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Untargeted fingerprinting of cider volatiles from different geographical regions by HS-SPME/GC-MS

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ABSTRACT

The volatomic fingerprint of ciders produced in different geographical regions from Madeira Island was established using headspace solid phase microextraction combined with gas chromatography mass spectrometry (HS-SPME/GC–MS) in order to explore the effects of geographical region on the volatile pattern ciders in addition to identify potential molecular geographic markers. A total of 107 volatile organic compounds (VOCs) belonging to different chemical families were identified from which 50 VOCs are common to all ciders analysed. Significant differences in the relative content of VOCs from ciders of different geographical regions were observed. The potential of the identified VOCs for ciders discrimination according to region was assessed through chemometric tools, such as principal components analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). The PCA showed significant differences among ciders from different island geographical regions. Fifteen VOCs responsible for ciders discrimination were identified by PLS-DA. Fifteen VOCs, namely five terpenoids, four alcohols, three acids and three esters, present variable importance in projection (VIP) values higher than one. Our findings provide relevant information related to volatile signature of ciders produced in Madeira Island, which may be a useful tool to cider-making process contributing to improve the quality of the final product. In addition, the geographical discrimination recognizes the unique and distinctive characteristics that will allow in the future to protect the quality and typicity of products originating in certain geographical regions.

1. Introduction

Apples are the most consumed fruits worldwide and grown especially in temperate regions. According to the literature, > 10,000 apple varieties are documented, resulting in an extensive range of properties namely resistance, sweetness, acidity and ripening [1]. The apples production in Madeira Island reached over 3300 tons per year and there are several varieties available, being apple tree from *Malus domestica* Borkh. (family Rosaceae), from hybrid origin, the most predominant [2]. Apples are good raw material for the production of value-added products, such as juices, cakes, concentrates, dried fruits and ciders [3].

Cider is a worldwide traditional beverage and, in recent years, its consumption increased remarkably. In Madeira Island, contrary to the observed in remaining country, the production through the traditional process of cider has never been stopped. The quality of the final product depend of each step involved in cider-making, being the apple selection the most important step. The quality of apples and their derivatives is the result of several factors, such as cultivars used and climatic conditions. Thus, comprehensive knowledge about the chemical composition of apple varieties and the effect of their blending is crucial for understanding which volatile organic compounds (VOCs) result in high-quality processed products [1].

Aroma profile is the utmost criteria in the assessment of cider quality and consumer acceptance, that is composed by a large number of VOCs belonging to different chemical families, such as alcohols, esters, acids, terpenoids, carbonyls compounds, among others [3]. The establishment of volatile signature of cider can provide important information related to raw materials and technological processes employed in the fermentation to guarantee cider quality and to avoid financial losses [4]. Currently, several studies have been performed related to the characterization of cider volatile profile [1,4–8]. In this context, Pello-Palmo et al. [5] characterized the new cider apple genotypes obtained from the Asturias breeding program by chemometric analysis of their VOCs with the purpose of selection and inclusion of

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those into the protected designation of origin (PDO) cider of Asturias and production of high quality apple derivates. Riekstina-Dolge and Kruma [4] compared the phenolic and volatile profile of commercial and experimental ciders, whereas Villiére et al. [6] explored the relationships between some parameters (e.g., apple blends, pressing conditions, pre-fermentation clarification, biomass reduction) of the French cider-making process and the odorant compounds of cider. Antón-Díaz et al. [8] evaluated the effect of different treatments involving contact with natural lees on the aromatic profile of cider, while Antón et al. [7] performed the aromatic profile of nine Asturias cider through chemical quantitative, gas chromatography-olfactometry (GC-O) and sensory analysis. The results obtained with previous study revealed the presence of 55 aromatic areas, and VOCs such as 3-methyl-2-butenol, 2-phenylethanol, ethyl 2-methylbutyrate, ethyl hexanoate, ethyl octanoate, hexanoic acid, octanoic acid, 2-phenylethyl acetate, 4-ethyl guaiacol and 4-ethyl phenol were considered as being part of the structure of cider aroma.

The purpose of the current research is to establish the volatile signature of cider from five different Madeira Island geographical regions using headspace solid phase microextraction (HS-SPME) tandem with gas chromatography–quadrupole mass spectrometry (GC–qMS). Chemometric tools, such as principal components analysis (PCA), partial least square-discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA) were applied to obtain insights into variations of the volatile signature and to identify the VOCs responsible for the discrimination among cider clusters and explain these results based on island geographic regions. As far as we know, this is the first time that volatile signature of cider produced in Madeira Island was analysed. Our study's findings could provide new opportunities to promote the cider-making with enhanced levels of odor and bioactive compounds, which will improve the quality of the final product. Moreover, regional government aims to valorize cider products through the certification as Protected Designation of Origin (PDO) product and their commercialization worldwide.

2. Material and methods

2.1. Reagents and standards

The VOCs standards used for identification of the target compounds were purchased from Sigma-Aldrich (Madrid, Spain), Acros Organics (Geel, Belgium) and Fluka (Buchs, Switzerland) with purity > 98%. The individual stock solutions were prepared in ethanol at a concentration of 500 mg L^{-1} and were stored at 4 °C. Sodium chloride (NaCl, 99.5%) and 3-octanol (internal standard, IS) were obtained from Sigma-Aldrich (Madrid, Spain), whereas the GC carrier gas, helium of purity 5.0 was obtained from Air Liquide, Portugal. The glass vials, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre and SPME holder for manual sampling were purchased from Supelco (Bellefonte, PA, USA). The alkane series, C_8 to C_{20}, with a concentration of 40 mg L^{-1} in *n*-hexane used to determine the kovat index (KI) was supplied from Fluka (Buchs, Switzerland).

2.2. Cider samples

Seven ciders (three samples from the same cellar, in total 21 samples) were produced in 2015 from five different Madeira Island geographical regions, namely Camacha (N 32° 40′ 47.47″; W 16° 51′ 1.04″, altitude 730 m), Santo da Serra (N 32° 43′ 20.29″; W 16° 49′ 14.83″, altitude 675 m), Machico (N 32° 43′ 54.62″; W 16° 47′ 28.64″, altitude 230 m), São Roque do Faial (N 32° 46′ 13.18″; W 16° 51′ 24.32″, altitude 269 m) and Jardim da Serra (N 32° 41′ 15.67″; W 16° 59′ 30.99″, altitude 746 m). Furthermore, ciders from Camacha and São Roque do Faial were obtained from the same geographic zone but from different cider producers, and for this reason for these geographical regions have two samples. These cider samples were obtained by monovarietal apple varieties, according to the particular conditions used by the respective cider producers. Briefly, the apples were washed with tap water for 10 min and continually pressed, filtered and held in a refrigerated tank for 24 h at 4 °C. Apple juice concentrate was diluted to a total concentration of 200 g L^{-1} (23°brix), and 80 mg L^{-1} SO_2 was added. Then, the apple juice was inoculated with 2% (*v*/v) of *Saccharomyces cerevisiae* during 18 days at 18 °C in a closed fermentation tank to produce apple cider. The final product was transported to laboratory in a cooler with ice and kept at −80 °C until the analysis.

