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Geographical differentiation of apple ciders based on volatile fingerprint

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ABSTRACT

With the globalization of food trade, it's traceability and genuineness becomes increasingly more difficult. Therefore, it is necessary to develop analytical tools to define the authenticity and genuineness of food-derived products. In the current work, headspace solid-phase microextraction followed by gas chromatography-mass spectrometry (HS-SPME/GC–MS) combined with chemometric tools was used to establish the volatile fingerprint of apple ciders produced in different geographical regions of Madeira Island, in order to define their typicity and to identify putative geographical markers. A total of 143 volatile organic compounds (VOCs) belonging to different chemical families have been identified, of which 28 were found in all apple ciders independently of geographical region. Esters, terpenic and furanic compounds presented on average a higher contribution for the total volatile fingerprint in cider produced in northern region of the Island, whereas alcohols, acids, volatile phenols, carbonyl compounds and lactones in cider from southern region. Considering the relative areas of the VOCs, 43 revealed statistically significant differences ($p < 0.001$) between geographical regions, and 11 between northern and southern regions. A clear differentiation among cider-producing regions was observed on the developed partial least squares-discriminant analysis (PLS-DA) model. Two alcohols (1-hexanol, 1-octanol), 6 esters (methyl acetate, (*Z*)-3-hexen-1-ol acetate, ethyl hexanoate, ethyl nonanote, ethyl octanoate, isoamyl octanoate) and 1 terpenic compound (limonene), can be considered putative geographic markers due to their discriminatory ability. The results obtained recognize the specific and typical geographical characteristics of the cider, which will allow the forthcoming guarantee for the construction of a sustainable platform for the establishment of the authenticity and typicality of the regional cider.

1. Introduction

Apple cider is a traditional alcoholic beverage fermented from apple juice, with increasing consumption and production worldwide. Apples (*Malus domestica*) and their derivates, particularly cider, pose a substantial impact in terms of global fruit cultivation, being the most ubiquitous and well-adapted fruit species in temperate regions (Delgado-Pelayo, Gallardo-Guerrero, & Hornero-Méndez, 2014). In 2017, across Madeira Island, around 130 ha were dedicated to the production of 2000 tons of apples, subsequently leading to 3328 hl of apple cider. Moreover, opposite to evidence in the remaining country, the cider-making by traditional process has never been discontinued in Madeira Island (Perestrelo, Silva, Silva, et al., 2019). The traditional cider-making started with the harvest of selected apple varieties (*e.g.*, Azedo, Branco, Calhau, Domingos, Festa, Rijo, Ribeiro, Vime and Verde apple varieties). Then, the apples are cleaned, crushed and pressing to obtain the fruit juice without solid parts. The fruit juice is submitted to a controlled fermentation (15 to 18 days) at 18 °C to obtain an ethanol content of 7–8% (v/v). Due to the edaphoclimatic and geographical characteristics associated with the organoleptic quality of the different endogenous varieties of cultivated apples, Madeira Island has all the natural conditions to produce excellent quality apple ciders. Based on this, the government entities encourage cider producers to maximize the potential of the cider and increase the production of apples and pears of regional varieties, which resulted in the construction of four new "sidrarias" and in the formation of a panel of tasters for the sensorial analysis of the product. The aroma and flavour of apple ciders rely on the quality of the apples used during cider-making, which is associated to several factors, namely cultivars diversity and geographical climatic conditions (Pello-Palma et al., 2017). The combination of the several factors influenced the final product with the release of volatile organic compounds (VOCs) that can be used for

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authentication purposes, having a considerable contribution on the aroma and flavour of ciders (Mangas, González, Rodríguez, & Blanco, 1996). Therefore, the analysis of VOCs content in ciders obtained from distinct apple varieties and from different growing regions, it is crucial to achieve a high-quality product without reducing the flavour, aroma or texture (Pizarro, Pérez-del-Notario, & González-Sáiz, 2009). In this sense, the authentication of food products is a procedure by which it is verified that the product matches the statements displayed on the label, including the raw material, the production techniques and the geographical origin (Vinci, Preti, Tieri, & Vieri, 2012).

Typically, gas chromatography-mass spectrometry (GC–MS) combined with solid-phase microextraction (SPME) is one of the most common analytical tool used to differentiate beverages and food products from different geographical origin, through the identification of slight analytical differences on volatile fingerprints and through the establishment of putative VOCs as discriminatory geographical markers (Danezis, Tsagkaris, Camin, Brusic, & Georgiou, 2016; Medina, Perestrelo, Santos, Pereira, & Câmara, 2019; Perestrelo, Barros, Rocha, & Câmara, 2014).

Regarding the research which assesses the authenticity and typicity of ciders using VOCs, Lobo et al. (Lobo, Antón-Díaz, Alonso, & Valles, 2016) described the volatile composition and olfactometric profiles of Asturian and Basque ciders as a putative marker for origin discrimination, while Picinelli et al. (2000) differentiated Asturian ciders from foreign ones through the analysis of volatile acidity, polyalcohols and predominant VOCs. Moreover, Uthurry et al. (2019) reported the physicochemical and sensorial characteristics of ciders with the purpose to discriminate industrial and homemade ciders from the Patagonia and Cuyo regions of Argentina. Also, Qin, Petersen, and Bredie (2018) characterized the flavour profiles of commercial ciders from the UK and Scandinavian region by sensory profiling and analysis of their volatile and non-volatile components. In another case, Nicolini et al. (2018) found significant differences between the elemental composition of French and Italian Alps ciders. More recently, several studies related to the characterization of the volatile fingerprint of apple cider and juice from Madeira Island were performed by using HS-SPME/GC–MS combined with chemometric tools (*e.g.*, principal component analysis (PCA), partial least squares – discriminant analysis (PLS-DA) (Medina et al., 2019; Perestrelo, Silva, Silva, et al., 2019). Indeed, Medina et al. (2019) established distinctive characteristics of apple juices according to Madeira Island apple varieties and geographical origin. The results showed that VOCs could be used as authenticity markers to verify variety and geographical origin of apple juices, providing local producers multiple benefits and legal protection against the misuse of products. Also, Perestrelo, Silva, Silva, et al. (2019) determined the volatile signature of apple ciders, obtained from Calhau apples (*Malus domestica*), from five different geographical regions of Madeira Island. A total of 107 VOCs was identified, from which 5 terpenoids, 4 alcohols, 3 acids and 3 esters presented variable importance in projection (VIP) values higher than one that could be used as putative geographical markers for cider samples.

The aim of the current work was to establish the volatile fingerprint of apple ciders obtained from Azedo, Calhau, Domingos, Festa, Golden, Marmelo, Pevide and Riscado apple varieties (*Malus domestica*), from different geographical origins of Madeira Island as a powerful tool to identify putative discriminatory geographical markers. In addition, compare the volatile fingerprint of apple ciders from the same apple variety and producer from two consecutive years (2018, 2019). To achieve the proposed goal, HS-SPME/GC–MS data combined with chemometric tools (*e.g.*, PCA, PLS-DA) will be used. The large number of analysed samples allow to obtain a more robust PLS-DA model and an evaluation of putative geographical markers related to environmental conditions (*e.g.*, altitude, climate). Furthermore, our study's outcomes could offer novel chances to encourage cider-making with improved content of odorants, as well as bioactive compounds, ultimately ensuring the production of high-quality products. Moreover, the results will be of outstanding importance for regional government entities to exploit the certification of regional cider as Protected Designation of Origin (PDO) and furthermore its worldwide commercialization.

2. Materials and methods

2.1. Reagents and material

Sodium chloride (NaCl, 99.5%) was obtained from Panreac (Spain, Barcelona), and ethanol (99.9%) was purchased from Fluka (Buchs, Switzerland). 3-Octanol, used as internal standard (IS), was obtained from Sigma-Aldrich (Madrid, Spain). The standards of VOCs, used for identification proposal, were purchased from Acros Organics (Geel, Belgium), Fluka (Buchs, Switzerland) and Sigma-Aldrich (Madrid, Spain) with high-purity grade (> 98%). Ultra-pure water was obtained from a Milli-Q® system (Millipore), and helium of purity 5.0, used as the GC carrier gas, was supplied from Air Liquide (Portugal). The SPME holder for manual sampling and the divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre with 50/30 µm film thickness, 2 cm length fiber, were purchased from Supelco (Bellefonte, PA, USA). The C_8 to C_{20} alkane series solution, (40 mg/L in n-hexane) used to calculate the kovat index (KI) was purchased from Fluka (Buchs, Switzerland).

2.2. Cider samples

Fifty-two apple ciders (3 samples for each lot, performing a total of 156 samples) produced in 2018 (n = 17, a total of 51) and 2019 (n = 35, a total of 105) in different geographical regions of Madeira Island (Table 1) were sampled and transported to the laboratory in a cooler with ice and kept at − 80 °C until the analysis. In order, to make the discussion of the results easier, the different geographical regions were organized in two groups, based on altitude: north (Santana, São Vicente) and south (Santa Cruz, Câmara de Lobos, Calheta). In addition, apple ciders originated from the same producer in two consecutive years were considered (2018 and 2019). Sample were obtained from monovarietal apple varieties, Azedo, Calhau, Domingos, Festa, Golden, Marmelo, Pevide and Riscado (*Malus domestica*), according to cider producers. Briefly, the apples were washed with water for 10 min and constantly pressed, filtered and held in a refrigerated tank at 4 °C for 24 h. The apple juice was diluted to a final concentration of 200 g/L (23° brix), and SO_2 (80 mg/L) was added. After this, 2% (v/v) of *Saccharomyces cerevisiae* was added into the apple juice during 18 days at 18 °C in a closed fermentation tank. It should be pointed out, that the starting point for alcoholic fermentation was the same for all producers.

2.3. Physicochemical parameters

The pH of apple ciders was determined using a pH meter (FE20 Plus, Mettler-Toledo) at 22 ± 1 °C, under continuous stirring (400 rpm). The total soluble solid (TSS) was measured by using a refractometer (Atago RX-1000) at 22 ± 1 °C and the result was expressed as °Brix. The titratable acidity (TA) was measured by titrating with 0.1 M NaOH to a pH end-point with phenolphthalein (pH = 8.1 ± 0.1). The results were expressed as gram of malic acid per liter of apple cider (g MA/L). All assays were carried out in triplicate.

2.4. Solid phase microextraction (SPME)

The SPME extraction conditions were adopted from a prior study carried out in our laboratory (Perestrelo, Silva, Silva, et al., 2019), with minor modifications. Shortly, in a 20 mL capped glass vial, 10 mL of sample, 10 µL of 3-octanol (IS at concentration of 2.5 µg/L) and 2 g of NaCl was added. Subsequently, solution was heated during 30 min at 50 °C in a thermostatic block, under constant stirring (800 rpm). All assays were carried out in triplicate.

