Original

Characterization and classification of pequi trees (*Caryocar brasiliense* Camb.) based on the profile of volatile constituents using headspace solid-phase microextraction – gas chromatography – mass spectrometry and multivariate analysis

Caracterização e classificação de pequizeiros (Caryocar brasiliense Camb.) baseadas no perfil de constituintes voláteis usando microextração em fase sólida no modo headspace – cromatografia a gás – espectrometria de massas e análise multivariada

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Abstract

In order to determine the variability of pequi tree (*Caryocar brasiliense* Camb.) populations, volatile compounds from fruits of eighteen trees representing five populations were extracted by headspace solid-phase microextraction and analyzed by gas chromatography-mass spectrometry. Seventy-seven compounds were identified, including esters, hydrocarbons, terpenoids, ketones, lactones, and alcohols. Several compounds had not been previously reported in the pequi fruit. The amount of total volatile compounds and the individual compound contents varied between plants. The volatile profile enabled the differentiation of all of the eighteen plants, indicating that there is a characteristic profile in terms of their origin. The use of Principal Component Analysis and Cluster Analysis enabled the establishment of markers (dendrolasin, ethyl octanoate, ethyl 2-octenoate and β-cis-ocimene) that discriminated among the pequi trees. According to the Cluster Analysis, the plants were classified into three main clusters, and four other plants showed a tendency to isolation. The results from multivariate analysis did not always group plants from the same population together, indicating that there is greater variability within the populations than between pequi tree populations.

Keywords: *chemometrics; biodiversity; Cerrado native fruit.*

Resumo

A fim de determinar a variabilidade de populações de pequizeiro (*Caryocar brasiliense* Camb.), compostos voláteis de frutos de dezoito árvores representando cinco populações foram extraídos por microextração em fase sólida no modo headspace e analisados por cromatografia a gás acoplada à espectrometria de massas. Setenta e sete compostos foram identificados, incluindo ésteres, hidrocarbonetos, terpenoides, cetonas, lactonas e álcoois. Vários compostos não haviam sido encontrados anteriormente no fruto. A quantidade total e a composição de voláteis variaram entre as plantas. O perfil volátil permitiu a diferenciação de todas as dezoito plantas, indicando que há um perfil característico em relação a sua origem. A aplicação da Análise de Componentes Principais e a Análise de *Cluster* permitiram estabelecer marcadores (dendrolasin, octanoato de etila, 2-octenoato de etila e β-cis-ocimeno) que discriminaram os pequizeiros. De acordo com a análise de *cluster*, as plantas foram classificadas em três grupos principais, com quatro outras plantas mostrando uma tendência ao isolamento. Os resultados da análise multivariada não agruparam sempre plantas da mesma população, indicando que há maior variabilidade dentro do que entre as populações de pequizeiro.

Palavras-chave: *quimiometria; biodiversidade; fruta nativa do Cerrado.*

1 Introduction

The Brazilian Cerrado is a savannah-like ecosystem that presents a large diversity of fruit species. These fruits stand out mainly because of their remarkable and unique sensory characteristics although they are still under-exploited commercially or scientifically. The pequi tree (*Caryocar brasiliense* Camb.) is a species that evokes considerable socioeconomic interest among the plants of this biome (LANDA et al., 2000). It produces a fruit of peculiar sensory quality and high lipid content, the pequi, which is explored by agricultural workers that inhabit the Cerrado region. In the northern region of Minas Gerais (Brazil), the harvesting and the trading of the pequi mobilize 50% of the population that resides in the countryside, representing 54,7% of the annual income of these workers (FAGUNDES; ARAÚJO; NUNES, 2007).

The pequi tree blooms from August to November, and the fruits begin ripening in mid-November and continue until the

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beginning of February (ALMEIDA, 1998). The yellow pulp of the pequi fruit is used as human food, in the manufacture of liquors and desserts, and for oil extraction (LIMA et al., 2007).