2.3. Solid phase microextraction (SPME)

The SPME procedure was adopted from a previous study performed in our laboratory [9]. Briefly, 10 mL of sample, 10 μL of 3-octanol (IS, 250 μg L^{-1}), 3 g of NaCl and a magnetic stirrer were added into a 20 mL amber glass. Then, the vial was capped with a PTFE-faced silicone septum and placed in a thermostatic block with a constant magnetic stirring (800 rpm) at 50 °C during 30 min. Subsequently, after extraction the fibre DVB/CAR/PDMS was withdrawn into the holder needle, removed from the vial and immediately introduced into the GC injector port for 6 min at 250 °C for thermal desorption of the analytes. DVB/CAR/PDMS coating (molecular weight ranging from 40 to 275) combines the absorption properties of the liquid polymer with the adsorption properties of porous particles, which contains macro (> 500 Å), *meso* (20–500 Å) and microporous (2–20 Å) and has bipolar properties. The mutually synergetic effect of adsorption and absorption of the stationary phase explains its high retention capacity [10]. All assays were done in triplicate.

2.4. Gas chromatography-mass spectrometry conditions

Chromatographic separations were performed using an Agilent 6890 N (Palo Alto, CA, USA) gas chromatograph system equipped with a BP-20 (30 m × 0.25 mm i.d. × 0.25 μm film thickness) fused silica capillary column supplied by SGE (Darmstadt, Germany) with helium (Helium N60, Air Liquid, Portugal) as carrier gas at a flow rate of 1 mL min^{-1} (column-head pressure: 13 psi). The injector temperature was fixed at 250 °C and a splitless injector equipped with an insert of 0.75 mm i.d. was used. The temperature program was programmed as follows: initial temperature 40 °C and a ramp of 3 °C min^{-1} to 220 °C and maintaining a constant temperature for 10 min at the end. The manifold, GC-qMS interface and quadrupole temperatures were held at 180, 220 and 180 °C, respectively. MS detection was performed in full scan in an Agilent 5975 quadrupole inert mass selective detector, the ion energy used for the electron impact (EI) was 70 eV and the source temperature was 180 °C. The electron multiplier was set to the auto tune procedure. The mass acquisition range, made in full scan mode, was 30–300 *m/z*, 1.9 spectra/s.

VOCs identification was achieved by the following ways:

(i) comparison the GC retention times and mass spectra with those of the standard, when available;
(ii) all mass spectra were also compared with the data system library (NIST, 2005 software, Mass Spectral Search Program v.2.0d; Nist 2005, Washington, DC);
(iii) kovat index (KI) values were determined according to the van den Dool and Kratz equation [11].

For the determination of the KI, a C_8–C_{20} n-alkanes series was used, and the values were compared, when available, with values reported in the literature for similar columns [12–14].

The VOCs concentration was estimated, semi quantitatively, using the added amount of 3-octanol (IS) according to the following equation: VOCs concentration = (VOC GC peak area / IS GC peak area) × IS concentration. This approach was already performed in a previous scientific study [14].

2.5. Statistical analysis

The obtained data was analysed with Metaboanalyst 4.0 [15], which included a data pre-processing to remove metabolites with missing values (MV) and normalization (data transformation by cubic root and data scaling by mean-center). The normalized data was further subjected to *one-way* ANOVA followed by Tukey's test for post-hoc multiple comparisons of means and multivariate statistical analysis namely, principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) to provide insights into the separations among the ciders from different island geographic regions under study and to detect VOCs that may indicate differences among the samples sets. Then, the model validation was evaluated through R^2 (represents goodness of fit), Q^2 (represents predictive ability), and a permutation test (1000 permutation). The significant differences in the model were assessed by calculating the *p*-values from the cross-validation analysis. Finally, Pearson's correlation was used to build the heat map of the five island geographic regions using the VOCs identified with the aim of identify clustering patterns.

3. Results and discussion

3.1. Establishment of volatile signature of ciders from different geographic region origin

The volatile signature of five ciders from different geographic regions of Madeira Island, namely Camacha (Cam), Machico (Mac), Santo da Serra (SSerra), Jardim da Serra (JSerra) and São Roque do Faial (SRFaial), was established using HS-SPME/GC-qMS. A total of 107 VOCs were tentatively identified in the seven cider samples (Table 1), being 50 VOCs common to all samples, by matching to NIST (resemblance percentage above 80%), by matching calculated KI values to literature values and/or by injection of authentic standards. The VOCs tentatively identified comprise 41 esters, 21 alcohols, 24 terpenoids, 13 acids, three carbonyl compound, four lactones and one volatile phenol. In ciders obtained from Camacha 92 VOCs were identified, whereas in ciders from Jardim da Serra and São Roque do Faial 74 and 70 VOCs were identified, respectively. A similar number of VOCs were identified in ciders from Machico and Santo da Serra, 65 and 64, respectively. Qualitatively, remarkable differences for esters and terpenoids among ciders from different island geographic regions were observed (Table 1), being ciders from Camacha the richest one and Santo da Serra the ciders the poorest. The VOCs extracted by HS-SPME technique have also been identified in other studies performed in ciders from Spain and China [1,3,6–8]. The relative concentration values ($\mu g\,L^{-1}$) of each VOC, grouped by chemical families, and relative standard deviation (% RSD) are listed in Table 1. The distribution of VOCs, according to its chemical family, is represented in Fig. 1. Taking into account the relative concentrations, the predominant chemical families in all ciders analysed were alcohols (47.3–61.3% for total volatile profile), esters (23.4–36.8%) and acids (12.7–17.5%). The contribution of the remaining chemical families identified, terpenoids, carbonyl compounds, lactones and volatile phenols for total volatile profile was lower than 2%.

The alcohols fraction was composed by aliphatic (e.g., 1-butanol, 3-methyl-1-butamol) and aromatic alcohols (e.g., 2-phenylethanol). The alcohols concentration is affected by medium composition, clarification technique and fermentation process. The total concentration of alcohols was 4877.21 and 5107.49 $\mu g\,L^{-1}$ for cider produced in Machico and Santo da Serra (Fig. 1), respectively, whereas for the remaining ciders analysed, the total concentration was higher than 7000 $\mu g\,L^{-1}$. As can be observed in Table 1, 3-methyl-1-butanol (concentration range, 3194.39–6413.62 $\mu g\,L^{-1}$) and 2-phenylethanol (1030.90–1920.53 $\mu g\,L^{-1}$) were the predominant alcohols identified in all ciders analysed. These two alcohols are well reported as regular compounds in fermented beverages, such as ciders, wines and whiskey, being 3-methyl-1-butanol associated to fruit notes (e.g., banana) and 2-phenylethanol with floral, honey and fragrant odours. According to Antón et al. [7], 3-methyl-1-butanol and 2-phenylethanol could be considered as being part of the structure of Asturias cider aroma. In addition, 2-butanol was only detected in cider from Jardim da Serra, whereas nonanol was only detected in cider from Camacha. On the other hand, 3-ethoxypropanol and octenol were only detected in cider from Machico and Santo da Serra.

