



Differential volatile organic compounds signatures of apple juices from Madeira Island according to variety and geographical origin



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ABSTRACT

One of the main food authenticity issues is related to products' false labelling concerning their variety or geographical origin. For this reason, the aim of this study was to establish distinctive characteristics for the discrimination of apple juices according to regional varieties (Rijo, Verde, Ribeiro and Azedo) and geographical origin (Prazeres and Santo da Serra (Madeira Island)) on the basis of their volatile pattern by headspace solid-phase microextraction combined with gas chromatography mass spectrometry (HS-SPME/GC–MS) combined with chemometric tools. The results obtained revealed a perfect discrimination between the different apple varieties, with Rijo apple juices as samples with the major relative concentration of ethanol, ethyl butanoate, ethyl 2-methylbutanoate and ethyl hexanoate. Moreover, this study allowed a geographical origin-based classification of Azedo apple juices, highlighting ethyl acetate, 2-methyl-1-propanol, ethyl hexanoate, and toluene (described for the first time in apple juices) as discriminatory features. This study demonstrated that volatile organic compounds (VOCs) could serve as authenticity indicators to verify variety and geographical origin of apple juices, providing local producers multiple benefits and legal protection against misuse of the products.

1. Introduction

Apples (*Malus domestica*) of regional varieties present a particular crop gene pool that must be preserved and promoted to guarantee biodiversity of genetic resources. Nevertheless, these regional varieties are not at all characterized in terms of the volatile and aromatic composition [1]. Flavour is one of the key drivers of consumers' appreciation of apple fruits and volatile organic compounds (VOCs) are the organic molecules responsible for these perceived odour and flavour attributes [2]. Over 300 VOCs have been measured in the aroma profile of apple fruit, some of them offer the characteristic apple aroma and others play a part in the aroma intensity [3]. However, their concentration rapidly may change during fruit ripening, due to variety/cultivar, geographical origin or production systems [4,5].

The diversity of this fruit and its widespread use has resulted in a rise of frauds. Many of the current major problems faced by governmental agencies and industries are caused by adulteration and food frauds. Indeed, the main authenticity issues in beverages are related to product's false labelling in terms of their variety/cultivar or geographical origin [6]. In this respect, it is increasingly necessary to specify the variety in ciders (fermented apple juice) in the same way as

in wines, so it would be really helpful to know the variety in apple juices. Indeed, it is essential the identification of compounds that are strongly associated to the odour/flavour descriptions present in complex matrices such as apples or apple juices. Due to these reasons, a multivariate analysis is more suited to explore the relationship between VOCs and the variety and geographical origin [2]. Chemometric tools with appropriate analytical techniques can be used for the unequivocal classification of apple juices and could monitor its traceability and authenticity [5]. To date, several techniques have been applied for this purpose: infra-red spectroscopy [7–9], atmospheric pressure chemical ionization mass spectrometry (APCI-MS) [6], gas chromatography (GC) and mass spectrometry (MS) [10–12]. Moreover, headspace solid-phase microextraction coupled with gas chromatography and mass spectrometry (HS-SPME/GC–MS) is able to detect flavour VOCs that are present at low concentration in complex matrices like apple juice and that may be the VOCs responsible for the discrimination between different apple juices. In addition, HS-SPME/GC–MS combined with chemometric methods enable targeted and untargeted approaches for food authentication purposes. Therefore, chemometric analysis of GC–MS profiles may be a suitable method for the classification of apple juices [13].

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The main objective of this work was to establish distinctive characteristics of apple juices according to regional apple varieties (Rijo, Verde, Ribeiro and Azedo) and geographical origin (Prazeres vs. Santo da Serra) on the basis of the global volatile pattern using HS-SPME/GC-MS combined with chemometric tools, characterizing the regional varieties, taking into account the agronomic importance to preserve locally adapted varieties and promoting the quality of traditional products. To the best of our knowledge, the discrimination of these apple juices according to regional apple varieties and Madeira Island origin has not been previously reported. Moreover, the regional government aims to valorize apple varieties as Protected Designation of Origin (PDO) or Traditional Speciality Guaranteed (TSG), which provide legal protection for local producers against the misuse of the products.

2. Experimental

2.1. Chemicals and reagents

All reagents and solvents used in this study were of analytical quality. Sodium chloride (NaCl, 99.5%) and calcium chloride (CaCl₂, > 99.0%) were supplied by Panreac (Spain, Barcelona). Ultra-pure water was obtained from a Milli-Q® system (Millipore).

Helium of purity 5.0 (Air Liquide, Portugal) was utilized as the GC carrier gas. The glass vials, SPME fiber (divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)) and SPME holder for manual sampling were purchased from Supelco (Bellefonte, PA, USA). The Kovats index (KI) was calculated through the injection of a series of C₈ to C₂₀ straight-chain *n*-alkanes (concentration of 40 mg L⁻¹ in *n*-hexane) produced by Fluka (Buchs, Switzerland), 3-octanol (internal standard, IS) and 1-butanol, 1-heptanol, 1-octanol, 1-propanol, (E)-2-hexenal, 2-methyl-butanol, 2-ethyl-1-hexanol, 2-methyl-1-propanol, 2-phenylethanol, acetaldehyde, benzaldehyde, ethanol, ethyl acetate, ethyl butanoate, ethyl decanoate, ethyl hexanoate, ethyl octanoate, ethyl propanoate, ethyl-3-hydroxybutanoate, hexyl acetate, and pentanol with purity up to 98% were obtained from Sigma-Aldrich (Madrid, Spain).

2.2. Apple juice samples

Fresh Rijo, Verde, Ribeiro, and Azedo apple varieties (*Malus domestica*) were directly provided by local producers of Prazeres (PRZ), a Madeira Island region, which is located within the coordinates: 32° 45' 4.76" N and 17° 12' 16.75" W, with an average temperature of 15.8 °C and mean annual rainfall of 665 mm and an altitude of 623 m. Azedo variety was also collected from Santo da Serra (SDS), located in the GPS coordinates: 32° 43' 20.52" N and 16° 49' 17.35" W with mean temperature: 15.5 °C, mean rainfall: 654 mm and altitude: 660 m. The varieties chosen for this study are the most cultivated varieties in PRZ and SDS regions. All samples of apple fruits, harvested in the year 2017, were visually inspected to ensure no apparent damage or disease.

Approximately three kilograms of apples were selected for each of the four varieties and they were collected from different plants in order to achieve a sample as representative as possible. Every fruit was carefully washed with tap water, cut into pieces (unpeeled fruits) and squeezed at room temperature (22 ± 1 °C) using a hand press juicer machine for apples. In order to have a representative portion of apple juice from a determined variety and region, several apples of the same region and same variety were used to make a whole juice. An amount (3%, w/v) of CaCl₂ was added to avoid enzyme browning according to a previous study [14]. The juice obtained was divided into aliquots of 50 mL and stored in sealed glass bottles at -80 °C until subsequent analysis. Prior to the analysis, each apple juice sample was defrosted overnight in a refrigerator at 4 °C in dark to avoid quality alteration. Although in industrial practice, apple juices are thermally processed, in this study, fresh (non-processed) apple juices were used in order to collect data about aroma descriptors of these juices that may be used in

the traditional cider-making process, thus generating useful information for producers.

2.3. Headspace solid-phase microextraction

The headspace solid-phase microextraction (HS-SPME) procedure was adopted from a previous study validated in our laboratory with apple fruit samples [15] with slight modifications. In short, 5 mL of apple juice, 5 µL of 3-octanol (IS at concentration of 2.94 µg L⁻¹), 2 g of NaCl and a magnetic stirrer were added into a 20 mL amber glass with a PTFE-faced silicone septum and placed in a thermostatic block with a constant magnetic stirring of 500 rpm.