Caldeira Júnior et al. (2007) interviewed rural inhabitants who reported that the pequi varies with regard to the amount of the pulp, color, taste, and aroma. Composition and content of aroma volatiles are related to the species cultivar or variety, depending largely upon genetic background (WANG et al., 2009). The use of secondary metabolites such as volatile compounds can contribute to the taxonomic classification of plant species (VIEIRA et al., 2001). These compounds can be extracted and analyzed by various techniques. Previously, all studies on the volatile composition of *Caryocar brasiliense* Camb. investigated the essential oils from the seeds and leaves (PASSOS et al., 2002) and the fruit pulp (MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009) using extraction techniques other than headspace-solid-phase microextraction (HS-SPME). HS-SPME has been used in combination with gas chromatography-mass spectrometry (GC-MS), and it has been successfully applied in the analysis of organic volatile and semi volatile compounds from food samples.

Multivariate analysis has been used in food quality evaluation to characterize varieties, species, or cultivars of fruits based on the volatile profile. This statistical analysis has been applied to the characterization of apricots (SOLIS-SOLIS et al., 2007), white grapes (ROCHA et al., 2007), peaches and nectarines (WANG et al., 2009), and citrus fruits (GONZÁLEZ-MAS et al., 2011).

The expansion of agricultural frontiers in the Cerrado region and the intensive exploitation for the production of charcoal from native species are endangering the preservation of the genetic variability of the pequi tree. In addition, the exhaustive extraction of the pequi fruit can cause loss of genetic material since almost all high quality fruits, which originate from superior genotypes, are collected and consumed or commercialized, thus preventing the natural reproduction from these fruits (MELO JÚNIOR et al., 2004). Consequently, the studies on pequi plants and populations to discover the differences among them are extremely relevant. The results of these studies can provide a scientific basis for the preservation of the species and individual trees. Therefore, the purpose of this study was to separate and identify volatile compounds present in the pequi pulp that could be used as parameters for the characterization of pequi plants and populations using HS-SPME techniques, followed by chromatographic analysis by GC-MS and the application of statistical multivariate methods such as PCA and the Cluster Analysis. In short, this research is an initial exploratory study that seeks to characterize the volatile compounds of pequi fruits in the headspace and verify the possibility of discriminating populations and plants using the data obtained.

2 Materials and methods

2.1 Samples

Mature fruits were collected during the 2007-2008 crop season. A total of eighteen samples of pequi fruits were collected from five populations, as described in Table 1. The plants were previously named according to the rural communities where they were found. These samples were used to study the composition of volatile compounds in the headspace. They were stored at –18 °C until extraction.

2.2 Sample extraction

The pequi pulp samples were cut in thin pieces using a knife, and 2.0 g of the pulp were transferred to 25 mL headspace vials. The vials were sealed with rubber septa and aluminum seals and kept in a freezer at –18 °C until analysis. The selected fruits did not show signs of contamination by insect larvae or evidence of decay. At least three fruits of each plant were selected, homogenized, and transferred to three different headspace vials to extract the volatile compounds.

2.3 SPME of volatile constituents

Polydimethylsiloxane-divinylbenzene (PDMS-DVB, 65 μm) SPME fibers (Supelco Co., Bellefonte, PA, USA) were used to collect and concentrate volatile components from the headspace above the pequi pulp. The conditioning of the fiber was performed at 250 °C for 30 minutes in the GC injection port according to the manufacturer's instructions. For each extraction, the headspace vial was placed in a heating plate at 60 °C for 10 minutes to achieve a constant temperature. The SPME fiber was exposed to the headspace for 30 minutes during sampling.

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

The fiber desorption was performed at 250 °C for 10 minutes in the chromatograph injector set in the splitless mode. Desorbed volatile compounds were separated and analyzed in a GC–MS Thermo Electron Trace GC Ultra chromatograph with a Polaris Q (Thermo Scientific, San Jose, CA) ion-trap

Table 1. Pequi tree samples, populations, and geographic origin.

Population	Rural community	Municipality	Plants[a]
1	Sambaíba	Januária	Ary, S I, S IV, S XVII, Tanasio, Milorde
2	Bom Jantar	Januária	BJ I, BJ II, BJ VI
3	São José	Campo Azul	CAM I, CAM II, CAM III, CAM IV
4	Morro Velho	Januária	Gera, MV X, MV XII, MV XVII
5	Angical	Brasília de Minas	Angical

[a]At least three fruits of each plant were extracted, homogenized, and analyzed in triplicate.

mass spectrometer, equipped with a 30 m × 0.25 mm (i.d.) × 0.25 μm film thickness TRACE TR-1MS fused-silica capillary column (Thermo Scientific, San Jose, CA).

Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was initially programmed at 40 °C (3 min). It was increased at 2.5 °C/min to 125 °C and then increased at 10 °C/min to 245 °C; the temperature was then held for 2 minutes.

Mass spectra were obtained by electron impact ionization (EI) at 70 eV and scanned using full scan mode in the range 30-400 m/*z*. The ion source temperature was 200 °C. The identification of volatile compounds was achieved by comparing the mass spectra against the data system library (NIST, 2005) and retention indexes (RI) calculated using n-alkane standards (C8-C40).

2.5 Multivariate analysis

The retention times and relative intensities of all the chromatographic peaks were obtained. Data for each plant were averages of three replications, i.e., three independent extractions of each sample and one GC injection of each extract. PCA and Cluster Analysis were performed to establish relationships between pequi trees and the chromatographic profile of the volatile compounds from the pequi pulp so as to detect clustering formations. STATISTICA 8.0 (StatSort, Inc., Tulsa, USA) software was used to perform these analyses.

3 Results and discussion

The PDMS-DVB fiber was able to extract and concentrate the volatile compounds from the eighteen pequi trees. A total of 77 volatile compounds were obtained, including 35 esters, 11 hydrocarbons, 11 terpenoids, three ketones, three lactones, and four alcohols (Table 2). Only twenty one of these compounds have been previously identified in the pequi by other authors (PASSOS et al., 2002; MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009) using extraction techniques other than HS-SPME.

The pequi plants differed with respect to the number and concentration of volatile compounds (Table 3). The data obtained indicated that ethyl hexanoate, ethyl octanoate, β-cis-ocimene, and dendrolasin were the main volatile compounds extracted by the HS-SPME-GC-MS technique.

Table 2. Pequi volatile compounds obtained by HS-SPME-GC-MS.

Number	Class	Compound	RI observed[a]	RI tabled[b]
1	Acids	Acetic acid	744	625
2		Pentanoic acid	820	880
3		2-methylpentanoic anhydride	1348	1347,5
4		Hexanoic anhydride	1539	1517[c]
5		Z-11-hexadecenoic acid	1954	1953
6	Alcohols	Phenylethyl alcohol	1082	1080
7		Butylated hydroxytoluene	1492	1494
8		Z-9-pentadecenol	1749	1763[c]
9		(E,E)-3,7,11,15-tetramethyl-1,6,10,14-hexadecatetraen-3-ol	2014	2064[c]
10	Aldehydes	3-(2,2,6-trimethyl-7-oxabiciclo[4.1.0]hept-1-il)-2-propenal	1401	1407[c]
11	Ketones	(E)-3-penten-2-one	770	735
12		2,5-dimethyl-4-hydroxy-3(2H)-furanone	1079	1075
13		2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl-1H-inden-1-one	1374	1371[c]
14		1-(2,6,6-trimethyl-1-cyclohexen-1-il)-3-buten-1-one	1467	1392
15		(E,Z)-α-farnesene	1552	1475
16		1-hydroxy-6-(3-isopropenyl-cycloprop-1-enyl)-6-methyl-heptan-2-one	1622	1649[c]
17	Esters	Ethyl butanoate*	804	800
18		Ethyl 2-methylbutanoate*	850	846
19		Methyl hexanoate*	908	905
20		Ethyl hexanoate*	986	982
21		Ethyl (E)-2-hexenoate*	1023	1038
22		Isopropyl hexanoate	1035	1046
23		Propyl hexanoate*	1080	1080
24		Ethyl heptanoate*	1084	1095
25		3-methylbutyl 3-methylbutanoate	1093	-[d]
26		Ethyl 3-hydroxyhexanoate*	1107	1134
27		Methyl octanoate	1110	1106

[a]Retention index calculated from the Van der Dool and Kratz equation (BABUSHOK et al., 2007); [b]Retention index obtained from the NIST Chemistry Webbook database (http://webbook.nist.gov/chemistry/); [c]Retention index estimated by the NIST (2005) spectra library; [d]Retention index not found. *Compounds previously found in the literature (PASSOS et al., 2002; MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009).

Table 2. Continued...