Esters are qualitatively the main chemical family and contribute positively to the general quality of cider samples being responsible for their fruity and floral sensory properties [7]. Perhaps, the majority of the esters are initially present in apple and apple juice, most of them are formed during fermentation step, through esterification process [3]. As can be observed in Table 1, among the esters identified, only 18 esters are common to all ciders analysed, being ethyl acetate (536.65–718.77 $\mu g\,L^{-1}$), ethyl hexanoate (203.63–785.01 $\mu g\,L^{-1}$), ethyl octanoate (916.40–3179.90 $\mu g\,L^{-1}$) and ethyl decanoate (433.55–1234.79 $\mu g\,L^{-1}$) the predominant esters, accounting on average 81.40% of total ester profile of cider samples. Ethyl hexanoate and ethyl octanoate were also reported as potential active aroma compounds Asturias ciders [7]. Ethyl 3-ethoxypropanoate, 2-ethylhexyl acetate, isopentyl hexanoate, butyl octanoate, methyl 2-hydroxybenzoate, ethyl 2-hydroxybenzoate and methyl jasmonate were only detected in cider from Camacha, whereas butyl acetate, methyl octanoate and methyl decanoate in cider from São Roque do Faial. On the other hand, ethyl 2-hydroisovalerate was only detected in cider from Jardim da Serra, whereas ethyl (E)-3-hexenoate, ethyl benzoate and ethyl 9-decenoate were only detected in ciders from Camacha and São Roque do Faial.

Acids contributed around 14.68% for total volatile signature of cider samples, being the lowest contribution observed in ciders from Jardim da Serra (13.51%), followed by São Roque do Faial (13.55%), Machico (14.86%), Santo da Serra (15.42%) and Camacha (15.93%). Acetic (296.01–617.81 $\mu g\,L^{-1}$), octanoic (390.12–1086.06 $\mu g\,L^{-1}$) and decanoic (387.50–1163.65 $\mu g\,L^{-1}$) acids were predominant in the studied cider samples, accounting on average with 94.26% of total acid profile of cider samples. A similar result was reported by Ye et al. [3], where hexanoic and octanoic acids (products of lipid oxidation) were the main acids in ciders from China. These two acids contribute with soapy, green and fatty notes to cider aroma. In addition, propanoic acid was only detected in cider from Camacha, whereas 2-methylpropanoic acid was detected in all ciders except in cider from São Roque do Faial.

Terpenoids represent an excellent example of VOCs from varietal origin, come directly from fruits, which may be used for authentication and/or typicality of cider samples. The ciders from Jardim da Serra seem to be the richest sample in terpenoids (1.70%), followed by Santo da Serra (0.95%), Camacha (0.94%), Machico (0.89%) and São Roque do Faial (0.52%). From the pool of terpenoids identified, Table 1, only five were detected in all ciders analysed, namely limonene oxide (0.78–71.80 $\mu g\,L^{-1}$), camphor (1.22–3.82 $\mu g\,L^{-1}$), vitispirane I (1.44–4.75 $\mu g\,L^{-1}$), vitispirane II (1.63–6.73 $\mu g\,L^{-1}$) and δ-cadinol (2.76–14.09 $\mu g\,L^{-1}$). From a sensorial point of view, terpenoids showed a positive contribution to aroma, with fruit, floral, woody and citrus notes [16].

Carbonyl compounds contribute on average with 0.10% for total volatile profile of cider samples. However, this chemical family was not detected in cider from São Roque do Faial (Table 1), whereas 3-hydroxybutan-2-one (1.21–4.86 $\mu g\,L^{-1}$) was only detected in ciders from Santo da Serra, Machico and Jardim da Serra. In addition, nonanal has been also identified in ciders from China, and its presence contribute positively to cider aroma with fruity or fatty-floral odours [3].

Lactones contribute on average to total volatile profile with 1.11%, being the highest contribution in cider from Machico (1.71%) and Santo da Serra (1.46%). Wine lactone (1.87–7.74 $\mu g\,L^{-1}$) was only detected in cider from Camacha and São Roque do Faial. This chemical family revealed a positive contribution to aroma, with caramel, fruity, sweet and coconut-like notes [17].

Table 1
Relative concentration of volatile organic compounds (VOCs) identified in cider from different island geographic zones using HS-SPME$_{DVB/CAR/PDMS}$/GC-qMS.