Before using the SPME fiber, the fiber was conditioned according to the manufacturer's instructions. Then, the fiber was exposed to the headspace for 45 min at 40 ± 1 °C. Subsequently, after extraction the fiber 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 VOCs. All analyses were carried out in triplicate (n = 3).

2.4. Gas chromatography-quadrupole mass spectrometry conditions

Chromatographic separations were performed using an Agilent 6890N (Palo Alto, CA, USA) gas chromatography 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 set up as it follows: initial temperature 40 °C, a ramp of 3 °C min⁻¹ to 220 °C and a constant temperature was kept 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 autotune procedure. The mass acquisition range, made in full scan mode, was 30–300 *m/z*.

VOCs identification was achieved by the following ways:

- (i) comparison of the GC retention times and mass spectra with those of the standard (Tables 1 and 2), when available;
- (ii) all mass spectra were also compared to the data system library (NIST, 2005 software, Mass Spectral Search Program v.2.0d; Washington, DC). The match factor criterion for identification was ≥ 90%;
- (iii) Kovats index (KI) values were determined according to the Van den Dool and Kratz equation [16].

A C₈–C₂₀ *n*-alkanes series was used to determinate the KI. The values were compared, when available, with values reported in the literature for similar columns [15,17] and databases available online (the pherobase and flavornet).

Semi-quantitative analysis was carried out and the VOCs concentration was estimated 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 of Madeira wines [18].

2.5. Statistical data elaboration

Before applying the chemometric approach, data from GC-qMS analyses were then median-normalised and Pareto-scaled [19]. Then, data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test for post-hoc multiple comparisons of means from four apple juices varieties data at *p*-value < 0.05 to identify significant

Table 1

The characteristics of the 66 discriminatory volatile organic compounds (VOCs) from the statistical analysis to compare four apple varieties from the same geographical region, Prazeres (Madeira Island).

RT ^a (min)	Id	Chem. family	Compound name	KI ^b	KI ^c	ANOVA- post-hoc Tukey		Relative concentration ($\mu\text{g L}^{-1}$) (mean \pm SD)			
						p-Value	FDR	Rijo	Verde	Ribeiro	Azedo
4.98	1	A	Acetaldehyde ^d	650	690	6.54E-08	6.02E-07	10.53 \pm 2.24	-	-	-
5.71	2	A	Propanal	803	801	1.16E-05	4.62E-05	0.32 \pm 0.08	-	-	-
7.20	5	E	Ethyl acetate ^d	890	907	5.97E-06	2.89E-05	3.62 \pm 0.80	-	-	-
7.45	6	K	2-Butanone	902	901	4.67E-05	1.38E-04	0.30 \pm 0.06	-	-	-
7.62	7	E	Methyl propionate	909	911	8.77E-05	2.28E-04	0.21 \pm 0.02	-	-	-
7.79	8	A	2-Methyl butanal ^d	915	916	2.75E-03	4.96E-03	0.44 \pm 0.11	-	3.33 \pm 0.29	2.53 \pm 0.90
8.13	9	Al	Ethanol ^d	928	929	2.12E-02	3.09E-02	187.42 \pm 34.50	-	1.20 \pm 0.14	1.90 \pm 0.37
8.65	10	F	2-Ethylfuran	945	950	8.33E-05	2.26E-04	-	1.33 \pm 0.01	3.21 \pm 0.42	2.20 \pm 0.32
8.67	11	D	2,4,5-Trimethyl-1,3-dioxolane	946	967	2.78E-06	1.71E-05	6.82 \pm 1.37	-	-	-
8.97	12	E	Ethyl propanoate ^d	956	959	4.91E-03	8.22E-03	15.96 \pm 3.74	-	-	-
9.65	15	A	Pentanal ^d	977	985	1.55E-04	3.58E-04	0.69 \pm 0.14	1.28 \pm 0.17	-	-
9.77	16	-	N.I. (m/z: 101, 73)	981	-	9.58E-03	1.52E-02	0.45 \pm 0.09	-	-	-
9.89	17	E	Methylbutanoate	985	982	6.66E-06	3.06E-05	3.40 \pm 0.80	-	-	-
10.57	18	K	4-Methyl-2-pentanone	1004	1008	3.45E-05	1.09E-04	0.30 \pm 0.07	-	-	-
10.75	19	E	Methyl 2-methylbutanoate	1009	1033	5.52E-06	2.82E-05	3.04 \pm 0.76	-	-	-
10.84	20	E	Isobutyl acetate	1011	1015	1.36E-08	1.74E-07	-	0.57 \pm 0.07	-	-
11.40	21	Al	1-Propanol ^d	1027	1037	1.66E-02	2.47E-02	10.59 \pm 1.90	-	4.06 \pm 1.36	3.37 \pm 0.17
11.81	23	E	Ethyl butanoate ^d	1037	1040	3.61E-04	7.91E-04	367.55 \pm 89.47	0.61 \pm 0.04	-	-
12.08	24	E	Propyl propanoate	1044	1056	2.14E-05	7.58E-05	0.58 \pm 0.008	-	1.18 \pm 0.16	-
12.47	25	E	Ethyl 2-methylbutanoate	1053	1050	4.84E-05	1.39E-04	212.10 \pm 52.12	0.67 \pm 0.02	1.41 \pm 0.15	-
13.20	27	E	Butyl acetate	1071	1075	1.26E-03	2.41E-03	2.04 \pm 0.10	9.92 \pm 0.98	-	-
14.12	30	A	2-Methyl-2-butenal isomer	1091	1100	5.12E-04	1.07E-03	-	-	4.22 \pm 0.31	4.90 \pm 0.42
14.72	31	E	Diethyl carbonate	1103	1083	1.31E-02	1.98E-02	1.42 \pm 0.28	-	-	-
15.53	32	E	2-Methyl-1-butanol acetate	1120	1145	8.52E-06	3.58E-05	1.50 \pm 0.04	335.05 \pm 17.64	2.21 \pm 0.19	-
15.63	33	E	Propyl butanoate	1122	1135	2.20E-03	2.98E-03	8.91 \pm 0.48	-	2.90 \pm 0.74	7.80 \pm 0.84
15.80	34	A	2-Pentenal isomer	1125	1131	7.72E-04	1.58E-03	-	0.67 \pm 0.05	-	-
16.24	35	Al	1-Butanol ^d	1133	1145	1.11E-07	9.28E-07	7.67 \pm 1.82	-	-	-
17.58	37	E	Ethyl 2-butenoate	1157	1152	2.02E-05	7.44E-05	3.68 \pm 0.83	-	-	-
17.62	38	E	2-Methylpropyl butanoate	1158	1152	4.15E-04	8.87E-04	-	-	9.88 \pm 0.82	6.33 \pm 0.64
18.22	39	E	Pentyl acetate	1168	1147	3.64E-06	1.97E-05	-	3.54 \pm 0.13	-	-
18.52	40	E	2-Methylpropyl-2-methylbutanoate	1173	1171	1.04E-02	1.62E-02	0.58 \pm 0.14	-	-	-
18.91	42	E	Methyl hexanoate	1180	1188	3.07E-06	1.77E-05	3.53 \pm 0.87	-	-	-
19.08	43	E	Pentyl propanoate	1183	1192	1.81E-04	4.07E-04	0.32 \pm 0.07	-	-	-
20.42	45	A	2-Hexenal isomer ^d	1204	1220	1.52E-08	1.74E-07	234.24 \pm 45.13	632.78 \pm 74.88	695.98 \pm 115.20	626.54 \pm 132.62
21.55	46	E	Ethyl hexanoate	1225	1220	3.16E-11	2.91E-09	248.45 \pm 60.81	-	-	-
21.64	47	E	Butyl 2-methylbutanoate	1227	1228	9.01E-07	5.92E-06	-	1.53 \pm 0.14	3.49 \pm 0.51	3.82 \pm 0.27
23.54	50	E	2-Methylbutyl butanoate	1260	1268	1.49E-03	2.80E-03	1.31 \pm 0.13	-	-	1.47 \pm 0.19
23.78	51	E	Hexyl acetate ^d	1265	1270	8.56E-06	3.58E-05	0.99 \pm 0.23	18.59 \pm 0.23	-	1.43 \pm 0.22
24.42	53	E	2-Methylbutyl-2-methylbutanoate	1275	1283	6.21E-07	4.76E-06	-	-	2.16 \pm 0.18	3.16 \pm 0.15
24.46	54	E	Butyl ethyl carbonate	1276	-	3.26E-05	1.07E-04	2.47 \pm 0.57	-	-	-
25.45	56	E	Ethyl 3-hexenoate	1291	1292	2.28E-02	3.09E-02	0.55 \pm 0.13	-	-	-
25.93	57	Al	2-Methyl-2-buten-1-ol	1299	1320	1.56E-04	3.58E-04	-	-	13.12 \pm 2.02	2.88 \pm 0.60
26.10	58	Al	2-Pentenol isomer	1302	1301	3.17E-09	5.84E-08	-	1.03 \pm 0.05	-	-
29.54	62	Al	3-Hexenol isomer	1364	1388	1.65E-03	3.04E-03	-	-	-	1.54 \pm 0.25
31.02	64	A	2,4-Hexadienal isomer	1389	1397	1.39E-04	3.46E-04	-	6.56 \pm 0.98	7.21 \pm 1.29	9.03 \pm 1.60
31.85	65	E	Butyl hexanoate	1403	1403	4.41E-09	6.76E-08	-	-	-	0.92 \pm 0.11
32.00	66	E	Hexyl butanoate	1406	1419	1.10E-09	4.69E-08	-	0.70 \pm 0.01	-	1.24 \pm 0.11
32.44	67	-	N.I. (m/z: 101, 69)	1414	-	3.78E-03	6.56E-03	174.64 \pm 42.70	1.79 \pm 0.08	-	-
33.05	68	E	Ethyl octanoate ^d	1426	1436	1.53E-09	4.69E-08	-	-	-	4.05 \pm 0.70
33.62	69	Al	1-Heptanol ^d	1436	1461	5.69E-03	9.35E-03	-	-	1.79 \pm 0.16	1.39 \pm 0.20
34.24	70	Al	2-Methyl-6-hepten-1-ol	1448	1480	2.02E-05	7.44E-05	3.95 \pm 0.81	-	-	-
35.49	71	Al	2-Ethyl-1-hexanol ^d	1470	1489	1.49E-04	3.58E-04	-	6.12 \pm 0.24	5.40 \pm 1.12	3.04 \pm 0.31
35.87	72	-	N.I. (m/z: 101, 45)	1477	-	1.23E-02	1.87E-02	43.01 \pm 10.28	-	-	-
37.14	73	E	Ethyl 3-hydroxybutanoate ^d	1499	1522	3.15E-05	1.07E-04	7.27 \pm 0.77	-	-	-
37.77	74	A	Benzaldehyde ^d	1511	1495	2.87E-02	4.07E-02	4.37 \pm 0.97	-	2.48 \pm 0.26	11.95 \pm 1.07
39.82	75	Al	1-Octanol ^d	1551	1526	6.67E-05	1.86E-04	5.13 \pm 0.66	-	-	-
39.97	76	E	Ethyl 3-(methylthio)propanoate	1554	1580	7.10E-07	5.03E-06	9.66 \pm 1.74	-	-	-
42.61	77	Al	5-Octenol isomer	1603	1608	8.94E-05	2.28E-04	4.49 \pm 0.87	-	-	-
44.53	79	E	Ethyl decanoate ^d	1642	1636	2.25E-08	2.30E-07	0.48 \pm 0.04	-	-	2.12 \pm 0.52
48.12	84	Al	3-(Methylthio)-1-propanol	1713	1704	2.85E-03	5.04E-03	0.78 \pm 0.08	-	-	-
50.37	87	T	α -Curcumene	1758	1772	7.89E-04	1.58E-03	-	-	-	0.69 \pm 0.01
50.48	88	-	N.I. (m/z: 157, 111)	1761	-	4.01E-05	1.23E-04	2.99 \pm 0.83	-	-	-