2.5. *Gas chromatography – Mass spectrometry (GC–MS) conditions*

The Agilent Technologies 6890 N Network gas chromatography was equipped with a BP20 (30 m × 0.25 mm i.d. × 0.25 μm) fused silica capillary column obtained from SGE (Darmstadt, Germany) and interfaced with an Agilent 5975 quadrupole inert mass selective detector (Palo Alto, CA, USA). After HS-SPME extraction, the fibre was introduced into the GC injector port for the thermal desorption of analytes at 250 °C during 6 min, and a splitless injector equipped with an insert of 0.75 mm i.d. was used. The column flow was constant at 1 mL/min by using helium as a carrier gas. The following chromatographic protocol was used before the MS analysis: 40 °C increased 3 °C/min to 220 °C with 10 min hold, for a total GC run time of 83 min. The ion source, GC-qMS interface and quadrupole temperatures were held at 180, 220 and 180 °C, respectively. MS acquisition was carried out in full scan mode (30 – 300 *m/z*) with electronic impact (EI) mode at 70 eV. The electron multiplier was set to auto tune procedure. The identification of VOCs was performed by manual interpretation of spectra, by matching the mass spectra with the data system library (NIST05, Mass Search Program v.2.0d; Washington, DC) with a similarity higher than 80%, by comparison with standards when available, and by KI values calculated based on the van den Dool and Kratz equation (van Den Dool & Dec. Kratz, 1963). The KI values were compared, when available, with values reported in the literature for similar columns. The relative peak area was determined by the ratio between the GC peak areas of the VOC's peak and the internal standard's peak (IS). The relative concentration of VOCs was estimated based on the added amount of 3-octanol (IS) and expressed as μg/L of 3-octanol equivalents.

2.6. *Statistical analysis*

Data processing was carried out using web-based application Metaboanalyst 4.0 developed by the University of Alberta, Canada (Worley & Powers, 2013). Prior to the statistical analysis, the data matrix was pre-processed to remove VOCs with missing values (MV), and then was normalized by cubic root and data scaling by auto-scaling. After normalization, a comparison of means of relative areas was achieved using a one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test at *p*-value < 0.001 to select the VOCs that were significant to distinguish the samples by geographical origin. Firstly, a principal component analysis (PCA), as an exploratory data analysis method, was used to visualize group tendencies based on geographical origin, using whole information contained in many variables, the VOCs fingerprint. Then, to investigate the differences in the VOCs levels of cider, PLS-DA was performed using the relative peak area of the VOCs selected by ANOVA. All the VOCs with variable importance in projection (VIP) values higher than 1.5 were considered as putative geographical markers. The efficiency and feasibility of the PLS-DA model were evaluated by R^2 (representing the goodness of fit), Q^2 (representing the predictive ability), and a permutation test (1000 permutations). The significant differences in the model were assessed by calculating the *p*-values obtained from the cross-validation analysis. For HCA, the squared Euclidean distance through Ward agglomeration method was used in the assignment of clusters.

3. Results and discussion

3.1. *Physicochemical parameters of apple ciders*

The physicochemical parameters of apple ciders from different geographical regions, namely pH, total soluble solid (TSS), alcohol degree % vol. 20 °C (TAV), titratable acidity (TA) and ratio of TSS/TA are shown in Table 2. A higher pH ($^>$ 4) value can impairs microbial stability, promotes the spoilage potential, and lead to severe flavour

Table 1

Geographic and climatic conditions of Madeira Island apple ciders.

Madeira Island	Province	Locality	Sample code	Latitude[a]	Longitude[a]	Altitude (m)[b]	Mean annual precipitation (mm)[b]		Mean annual temperature (°C)[b]	
							2018	2019	2018	2019
North	Santana	São Roque do Faial	A001, A002, A005, A016, A001_19, A007_19, A012_19, A019_19, A021_19, A023_19; A024_19, A029_19	32°46′15″N	16°51′25″W	260	1309.3	752.5	15.9	16.5
	São Vicente	São Vicente	A020, A021, A002_19, A003_19, A010_19	39°27′52″N	8°11′53″W	271	1601.2	712.0	18.1	18.4
		Ponta Delgada	A026, A005_19	37°43′59N	25°40′00″W	261	1219.4	678.9	16.1	17.2
South	Santa Cruz	Camacha	A006_19	32°40′45″N	16°50′41″W	700	1347.2	723.6	15.4	16.7
		Santo da Serra	A006, A007, A017, A025_19, A027_19, A033_19	32°43′21″N	16°49′17″W	660	1417.7	940.3	13.8	14.4
	Câmara de Lobos	Jardim da Serra	A015, A022, A024, A025, A028, A031, A004_19, A009_19, A013_19, A015_19, A017_19, A018_19, A020_19, A022_19, A026_19, A028_19, A030_19, A031_19, A032_19, A034_19, A035_19	32°41′11″N	16°59′24″W	702	660.1	369.3	16.9	17.6
	Calheta	Prazeres	A008, A008_19, A011_19	32°45′4.76″N	17°12′16.75″W	623	530.4	413.1	16.3	17.5
		Fajã da Ovelha	A014_19, A016_19	32°46′28″N	17°14′04″W	480	568.9	374.4	17.9	18.6

[a] Information obtained from www.google.com/maps.

[b] Information obtained from https://es.climate-data.org/.

Table 2
Chemical characteristics of apple ciders from different geographical regions: pH, total soluble solids (TSS), sugar content, total alcoholic degree (TAV, % vol. 20 °C), titratable acidity (TA) and ratio of TSS/TA.

Province	Locality	Sample code	Apple variety	pH[a]	TSS (°Brix)[a]	Sugar g/L[a]	TAV (%vol)[a]	TA (g/L)[a]	TSS/TA
Santana	São Roque do Faial	A001	Calhau	3.55	17.8	167	9.92	8.54	2.08
		A007_19	Calhau	3.53	15.0	136	8.08	7.58	1.98
		A002	Calhau	3.57	16.2	149	8.86	7.46	2.17
		A029_19	Calhau	3.41	13.3	118	6.99	7.04	1.89
		A005	Calhau	3.52	14.5	131	7.76	7.85	1.85
		A012_19	Calhau	3.49	17.4	163	9.66	9.01	1.93
		A016	Calhau	3.34	14.3	128	7.63	8.09	1.77
		A019_19	Calhau	3.40	14.0	125	7.43	7.59	1.84
		A001_19	Domingos	3.45	17.7	166	9.86	6.03	2.94
		A021_19	Golden	3.46	14.8	134	7.95	8.05	1.84
		A023_19	Domingos	3.39	14.4	130	7.69	7.79	1.85
		A024_19	Festa	3.59	14.6	132	7.82	7.06	2.07
São Vicente	São Vicente	A020	Golden	3.37	17.3	162	9.59	9.91	1.75
		A002_19	Golden	3.35	16.0	147	8.73	7.55	2.12
		A021	Calhau	3.26	14.9	135	8.01	7.08	2.10
		A003_19	Calhau	3.57	14.1	126	7.50	6.34	2.22
		A010_19	Calhau	3.49	17.4	163	9.66	7.59	2.29
	Ponta Delgada	A026	Golden	3.41	17.6	165	9.79	8.03	2.19
		A005_19	Golden	3.50	15.7	144	8.54	7.67	2.05
Santa Cruz	Camacha	A006_19	Calhau	3.39	13.4	119	7.05	6.99	1.92
	Santo da Serra	A006	Riscado	3.32	13.2	117	6.92	6.56	2.01
		A025_19	Riscado	3.41	13.7	122	7.24	6.48	2.11
		A007	Pevide	3.42	15.1	137	8.15	7.32	2.06
		A027_19	Pevide	3.28	13.2	117	6.92	7.40	1.78
		A017	Calhau	3.74	15.6	143	8.47	5.76	2.71
		A033_19	Calhau	3.35	13.8	123	7.30	7.32	1.89
Câmara de Lobos	Jardim da Serra	A015	Festa	3.42	14.1	126	7.50	5.97	2.36
		A004_19	Festa	3.21	14.7	133	7.88	5.98	2.46
		A022	Azedo	3.57	13.8	123	7.30	5.79	2.38
		A009_19	Azedo	3.35	16.8	156	9.26	6.02	2.79
		A024	Domingos	3.32	14.7	133	7.88	7.43	1.98
		A013_19	Domingos	3.49	13.5	120	7.11	6.95	1.94
		A025	Domingos	3.59	15.7	144	8.54	7.80	2.01
		A015_19	Domingos	3.47	13.6	121	7.18	7.14	1.90
		A028	Domingos	3.27	14.2	127	7.56	7.03	2.02
		A017_19	Domingos	3.55	13.4	119	7.05	6.95	1.93
		A031	Festa	3.28	15.2	138	8.21	7.14	2.13
		A018_19	Festa	3.56	14.5	131	7.76	7.37	1.97
		A020_19	Festa	3.70	15.1	137	8.15	6.69	2.26
		A022_19	Golden	3.52	15.1	137	8.15	7.14	2.11
		A026_19	Golden	3.51	12.8	112	6.66	7.32	1.75
		A028_19	Domingos	3.48	13.5	120	7.11	7.90	1.71
		A030_19	Marmelo	3.47	14.8	134	7.95	6.59	2.25
		A031_19	Marmelo	3.49	13.3	118	6.99	6.78	1.96
		A032_19	Marmelo	3.40	15.5	142	8.41	7.08	2.19
		A034_19	Calhau	3.61	12.3	107	6.35	7.46	1.65
		A035_19	Calhau	3.54	12.2	106	6.28	7.09	1.72
Calheta	Prazeres	A008	Golden	3.22	15.5	142	8.41	7.21	2.15
		A008_19	Golden	3.49	13.9	124	7.37	7.39	1.88
		A011_19	Azedo	3.45	12.9	113	6.73	7.68	1.68
	Fajã da Ovelha	A014_19	Golden	3.61	14.0	125	7.43	6.56	2.13
		A016_19	Golden	3.65	13.3	118	6.99	6.98	1.91

[a] % RSD values lower than 5% for all assays.

complications. A desirable apple juice pH range for cider-making should be from 3.2 to 3.8. On average, the highest pH was determined in Calhau apples (3.49 ± 0.10), whereas the lowest in Pevide apples (3.35 ± 0.06). For the apple cider studied, the pH ranged from 3.21 to 3.74, which is appropriate for the cider-making since pH does not exceed 4. In ciders, the most abundant organic acids are malic acid and citric acid, representing 90% of all organic acids (Lachowicz, Oszmiański, Uździcka, & Chmielewska, 2019), therefore the TA is usually expressed in equivalents of malic acid (MA) per liter. As observed in Table 2, on average, the lowest TA was estimated for Azedo apples (6.50 ± 0.23 g(MA)/L), and the highest for Golden apples (7.63 ± 0.42 g(MA)/L). The TSS was in the range from 106 ± 3.18 to 167 ± 5.21 g/L, with a mean value of 133 ± 4.36 g/L. This difference can be a result of using apple cultivar, since the Riscado (119 ± 1.56 g/L) and golden (136 ± 2.14 g/L) apple showed the lowest and the highest TSS content. The maximum TSS and the minimum TA are key parameters to the cider quality evaluation, and equally play an important role in cider stability. According to the obtained data, the ratio of TSS/TA ranged from 1.65 to 2.94, which is in agreement with the ratio reported in the literature (TSS/TA ranged from 1.5 to 3.8) (Włodarska, Pawlak-Lemańska, Górecki, & Sikorska, 2017).