Peak n°	RT (min)[a]	KI_{cal}[b]	KI_{lit}[c]	Chemical families	Relative concentration (μg L^{-1}) (%RSD)													
					Cam		Cam1		*S. Serra*		Machico		S.R. Faial		S.R. Faial 1		J. Serra	
Esters																		
1	2.91	955	907	Ethyl acetate[d]	659.54	(18)	546.35	(9)	718.77	(1)	697.58	(2)	692.86	(7)	536.65	(14)	496.72	(4)
3	4.35	1039	1015	Isobutyl acetate	–		–		16.86	(2)	22.19	(4)	–		–		–	
5	4.68	1055	1028	Ethyl butanoate[d]	19.91	(10)	15.79	(4)	2.36	(12)	1.92	(3)	29.93	(11)	28.72	(5)	–	
6	5.29	1081	1050	Ethyl 3-methylbutanoate	10.12	(8)	38.93	(4)	4.58	(1)	4.73	(1)	5.45	(16)	3.50	(8)	–	
7	5.34	1084	1075	Butyl acetate[d]	–		–		–		–		8.74	(8)	1.85	(11)	–	
9	6.51	1125	1117	Isoamyl acetate[d]	406.55	(9)	386.86	(2)	282.69	(1)	334.78	(2)	392.54	(10)	217.19	(3)	141.03	(2)
13	10.04	1222	1220	Ethyl hexanoate[d]	785.01	(10)	406.45	(20)	205.75	(4)	254.45	(1)	457.36	(11)	352.75	(2)	203.63	(3)
15	12.40	1285	1304	Ethyl 3-ethoxypropanoate	1.93	(8)	2.76	(11)	–		–		–		–		–	
16	12.67	1291	1292	Ethyl (E)-3-hexenoate	6.20	(2)	4.16	(9)	–		–		1.26	(7)	0.74	(8)	–	
17	13.23	1305	1328	(E)-3-Hexen-1-ol acetate	7.44	(7)	10.43	(11)	5.12	(2)	4.16	(3)	13.30	(9)	2.25	(6)	1.16	(13)
20	13.87	1320	1305	Ethyl 2-hexenoate[d]	4.74	(11)	5.27	(4)	–		–		1.64	(8)	1.57	(6)	1.62	(18)
21	13.99	1331	1327	(Z)-2-Hexen-1-ol acetate	5.76	(3)	1.49	(9)	–		–		–		–		–	
22	14.67	1339	1358	Ethyl lactate[d]	197.99	(10)	138.13	(14)	100.90	(12)	64.63	(3)	69.43	(10)	117.58	(4)	238.47	(5)
25	15.53	1263	1270	Hexyl acetate[d]	40.63	(6)	28.80	(2)	14.90	(2)	14.43	(3)	43.83	(11)	36.35	(5)	9.90	(2)
28	16.10	1370	–	2-Ethylhexyl acetate	1.01	(5)	0.96	(3)	–		–		–		–		–	
30	16.16	1372	1389	Methyl octanoate[d]	–		–		–		–		0.98	(5)	2.63	(8)	–	
34	17.80	1405	1394	Ethyl 2-hydroxyisovalerate	–		–		–		–		–		–		3.16	(2)
35	18.16	1416	1436	Ethyl octanoate[d]	2420.97	(8)	2373.34	(2)	1024.43	(3)	1026.05	(6)	2819.08	(10)	3179.90	(2)	916.40	(13)
37	19.19	1441	–	Isopentyl hexanoate	3.42	(9)	2.51	(6)	–		–		–		–		–	
48	22.30	1518	1483	Ethyl 3-hydroxybutanoate[d]	5.16	(12)	6.34	(4)	0.45	(8)	0.77	(6)	2.73	(6)	3.08	(13)	1.61	(10)
51	22.61	1526	1551	2-Ethyl hydroxycaproate	5.28	(11)	2.02	(11)	0.48	(16)	0.39	(8)	1.26	(8)	1.63	(8)	10.88	(1)
53	23.04	1536	1533	Hexyl butanoate	12.79	(15)	8.34	(3)	–		–		4.95	(11)	3.98	(3)	–	
60	24.76	1574	1591	Methyl decanoate[d]	–		–		–		–		1.08	(9)	1.77	(19)	–	
66	26.58	1617	1636	Ethyl decanoate[d]	1106.31	(13)	1015.39	(4)	434.14	(3)	434.62	(12)	982.53	(8)	1234.79	(3)	433.55	(19)
67	26.85	1625	1610	Butyl octanoate	8.80	(1)	7.06	(5)	–		–		–		–		–	
69	27.35	1639	1648	Ethyl benzoate[d]	46.75	(7)	31.91	(1)	–		–		58.62	(10)	44.56	(2)	–	
71	28.07	1659	1689	Diethyl succinate[d]	79.69	(3)	90.40	(2)	34.06	(1)	36.46	(10)	140.00	(14)	113.10	(8)	111.07	(8)
72	28.39	1668	1694	Ethyl 9-decenoate[d]	99.09	(10)	114.36	(3)	–		–		226.52	(10)	162.84	(2)	–	
74	29.34	1693	1664	Ethyl 3-hydroxyhexanoate[d]	2.21	(13)	1.01	(20)	0.84	(19)	0.82	(15)	1.51	(8)	2.56	(18)	5.34	(12)
78	31.42	1754	–	Methyl 2-hydroxybenzoate	1.45	(11)	1.29	(12)	–		–		–		–		–	
79	32.07	1773	1775	Ethyl phenylacetate	28.74	(12)	18.95	(7)	2.80	(2)	2.70	(13)	3.08	(7)	2.75	(1)	1.73	(8)
80	32.47	1784	–	Dibuthyl succinate	2.73	(9)	6.79	(2)	0.75	(4)	0.85	(14)	1.42	(8)	1.30	(9)	2.11	(14)
81	32.73	1791	–	Ethyl 2-hydroxybenzoate	1.23	(2)	0.92	(5)	–		–		–		–		–	
83	34.32	1838	1837	Ethyl dodecanoate[d]	78.33	(12)	41.96	(3)	29.84	(3)	28.80	(6)	62.80	(6)	77.61	(18)	57.14	(15)
85	34.99	1857	1821	Benzyl propanoate	27.11	(13)	56.16	(15)	30.50	(18)	30.97	(18)	6.70	(15)	15.03	(4)	7.75	(18)
86	35.58	1873	1849	Benzyl butanoate	7.58	(6)	2.78	(16)	4.10	(4)	4.33	(5)	12.14	(3)	17.85	(11)	4.23	(13)
87	35.87	1800	1803	2-Phenylethyl acetate[d]	9.23	(12)	8.13	(2)	–		–		–		–		1.74	(10)
97	43.13	2193	2189	Phenylethyl benzoate	5.18	(15)	3.93	(4)	–		–		9.08	(4)	12.26	(4)	6.20	(12)
102	48.08	2308	2328	Diethyl tartrate	3.25	(6)	2.96	(2)	–		–		1.96	(18)	1.28	(11)	0.95	(13)
Alcohols																		
2	3.15	968	929	Ethanol	360.88	(13)	302.58	(6)	182.12	(4)	204.42	(18)	313.70	(6)	348.77	(13)	126.13	(9)
4	4.66	1054	1099	2-Butanol[d]	–		–		–		–		–		–		29.78	(1)
8	6.12	1113	1085	2-Methylpropanol[d]	41.99	(10)	69.79	(17)	29.70	(7)	27.50	(1)	23.38	(12)	16.05	(4)	40.63	(2)
10	7.87	1165	1145	1-Butanol[d]	4.24	(8)	10.52	(15)	3.62	(5)	3.42	(3)	22.51	(13)	23.87	(1)	8.50	(8)
12	9.51	1206	1206	3-Methylbutanol[d]	6413.62	(11)	5273.29	(12)	3423.92	(5)	3194.39	(2)	5930.22	(9)	5780.68	(2)	5595.16	(4)
19	13.51	1312	1332	2-Heptanol[d]	5.39	(8)	7.73	(8)	–		4.65	(4)	–		–		–	
23	15.15	1350	1360	1-Hexanol[d]	183.12	(4)	266.92	(10)	55.50	(1)	55.79	(1)	267.68	(5)	266.70	(1)	48.04	(2)
24	15.23	1352	1386	(E)-3-Hexenol	1.35	(13)	3.71	(19)	1.08	(4)	1.01	(15)	0.97	(2)	0.73	(8)	0.87	(4)
26	15.81	1364	1379	3-Ethoxypropanol	–		–		2.74	(12)	0.38	(2)	–		–		–	
29	16.11	1371	1391	(Z)-3-Hexenol	40.24	(10)	50.65	(13)	16.14	(3)	16.68	(6)	9.31	(6)	6.56	(8)	16.63	(5)
33	17.69	1383	1388	Octanol	133.81	(12)	218.89	(2)	237.52	(2)	222.06	(15)	19.77	(10)	10.78	(1)	16.77	(8)
42	20.48	1475	1487	2-Ethylhexanol[d]	19.16	(9)	1.52	(3)	1.34	(5)	1.40	(10)	13.50	(9)	13.32	(4)	4.83	(7)
47	21.71	1503	1502	Nonanol	74.64	(12)	39.18	(9)	–		–		–		–		–	
50	22.43	1521	1546	(*R*,*S*)-2,3-Butanediol	8.79	(10)	1.40	(13)	2.07	(1)	2.01	(7)	2.52	(4)	6.94	(16)	10.68	(10)
54	23.23	1540	1535	2-Nonanol[d]	12.36	(2)	15.02	(8)	1.81	(2)	1.76	(3)	11.11	(9)	8.22	(8)	1.89	(6)
57	23.93	1556	1583	(*R*,*R*)-2,3-Butanediol	6.44	(18)	1.29	(11)	1.02	(16)	1.06	(4)	1.30	(5)	2.06	(14)	17.57	(13)
68	27.23	1635	1610	Octenol	–		–		9.56	(2)	9.24	(6)	–		–		–	
75	29.65	1701	1723	Methionol[d]	10.89	(1)	7.97	(8)	–		–		4.01	(13)	1.64	(7)	–	
77	31.28	1750	1765	1-Decanol[d]	16.29	(14)	10.65	(17)	2.60	(9)	1.40	(5)	7.42	(10)	8.55	(8)	9.75	(5)
90	37.94	1898	1925	2-Phenylethanol[d]	1920.53	(9)	1444.04	(7)	1132.71	(1)	1126.46	(1)	1614.67	(4)	1466.45	(1)	1030.90	(3)

