101/1105	1101/1004	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
8	p-Menth-1-en-4-ol	14.1	1181	1179	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d
9	Methyl salicylate	14.5	1192	1192	d	d	d		d		d		d	d		d	d	d	d	d
10	Ethyl caprylate	14.7	1196	1197	d		d	d	d		d	d	d	d	d		d		d	d
11	Dec-2-enal	17.6	1263	1263	d	d		d	d	d	d	d	d	d	d	d	d	d	d	d
12	Methyl geranate	20.0	1320	1322	d	d	d		d	d	d		d	d			d		d	d
13	Isoledene	22.0	1367	1373		d	d													d
14	Copaene	22.2	1372	1377		d				d		d						d		d
15	Caryophyllene	24.0	1414	1420	d	d	d		d		d	d			d			d	d	d
16	γ-Elemene	24.4	1425	1433	d	d		d	d		d	d			d			d	d	
17	(+)-Aromadendrene	24.7	1433	1442		d						d			d					d
18	Unidentified compound	26.7	1482			d														d
19	Viridiflorene	26.9	1485	1493		d														
20	α-Selinene/ epizonarene/α-Muurolene	27.1-27.2	1491-1493	1496/1497/1499		d	d													d
21	γ-Cadinene	27.8	1507	1513		d		d	d			d			d					d
22	δ-Cadinene	28.0	1513	1524	d	d	d	d	d	d	d	d		d	d		d	d	d	d
23	Unidentified compound	28.5	1527		d	d						d								d
24	Unidentified compound	28.7	1530		d	d	d	d	d	d	d	d		d	d		d	d	d	d
25	Eudesma-3,7(11)-diene	28.8	1535	1542	d	d	d	d	d	d	d	d			d		d	d	d	d

RT: retention time in minutes. [a]The numbers of the peaks refer to the compounds in Figure 2; [b]Linear retention index calculated from experimental results; [c]Linear retention index on DB-5 (or equivalent) column from literature (Avsar et al., 2004; Baccouri et al., 2007; Balbontín et al., 2007; Dehghan et al., 2007; Flamini et al., 2003, 2007; Karioti et al., 2003; Macchioni et al., 2003; Masoudi et al., 2006).

α -muurolene, γ-cadinene, δ-cadinene, unidentified compound (28.5), unidentified compound (retention time = 28.7 min) and eudesma-3,7 (11)-diene, respectively numbered 13 to 25 in Figure 2 and Table 1. Compound weights are shown in Table S1. The groups separated by PC2 show that terpenes and esters are located in different quadrants and classify the different accessions. The esters in positive PC2 are the predominant compounds in which 68.42% of the accessions are located in this quadrant. Terpenes predominate in negative PC2, with 31.58% of accessions located in this quadrant. PC1 has 94.74% of the accessions in the negative quadrant, while 5.26% (which represents the EPAMIG-C02 accession) were located in the positive quadrant due to the compounds corresponding to the peaks from 13 to 25.

Finally, hierarchical cluster analysis was used with Mahalanobis distance and showed cluster formations presented on the HCA graph, identifying similarity (Figure 6). The distances of the different *S. tuberosa* accessions present similarities between them in the Figure 6, with the predominant compounds for the EPAMIG-C02 accession being grouped terpenes and the esters presented another grouping.

The results showed that the fruits of 16 accessions show differences in physical aspects, as may be observed in Figure 1, and differences in the production of secondary metabolites. Therefore, we believe that this study will contribute to expanding scientific knowledge about the species.

Regarding the flavor, the volatile compound identification in *S. tuberosa* fruits from 16 different accessions and its classification by principal components provide valuable chemical information to select fruits with better quality parameters along with other studies on the species. In addition, it can help to identify molecules responsible for the characteristic flavor of *S. tuberosa*, which is useful for both the development of artificial flavorings and for the quality control of products derived from the fruit.