(continued on next page)

Table 1 (continued)

RT ^a (min)	Id	Chem. family	Compound name	KI ^b	KI ^c	ANOVA- post-hoc Tukey		Relative concentration ($\mu\text{g L}^{-1}$) (mean \pm SD)			
						p-Value	FDR	Rijo	Verde	Ribeiro	Azedo
55.18	89	E	Ethyl 3-hydroxydodecanoate	1864	–	9.02E–03	1.45E–02	32.95 \pm 5.15	–	–	–
55.90	90	Al	2-Phenylethanol ^d	1880	1859	4.06E–03	6.92E–03	–	–	–	6.73 \pm 0.57
57.44	91	–	N.I. (<i>m/z</i> : 117, 71)	1916	–	1.17E–03	2.28E–03	97.76 \pm 15.63	–	–	–
58.31	92	SC	Benzothiazole	1937	1952	2.50E–09	3.75E–08	–	–	–	3.64 \pm 0.86

(A): Aldehyde, (AL): Alcohol, (D): Dioxolane, (E): Ester, (F): Furan (K): Ketone, (SC): Sulfur compounds, (T): Terpenoid, (FDR): False Discovery Rate. –: not detected. N.I: not identified. The mean concentration of three replicates is relative to internal standard (3-octanol).

^a Retention time (min).

^b Kovat index relative *n*-alkanes (C₈ to C₂₀) on a BP-20 capillary column.

^c Kovat index relative reported in literature for equivalent capillary column [15,17] and databases available online (the pherobase and flavornet).

^d Identified using pure standards.

differences. In addition, Student's *t*-test was performed to distinguish between Azedo variety apple juices from two geographical origins.

Besides, principal component analysis (PCA) was used for unsupervised analysis and partial least squares-discriminant analysis (PLS-DA) for supervised analysis. All the features with variable importance in the projection (VIP) score ≥ 1.6 and differentially expressed in the univariate analysis were considered to be potential candidates for the discrimination of apple juices varieties. Receiver Operating Characteristics (ROC) curves were used to evaluate the classification capability of a model. They are plots of sensitivity *versus* 1-specificity for a binary classification model [20]. Hierarchical clustering analysis (HCA) was generated by Euclidean distance through Ward agglomerative method in order to identify clustering patterns. Statistical analysis was performed using web-based application MetaboAnalyst v. 4.0, created at the University of Alberta, Canada [21].