(*continued on next page*)

Table 1 (*continued*)

Peak n°	RT (min)[a]	KI_{cal}[b]	KI_{lit}[c]	Chemical families	Relative concentration (μg L^{-1}) (%RSD)											
					Cam		Cam1		*S. Serra*		Machico		S.R. Faial		S.R. Faial 1		J. Serra	
91	39.24	1956	1952	Tridecanol	5.07	(6)	4.76	(12)	4.05	(10)	3.59	(13)	5.49	(13)	4.96	(3)	4.87	(9)
Acids																		
36	18.51	1425	1450	Acetic acid[d]	519.12	(8)	460.80	(11)	472.45	(6)	380.50	(6)	296.01	(16)	311.16	(9)	617.81	(6)
49	22.36	1520	1523	Propanoic acid	10.09	(1)	9.06	(2)	–		–		–		–		–	
55	23.52	1547	1572	2-Methylpropanoic acid[d]	33.84	(10)	28.32	(3)	4.83	(1)	5.77	(19)	–		–		26.19	(6)
63	25.97	1600	1619	Butanoic acid[d]	3.21	(2)	2.09	(9)	2.02	(9)	2.39	(12)	1.09	(2)	6.58	(5)	3.08	(2)
70	27.56	1645	1665	3-Methylbutanoic acid[d]	29.61	(12)	31.82	(11)	22.27	(6)	23.60	(16)	9.69	(13)	9.19	(7)	18.33	(11)
82	34.29	1837	1850	Hexanoic acid[d]	137.40	(7)	72.27	(5)	53.80	(3)	53.90	(1)	64.08	(2)	37.52	(4)	31.60	(5)
89	36.69	1937	1962	(E)-2-Hexenoic acid	–		–		–		–		7.34	(15)	5.49	(9)	4.97	(10)
96	41.82	2148	2083	Octanoic acid[d]	914.48	(7)	1070.82	(2)	562.65	(2)	557.14	(6)	1086.06	(13)	1071.29	(14)	390.12	(8)
100	45.20	2259	2202	Nonanoic acid	0.51	(9)	0.66	(13)	0.67	(16)	0.70	(3)	0.36	(12)	0.73	(15)	1.55	(9)
103	48.56	2311	2307	Decanoic acid[d]	962.90	(11)	1163.65	(2)	387.50	(11)	387.61	(16)	635.69	(14)	962.44	(10)	436.98	(16)
105	53.07	2332	–	Benzenecarboxilic acid	–		–		1.40	(5)	1.59	(7)	–		–		1.01	(13)
106	54.80	2340	2361	Dodecanoic acid[d]	26.40	(16)	11.66	(5)	1.12	(14)	1.42	(1)	4.39	(15)	7.84	(3)	1.83	(14)
107	67.81	2392	2407	Undecylic acid	4.72	(12)	2.70	(8)	–		–		2.61	(4)	9.12	(15)	1.52	(2)
Terpenoids																		
11	8.76	1187	1214	Eucalyptol[d]	1.76	(13)	1.33	(3)	–		–		–		–		5.90	(2)
18	13.38	1308	1337	(*E*)-Rose oxide	5.93	(17)	4.90	(2)	–		–		–		–		13.45	(10)
27	16.00	1368	1373	(*Z*)-Rose oxid	–		–		–		–		–		–		4.71	(8)
32	16.74	1382	1423	(E)-Linalool oxide	0.95	(12)	1.58	(10)	–		–		–		–		2.24	(15)
38	19.22	1443	1449	Dihydrolinalool	8.50	(18)	12.50	(12)	–		–		10.86	(13)	15.40	(3)	–	
39	19.40	1448	1467	(Z)-Linalool oxide	1.74	(2)	0.86	(6)	1.69	(3)	1.62	(3)	–		–		5.56	(6)
40	19.97	1462	1474	Menthone	1.93	(3)	1.72	(4)	–		–		–		–		8.01	(9)
41	20.16	1467	1467	Limonene oxide	35.42	(6)	47.05	(3)	71.80	(1)	61.45	(14)	2.63	(11)	0.78	(11)	4.53	(6)
44	21.18	1491	1491	Camphor	3.82	(5)	3.47	(6)	2.87	(1)	2.57	(8)	1.22	(1)	2.13	(4)	2.08	(5)
45	21.47	1498	1531	Vitispirane I	4.38	(11)	4.75	(5)	3.36	(2)	3.25	(8)	1.44	(7)	4.58	(6)	4.25	(5)
46	21.57	1501	1534	Vitispirane II	5.56	(7)	6.03	(11)	3.05	(7)	3.80	(4)	1.63	(17)	5.49	(8)	6.73	(1)
52	22.88	1530	1537	Linalool[d]	–		–		0.62	(5)	0.71	(11)	–		–		2.17	(3)
56	23.72	1551	1580	Bornyl acetate	–		–		–		–		7.24	(8)	8.11	(8)	–	
58	24.44	1559	1569	Linalyl acetate	8.04	(17)	13.46	(8)	–		–		–		–		–	
59	24.60	1567	1570	Isocaryophyllene	2.19	(2)	3.83	(6)	–		–		–		–		–	
61	24.80	1575	1574	Fenchyl alcohol	4.63	(10)	3.86	(5)	–		–		–		–		–	
64	26.27	1610	1626	Menthol	1.11	(18)	1.94	(2)	–		–		–		–		2.91	(3)
65	26.34	1608	1632	Pulegone	1.95	(3)	1.81	(3)	0.52	(5)	–		–		–		–	
73	28.58	1673	1669	α-Terpineol[d]	7.67	(3)	6.73	(4)	1.31	(3)	1.35	(14)	–		–		18.46	(6)
76	30.16	1716	–	TDN	0.97	(2)	1.62	(9)	–		–		–		–		–	
84	34.59	1844	1840	Geranyl acetone[d]	47.35	(13)	30.35	(8)	–		–		31.21	(16)	29.25	(5)	3.11	(11)
88	36.47	1881	1880	Citronellyl valerate	2.31	(12)	1.59	(6)	0.46	(2)	1.76	(5)	1.22	(7)	2.35	(4)	2.89	(1)
92	39.66	1970	1974	Methyl jasmonate	1.58	(5)	1.24	(12)	–		–		–		–		–	
93	39.86	1981	2009	Nerolidol[d]	1.28	(2)	0.92	(3)	–		–		–		–		–	
95	41.16	2125	2134	δ-Cadinol	8.68	(16)	5.22	(7)	7.37	(1)	8.22	(4)	12.76	(12)	14.09	(16)	2.76	(6)
Carbonyl compounds																		
14	12.18	1278	1272	3-Hydroxybutan-2-one	–		–		1.34	(10)	1.21	(2)	–		–		4.86	(12)
31	16.29	1374	1385	Nonanal[d]	4.26	(12)	6.11	(7)	5.75	(9)	4.12	(7)	–		–		0.56	(5)
43	20.81	1483	1484	Decanal[d]	1.80	(18)	7.05	(8)	7.17	(4)	4.97	(12)	–		–		8.61	(9)
Lactones																		
62	25.70	1594	1618	Butyrolactone[d]	1.41	(10)	0.89	(19)	0.37	(5)	0.42	(6)	1.27	(1)	1.52	(4)	2.56	(4)
94	40.63	2107	2103	γ-Decalactone[d]	70.29	(1)	69.97	(2)	35.16	(3)	37.57	(5)	57.90	(14)	55.17	(18)	31.11	(12)
98	43.90	2218	2209	Wine lactone	7.74	(7)	6.09	(7)	–		–		1.87	(11)	5.52	(13)	–	
101	45.44	2267	2241	δ-Decenolactone	97.33	(3)	124.26	(2)	107.65	(2)	124.83	(10)	78.28	(5)	54.63	(2)	62.08	(13)
Volatile phenols																		
99	45.13	2257	2250	Eugenol[d]	5.49	(3)	6.46	(4)	0.59	(2)	0.93	(18)	6.64	(8)	4.53	(13)	3.90	(11)
104	51.80	2326	2296	Syringol	–		–		–		0.95	(12)	3.57	(13)	6.89	(18)	1.10	(12)