3.2. *Volatile fingerprint of apple ciders*

Fifty-two samples (156 collections) from 5 geographical regions were analysed using HS-SPME/GC-qMS methodology. Table 3 reports the frequency of occurrence (% FO) of identified VOCs in apple cider from different geographical regions. In qualitative terms, a total of 143 VOCs were identified from a widespread of chemical families, namely

Table 3
VOCs identified in apple ciders from different geographical regions and frequency of occurrence in percentage (%FO).

RT (min)[a]	KI_{cal}[b]	KI_{Lit}[c]	Peak n°	Chemical families	VOCs ID[d]	North	South
				Alcohols			
13.69	980	980	10	2-Butanol	MS, ST	21	30
14.37	994	993	11	1-Propanol	MS, ST	79	88
16.91	1046	1048	17	2-Methyl-1-propanol	MS, ST	95	100
20.02	1097	1094	21	1-Butanol	MS, ST	100	100
23.58	1161	1163	27	3-Methyl-1-butanol	MS, ST	100	100
25.99	1200	1205	30	1-Pentanol	MS	53	55
29.71	1266	1268	41	2-Penten-1-ol	MS	26	30
29.94	1270	1280	42	2-Heptanol	MS, ST	47	70
30.48	1279	1282	45	4-Methyl-1-pentanol	MS	37	48
32.02	1305	1308	49	1-Hexanol	MS, ST	100	100
32.63	1317	1326	50	(*E*)-3-Hexen-1-ol	MS, ST	42	64
33.87	1340	1342	52	(*Z*)-3-Hexen-1-ol	MS, ST	100	100
35.55	1370	1371	56	2-Hexen-1-ol	MS	0	15
37.74	1410	1405	63	1-Heptanol	MS	42	67
38.16	1428	1428	64	1-Octen-3-ol	MS	21	33
39.66	1448	1446	70	2-Ethyl-1-hexanol	MS, ST	74	85
40.80	1469	1477	73	3-Ethyl-4-methylpentanol	MS	47	48
41.17	1476	1483	75	2-Nonanol	MS	42	52
41.47	1482	1487	76	4-Hepten-1-ol	MS	16	21
42.60	1501	1492	80	2,3-Butanediol	MS, ST	37	18
43.29	1509	1507	83	1-Octanol	MS, ST	68	85
46.77	1546	1536	87	(*E*)-Hept-2-en-1-ol	MS	21	36
48.58	1624	1624	92	1-Nonanol	MS	47	58
51.85	1692	1687	101	3-(Methylthio)-1-propanol	MS, ST	63	73
52.02	1695	1687	102	4-Tert-butylcyclohexanol	MS	11	24
53.60	1731	1726	104	2-Undecanol	MS	21	24
54.79	1758	1760	106	1-Decanol	MS	5	12
56.19	1789	1789	110	1,3-Propanediol	MS	0	12
59.52	1882	1881	120	Benzyl alcohol	MS, ST	100	100
61.17	1907	1905	124	2-Phenylethyl alcohol	MS, ST	100	100
				Esters			
7.36	798	796	2	Methyl acetate	MS, ST	53	36
8.76	849	850	3	Ethyl acetate	MS, ST	100	100
11.10	918	910	6	Ethyl propanoate	MS, ST	84	73
11.41	926	929	7	Ethyl isobutanoate	MS	74	73
12.25	947	944	8	Propyl acetate	MS	32	24
13.36	973	976	9	Isobutyl acetate	MS	84	82
14.45	995	1000	12	Ethyl butanoate	MS, ST	100	100
15.19	1014	1009	14	Ethyl 2-methylbutanoate	MS	100	100
15.92	1028	1031	15	Ethyl 3-methylbutanoate	MS	100	100
16.14	1032	1036	16	Butyl acetate	MS, ST	11	12
18.79	1078	1080	19	Propyl propanoate	MS	95	100
19.46	1089	1096	20	Isoamyl acetate	MS, ST	47	36
20.92	1114	1113	22	Ethyl pentanoate	MS	26	15
22.50	1143	1142	24	Methyl hexanoate	MS	26	21
22.86	1149	1153	25	Propyl butanoate	MS	32	48
25.18	1187	1183	28	Ethyl hexanoate	MS, ST	100	100
25.52	1193	1192	29	Isoamyl propanoate	MS	37	33
27.04	1219	1210	33	Pentyl propanoate	MS	37	33
27.46	1227	1235	34	Hexyl acetate	MS, ST	100	100
27.77	1233	1227	35	Butyl 2-methylbutanoate	MS	42	45
27.94	1236	1229	36	Butyl butanoate	MS	16	27
28.78	1251	1258	38	Isoamyl butanoate	MS	5	9
29.18	1258	1267	40	2-Methylbutyl 2-methylbutanoate	MS	53	52
30.15	1274	1275	44	(*Z*)-3-Hexen-1-ol acetate	MS, ST	16	39
31.01	1288	1287	46	Ethyl 3-hexenoate	MS, ST	53	70
31.71	1299	1309	48	Ethyl lactate	MS, ST	84	85
34.34	1349	1355	53	Methyl octanoate	MS	11	24
36.50	1387	1389	60	Ethyl 3-hydroxy-3-methylbutanoate	MS	58	39
36.82	1393	1392	61	Ethyl octanoate	MS, ST	100	100
38.35	1432	1433	65	Isobutyl lactate	MS	37	39
42.33	1497	1503	77	Isoamyl hexanoate	MS, ST	26	27
42.86	1506	1509	82	Ethyl nonanoate	MS, ST	5	33
44.30	1520	1527	84	Ethyl 2-hydroxyhexanoate	MS	63	79
47.74	1605	1608	90	Ethyl decanoate	MS, ST	100	100
48.75	1627	1625	93	Isoamyl octanoate	MS	42	82
49.68	1647	1655	95	Diethyl succinate	MS, ST	100	100
49.74	1648	1650	96	Ethyl benzoate	MS	0	15
50.41	1662	1668	99	Ethyl 9-decenoate	MS	63	79
55.40	1772	1775	107	Ethyl phenylacetate	MS	89	97
56.89	1806	1805	112	2-Phenylethyl acetate	MS, ST	100	100
56.86	1805	1812	113	Butyl decanoate	MS	5	9
57.63	1828	1827	115	Ethyl dodecanoate	MS, ST	100	100

(*continued on next page*)

Table 3 (*continued*)

RT (min)[a]	KI$_{cal}$[b]	KI$_{Lit}$[c]	Peak n°	Chemical families	VOCs ID[d]	North	South
58.53	1854	1864	118	Ethyl 3-hydroxydodecanoate	MS	16	36
59.92	1894	1895	121	Docyl acetate	MS	11	30
60.07	1885	1886	122	Ethyl benzenepropanoate	MS	37	36
60.41	1892	1897	123	Propyl dodecanoate	MS	100	100
62.31	1931	1923	125	Benzyl benzoate	MS	26	6
62.42	1932	1926	126	Ethyl tridecanoate	MS	21	33
66.65	2017	2023	132	Diethyl dl-malate	MS	11	36
70.75	2099	2094	134	Ethyl tetradecanoate	MS, ST	63	70
74.92	2126	2126	137	Ethyl pentadecanoate	MS, ST	100	100
77.69	2209	2215	139	Ethyl hexadecanoate	MS, ST	37	39
79.87	2218	2221	143	Hexyl salicylate	MS	5	9
				Acids			
39.65	1447	1448	69	Acetic acid	MS, ST	79	73
45.56	1534	1533	85	2-Methylpropanoic acid	MS	16	24
50.16	1657	1644	97	2-Methylbutanoic acid	MS	32	36
59.02	1868	1868	119	Hexanoic acid	MS, ST	100	100
62.89	1942	1943	127	2-Ethylhexanoic acid	MS, ST	5	24
68.08	2046	2046	133	Octanoic acid	MS, ST	100	100
76.79	2205	2206	138	4-Ethyloctanoic acid	MS, ST	100	100
79.78	2218	2227	142	Decanoic acid	MS, ST	16	39
				Carbonyl compounds			
5.58	721	723	1	Acetaldehyde	MS, ST	89	73
9.17	862	866	4	2-Butanone	MS, ST	11	18
9.72	879	884	5	3-Methyl-butanal	MS, ST	32	33
22.32	1139	1141	23	3-Heptanone	MS, ST	21	39
26.52	1210	1210	31	3-Octanone	MS, ST	32	39
28.61	1248	1248	37	Octanal	MS, ST	11	21
28.82	1254	1258	39	3-Hydroxy-2-butanone	MS, ST	26	30
31.44	1295	1301	47	6-Methyl-5-hepten-2-one	MS	16	18
34.37	1350	1350	54	2-Nonanone	MS, ST	11	18
34.98	1360	1361	55	Nonanal	MS, ST	53	76
36.24	1383	1389	58	2,4-Hexadienal	MS	47	64
40.59	1465	1469	72	Decanal	MS, ST	58	55
42.36	1498	1486	78	Benzaldehyde	MS, ST	21	42
56.81	1803	1803	111	Tridecanal	MS	11	24
57.71	1830	1831	116	4-Propyl-benzaldehyde	MS	58	70
79.16	2215	2219	140	Hexadecenal	MS	5	6
				Terpenic compounds			
23.11	1153	1153	26	Limonene	MS, ST	47	9
35.87	1376	1388	57	(*E*)-Linalool oxide	MS	26	33
38.96	1434	1428	67	(*Z*)-Linalool oxide	MS	16	36
42.79	1504	1506	81	Linalool	MS, ST	37	21
46.15	1540	1547	86	4-Terpineol	MS	58	64
47.46	1554	1563	89	β-Terpineol	MS, ST	21	6
50.86	1672	1669	100	α-Terpeniol	MS, ST	95	100
53.25	1723	1730	103	α-Curcumene	MS	37	24
55.59	1776	1783	109	Geraniol	MS, ST	5	15
66.23	2009	2008	130	Nerolidol	MS	100	100
				C$_{13}$ Norisoprenoids			
57.22	1815	1814	114	β-Damascenone	MS, ST	100	100
58.40	1850	1848	117	Geranyl acetone	MS, ST	63	76
				Furanic compounds			
36.25	1383	–	59	Tetrahydro-2,5-dimethoxyfuran	MS	16	30
38.83	1431	1431	66	2-Furfural	MS, ST	11	15
47.23	1551	1553	88	Methyl 2-furoate	MS, ST	11	18
48.88	1630	1623	94	2-Furanmethanol	MS	5	6
				Lactones			
48.11	1613	1611	91	Butyrolactone	MS, ST	42	36
63.68	1958	1957	129	(*Z*)-Wiskey lactone	MS, ST	11	21
				Volatile phenols			
63.21	1945	1945	128	4-Methyl guaiacol	MS	0	9
66.42	2012	2010	131	4-Ethyl guaiacol	MS, ST	100	100
72.06	2108	2116	135	Eugenol	MS, ST	100	100
72.25	2109	2110	136	4-Ethyl phenol	MS, ST	100	100
79.23	2218	2218	141	4-Vinylguaiacol	MS	32	27
				Miscellaneous			
14.77	1005	1011	13	Toluene	MS, ST	11	33
17.76	1061	1070	18	2-Ethyl anisole	MS	68	48
26.84	1216	1224	32	Styrene	MS	100	100
30.13	1273	1269	43	4-Ethyl toluene	MS	21	9
33.36	1330	1335	51	2-Ethyl p-xylene	MS	32	42
37.32	1401	1400	62	1,2,4,5-Tetramethyl-benzene	MS	47	64
39.64	1447	1446	68	3-Methylanisole	MS	16	27
40.35	1461	1456	71	1,2,3,5-Tetramethyl-benzene	MS	21	45
41.07	1474	1468	74	4-Methylindan	MS	5	12