3. Results and discussion

3.1. Classification of apple juices according to variety

In the present assay, four apple varieties (Rijo, Verde, Ribeiro and Azedo) from the same geographical region (Prazeres: PRZ) were investigated. The Fig. 1 shows a representative GC-qMS chromatogram (total ion chromatogram (TIC)) of the volatile pattern of apple juices investigated in this study indicating the most representative VOCs. Differences in VOCs profile among four varieties from PRZ and Azedo variety from PRZ and SDS can be observed. In addition, the VOCs and their relative concentrations were significantly different in apple juices (Table 1). Statistically significant differences were found for a very large number of VOCs (66 VOCs, namely 33 esters, 13 alcohols, nine aldehydes, two ketones, one dioxolane, one furane, one sulfur compound, one terpenoid and five unknown compounds). Overall, the esters and alcohols were the predominant chemical families of VOCs in

apple juices. As it can be observed in Table 1, a high inter-varietal heterogeneity was found and the most distinguished apple variety was Rijo with the highest amount of statistically significant features (46 VOCs) and Azedo, Ribeiro and Verde varieties presented 25, 18 and 17 VOCs, respectively. The heterogeneity among varieties in terms of number, type and VOCs concentration has been previously reported in apple fruits in several studies [22,23]. As for the discriminatory metabolites (Table 1), the main VOC was 2-hexenal isomer that presented a similar concentration in Verde, Ribeiro and Azedo varieties ranged from 626 to 695 $\mu\text{g L}^{-1}$, whereas in Rijo variety 248 $\mu\text{g L}^{-1}$ was identified. 2-Hexenal isomer has been described as one of the major contributors to the green odour of apple fruit and juice [24]. As it can be observed in Table 1, the predominant VOCs present in apple juices depending on the apple variety. Thus, for example, in Rijo variety, the predominant VOCs belong to esters (ethyl propanoate (12), ethyl butanoate (23), ethyl-2-methylbutanoate (25), ethyl hexanoate (46)) and alcohols (ethanol (9)). Verde variety registered the major relative concentration of esters: 2-methyl-1-butanol acetate (32) with 335 $\mu\text{g L}^{-1}$ and hexyl acetate (51) with 18 $\mu\text{g L}^{-1}$ as relative concentration level. Ribeiro variety was highlighted by its relative concentration of 2-methyl-2-butenol (57), an alcohol with a green oily odour. In the same way, Azedo variety showed a higher relative concentration of benzaldehyde (74) (11.95 $\mu\text{g L}^{-1}$) than the other ones, bringing a bitter almond aroma. Nonetheless, the level of benzaldehyde detected in Azedo variety was far below the value of its odour threshold (350–3500 $\mu\text{g L}^{-1}$). Consequently, this VOC might probably be used as discriminant marker but no as compound of influence for sensorial properties. The PLS-DA results showed a clear differentiation among apple juices of four different apple varieties (Rijo, Verde, Ribeiro and Azedo) from the same geographical region (PRZ) (Fig. 2). The first two components obtained from PLS-DA explained 67.2% of the total variance, respectively (Fig. 2A). In addition, the Variable Importance in Projection (VIP) values from PLS-DA with (VIP) score ≥ 1.6 were used

Table 2

Significant features identified by Student's *t*-test in apple juices of Azedo variety from two geographical regions (Azedo PRZ vs. Azedo SDS).

RT ^a (min)	Id	Chemical Family	Compound name	KI ^b	KI ^c	Student's <i>t</i> -test		Relative concentration ($\mu\text{g L}^{-1}$)	
						p-Value	FDR	AZEDO PRZ	AZEDO SDS
7.20	5	Ester	Ethyl acetate ^d	890	907	5.44E–03	4.83E–02	–	9.45 \pm 0.87
10.43	93	–	N.I (<i>m/z</i> : 41,40)	1000	–	6.17E–03	4.83E–02	–	42.38 \pm 0.63
11.92	94	Aromatic hydrocarbon	Toluene	1040	1042	1.70E–05	8.03E–04	–	80.79 \pm 14.62
13.98	29	Alcohol	2-Methyl-1-propanol ^d	1088	1085	1.91E–03	2.99E–02	–	1.82 \pm 0.32
21.56	46	Ester	Ethyl hexanoate ^d	1225	1220	4.02E–03	4.72E–02	–	4.26 \pm 0.61

–: not detected. N.I: not identified. The mean concentration of three replicates is relative to internal standard (3-octanol).

^a Retention time (min).

^b Kovat index relative *n*-alkanes (C₈ to C₂₀) on a BP-20 capillary column.

^c Kovat index relative reported in literature for equivalent capillary column and databases available online (the pherobase and flavornet), [15,17].

^d Identified using pure standards.

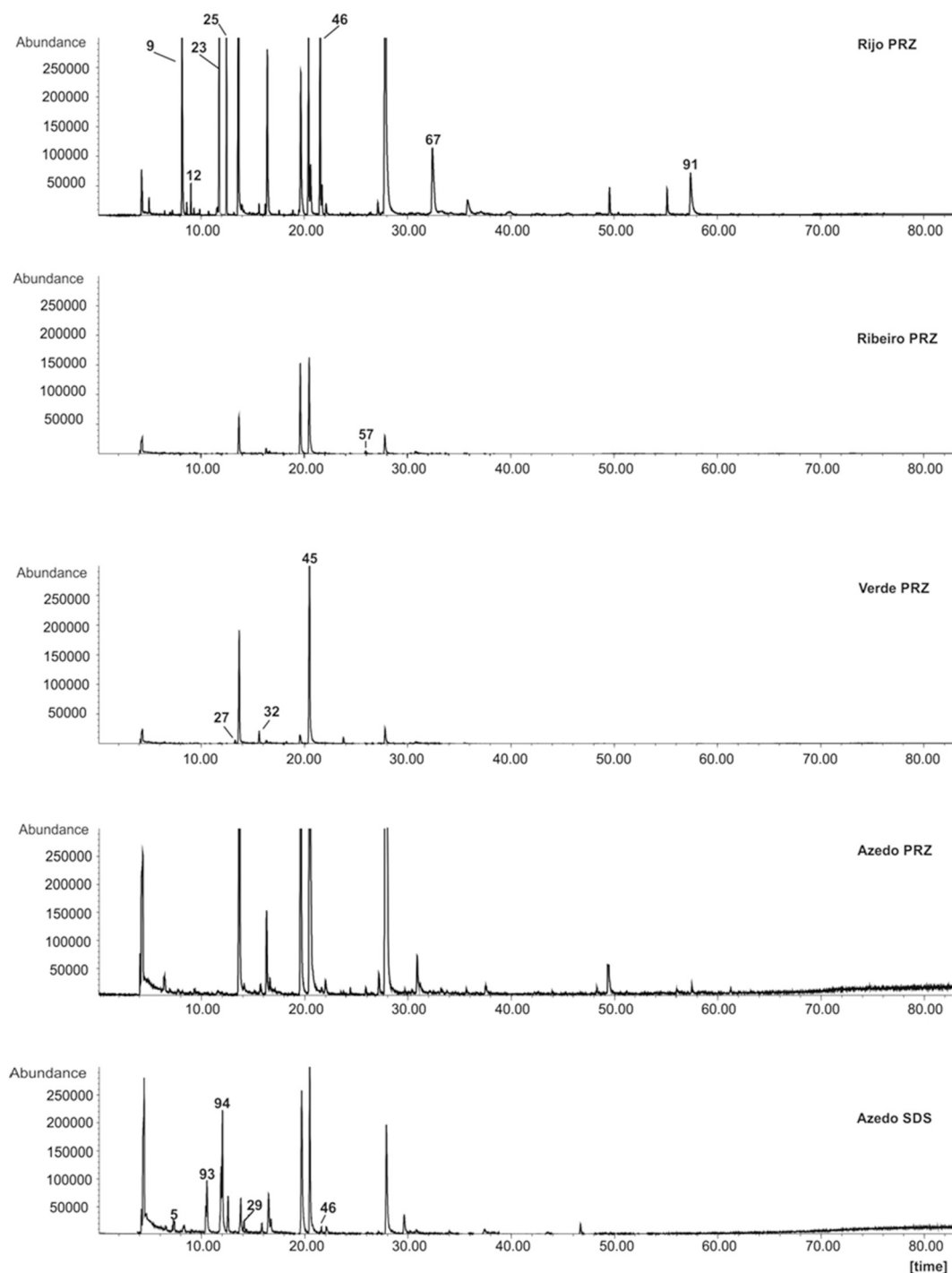


Fig. 1. The GC-MS profile of a representative sample of different regional apple varieties. The correspondence between peak numbering and VOC assignment is shown in Tables 1 and 2.