- Not detected.

TDN: 1,1,6-Trimethyl-1,2-dihydronaphthalene.

Wine lactone: (3S,3aS,7aR)-3,6-Dimethyl-3a,4,5,7a-tetrahydro-3H-1-benzofuran-2-one.

[a] Retention time (min).

[b] Kovat index relative *n*-alkanes (C_8 to C_{20}) on a BP-20 capillary column.

[c] Kovat index relative reported in literature for equivalent capillary column [11–13].

[d] Identified using pure standards.

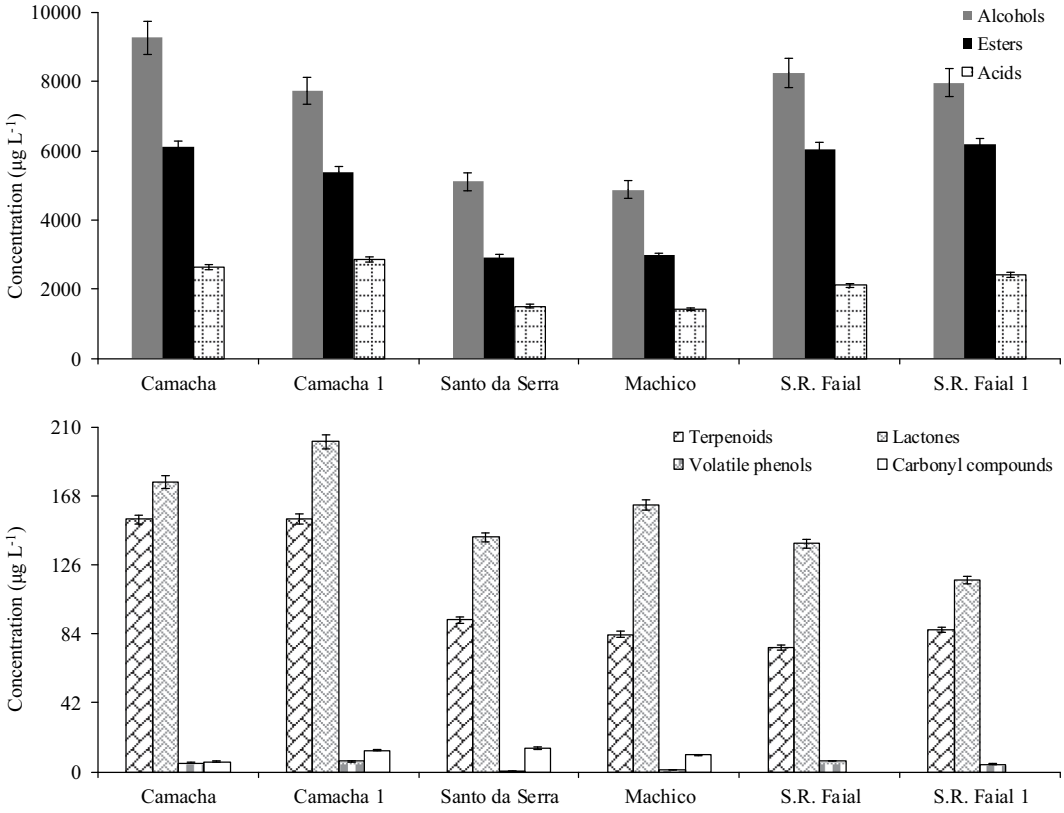

Fig. 1. Volatile signature of cider from different island geographical regions. The error bars represent the standard deviation for each measurement (n = 3).

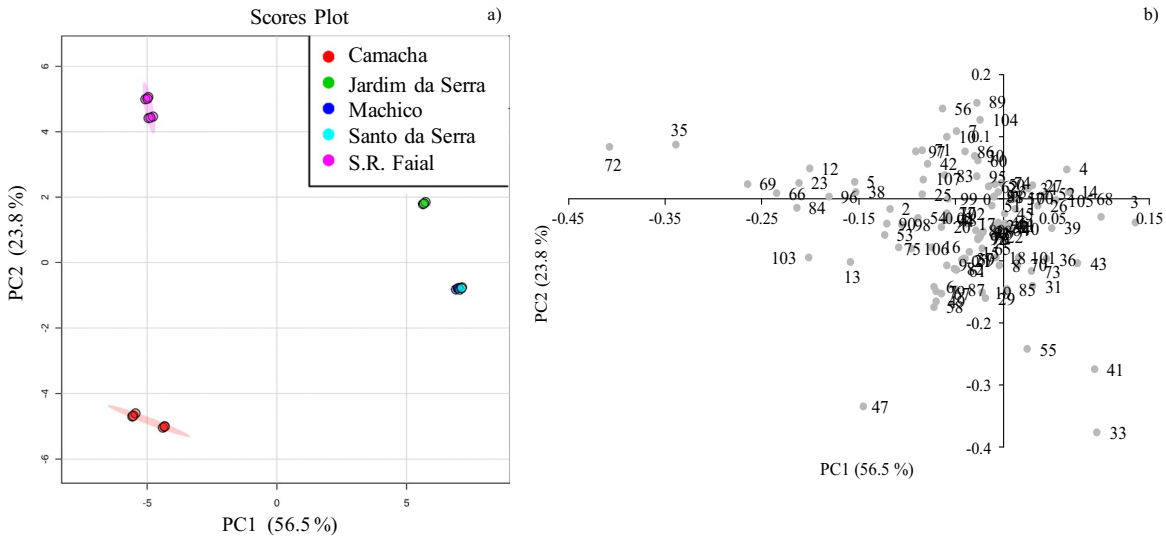

Fig. 2. PCA of the volatile signature of cider from different island geographical regions (n = 3 for each data point). **a)** PC1 × PC2 score scatter plot and **b)** loading weight plot (attribution of the peak number is shown in Table 1). SRFaial – São Roque do Faial.