Number	Class	Compound	RI observed[a]	RI tabled[b]
28		1-methylpropyl hexanoate	1119	1117
29		2-methylpropyl hexanoate	1135	1136
30		Ethyl 4-octenoate*	1172	1173[c]
31		Ethyl octanoate*	1183	1183
32		Ethyl benzenoacetate	1213	1213
33		Ethyl 2-octenoate*	1227	1243
34		Isopentyl hexanoate*	1236	1254
35		2-methylbutyl hexanoate	1239	1235
36		Ethyl 6-methyl-2,4-heptadienoate	1253	1296[c]
37		Pentyl hexanoate	1272	1282
38		2-ethylbutyl hexanoate	1278	1281
39		Hexyl hexanoate	1371	1370
40		Diethyl hexanedioate	1379	1354
41		Ethyl decanoate*	1382	1377
42		Ethyl trans-2-decenoate	1434	1389[c]
43		3-methylbutyl octanoate	1438	1450
44		Ethyl (E,Z)-2,4-decadienoate*	1456	1465
45		Ethyl dodecanoate*	1583	1592
46		Ethyl tetradecanoate	1780	1778
47		Methyl hexadecanoate	1914	1914
48		Ethyl hexadecanoate	1981	1985
49		Methyl (E)-9-octadecenoate	2084	2084
50		Ethyl (Z,Z)-9,12-octadecadienoate	2128	2155
51		Ethyl (E)-9-octadecenoate	2152	2185[c]
52	Hydrocarbons	4-methyl-1-decene	1053	1052
53		2-methyldecane	1065	1063
54		Undecane*	1099	1100
55		2,5,9-trimethyldecane	1129	1121[c]
56		2,3,5,8-tetramethyldecane	1170	1156[c]
57		9-(1-methylethylidene)-1,5-cycloundecadiene	1546	1544[c]
58		3-nonyl-cyclohexene	1557	1558[c]
59		Octadecane*	1802	1800
60		Nonadecane	1905	1900
61		Eicosane*	2003	2000
62		Heneicosane*	2099	2100
63	Lactones	δ-octalactone	1232	1240
64		δ-decalactone	1453	1461
65		δ-tetradecalactone	1879	1893
66	Pyrazines	2-ethyl-3,6-dimethylpyrazine	1061	1061
67	Terpenoids	3-methyl-4-methylene-bicyclo[3.2.1]oct-2-ene	990	1000[c]
68		α-felandrene	999	998
69		Ocimene	1028	1032
70		β-cis-ocimene*	1038	1040
71		1,3,8-*p*-menthatriene	1115	1111
72		2,6-dimethyl-2,4,6-octatriene	1118	1118
73		*p*-mentha-1,5-dien-8-ol	1144	1141
74		β-cyclocitral	1188	1195
75		Germacrene D*	1476	1478
76		Chilosciphone	1563	1576
77		Dendrolasin	1575	1589

[a]Retention index calculated from the Van der Dool and Kratz equation (BABUSHOK et al., 2007); [b]Retention index obtained from the NIST Chemistry Webbook database (http://webbook.nist.gov/chemistry/); [c]Retention index estimated by the NIST (2005) spectra library; [d]Retention index not found. *Compounds previously found in the literature (PASSOS et al., 2002; MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009).

Table 3. Pequi trees, total amount of extracted compounds, main identified compounds, and their relative intensities.

Plant	Total amount of volatile compounds	Main volatile compounds	Mean relative intensity (%)[a]
Angical	40	Ethyl hexanoate	100.00
		Ethyl octanoate	17.80
		β-cis-ocimene	12.07
		2-ethyl octenoate	2.69
		Dendrolasin	0.22
Ary	37	Ethyl hexanoate	100.00
		Dendrolasin	26.91
		β-cis-ocimene	19.33
		Ethyl octanoate	8.80
		2-ethyl octenoate	1.80
BJ I	29	Ethyl hexanoate	100.00
		Ethyl octanoate	36.53
		β-cis-ocimene	27.59
		Dendrolasin	12.39
		2-ethyl octenoate	5.95
BJ II	37	Ethyl hexanoate	100.00
		β-cis-ocimene	53.86
		Ethyl octanoate	47.87
		Dendrolasin	19.47
		2-ethyl octenoate	6.47
BJ VI	34	Ethyl hexanoate	100.00
		Ethyl octanoate	43.01
		β-cis-ocimene	10.04
		2-ethyl octenoate	9.01
		Dendrolasin	4.24
CAM I	36	Ethyl hexanoate	100.00
		Dendrolasin	75.09
		β-cis-ocimene	28.36
		Ethyl octanoate	21.83
		2-ethyl octenoate	3.64
CAM II	33	Ethyl hexanoate	100.00
		Ethyl octanoate	34.22
		β-cis-ocimene	7.61
		2-ethyl octenoate	4.70
		Dendrolasin	0.28
CAM III	29	Ethyl hexanoate	100.00
		Ethyl octanoate	20.83
		β-cis-ocimene	11.69
		2-ethyl octenoate	8.38
		Dendrolasin	5.04
CAM IV	42	Ethyl hexanoate	100.00
		β-cis-ocimene	37.28
		Ethyl octanoate	17.54
		2-ethyl octenoate	3.68
		Dendrolasin	0.58
Gera	45	Ethyl hexanoate	100.00
		Dendrolasin	36.44
		Ethyl octanoate	12.71
		β-cis-ocimene	5.19
		2-ethyl octenoate	1.73