Furthermore, previous studies have been conducted on this topic since the volatile compounds may be involved in

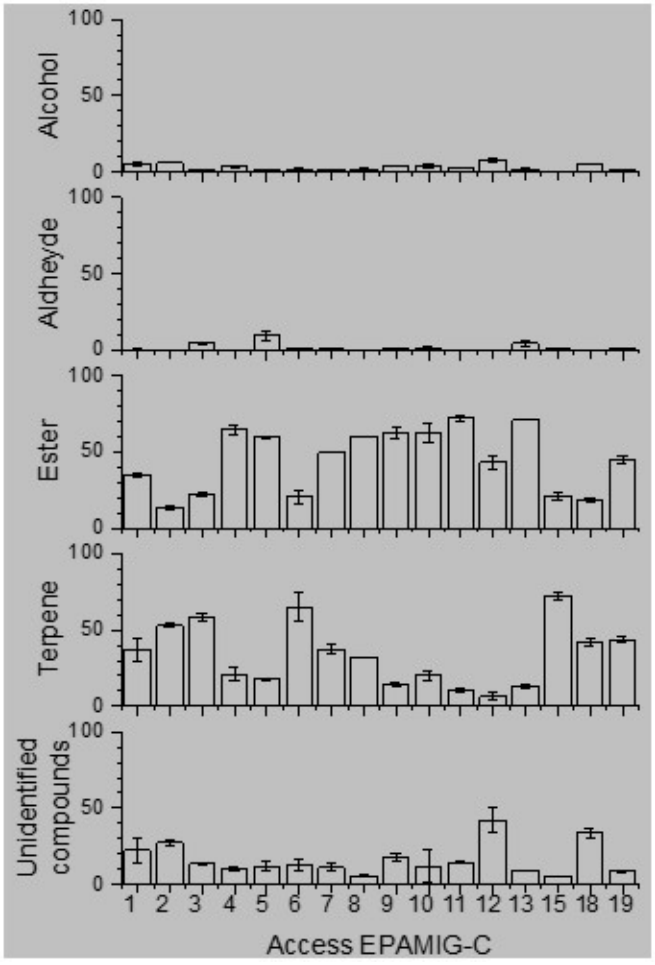

Figure 3. Relative chromatographic areas of the main chemical classes identified in the volatile compounds from 16 *S. tuberosa* fruit accessions.

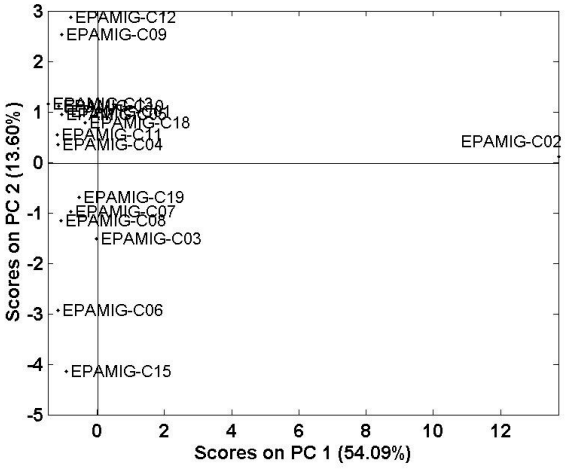

Figure 4. Scores graph showing the disposition in the PC1 and PC2 quadrants, separating the accession by the influence and similarity of the identified compounds considering the percentage presented in the identification.

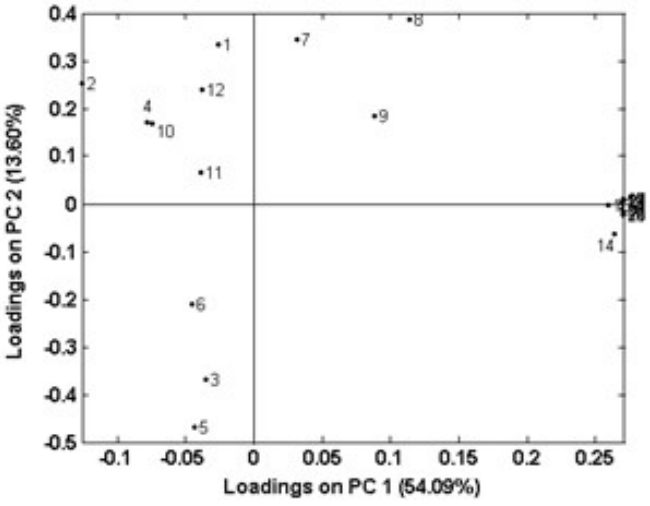

Figure 5. Loadings graph showing the influences of the peaks of compounds responsible for the separation of the accessions in the first two principal components. The numbering indicates the corresponding compound from Table 1.