3.2. Statistical and multivariate data analysis

In order to evaluate the performance of HS-SPME/GC–MS to discriminate ciders from different geographic regions in terms of volatile signature, PCA and PLS-DA were applied as multivariate analysis. The dataset composed by 107 VOCs and 21 samples (7 samples × 3 bottles) from five island geographic regions was tested by PCA. This analysis is an unsupervised method that was performed to visualize the similarity/difference among cider samples profile. PCA score plot and loading plot from cider samples analysed are showed in Fig. 2a and b, respectively. The variances of PC1 and PC2 were 56.5 and 23.8%, respectively, representing 80.3% of the total VOCs variability of data. The cider from Camacha, projected in PC1 and PC2 negative, are mainly characterized by ethyl hexanoate (13), decanoic acid (103) and nonanol (47), whereas ciders from São Roque do Faial placed in PC1 negative and PC2 positive by ethyl octanoate (35) and ethyl 9-decenoate (72). The ciders from Jardim da Serra, PC1 and PC2 positive, is characterized by 2-butanol (4), whereas the ciders from Machico and Santo da Serra (PC1 positive and PC2 negative) are characterized by octanol (33), limonene oxide (41) and 2-methylpropanoic acid (55).

Additionally, the PLS-DA (Fig. 3a-d) was used as a supervised clustering method and 15 differently expressed VOCs were found with

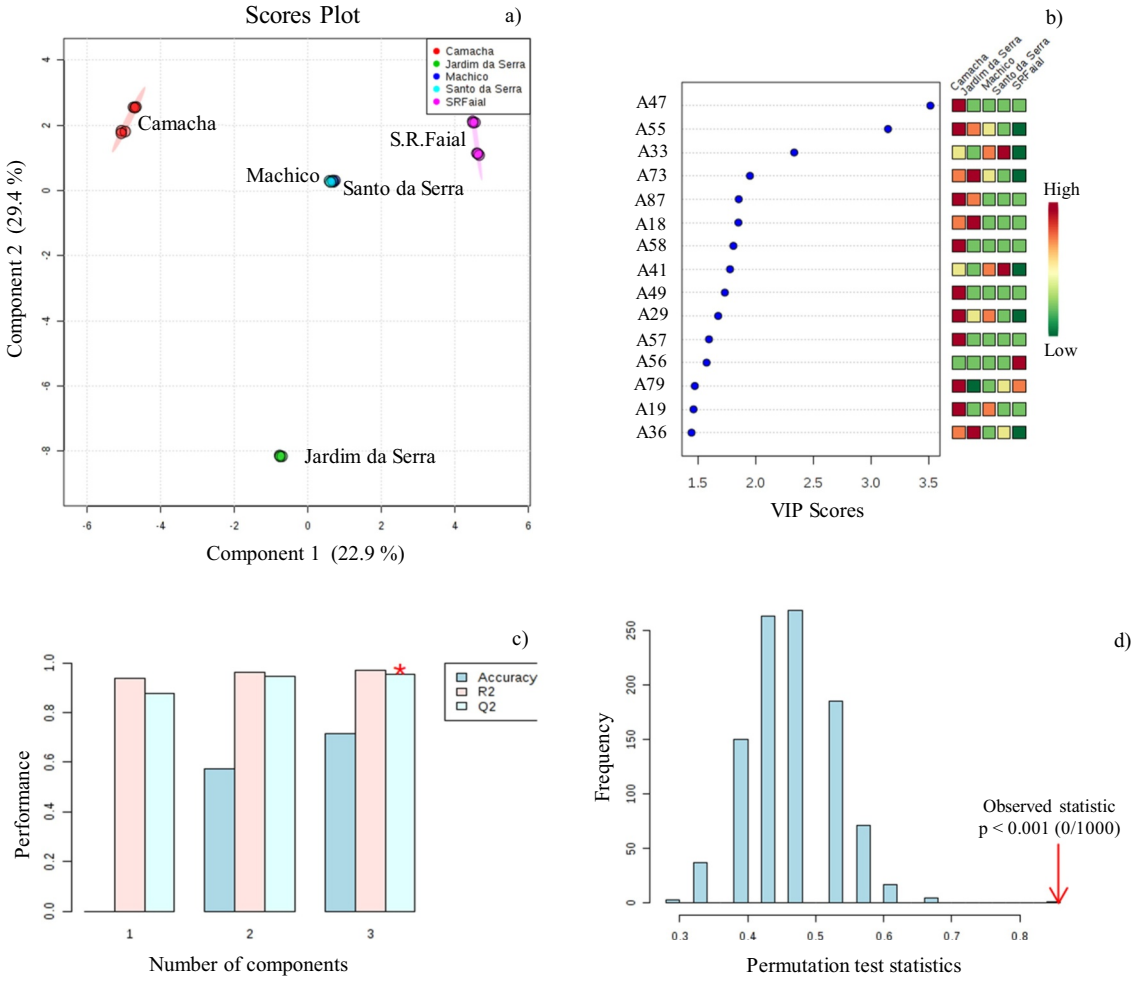

Fig. 3. PLS-DA of the volatile signature of cider from different island geographical regions (n = 3 for each data point). **a)** score scatter plot, **b)** VIP scores, **c)** 10-fold cross-validation performance and **d)** model validation by permutation test based on 1000 permutations of VOCs obtained by GC-qMS of cider samples (attribution of the peak number is shown in Table 1). SRFaial – São Roque do Faial.

presented VIP score higher than 1, being the most relevant for explaining the discrimination of ciders from different geographic regions, namely (*E*)-rose oxide (18), 2-heptanol (19), (Z)-3-hexenol (29), octanol (33), acetic acid (36), limonene oxide (41), nonanol (47), propanoic acid (49), 2-methylpropanoic acid (55), bornyl acetate (56), linalyl acetate (58), butyl octanoate (67), α-terpineol (73), ethyl phenylacetate (79), and 2-phenylethyl acetate (87). In accordance with our findings, 3-hexenol was previously reported by Gan et al. [18] to discriminate among monovarietal apple juices from different geographic origins (New Zealand, South Africa and Chile). In addition, previous reports have informed about α-terpineol as one of the VOCs accountable for geographic origin discrimination in honey samples [19] and in grapes from different Madeira Island regions [20]. To confirm the robustness of the model, a random permutation test with 1000 permutations was performed with PLS-DA (Fig. 3d). The permutation test yielded an R^2 (represents goodness of fit) of 0.938 and a Q^2 (represents predictive ability) of 0.868 indicating that the model is not over fitted (the difference between R^2 and Q^2 < 0.3) and have a relative good predictive ability to distinguish all cider samples.