[a]Relative intensity was normalized according to the most abundant volatile compound (ethyl hexanoate); mean values corresponding to n = 3 measurements.

Table 3. Continued...

Plant	Total amount of volatile compounds	Main volatile compounds	Mean relative intensity (%)[a]
Milorde	31	Ethyl hexanoate	100.00
		Ethyl octanoate	36.90
		Dendrolasin	24.41
		β-cis-ocimene	22.26
		2-ethyl octenoate	6.24
MV X	33	Ethyl hexanoate	100.00
		Dendrolasin	37.93
		β-cis-ocimene	29.98
		Ethyl octanoate	24.41
		2-ethyl octenoate	3.43
MV XII	22	Ethyl hexanoate	100.00
		β-cis-ocimene	34.34
		Ethyl octanoate	10.68
		2-ethyl octenoate	1.82
MV XVII	21	Ethyl hexanoate	100.00
		β-cis-ocimene	18.36
		Ethyl octanoate	3.03
		2-ethyl octenoate	0.45
S I	28	Ethyl hexanoate	100.00
		Ethyl octanoate	41.89
		Dendrolasin	15.21
		2-ethyl octenoate	8.41
		β-cis-ocimene	5.16
S IV	31	Ethyl hexanoate	100.00
		Ethyl octanoate	5.31
		β-cis-ocimene	5.25
		2-ethyl octenoate	0.79
		Dendrolasin	0.37
S XVIII	43	Ethyl hexanoate	100.00
		β-cis-ocimene	30.77
		Ethyl octanoate	16.34
		2-ethyl octenoate	2.37
		Dendrolasin	0.15
Tanasio	37	Ethyl hexanoate	100.00
		Ethyl octanoate	56.62
		β-cis-ocimene	32.01
		2-ethyl octenoate	14.51
		Dendrolasin	2.69

[a]Relative intensity was normalized according to the most abundant volatile compound (ethyl hexanoate); mean values corresponding to n = 3 measurements.

Ethyl hexanoate was the compound with the highest relative intensity in all chromatographic profiles. This finding is in agreement with other studies. It has been reported that ethyl hexanoate and ethyl octanoate were found in higher percentages in the pequi pulp and in the pequi seed oil (PASSOS et al., 2002; MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009).

3.1 Esters

The esters were the most abundant volatile components found in the headspace extract of the pequi pulp, accounting for 45.5% of the total volatile components. These compounds are important constituents of the aroma of many fruits (BELITZ; GROSCH; SCHIEBERLE, 2009). Furthermore, it has been reported in previous studies (MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009) that esters were the most abundant compounds in the volatile profile of the pequi pulp.

Although 35 different esters were identified in this work, only methyl hexanoate, ethyl hexanoate, (E)-2-ethyl hexenoate, ethyl octanoate, ethyl (E)-2-octenoate, isopentyl hexanoate, ethyl decanoate, ethyl dodecanoate, ethyl hexadecanoate were common to all samples. Ethyl dodecanoate has not been previously found in the pequi pulp (MAIA; ANDRADE; SILVA,

2008; DAMIANI et al., 2009), but only in the pequi oil seed (PASSOS et al., 2002).