(*continued on next page*)

Table 3 (*continued*)

RT (min)[a]	KI_{cal}[b]	KI_{Lit}[c]	Peak n°	Chemical families	VOCs ID[d]	North	South
42.39	1499	1501	79	4-Ethylanisole	MS	11	18
50.21	1658	1660	98	4-Vinylanisole	MS	37	33
53.75	1735	1730	105	Naphthalene	MS	100	97
55.40	1772	1771	108	2-Acetylthiophene	MS	26	3

–: Not detected.

[a] Retention time (min) determined in a BP-20 capillary column.

[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 in Pubmed database.

[d] VOCs identification using mass spectra of the NIST database (MS) and standard (ST).

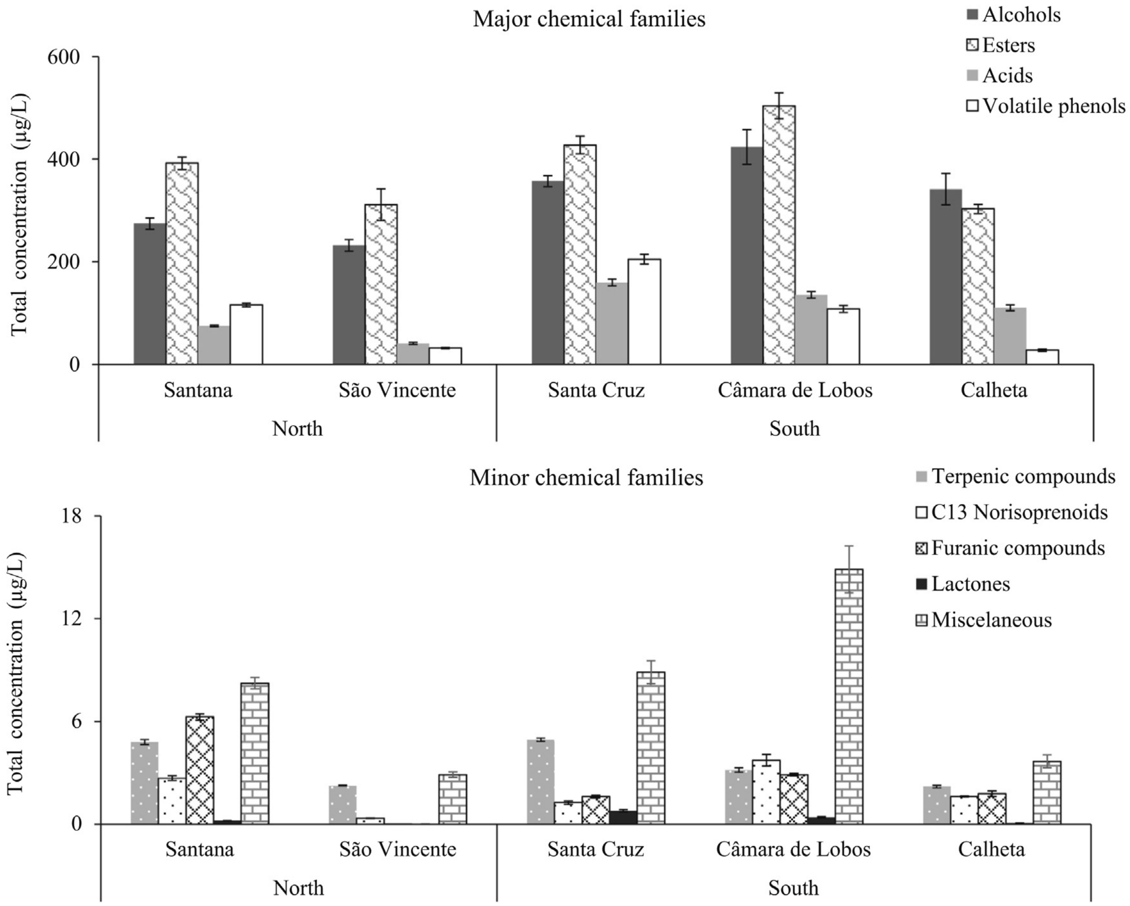

Fig. 1. Total relative concentration (µg/L) of chemical families identified in apple ciders from different geographical regions. The error bars represent the standard deviation for each measurement (n = 3).

30 alcohols, 53 esters, 8 acids, 16 carbonyl compounds, 10 terpenic compounds, 2 C_{13} norisoprenoids, 4 furanic compounds, 2 lactones, 5 volatile phenols, and 13 miscellaneous. As many as 28 of the 143 identified VOCs showed a FO of 100%, namely 6 alcohols, 13 esters, 3 acids, 1 terpenic compounds, 1 C_{13} norisoprenoid, 3 volatile phenols and 1 miscellaneous. Four of VOCs were identified only in apple ciders produced in southern regions of Madeira Island, namely 2-hexen-1-ol, 1,3-propanediol, ethyl benzoate and 4-methyl guaiacol. On the other hand, 2-penten-1-ol, (*E*)-hept-2-en-1-ol, 1-nonanol, butyl acetate, isoamyl propanoate, ethyl 3-hexenoate, ethyl nonoate, butyl decanoate, docyl acetate, ethyl tridecanoate, ethyl hexadecanoate, 2-methylpropanoic acid, 2-ethylhexanoic acid, decanoic acid, 3-octanone, 3-hydroxy-2-butanone, nonanal, tetrahydro-2,5-dimethoxyfuran and toluene have not been identified in São Vicente apple ciders, whereas 2,3-butandiol, propyl acetate, octanal and β-terpineol were not identified in the apple ciders produced in Calheta. A high number f these VOCs have been reported earlier in different cider types, as well as other cider derivatives (Lobo et al., 2016; Medina et al., 2019; Nicolini et al., 2018; Perestrelo, Silva, Silva, et al., 2019; Picinelli et al., 2000).

In semi-quantitative terms, esters (on average 381 ± 18.8 µg/L of the total relative concentration of volatile fingerprint), alcohols (on average 313 ± 21.5 µg/L), acids (96.3 ± 4.52 µg/L), volatile phenols (93.5 ± 2.60 µg/L) and carbonyl compounds (15.0 ± 1.03 µg/L) were the chemical families with the highest contribution to the volatile fingerprint of the investigated apple ciders (Fig. 1). The contribution of the remaining chemical families for the total volatile fingerprint was lower than 1%.

The esters were the major chemical family of VOCs, contributing with 46.7 ± 2.04 and 38.4 ± 1.56% of the total volatile fingerprint of apple ciders produced in the north and south of Madeira Island. Apple ciders from Câmara de Lobos seem to be the richest in esters (504 ± 20.7 µg/L), followed by apple ciders produced in Santa Cruz

Table 4
Important volatile organic compounds identified by t-tests ($p < 0.001$) between northern and southern Madeira Island regions.

VOCs	t stat	p value	−log10(p)	FDR
Isoamyl octanoate	−5.52	1.38E−07	6.86	5.80E−06
Ethyl octanoate	−4.99	1.65E−06	5.78	3.43E−05
Methyl acetate	4.90	2.45E−06	5.61	3.43E−05
Limonene	4.60	8.67E−06	5.06	6.68E−05
Ethyl nonanoate	−4.59	9.01E−06	5.05	6.68E−05
1,2,4,5-Tetramethyl-benzene	−4.56	1.02E−05	4.99	6.68E−05
1-Hexanol	−4.54	1.11E−05	4.95	6.68E−05
Ethyl hexanoate	−4.48	1.43E−05	4.84	7.51E−05
1-Octanol	−4.36	2.41E−05	4.62	1.12E−04
(*Z*)-3-Hexen-1-ol acetate	−4.13	5.96E−05	4.22	2.50E−04
Benzyl benzoate	4.08	7.27E−05	4.14	2.77E−04
Nonanal	−4.00	9.72E−05	4.01	3.40E−04
Octanoic acid	−3.90	1.42E−04	3.85	4.51E−04
Ethyl 2-hydroxyhexanoate	−3.89	1.50E−04	3.82	4.51E−04

FDR – False discover rate.