in this study to obtain 15 features with the greatest discriminating power to distinguish the four varieties studied (Fig. 2B). ROC approach was used to evaluate the performance of classification model based on VOCs. The area under the curve (AUC) ranged between 0.8 and 1 (perfect classifier) as shown in Fig. S1. Among these significant features that contribute to the juices' classification according to apple variety, nine esters (ethyl 2-methylbutanoate (25), butyl acetate (27), 2-methyl-1-butanol acetate (32), propyl butanoate (33), 2-methylpropyl butanoate (38), 2-methylbutyl-2-methylbutanoate (53), butyl hexanoate (65), ethyl octanoate (68) and ethyl decanoate (79)), three aldehydes (2-methyl-2-butenal isomer (30), 2-hexenal isomer (45) and

benzaldehyde (74)), one sulfur compound (benzothiazole (92)), one alcohol (2-methyl-2-butenol (57)) and one unknown compound (not identified (67), m/z : 101, 69) were obtained. The contribution of VOCs to the flavour has been investigated in previous studies in order to establish relationships between VOCs and sensory attributes [2,25]. Each class of VOCs attributes a typical odour characteristic to juices [3,26]. All the ester markers, resulting from VIP and PLS-DA as potential variables contributing to the classification of apple juices from four varieties, are described to have apple-like impact odorants, with fruity, sweet and green apple aroma. Thus, ethyl 2-methylbutanoate (25), detected in high relative concentration in Rijo variety, is correlated

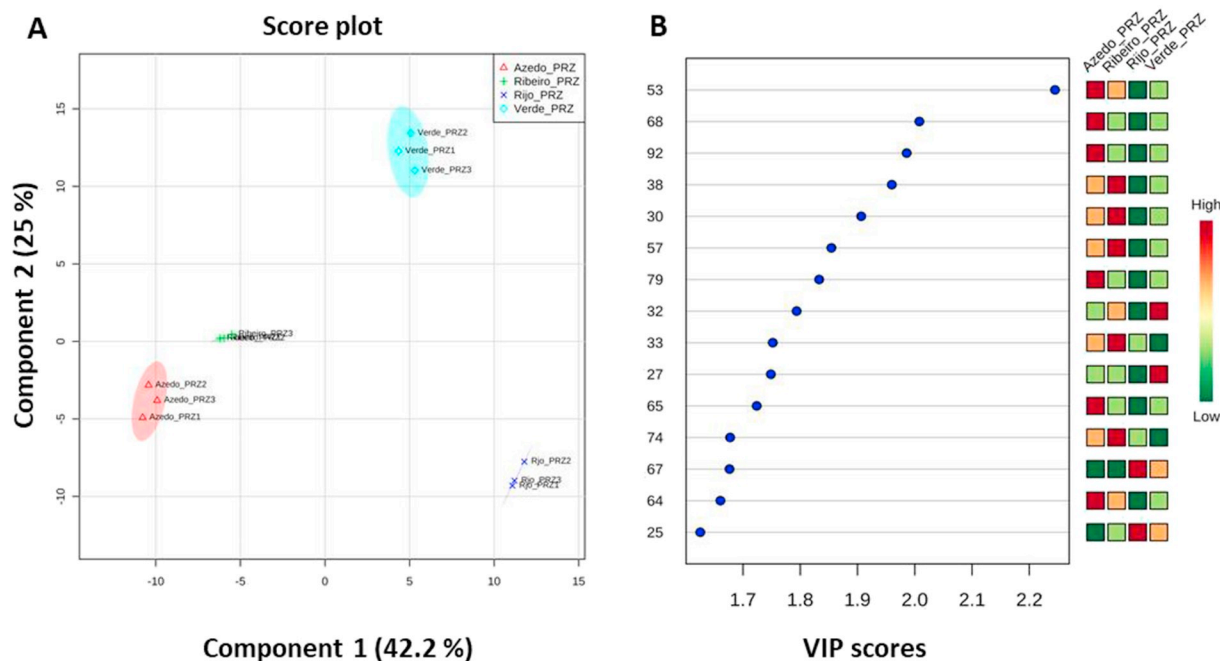


Fig. 2. A. Partial least square-discrimination analysis (PLS-DA) score plot applied to VOCs obtained by HS-SPME-GC-MS technique for the differentiation of apple juice samples on the basis of apple variety. B. Selected VOCs based on VIP score contributing to the variance observed in the PLS-DA model. The numbers in the VIP graph are according to Table 1.

with sweet, green, apple and fruity attributes. This VOC has been already described by other authors as a significant contributor to the distinction of apple varieties [10,22]. In the same way, butyl acetate (27), 2-methyl-1-butanol acetate (32), and hexyl acetate (51): statistically significant features from ANOVA (Table 1) were detected in a higher concentration in Verde apple variety than in the other ones. These features have been recently reported as VOCs that allowed the discrimination among apple juices prepared from Braeburn, Golden Delicious, GrannySmith, Jazz and Pink Lady varieties by atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and chemometric tools [6], which is in line with our findings. Moreover, butyl hexanoate (65) is only present in Azedo variety, through in a very low relative concentration ($0.92 \mu\text{g L}^{-1}$), it might contribute with a green apple aroma. Butyl hexanoate has also allowed the discrimination between different varieties of passion fruits: Orange (*Passiflora ligularis*) and Lemon (*P. edulis* var. *panama gold*) passion fruits that were also grown in Madeira Island [27], consequently, butyl hexanoate may be considered as a potential marker for authentication purposes that can differentiate among fruit varieties grown in Madeira Island. Another ester, ethyl octanoate (68), with fruity, sweet, banana and pear aromas, was only identified in Azedo variety ($\sim 4.05 \mu\text{g L}^{-1}$). Moreover, this compound was previously reported as a discriminant marker to distinguish Chardonnay wines from other varieties with a correct classification of 74% [28].

Regarding the aldehyde from VIP and PLS-DA results of this study, benzaldehyde with bitter almond as an aroma descriptor presented the major concentration level in Azedo variety ($11.95 \mu\text{g L}^{-1}$). Our findings are in accordance with a recent report that informed about the discriminatory power of this compound when apple juices from three apple varieties (Nesta, Panaia and Cipolla) were investigated [25]. Apart from that, as it can be seen in Table 1, five esters (ethyl acetate (5), ethyl propanoate (12), isobutyl acetate (20), ethyl butanoate (23) and ethyl 3-hydroxybutanoate (73)) and two alcohols (1-propanol (21) and 1-butanol (35)) were statistically significant as metabolites able to distinguish among apple juices from the four apple varieties. These results coincide with those obtained previously by Moragó et al. [25], where some correspondences between the sensory and aromatic data

were observed. For example, 1-butanol was associated with sharp olfactory notes in cider and in this assay was only represented by Rijo variety. Coincidentally, in several regions from Madeira Island, the traditional cider production is elaborated with Rijo variety. Nowadays, other regional varieties are being included in the cider production process in order to obtain an identifying mark of regional varieties and to gain the Protected Designations of Origin (PDO) status. For this purpose, it is necessary to characterise the volatile pattern of each apple variety since, depending on the chosen apple variety, the aroma of the final product (juice or cider) may vary greatly. In the present study, a sulfur compound (benzothiazole (92)) identified only in Azedo apple juice (Table 1), has been recently linked to the sweet fruity descriptor in apple fruits that may elicit odour-induced enhancement of sweetness perception [29]. Besides, three decades ago, this VOC was already identified in apple juice samples from Kogyoku (American Jonathan) apples [30].