3.2 Hydrocarbons

A total of 11 different hydrocarbons were identified, i.e., 14.3% of all the volatile compounds from the pequi headspace consisted of hydrocarbons although all the hydrocarbons were not common to all samples. However, 10-carbon compounds such as 2,3,5,8-tetramethyldecane, along with 2,5,9-trimethyldecane, were common to almost all plants, but they were not present in the chromatographic profile of the headspace extract from MV XVII. These two hydrocarbons were found for the first time in the pequi pulp. 2,3,5,8-tetramethyldecane was identified in the essential oils of *Panax japonicas* roots (ZHANG; ZHANG; SUN, 2011), and 2,5,9-trimethyldecane was found in the profile of volatile compounds from the leaves of *Gentiana asclepiadea* (GEORGIEVA et al., 2005). Among all hydrocarbons identified, only heneicosane, eicosane, octadecane, and undecane have been previously identified in the pequi by other authors (PASSOS et al., 2002; MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009).

3.3 Terpenoids

In the analysis of these pequi plants, terpenoids contributed with 14.3% of the total volatile compounds. β-cis-ocimene was common to all plants, and it has been found in the volatile fraction of the pequi by other authors (PASSOS et al., 2002; MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009). Terpenes stimulate a broad spectrum of flavors in foods (BELITZ; GROSCH; SCHIEBERLE, 2009). Another terpenoid compound that can be highlighted is dendrolasin, which was found in the pequi for the first time, but it has been previously identified in the volatile profile of red apples in the Marche region (Italy) (FRATERNALE et al., 2011) and in *Capsicum* fruits (RODRÍGUEZ-BURRUEZO et al., 2010).

3.4 Other compounds

Other compounds such as lactones, pyrazines, organic acids, ketones, alcohols, and aldehydes were also recovered from the headspace above the pequi pulp and were subsequently separated and identified by GC-MS. These other compounds contribute to 25.9% of the total number of volatile compounds and, among them, all the compounds were found in the pequi for the first time (PASSOS et al., 2002; MAIA; ANDRADE; SILVA, 2008; DAMIANI et al., 2009). None of these compounds was common to all plants.

3.5 Multivariate analysis

PCA was performed using the data of the eighteen pequi plants and the 77 volatile compounds. The ethyl hexanoate data were excluded because the relative abundance was 100% for all samples. The analysis was applied to the normalized intensities of the peaks. The cumulative percentage contribution of variance of the first three principal components (PCs) was 96.9%, which was sufficiently high to represent all of the variables. PC1 represented 45.9% of the total variance; PC2 explained 33.0%, and PC3, 18.0%.

PCA analysis in two dimensions, PC1 and PC2, presented the distribution of each plant. The scatter plot of PCA scores of all the eighteen pequi plants is shown in Figure 1a, and the corresponding loading plot establishing the relative importance of the variables (volatile compounds) is shown in Figure 1b.

The positions of the pequi plants in the scatter plot of PCA scores (Figure 1a) indicated that the scores of some plants presented a tendency to isolation. In PC1, CAM I was displaced in a positive direction, along with Gera, Ary, MV X, Milorde, and BJ II. As shown in Table 2, the plants that exhibited higher dendrolasin concentrations are as follows: CAM I (75.09%), MV X (37.93%), Gera (36.44%), Ary (26.91%), and S I (15.21%). Ary and S I belong to the same population (Population 1) as Gera, and MV X belong to Population 4. This result indicates that