(427 ± 25.3 µg/L), Santana (392 ± 19.4 µg/L), São Vicente (311 ± 12.9 µg/L) and Calheta (303 ± 15.9 µg/L). They are formed by yeasts during fermentation and can impart a pleasant flavour like fruits and floral in overall cider aroma. Within ciders, ethyl esters derived from ethanol (*e.g.*, ethyl butanoate, ethyl hexanoate, ethyl octanoate), and acetates derived from acetic acid (*e.g.*, ethyl acetate, hexyl acetate) are the dominant VOCs in this chemical family (Nešpor, Karabín, Štulíková, & Dostálek, 2019). Ethyl acetate (on average, the relative concentration in apple cider produced in northern and southern of Madeira Island was 113 ± 2.63 and 95.4 ± 3.45 µg/L, respectively) is the most predominant ester, followed, in lower, extent by ethyl octanoate (66.7 ± 1.96 and 110 ± 6.14 µg/L), ethyl hexanoate (24.7 ± 0.96 and 42.2 ± 1.27 µg/L) and ethyl decanoate (31.1 ± 1.78 and 34.6 ± 2.97 µg/L). From these, ethyl octanoate and ethyl hexanoate showed significant differences ($p < 0.001$) between northern and southern of Madeira Island regions (Table 4). The relative concentration of ethyl hexanoate and ethyl octanoate was above of its odour threshold (OT = 14 and 5 µg/L determined in water/10% ethanol solution at pH 3.2 (Mestre et al., 2019), respectively). Consequently these VOCs can contribute positively with fruits, apple, pear, and pineapple notes to sensorial properties of apple ciders, as well as might probably be used as discriminant markers. Moreover, Antón, Suárez Valles, García Hevia, and Picinelli Lobo (2014) reported ethyl hexanoate and ethyl octanoate as putative VOC odorants of Asturias cider.

Alcohols are formed through α-keto acids produced either anabolically via the biosynthetic route from the carbon source, or catabolically, via the degradation of amino acids (Ehrlich pathway) (Sun et al., 2019). The occurrence of this chemical family at concentrations higher than 400 mg/L can impart a strong, pungent smell and taste. However, if lower than 300 mg/L can contribute positively to the overall aroma with a fruity character (González-Robles & Cook, 2016). The alcohols contribution for the total volatile fingerprint is similar among the apple ciders in the north and south of Madeira Island, 33.8 ± 1.98 and 35.8 ± 2.86%, respectively. The alcohols' contribution is strongly influenced by 3-methylbutan-1-ol (on average, the relative peak area in apple cider produced in northern and southern of Madeira Island was

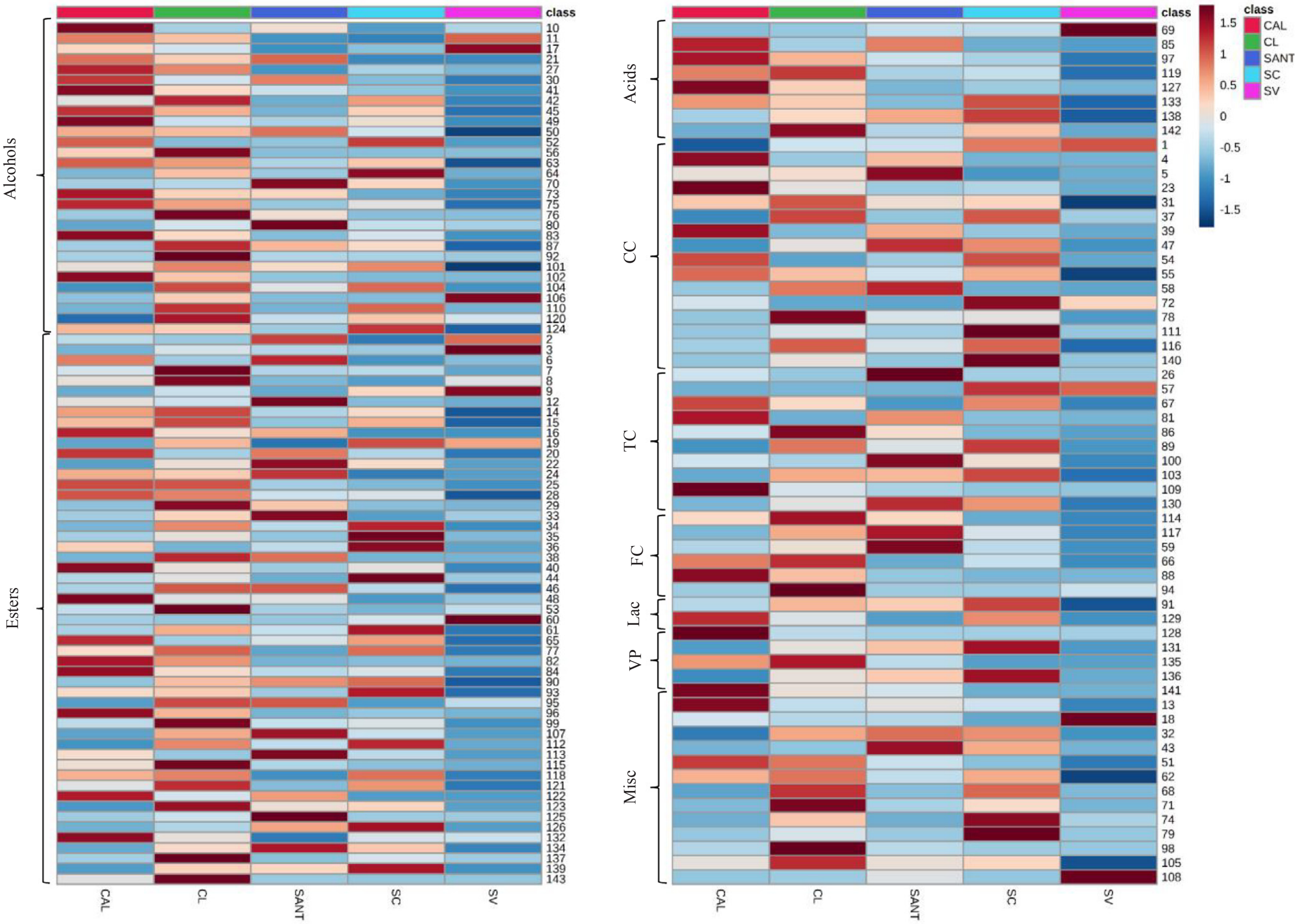

Fig. 2. Heatmap of the mean relative concentration of volatile organic compounds identified in apple ciders by geographical regions (peak number attribution is shown in Table 3). CC – Carbonyl compounds; TC – Terpenic compounds; FC – Furanic compounds; Lac – Lactones; VP – Volatile phenols; Misc - Miscellaneous.

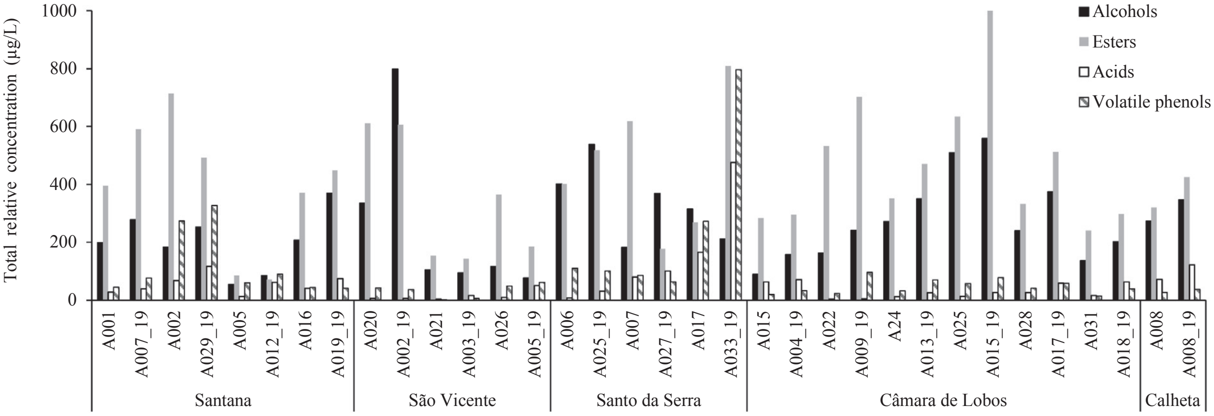

Fig. 3. Comparison of total relative concentration (µg/L) by major chemical families identified in apple ciders produced in 2018 and 2019.

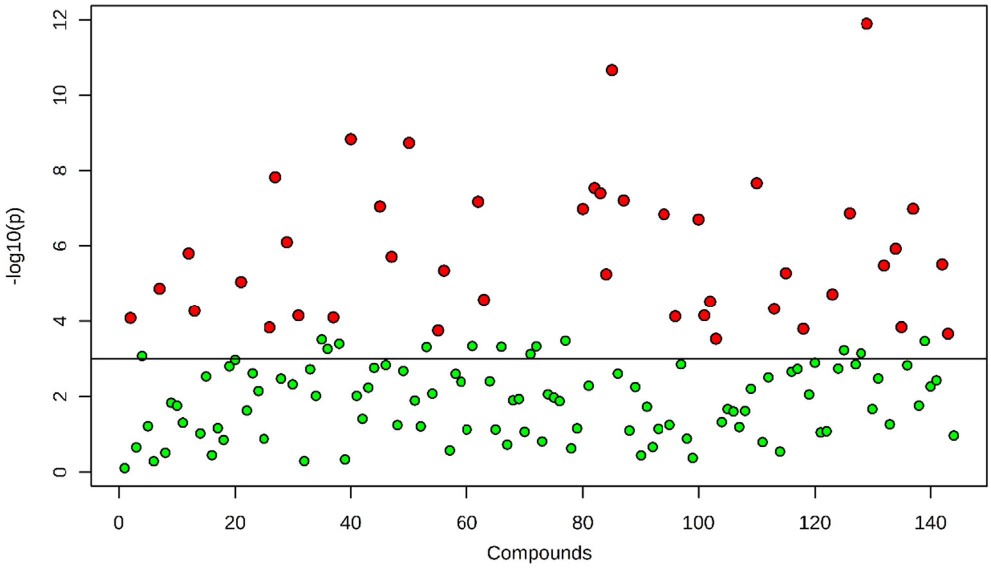

Fig. 4. One-Way Analysis of Variance (ANOVA) followed by post-hoc Tukey's test at *p*-value < 0.001. Red – VOCs statistically significant among apple ciders from different geographical regions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

147 ± 10.3 and 260 ± 24.6 µg/L, respectively), followed by phenylethyl alcohol (51.4 ± 2.09 to 68.3 ± 1.46 µg/L), 1-hexanol (29.5 ± 2.14 to 36.8 ± 3.07) and 2-methyl-1-propanol (16.2 ± 1.96 and 15.7 ± 1.06 µg/L). 3-Methyl-1-butanol and 2-phenylethyl alcohol are widely reported in fermented beverages, including ciders (Antón et al., 2014; Perestrelo, Silva, Silva, et al., 2019), beers (Andrés-Iglesias, Blanco, García-Serna, Pando, & Montero, 2016) and wines (Perestrelo, Silva, & Câmara, 2019), to which are associated by banana and rose-like odours, respectively. Nonetheless, the relative concentration of 3-methyl-1-butanol and 2-phenylethyl alcohol was below of their OTs, 30,000 and 14000 µg/L (Mestre et al., 2019), respectively. Therefore, these alcohols cannot influence the apple cider sensorial properties. 1-Hexanol is a typical VOC from apple fruits and is formed during the enzymatic oxidation process of linoleic acid (Medina et al., 2019), and showed significant differences ($p < 0.001$) between northern and southern of Madeira Island regions (Table 3). Nonetheless, the relative concentration of 1-hexanol in apple cider was below the value of its OT (2500 µg/L (Mestre et al., 2019)). So, this VOC probably will be used as discriminant marker but no as compound of influence for sensorial properties.