As for the VOC: 2,4,5-trimethyl-1, 3-dioxolane (11) has only been identified in Rijo apple juices at the concentration of $6.82 \mu\text{g L}^{-1}$. This VOC has not been frequently reported in this matrix, however it appears in Food Database (FDB019998) as a flavour constituent of wine, beer, and cider. The Volatile Compound in Food (VCF) online database registers that, five decades ago, this compound was identified in apple processed (*Malus* species juices) [31]. Furthermore, it should also be highlighted that α -curcumene (87), the only terpenoid that was statistically significant, was identified only in Azedo PRZ juices, this VOC is an unusual VOC measured in apple fruits but, recently, this sesquiterpenoid has been identified in Starkrimson apple variety as a new aroma marker in this fruit responsible for its characteristic herbal odour [32].

Some VOCs were in relatively minor levels, however these may be potent odorants of apple juice. As stated by Wu et al. [26] not only the major VOCs but also the minor ones ought to be considered as representative indices for classification and discrimination of apple juices from different varieties. In addition, it was also performed the heatmap clustering based on significant features from ANOVA and Tukey post-hoc test to display the distribution of the data and to compare the respective relatively-quantified levels of VOCs throughout four apple

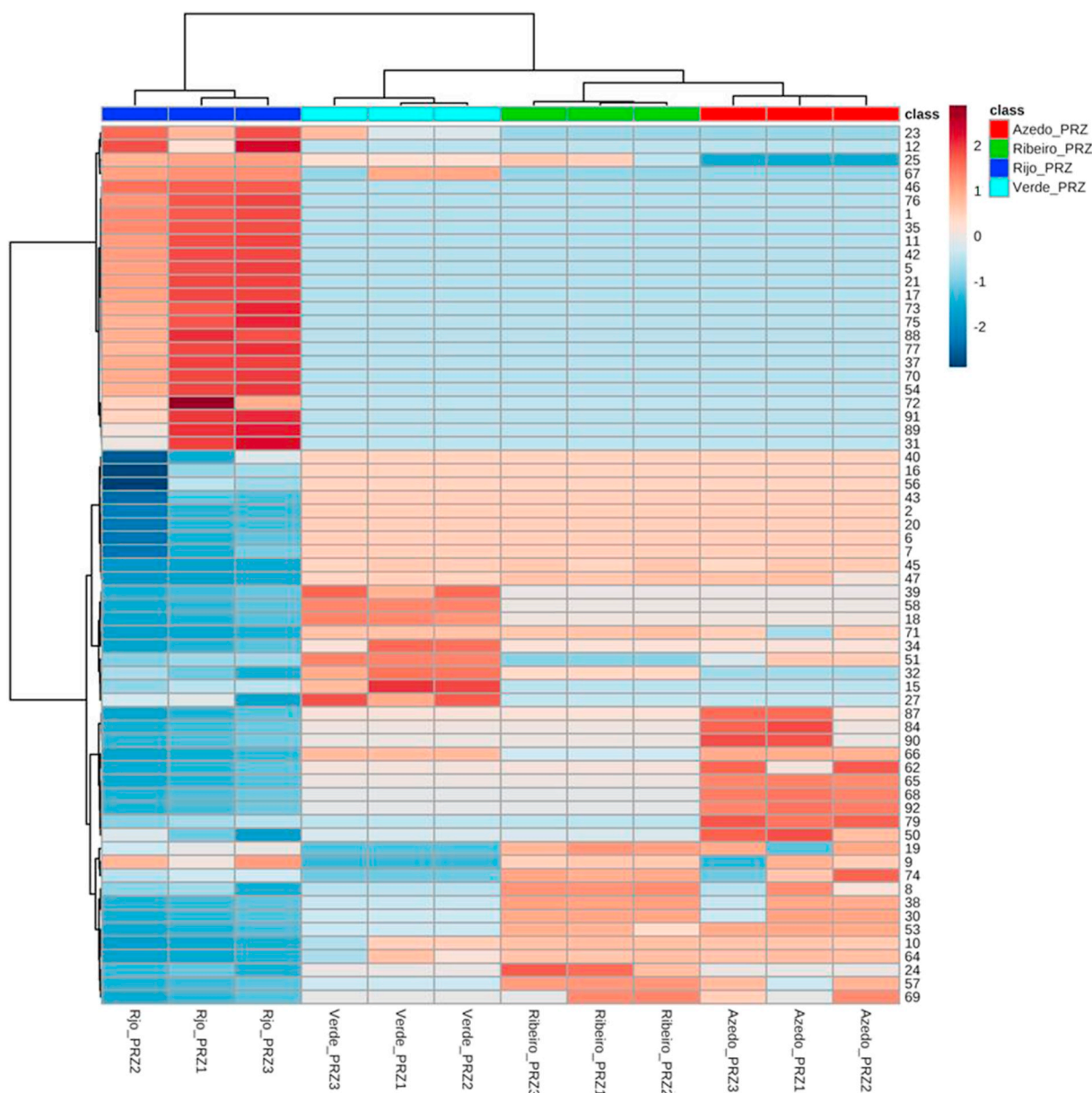


Fig. 3. Hierarchical clustering analysis (HCA) performed using VOCs profiles of four varieties of apples (Azedo, Ribeiro, Verde and Rijo). The heatmap was generated using the significant VOCs from ANOVA and Tukey *post hoc* test (Table 1). The rows in the heatmap represent VOCs and the columns indicate samples. The colour gradient ranging from dark blue through white to dark red represents low, middle and high abundance of a VOC. The HCA were performed by Euclidean distance through Ward agglomerative method. The correspondence between peak numbering and VOC assignment is shown in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

varieties (Fig. 3).

3.2. Discrimination of apple juices by geographical origin

The sensory quality of apple fruits changes not only from one cultivar/variety to another, but also according to the geographical origin [22]. In this respect, the principal component analysis (PCA) has allowed the visualization of significant differences among the Azedo variety from two geographical island regions (PRZ and SDS) and has also enabled the determination of significant variables (VOCs) that contribute the most for such differences. Thus, an effective separation according to geographical origin of apple juices was achieved (Fig. 4A), the variance of PC1 and PC2 was 49.8 and 24.5%, respectively, representing 74.3% of the total VOCs variability of data, allowing a good differentiation of the monovarietal apple juices.

Therefore, ROC analysis was performed for two groups comparison (PRZ vs. SDS), showing an AUC > 0.88 (excellent discriminatory ability) as shown in Fig. S2.

In addition, Student's *t*-test identified five VOCs as differential features between apple juices (Azedo PRZ vs. Azedo SDS) from the experimental data set (Table 2). Moreover, the chromatographic profiles of Azedo from different geographical origin are shown in Fig. 1, where the differences in the specific VOCs can be observed. Two esters, one alcohol, one aromatic hydrocarbon, and one unknown compound (not identified) were found as significant features (Table 2). These VOCs were only identified in Azedo SDS apple juice. As previously mentioned, ethyl acetate (5) was identified as a significant feature for the discrimination of four apple varieties investigated and this VOC was also identified as a metabolite was able to distinguish apple juices according to geographical origin by Student's *t*-test (Table 2). This VOC