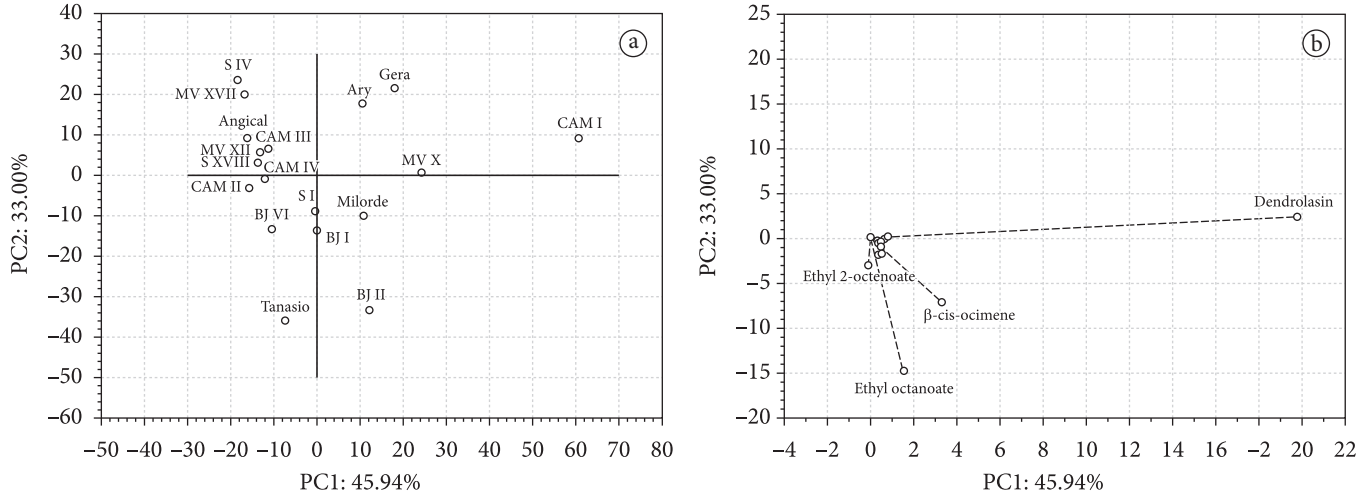

Figure 1. a) Score plot of the pequi plants; b) Loading plot.

the first PC discriminated adequately the plants that exhibited higher concentrations of dendrolasin from the others.

In PC2, the plants Tanasio, BJ II, BJ VI, BJ I, Milorde, S I, and CAM II exhibited displacement in a negative direction. These plants presented higher ethyl octanoate concentrations, as shown in Table 2: Tanasio (56.62%), BJ II (47.87%), BJ VI (43.01%), S I (41.89%), Milorde (36.90%), BJ I (36.53%), and CAM II (34.22%). In this case, the plants BJ I, BJ II, and BJ VI were allocated to the same population (Population 2, rural community of Bom Jantar), but BJ II was more isolated from the other samples and closer to Tanasio. Milorde, Tanasio, and S I also belong to the same population; however, like presented above, Tanasio is more isolated and closer to BJ II than to the plants of Population 1 (rural community of Sambaíba). This result indicates that the second PC separated the plants that contained higher ethyl octanoate concentrations.

The loading plot (Figure 1b) shows the variables that had the highest influence on the principal components. These variables were dendrolasin, β-cis-ocimene, ethyl octanoate, and ethyl 2-octenoate. These volatile compounds were selected as markers for the discrimination of pequi plants.

The results of the Cluster Analysis (Figure 2) indicated a tendency to a formation of three main clusters, and the largest group was composed of S IV, MV XVII, CAM II, Angical, BJ VI, CAM II, S I, MV XII, S XVIII and CAM IV. The second group included only Milorde and BJ I. Finally, the last group was formed by Gera and Ary. The other plants, Tanasio, BJ II, CAM I, and MV X showed a tendency to isolation. CAM I had the highest content of dendrolasin, followed by MV X. This information could explain its lack of similarity with the other plants.

The formation of clusters did not reflect the distribution of populations; plants of the same population were allocated in different clusters. This result indicates that there is greater variability within the populations than between populations, i.e., plants from different regions have similar characteristics, and plants of the same region have different characteristics.

Similar results for the application of multivariate methods for the characterization of fruit varieties are found in the literature. SOLIS-SOLIS et al. (2007) were able to discriminate eight varieties of apricots (*Prunus armeniaca*) by SPME and other aroma extraction techniques using PCA and factorial discriminate analysis (FDA). These authors also verified that the concentrations of volatile compounds depend on the variety and the extraction technique used (SOLIS-SOLIS et al., 2007). Similarly, the volatile composition of musts from four white grape (*Vitis vinifera* L.) varieties was analyzed, and each exhibited different volatile composition patterns, which suggested that it was possible to establish volatile markers for their characterization after the application of multivariate analyses (ROCHA et al., 2007). Wang et al. (2009) investigated