Acids are formed through the metabolism of lipids by yeast. At concentrations higher than their odour thresholds typically imparts unpleasant flavours (e.g., fatty, sweat, rancid, cheese) (Tesnière, 2019). The contribution of this chemical family to the total volatile fingerprint of apple cider produced in northern and southern regions of Madeira Island is significantly different, 7.42 ± 0.26 and 12.8 ± 0.78%, respectively. The high relative concentration of octanoic (27.1 ± 0.97 and 72.4 ± 6.89 µg/L for northern and southern regions of Madeira Island, respectively) and decanoic (15.9 ± 2.05 and 30.6 ± 1.13 µg/L) acids explains the difference observed in apple ciders from northern and southern regions. Their ratio, between northern and southern, is almost 2.68 and 1.92 times higher, respectively. Nevertheless, since their relative concentration was below their OTs, 3000 and 10000 mg/L (Mestre et al., 2019), respectively, any influence on apple cider sensorial properties is no expected. Moreover, 2-methylpropanoic, 2-ethylhexanoic and decanoic acids were not detected in any of apple ciders produced in São Vicente.

Volatile phenols are produced by decarboxylation of hydroxycinnamic acids (Lentz, 2018), and represent 8.98 ± 0.49 and 9.84 ± 0.56% of the total volatile fingerprint of apple ciders produced in the northern and southern regions of Madeira Island, respectively. Apple ciders from Santa Cruz seem to be the richest in volatile phenols (205 ± 20.6 µg/L), followed by apple ciders produced in Santana (115 ± 2.98 µg/L), Câmara de Lobos (108 ± 1.79 µg/L), São Vicente (31.7 ± 1.09 µg/L) and Calheta (27.6 ± 0.98 µg/L). From the pool of volatile phenols identified, 4-ethyl guaiacol (the average relative peak area in apple cider produced in northern and southern regions of Madeira Island was 35.4 ± 1.73 and 33.6 ± 2.75 µg/L, respectively) and

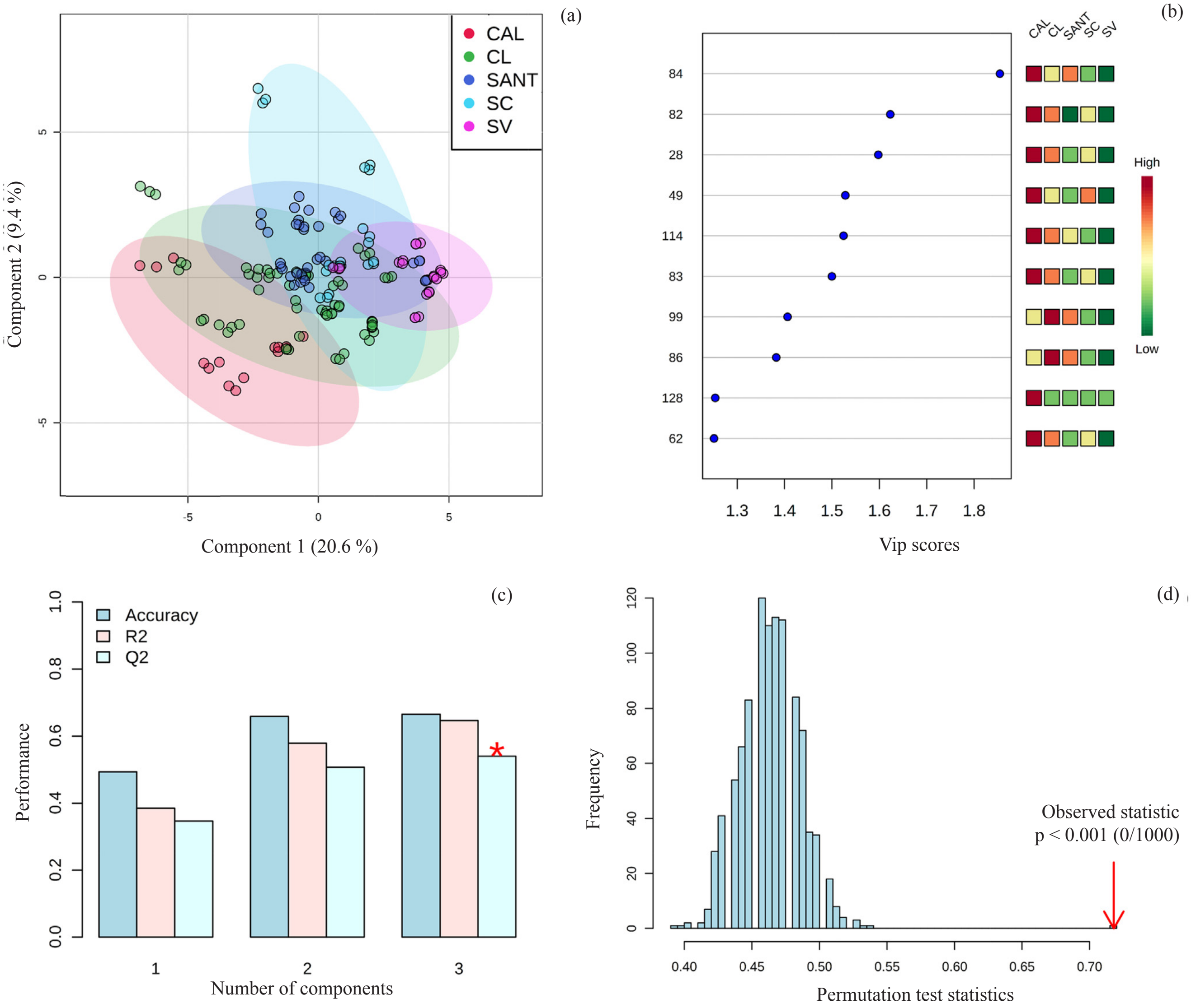

Fig. 5. PLS-DA of the volatile fingerprinting of apple ciders from different island geographical regions (n = 3 for each data point). a) score scatter plot, b) VIP scores, c) 10-fold cross-validation performance and d) model validation by permutation test based on 1000 permutations of VOCs obtained by GC-qMS of apple cider samples (peak number attribution is shown in Table 3). CAL – Calheta, CL – Câmara de Lobos, SANT – Santana, SC – Santa Cruz, SV – São Vicente.

4-ethyl phenol (46.4 ± 2.79 and 49.9 ± 3.98 µg/L) were the most abundant, with FO of 100%. The OT determined in water/10% ethanol solution at pH 3.2 for 4-ethyl guaiacol and 4-ethyl phenol is 33 and 440 µg/L (Nikfardjam, May, & Tschiersch, 2009), respectively. Their presence imparts horsy, barnyard, smoky and medicinal aromatic odours when present at concentration above their OTs. Specific case is the apple cider produced in Santa Cruz (A033_19), since the relative concentrations of the above mentioned volatile phenols were above their OTs (333 ± 25.1 and 489 ± 41.3 µg/L for 4-ethyl guaiacol and 4-ethyl phenol, respectively). Moreover, the relative concentration of 4-ethyl guaiacol was above its OT in 29% of apple ciders analysed. In addition, 5-methyl guaiacol was only found in apple ciders produced in Câmara de Lobos (FO of 5%) and Calheta (FO of 40%), whereas 4-vinylguaiacol was not detected in apple ciders from Santa Cruz.

In summary, the mean relative concentration of VOCs identified in apple ciders by geographical regions is showed in Fig. 2. As can be observed, apple ciders from São Vicente seem to be the poorest in all chemical families identified, which may be a result of the mean annual temperature (Table 1) reported for this geographical region. According to Ubalde, Sort, Zayas, and Poch (2010) high temperature affects the fruit development and, consequently accumulation of compounds. On the other hand, apple ciders from Câmara de Lobos seem to be the richest in esters (504 ± 20.7 µg/L), alcohols (424 ± 10.5 µg/L) and C_{13} norisoprenoids (3.74 ± 0.45 µg/L), whereas apple ciders from Santa Cruz have highest moiety of volatile phenols (205 ± 20.6 µg/L), acids (159 ± 6.92 µg/L) and carbonyl compounds (25.2 ± 1.49 µg/L). Moreover, related to terpenic compounds no remarkable differences were observed between apple ciders produced in Santana (4.80 ± 0.97 µg/L) and Santa Cruz (4.94 ± 1.28 µg/L).

Taking into account apple ciders obtained from the same produced from two consecutive years, 2018 and 2019, it was possible to observe that qualitative the profile is quite similar, whereas the semi-quantitative is different (Fig. 3). For esters, alcohols, acids and volatile phenols the total relative average concentrations increased between 2018 and 2019, except for apple ciders produced in São Vicente. This can be explained by the differences observed between samples A026 and A005_19. A possible explanation for the increase in total relative concentration of these chemical families can be supported by the lower mean annual precipitation observed in 2019 (Table 1). A similar pattern was reported by Ubalde et al. (2010) who observed an increase in compound accumulation as a result of water limitation on skin/pulp ratio in driest year.