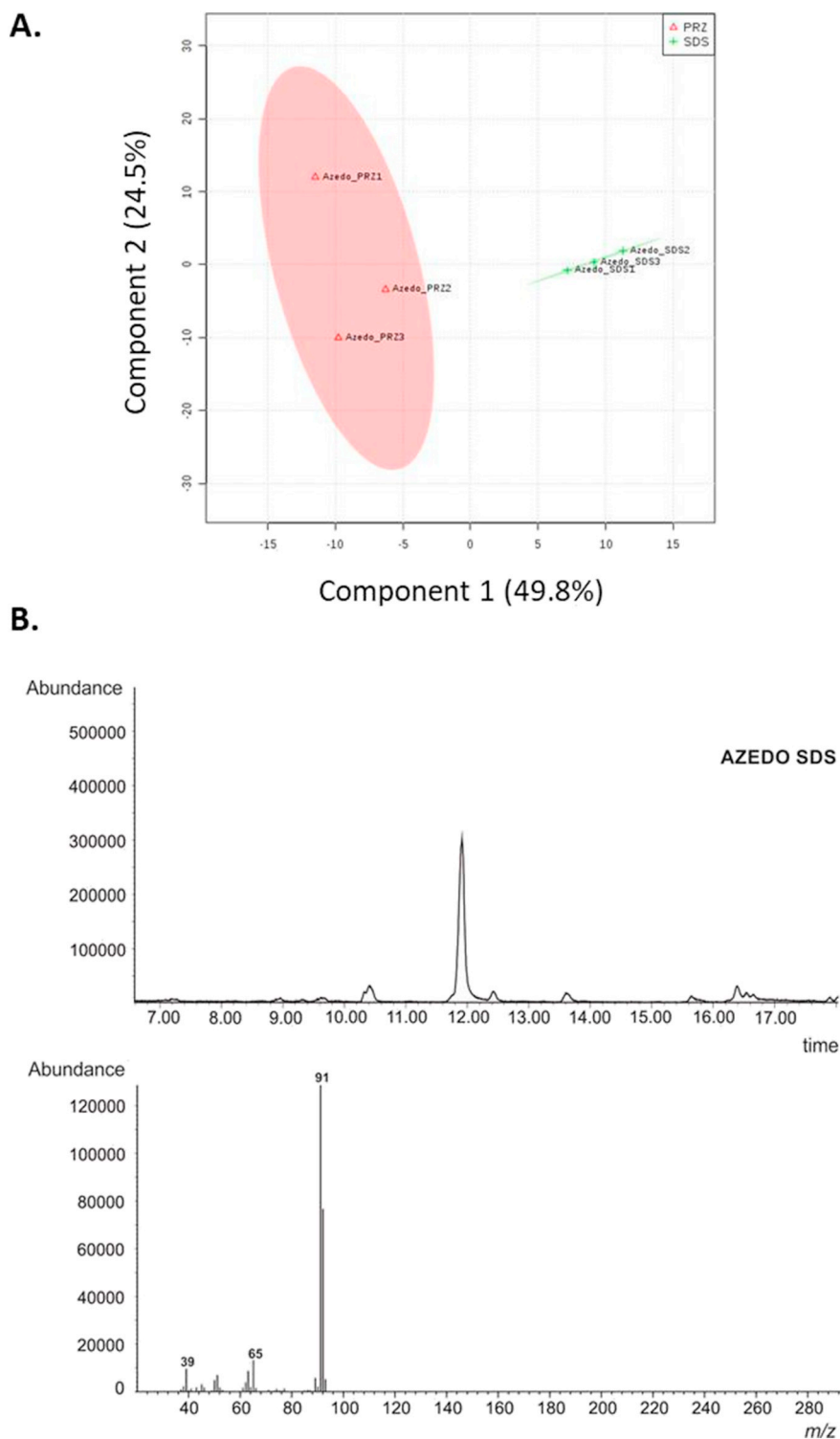


Fig. 4. A. PCA score plot on the basis of Azedo apple juice for discrimination on the basis of geographical origin (PRZ: Prazeres and SDS: Santo da Serra). B. SIM chromatogram and mass spectrum of toluene in Azedo SDS apple juices.

was reported as a significant feature for the geographical discrimination of several matrices, like extra virgin olive oils [33] or two kinds of geographical origin protected Chinese vinegars [34]. Indeed, identical molecules have been described as discriminant “geographical origin markers” in different matrices [5]. In our study, another ester, ethyl hexanoate, was found in Azedo SDS apple juices at $4.26 \mu\text{g L}^{-1}$, but in Azedo PRZ it was not identified. This VOC was previously identified in apple juice [6,10], in cider [35], in apple brandy [36] and in apple

fruits [37] with fruity odour notes. Moreover, a previous study informed that ethyl hexanoate was one of the most important contributor for the differentiation of apple fruits from three regions of Madeira Island (Ponta do Pargo, Porto Moniz and Santo da Serra), detecting high levels in apples (pulp) from Santo da Serra [15], which agrees with our findings. Hence, this VOC may be a good potential geographical marker to establish apples’ authenticity. Nonetheless, ethyl hexanoate and 2-methyl-1-propanol are highly dependent on the degree of ripeness of

the fruits. Thus, analysis of a larger set of Azedo apples (from different stages of ripening) is needed to show that the geographical origin is correlated with these compounds.

Furthermore, in the current study, an aromatic hydrocarbon (toluene) was identified for the first time in apple juices. This VOC with a green, fatty and lard aroma was only found in Azedo SDS apple juices ($80.79 \mu\text{g L}^{-1}$). Recently, this metabolite was for the first time reported in apple samples by metabolomic approach through SPME-two dimensional gas chromatography-time-of-flight mass spectrometry (GC x GC-ToFMS) by Risticvic et al. [38]. Moreover, the chromatogram and mass spectrum profile for the identification of toluene in Azedo SDS apple juices showed the fragment ions m/z : 92, 91, 65, and 39 (as shown in Fig. 4B), which are the same ions found in previous reports [38,39] and registered in the HMDB database (HMDB0034168). Furthermore, the mechanism of conversion of benzyl alcohol to toluene in fruit juices has also been reported [39]. These authors informed that the oxidative-reduction nature of benzoic acid transformation to benzyl alcohol and toluene may cause the toluene formation in fruit juices containing benzoates. Borachova et al. [39] reported toluene in orange juices but not in apple juices and they discussed that this fact may be caused the presence of phenolic compounds of redox systems, the main condition of forming toluene in fruit juices. In addition, they established that the differences in the reducing ability of oxidation-reduction systems of apple and orange juices may affect their different types of spontaneous redox reactions. Despite this, the current study identified toluene in apple juices from a specific geographical area (SDS), perhaps because apple phenolic compounds can be influenced by geographical origin as was previously reported [40] and this may alter the toluene formation. Additionally, toluene has already been reported as a metabolite for the discrimination of cashews (*Anacardium occidentale* L.) according to geographical origin [41] and for the classification of unroasted *Coffea arabica* and *C. canephora* beans from different countries [42]. Although the biochemical pathway for the production of toluene in plants is uncertain, previous report indicated that toluene may be synthesized by the plants like sunflower and pine as a reaction to their environment [43]. Besides, toluene was also reported in essential oils from different genera of plants [44,45]. At this point, the origin of toluene in plants is unclear, it may be passively absorbed from the air into waxy tissues of plant (contamination) as reported by previous study in citrus peel [46] or it could be produced by decomposition of other molecules, either through the normal metabolism of plants, or during digestion by fungi and bacteria [43]. As mentioned above, toluene was found in apple juices with a relative concentration of $80.79 \mu\text{g L}^{-1}$ (ppb), the safe exposure limits for this VOC are usually in the range of 10 to 100 ppm [47]. Although the concentration of toluene is presumably harmless, but because this compound has not previously described in apple juices, further studies are required not only to conclude a correlation between this VOC and geographical origin of apples, but also to verify that a contamination of samples has not occurred.