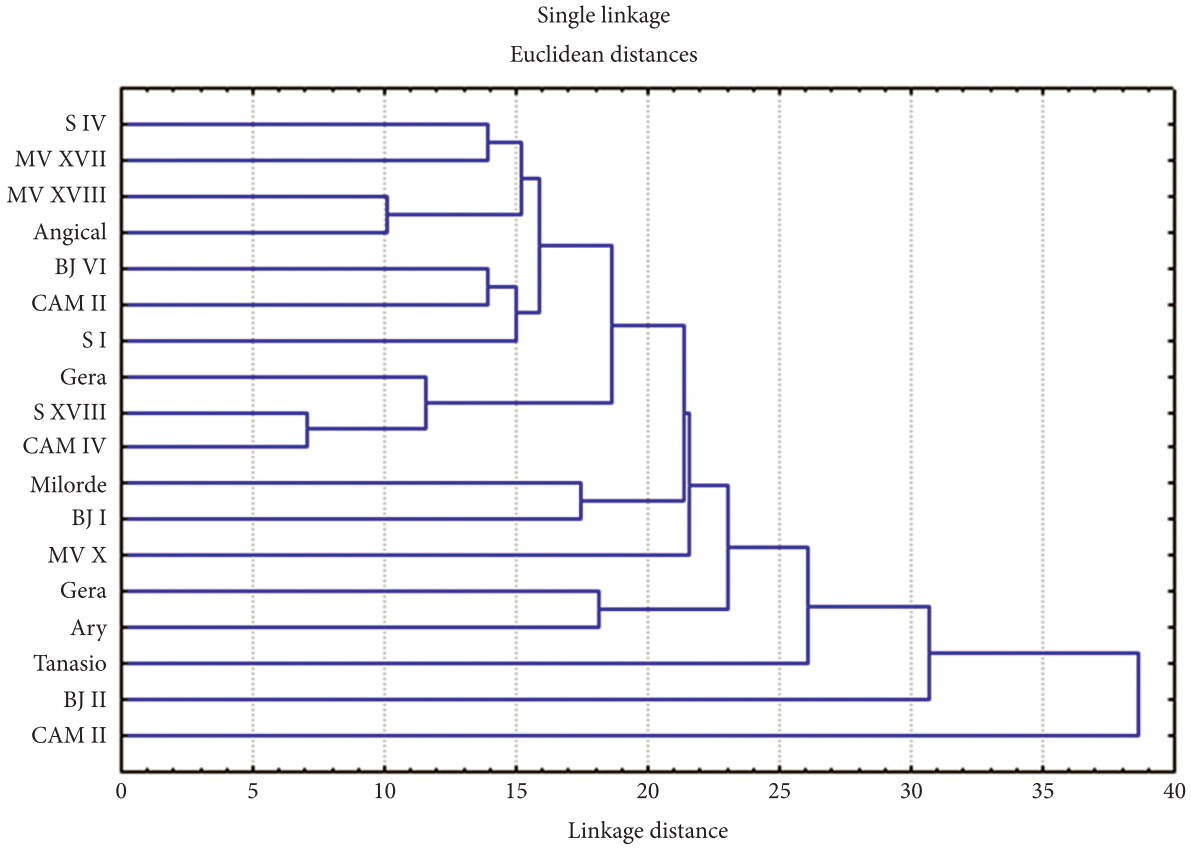

Figure 2. Dendrogram of the Cluster Analysis.

characteristics of the volatiles of peaches and nectarines by HS-SPME-GC-MS, and the results indicated that the composition and concentration of volatiles depended on the genotypic background and germplasm origin. The volatile composition of fruits from four Citrus varieties involving four different species was studied by González-Mas et al. (2011). Their findings supported the conclusion that the volatile profile was able to differentiate among all the varieties and revealed complex interactions between them, including the participation in the same biosynthetic pathway.

In this study, the results of the PCA and Cluster analyses indicated that there is a great variability in concentration and type of volatile compounds among the individuals studied. Therefore, these compounds were used to discriminate among plants and populations providing a scientific basis to support the preservation of the species.

4 Conclusions

This study showed that the use of HS-SPME with GC-MS enabled the characterization of pequi plants on the basis of their volatile compounds. Some volatile compounds have previously been identified in the pequi fruit. Several other compounds were identified for the first time in this study. Differences were observed in the volatile composition of the eighteen pequi plants studied, indicating that there is a characteristic profile corresponding to the genetic origin. The compounds ethyl octanoate, dendrolasin, β-cis-ocimene, and ethyl-2-octenoate were probably the volatile markers that contribute to discriminate the pequi tree plants. These results indicated that the plants differ from each other with regard to the profile of the volatile compounds, and this difference might be related to the sensory quality of the fruit. It can also be pointed out that there was greater variability within the populations than between pequi tree populations regarding the type and concentration of the volatile compounds present in the fruit. This finding was supported by the results of PCA and Cluster Analysis.

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