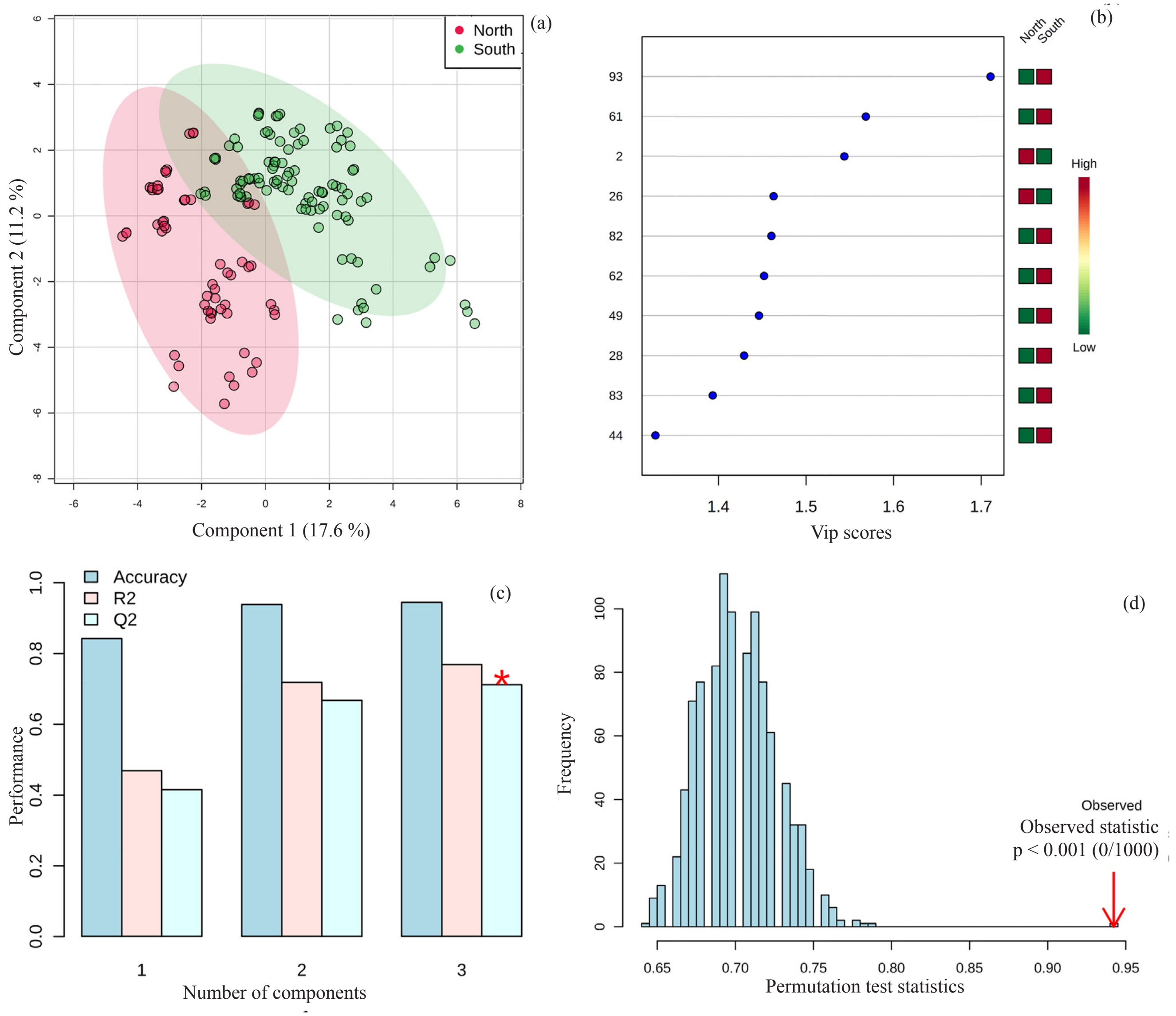

Fig. 6. PLS-DA of the volatile fingerprinting of apple ciders from north and south of Madeira Island (n = 3 for each data point). a) score scatter plot, b) VIP scores, c) 10-fold cross-validation performance and d) model validation by permutation test based on 1000 permutations of VOCs obtained by GC-qMS of apple cider samples (peak number attribution is shown in Table 3).

3.3. Statistical analysis

Statistical analysis was carried out to evaluate the potential to use the volatile fingerprint, as a suitable discriminatory tool for apple ciders, according to their geographical regions, and to guarantee their authenticity and typicity. The one-way ANOVA, followed by post-hoc Tukey's test at p-value < 0.001, was performed to select the VOCs that have statistically significant differences. Forty-three of the identified VOCs showed significant differences among apple ciders from different geographical regions (Fig. 4).

Statistically different VOCs were submitted to PCA analysis that allows to obtain a preliminary overview of similarities and differences among apple cider samples studied. In addition, PCA can show if geographical regions execute a remarkable influence on VOCs relative peak area. To further understand the differences among apple ciders of different geographical origins, a PLS-DA model was developed. Three significant components described 0.6470 of the goodness of fit (R^2 = 64.70%) and predicted ability of 0.5619 (Q^2 = 56.19%) based on crossing-validation. Nevertheless, the score plot (Fig. 5a) did not showed a clear separation of apple ciders based on geographical regions.

Therefore, a novel PLS-DA model was built, and a clear separation was observed between apple ciders produced in the northern and southern regions of Madeira Island (Fig. 6a). Combining the VIP values higher than 1 (Fig. 6b), with the loading plot, 10 VOCs were selected as putative geographical markers for the studied apple ciders. These geographical markers included (Z)-3-hexen-1-ol acetate, 1-octanol, ethyl hexanoate, 1-hexanol, 1,2,3,5-tetramethyl-benzene, ethyl nonanoate , limonene, methyl acetate, ethyl octanoate and isoamyl octanoate). The mean concentration of these VOCs, except methyl acetate, was higher in apple ciders produced in southern region of Madeira Island compared to the samples from north, as can be observed in Fig. 7. In agreement with our findings, (Z)-3-hexen-1-ol acetate, 1-hexanol, and limonene were previously reported as discriminatory geographical markers for apple varieties, with a high prediction accuracy (over 90%) (Giannetti, Boccacci Mariani, Mannino, & Marini, 2017). Ethyl octanoate was reported as a discriminatory marker to distinguish Chardonnay wines from other wines with a classification of 74% (Lukić & Horvat, 2017). Ethyl hexanoate, ethyl octanoate and 1-hexanol were also reported as putative geographical markers for grapes (Gambetta,

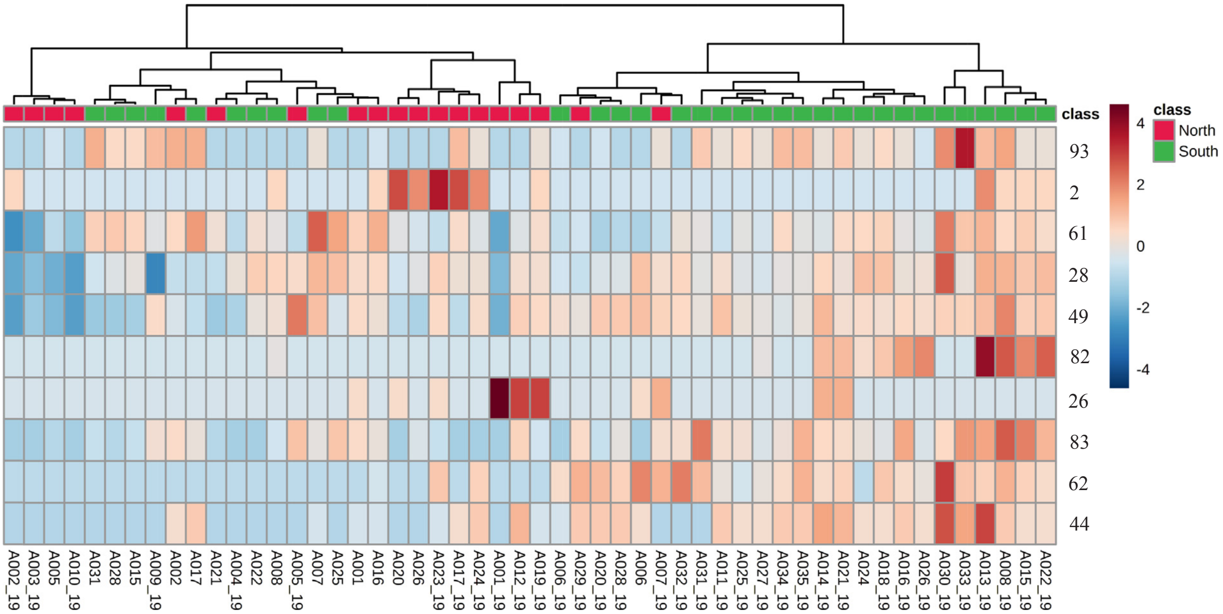

Fig. 7. Hierarchical cluster analysis (HCA). The heatmap of the putative markers identified in all apple ciders were generated by average algorithm and Pearsońs distance analysis (peak number attribution is shown in Table 3).

Cozzolino, Bastian, & Jeffery, 2017).

To evaluate the robustness of the model, a random permutation test with 1000 permutations was performed with PLS-DA model (Fig. 6c-d). Three significant components described 0.7681 of the goodness of fit ($R^2 = 76.81\%$) and a predicted ability of 0.7096 ($Q^2 = 70.96\%$) based on crossing-validation. Nevertheless, two significant components were sufficient to discriminate samples. The difference between R^2 and Q^2 was lower than 0.3 ($R^2 - Q^2 = 0.06$), which indicated that the model is not over fitted, and showed an acceptable predictive ability for discrimination of apple ciders from different geographical regions.

Fig. 7 displays the resulting dendrogram related to the heat-map constructed by Pearson's correlation, providing instinctive visualization of the dataset, which is often applied to recognize samples or features that are remarkably high or low. An analogous colour tone to the heat-map specifies the area, taking into account the VOCs relative peak area, with VIP values higher than 1, that a group of samples is similar. The obtained result showed that volatile fingerprint of apple ciders presents some differences among geographical regions. This difference could be a result of climate conditions, altitude and geographic location, which may result in an inhibition of the activity of certain odour related enzymes (Qin et al., 2017). It has been reported that geographic location (*e.g.*, longitude, latitude) and climatic factors (*e.g.*, mean annual temperature) have affect the volatile fingerprint of Fuji apples in the different regions of China (Qin et al., 2017). Furthermore, several studies have reported that grape volatile fingerprint is associated to environmental conditions, namely altitude, soil, topography, macro, meso and microclimate (de Oliveira et al., 2019; De Santis, Frangipane, Brunori, Cirigliano, & Biasi, 2017; Koundouras, 2018; Perestrelo et al., 2014; Young et al., 2016).