The *p* values obtained by *one-way* ANOVA with post-hoc Tukey test ($p < 0.05$) indicated that from the pool of the 107 VOCs previously identified, only two VOCs (vitispirane (45) and butanoic acid (63)) were not statistically significant different among the ciders from different island geographic regions (Supplementary Table S1). On the other hand, ethyl 3-hydroxybutanoate (48), 3-methylbutanoic acid (70) and γ-decalactone (94) were significantly different among all ciders analysed. Moreover, Fig. 4 shows the resulting dendrogram associated with heat map constructed using Pearson's correlation, providing intuitive visualization of the dataset, and it often is applied to identify samples or features that are unusually high or low. An analogous color tone to the heat-map indicates the area, a group of samples, taking into account the concentration of the analysed VOCs is similar. However, apart from geographic origin, other factors such as sample storage and processing may influence on volatile signature [21].

4. Conclusions

The volatile signature of cider from different geographic of Madeira island regions was established for the first time using HS-SPME/GC–MS methodology. A total of 107 VOCs belonging to different chemical families were identified, namely 41 esters, 21 alcohols, 24 terpenoids, 13 acids, 3 carbonyl compound, 4 lactones and 1 volatile phenol, being only 50 VOCs common to all ciders analysed. The *p* values obtained by *one-way* ANOVA with post-hoc Tukey test ($p < 0.05$) indicate that from the pool of the 107 VOCs previously identified, only vitispirane (45) and butanoic acid (63) were not statistically significant different among the ciders analysed. The application of chemometrics tools enabled the visualization of clustering tendencies between different island geographic regions, namely Camacha, Santo da Serra, Machico, São Roque do Faial and Jardim da Serra, as well as the VOCs responsible for discrimination of each cider. Considering the PLS-DA results, 15 discriminatory VOCs were found, with VIP values higher than one, namely

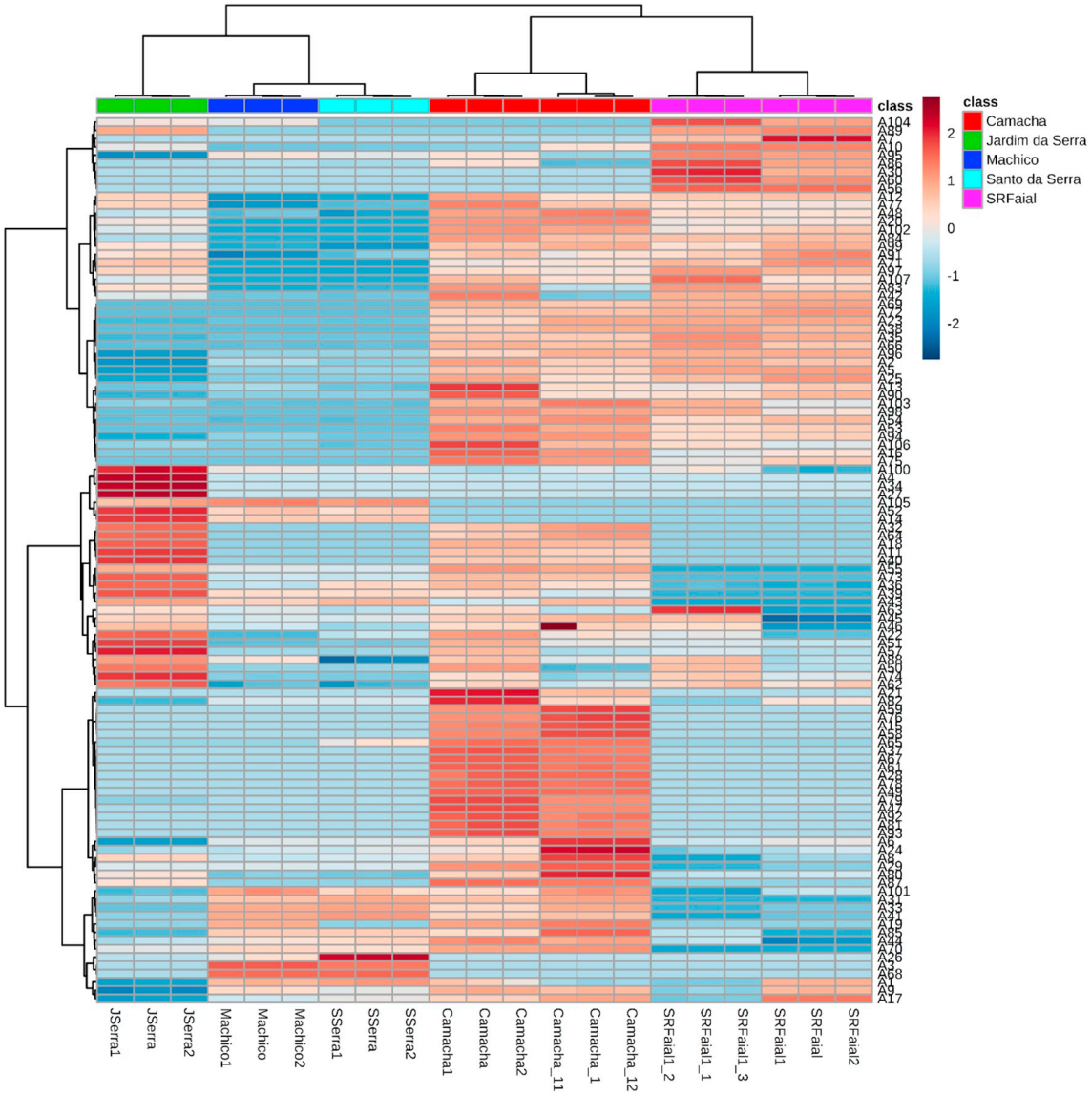

Fig. 4. Hierarchical cluster analysis (HCA). The heat maps of the 107 VOCs identified in all ciders samples were generated by average algorithm and Pearson distance analysis (attribution of the peak number is shown in Table 1). SRFaial – São Roque do Faial.

five terpenoids, four alcohols, three acids and three esters. Our findings provide relevant information related to volatile signature of ciders produced in Madeira Island, and could open up new opportunities to promote the cider-making process with enhanced levels of odor compounds; consequently, it improves the quality of the final product. In addition, the geographical discrimination recognized the unique and distinctive characteristics to protect the quality and typically of products originating in certain geographical regions. The regional government aims to valorize cider products through the certification as Protected Designation of Origin (PDO) product and their commercialization worldwide.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2019.05.028.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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