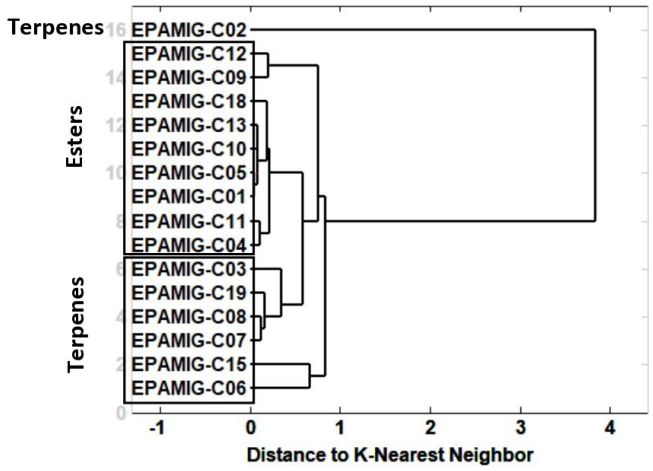

Figure 6. Cluster showing the similarity between the EPAMIG accessions and showing the compounds responsible for these similarities according to loads.

the attractiveness of fruits to pests (Cruz-López et al., 2006; Hernández-Sánchez et al., 2001; Malo et al., 2012; Nishida et al., 2000). Moreover, the main biological agents which may cause damage to cultures of *S. tuberosa* were recently compiled in a review study, in which *Anastrepha obliqua* and *Ceratitis capitata* were found to be among the insects that may cause fruit damage (Costa et al., 2019).

Studies about the influence of volatile compounds in attraction by *Anastrepha obliqua* and by the other Anacardiaceae (family of *Spondias* and other species) have been conducted (Cruz-López et al., 2006; Malo et al., 2012). It was demonstrated that a mixture of some volatiles (myrcene, α-pinene, and *trans*-β-ocimene) present in *Mangifera indica* (mango) may attract insects with the same intensity of fruit extracts (Malo et al., 2012). In this study, α-pinene and ocimene isomers were two of three compounds responsible for the clustering of EPAMIG-C03, EPAMIG-C06, EPAMIG-C07, EPAMIG-C08, EPAMIG-C15 and EPAMIG-C19. On the other hand, studies about volatile compounds from *S. Mombim* found nine other compounds which attracted this bug (Cruz-López et al., 2006). Of these, 3 were identified in this work in the fruits of *S. tuberosa*: ethyl butyrate, ethyl hexanoate, ethyl octanoate. All of these are part of the group of compounds that influence the clustering of EPAMIG-C01, EPAMIG-C04, EPAMIG-C05, EPAMIG-C09, EPAMIG-C10, EPAMIG-C11, EPAMIG-C12, EPAMIG-C13 and EPAMIG-C18. This information may be related to the vulnerability of *S. tuberosa* to this pest.

In addition, studies indicate that α-copaene and limonene may be an attractive compound for *C. capitata* (Hernández-Sánchez et al., 2001; Nishida et al., 2000). In this sense, is important to remark that copaene is one of the compounds responsible for separating the EPAMIG-C02 accessions from the others, and limonene is responsible for clustering the EPAMIG-C03, EPAMIG-C06, EPAMIG-C07, EPAMIG-C08, EPAMIG-C15 and EPAMIG-C19 accessions.

Therefore, it is observed that the comparison of the compounds identified in this work and the scientific literature suggest that there may be different attractiveness for these pests considering the existing genetic variability. However, specific studies on the topic will be needed for the species which may evaluate this possibility.

4 Conclusion

This study identified and compared the volatile profile of *S. tuberosa* fruits from 16 different accessions. The main chemical classes in most of the fruits were esters and terpenes. This is the first work identifying and comparing volatile compounds in different *S. tuberosa* fruits.

Also, to the best of our best knowledge, there are no other studies which compare the presence of any class or chemical compound in fruits from different plants of *S. tuberosa* species.

In this sense, this study contributes to increasing the knowledge about this important species of the Caatinga, in addition to providing information that may be useful to understand the taste and interaction with other species considering the genetic variability of the fruit. The PCA and HCA were able to show that two classes of compounds, terpenes and esters, were responsible for grouping the different accessions.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Loadings values as a function of the variables in the different principal components.

Figure S1. Eigenvalues graph, showing behavior in principal componentes.

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