4. Conclusions

HS-SPME/GC-qMS data combined with chemometric tools enabled a comprehensive knowledge of the different apple ciders produced in Madeira Island. A total of 143 VOCs were identified in the investigated apple ciders, belonging to different chemical families, and only 28 VOCs from these were common to all apple ciders. Moreover, remarkable differences in terms of qualitative and semi-quantitative profile was obtained, which means that geographical origin and climatic conditions (e.g., precipitation and temperature) have a significant impact on volatile fingerprint. The obtained results revealed that a combination of volatile fingerprint data with chemometric tools provides a powerful tool with a greater ability to accurately discriminate apple ciders from different geographical regions. The developed PLS-DA model showed a clear differentiation among apple ciders, giving 6 esters, 2 alcohols and 1 terpenic compound putative geographical markers.

An additional study combining GC–MS with other analytical systems would spread the coverage of apple ciders fingerprint and provide more geographical markers for classification of apple ciders. The results highlight the potential of the used strategy for defining authenticity of analysed apple ciders and, thuse, the possibility of a future recognition of products from Madeira Island as Protected Designation of Origin (PDO) or Traditional Speciality Guaranteed (TSG).

CRediT authorship contribution statement

António Sousa: Methodology, Writing - original draft. **José Vareda:** Methodology, Writing - original draft. **Regina Pereira:** . **Catarina Silva:** Writing - review & editing. **José S. Câmara:** Writing - review & editing. **Rosa Perestrelo:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare there are no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109550.

References

Andrés-Iglesias, C., Blanco, C. A., García-Serna, J., Pando, V., & Montero, O. (2016). Volatile compound profiling in commercial lager regular beers and derived alcohol-free beers after dealcoholization by vacuum distillation. *Food Analytical Methods, 9*(11), 3230–3241.

Antón, M. J., Suárez Valles, B., García Hevia, A., & Picinelli Lobo, A. (2014). Aromatic profile of ciders by chemical quantitative, gas chromatography-olfactometry, and sensory analysis. *Journal of Food Science, 79*(1), S92–S99.

Danezis, G. P., Tsagkaris, A. S., Camin, F., Brusic, V., & Georgiou, C. A. (2016). Food Authentication: Techniques, Trends & Emerging Approaches. *Trends in Analytical Chemistry, 85*, 123–132.

de Oliveira, J. B., Egipto, R., Laureano, O., de Castro, R., Pereira, G. E., & Ricardo-da-Silva, J. M. (2019). Climate effects on physicochemical composition of Syrah grapes at low and high altitude sites from tropical grown regions of Brazil. *Food Research International, 121*, 870–879.

De Santis, D., Frangipane, M. T., Brunori, E., Cirigliano, P., & Biasi, R. (2017). Biochemical markers for enological potentiality in a grapevine aromatic variety under different soil types. *American Journal of Enology and Viticulture, 68*(1), 100–111.

Delgado-Pelayo, R., Gallardo-Guerrero, L., & Hornero-Méndez, D. (2014). Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties. *Food Research International, 65*(Part B), 272–281.

Gambetta, J. M., Cozzolino, D., Bastian, S. E. P., & Jeffery, D. W. (2017). Exploring the effects of geographical origin on the chemical composition and quality grading of Vitis vinifera L. Cv. chardonnay grapes. *Molecules, 22*(2), 218–245.

Giannetti, V., Boccacci Mariani, M., Mannino, P., & Marini, F. (2017). Volatile fraction analysis by HS-SPME/GC-MS and chemometric modeling for traceability of apples cultivated in the Northeast Italy. *Food Control, 78*, 215–221.

González-Robles, I. W., & Cook, D. J. (2016). The impact of maturation on concentrations of key odour active compounds which determine the aroma of tequila. *Journal of the Institute of Brewing, 122*(3), 369–380.

Koundouras, S. (2018). Environmental and viticultural effects on grape composition and wine sensory properties. *Elements, 14*(3), 173–178.

Lachowicz, Oszmiański, Uździcka, & Chmielewska. (2019). The influence of yeast strain, β-cyclodextrin, and storage time on concentrations of phytochemical components, sensory attributes, and antioxidative activity of novel red apple ciders. Molecules, 24(13), 2477. https://doi.org/10.3390/molecules24132477.

Lentz, M. (2018). The Impact of Simple Phenolic Compounds on Beer Aroma and Flavor. *Fermentation, 4*(1), 20–33.

Lobo, A. P., Antón-Díaz, M. J., Alonso, J. J. M., & Valles, B. S. (2016). Characterization of Spanish ciders by means of chemical and olfactometric profiles and chemometrics. *Food Chemistry, 213*, 505–513.

Lukić, I., & Horvat, I. (2017). Differentiation of commercial PDO wines produced in Istria (Croatia) according to variety and harvest year based on HS-SPME-GC/MS volatile aroma compounds profiling. *Food Technology and Biotechnology, 55*(1), 95–108.

Mangas, J., González, M., Rodríguez, R., & Blanco, D. (1996). Solid-phase extraction and determination of trace aroma and flavour components in cider by GC-MS. *Chromatographia, 42*(1–2), 101–105.

Medina, S., Perestrelo, R., Santos, R., Pereira, R., & Câmara, J. S. (2019). Differential volatile organic compounds signatures of apple juices from Madeira Island according to variety and geographical origin. *Microchemical Journal, 150*, 104094.

Mestre, M. V., Maturano, Y. P., Gallardo, C., Combina, M., Mercado, L., Toro, M. E., … Dellacassa, E. (2019). Impact on sensory and aromatic profile of low ethanol Malbec wines fermented by sequential culture of Hanseniaspora uvarum and Saccharomyces cerevisiae native yeasts. *Fermentation, 5*(3), 65. https://doi.org/10.3390/fermentation5030065.

Nešpor, J., Karabín, M., Štulíková, K., & Dostálek, P. (2019). An HS-SPME-GC-MS method for profiling volatile compounds as related to technology used in cider production. *Molecules, 24*(11), 2117–2131.

Nicolini, G., Román, T., Carlin, S., Malacarne, M., Nardin, T., Bertoldi, D., & Larcher, R. (2018). Characterisation of single-variety still ciders produced with dessert apples in the Italian Alps. *Journal of the Institute of Brewing, 124*, 457–466.

Nikfardjam, M. P., May, B., & Tschiersch, C. (2009). 4-Ethylphenol and 4-ethylguaiacol contents in bottled wines from the German "Württemberg" region. *European Food Research and Technology, 230*(2), 333–341. https://doi.org/10.1007/s00217-009-1174-1.

Pello-Palma, J., González-Álvarez, J., Gutiérrez-Álvarez, M. D., Dapena de la Fuente, E., Mangas-Alonso, J. J., Méndez-Sánchez, D., … Arias-Abrodo, P. (2017). Determination of volatile compounds in cider apple juices using a covalently bonded ionic liquid coating as the stationary phase in gas chromatography. *Analytical and Bioanalytical Chemistry, 409*(11), 3033–3041.

Perestrelo, R., Barros, A. S. A. S., Rocha, S. M., & Câmara, J. S. (2014). Establishment of the varietal profile of Vitis vinifera L. grape varieties from different geographical regions based on HS-SPME/GC-qMS combined with chemometric tools. *Microchemical Journal, 116*, 107–117.

Perestrelo, R., Silva, C., & Câmara, J. S. (2019). Madeira wine volatile profile. A platform to establish madeira wine aroma descriptors. *Molecules, 24*, 3028–3045.

Perestrelo, R., Silva, C. L., Silva, P., Medina, S., Pereira, R., & Câmara, J. S. (2019). Untargeted fingerprinting of cider volatiles from different geographical regions by HS-SPME/GC-MS. *Microchemical Journal, 148*, 643–651.

Picinelli, A., Suárez, B., Moreno, J., Rodríguez, R., Caso-García, L. M., & Mangas, J. J. (2000). Chemical characterization of Asturian cider. *Journal of Agricultural and Food Chemistry, 48*(9), 3997–4002.

Pizarro, C., Pérez-del-Notario, N., & González-Sáiz, J. (2009). Headspace solid-phase microextraction for direct determination of volatile phenols in cider. *J. Sep. Science, 32*(21), 3746–3754.

Qin, L., Wei, Q. P., Kang, W. H., Zhang, Q., Sun, J., & Liu, S. Z. (2017). Comparison of volatile compounds in "Fuji" apples in the different regions in China. *Food Science and Technology Research, 23*(1), 79–89.

Qin, Z., Petersen, M. A., & Bredie, W. L. P. (2018). Flavor profiling of apple ciders from the UK and Scandinavian region. *Food Research International, 105*, 713–723.

Sun, Z. G., Wang, M. Q., Wang, Y. P., Xing, S., Hong, K. Q., Chen, Y. F., … Xiao, D. G. (2019). Identification by comparative transcriptomics of core regulatory genes for higher alcohol production in a top-fermenting yeast at different temperatures in beer fermentation. *Applied Microbiology and Biotechnology, 103*(12), 4917–4929.

Tesnière, C. (2019, October 1). Importance and role of lipids in wine yeast fermentation. Applied Microbiology and Biotechnology. Springer Verlag.

Ubalde, J. M., Sort, X., Zayas, A., & Poch, R. M. (2010). Effects of soil and climatic conditions on grape ripening and wine quality of Cabernet Sauvignon. *Journal of Wine Research, 21*(1), 1–17. https://doi.org/10.1080/09571264.2010.495851.

Uthurry, C. A., Susca, M. J., Fontanini, J. M., Gresia, J. A., Bezic, C. A., Caponi, A. M., & Franchi, M. L. (2019). Physicochemical and sensorial characterisation of Argentine ciders. *Journal of the Institute of Brewing, 125*(4), 433–442.

van Den Dool, H., & Dec. Kratz, P. (1963). A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. Journal of Chromatography A, 11, 463–471.

Vinci, G., Preti, R., Tieri, A., & Vieri, S. (2012). Authenticity and quality of animal origin food investigated by stable-isotope ratio analysis. *J Sci Food Agric, 93*(3), 439–448.

Włodarska, K., Pawlak-Lemańska, K., Górecki, T., & Sikorska, E. (2017). Classification of commercial apple juices based on multivariate analysis of their chemical profiles. *International Journal of Food Properties, 20*(8), 1773–1785. https://doi.org/10.1080/10942912.2016.1219367.

Worley, B., & Powers, R. (2013). Multivariate analysis in metabolomics. *Current. Metabolomics, 1*(1), 92–107.

Young, P. R., Eyeghe-Bickong, H. A., du Plessis, K., Alexandersson, E., Jacobson, D. A., Coetzee, Z., … Vivier, M. A. (2016). Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. Plant Physiology, 170(3), 1235–1254. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26628747.