4. Conclusions

The use of HS-SPME/GC-MS combined with chemometric tools is a powerful analytical option for the differentiation of apple juices based on regional varieties as well as geographical origin. As for the apple varieties, the Rijo variety showed a higher number of VOCs than the other ones, having a complex volatile pattern with the major relative concentration of ethanol (9), ethyl butanoate (23), ethyl 2-methylbutanoate (25) and ethyl hexanoate (46), that bring sweet and fruity aroma descriptors. This information can be very useful while considering selection criteria for the best apple variety for obtaining quality final apple-based products. In the same way, the present study shows the important relationship between some descriptors and the classification of monovarietal apple juices according to the geographical origin, highlighting ethyl acetate (5), 2-methyl-1-propanol (29), ethyl hexanoate (46), and toluene (94) as discriminatory significant features.

Nevertheless, an increased number of samples, as well as samples from more than two geographical regions and from different stages of ripening should be investigated to reach a valid conclusion.

Therefore, present findings can potentially have practical importance for both producers and suppliers while defining the varietal and regional typicity and authenticity of the apple juices investigated. These may serve as a well and open-minded theory for an improved management and control in order to guarantee the origin and identity of foodstuffs and, therefore, the possibility of a future recognition as Protected Designation of Origin (PDO) or Traditional Speciality Guaranteed (TSG) products from Madeira Island.

Declaration of Competing Interest

The authors declare 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.microc.2019.104094>.

References

- [1] R. Guiné, S. Andrade, A. Correia, A. Jordão, A. Lopes, D. Ferreira, Evaluation of textural properties in apples of regional varieties, *Int. J. Food Prop.* 14 (2011) 331–338.
- [2] E. Aprea, M.L. Corollaro, E. Betta, I. Endrizzi, M.L. Demattè, F. Biasioli, F. Gasperi, Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavour, *Food Res. Int.* 49 (2012) 677–686.
- [3] J. Dixon, E.W. Hewett, Factors affecting apple aroma/flavour volatile concentration: a review, *New Zeal. J. Crop Hort.* 28 (2000) 155–173.
- [4] E. Cubero-Leon, R. Peñalver, A. Maquet, Review on metabolomics for food authentication, *Food Res. Int.* 60 (2014) 95–107.
- [5] S. Medina, J.A. Pereira, P. Silva, R. Perestrelo, J.S. Câmara, Food fingerprints – a valuable tool to monitor food authenticity and safety, *Food Chem.* 278 (2019) 144–162.
- [6] H.-H. Gan, C. Soukoulis, I. Fisk, Atmospheric pressure chemical ionisation mass spectrometry analysis linked with chemometrics for food classification – a case study: geographical provenance and cultivar classification of monovarietal clarified apple juices, *Food Chem.* 146 (2014) 149–156.
- [7] J.D. Kelly, G. Downey, Detection of sugar adulterants in apple juice using Fourier transform infrared spectroscopy and chemometrics, *J. Agric. Food Chem.* 53 (2005) 3281–3286.
- [8] L. León, J.D. Kelly, G. Downey, Detection of apple juice adulteration using near-infrared transmittance spectroscopy, *Appl. Spectrosc.* 59 (2005) 593–599.
- [9] S. Sivakesava, J. Irudayaraj, R. Korach, Detection of adulteration in apple juice using mid infrared spectroscopy, *Appl. Eng. Agric.* 17 (2001) 815.
- [10] Guo, T. Yue, Y. Yuan, Feature selection and recognition from nonspecific volatile profiles for discrimination of apple juices according to variety and geographical origin, *J. Food Sci.* 77 (2012) C1090–C1096.
- [11] L.M. Reid, C.P. O'Donnell, J.D. Kelly, G. Downey, Preliminary studies for the differentiation of apple juice samples by chemometric analysis of solid-phase microextraction – gas chromatographic data, *J. Agric. Food Chem.* 52 (2004) 6891–6896.
- [12] G.R. Schmutzer, A.D. Magdas, L.I. David, Z. Moldovan, Determination of the volatile components of apple juice using solid phase microextraction and gas chromatography–mass spectrometry, *Anal. Lett.* 47 (2014) 1683–1696.

- [13] J. Yi, B.T. Kebede, D.N. Hai Dang, C. Buvé, T. Grauwet, A. Van Loey, X. Hu, M. Hendrickx, Quality change during high pressure processing and thermal processing of cloudy apple juice, *LWT Food Sci. Technol.* 75 (2017) 85–92.
- [14] D.M. Barrett, E. Garcia, Preservative treatments for fresh-cut fruits and vegetables, *Fresh-Cut Fruits and Vegetables*, CRC Press, 2002, pp. 273–309.
- [15] L. Ferreira, R. Perestrelo, M. Caldeira, J.S. Câmara, Characterization of volatile substances in apples from rosaceae family by headspace solid-phase microextraction followed by GC-qMS, *J. Sep. Sci.* 32 (2009) 1875–1888.
- [16] H. Van den Dool, A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography, *J. Chromatogr. A* 11 (1963) 463–471.
- [17] F. Bianchi, M. Careri, A. Mangia, M. Musci, Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: database creation and evaluation of precision and robustness, *J. Sep. Sci.* 30 (2007) 563–572.
- [18] V. Pereira, J. Cacho, J.C. Marques, Volatile profile of Madeira wines submitted to traditional accelerated ageing, *Food Chem.* 162 (2014) 122–134.
- [19] B. Worley, R. Powers, Multivariate analysis in metabolomics, *Current Metabolomics* 1 (2013) 92–107.
- [20] D. Ballabio, V. Consonni, Classification tools in chemistry. Part 1: linear models. PLS-DA, *Anal. Methods* 5 (2013) 3790–3798.
- [21] J. Chong, O. Soufan, C. Li, I. Caraus, S. Li, G. Bourque, D.S. Wishart, J. Xia, *MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis*, *Nucleic Acids Res.* 46 (2018) W486–w494.
- [22] E. Mehinagic, G. Royer, R. Symoneaux, F. Jourjon, C. Prost, Characterization of odor-active volatiles in apples: influence of cultivars and maturity stage, *J. Agric. Food Chem.* 54 (2006) 2678–2687.
- [23] E.A. Mureşan, S. Muste, S.A. Socaci, R.A. Vlaic, E. Racolţa, V. Mureşan, Volatile compounds profile during storage of Jonathan, Starkrimson and Golden delicious apple varieties, *Food Sci. Technol.* 71 (2014) 173–178.
- [24] R.-D. Rita, K. Zanda, K. Daina, S. Dalija, Composition of aroma compounds in fermented apple juice: effect of apple variety, fermentation temperature and inoculated yeast concentration, *Procedia Food Sci* 1 (2011) 1709–1716.
- [25] E. Maragò, M. Michelozzi, L. Calamai, F. Camangi, L. Sebastiani, Antioxidant properties, sensory characteristics and volatile compounds profile of apple juices from ancient Tuscany (Italy) apple varieties, *Eur. J. Hort. Sci.* 81 (2016) 255–263.
- [26] H. Wu, J. Wang, T. Yue, Y. Yuan, Variety-based discrimination of apple juices by an electronic nose and gas chromatography-mass spectrometry, *Int. J. Food Sci. Technol.* 52 (2017) 2324–2333.
- [27] P. Porto-Figueira, A. Freitas, C.J. Cruz, J. Figueira, J.S. Câmara, Profiling of passion fruit volatiles: an effective tool to discriminate between species and varieties, *Food Res. Int.* 77 (2015) 408–418.
- [28] I. Lukić, I. Horvat, Differentiation of commercial PDO wines produced in Istria (Croatia) according to variety and harvest year based on HS-SPME-GC/MS volatile aroma compound profiling, *Food Technol. Biotech.* 55 (2017) 95–108.
- [29] E. Aprea, M. Charles, I. Endrizzi, M.L. Corollaro, E. Betta, F. Biasioli, F. Gasperi, Sweet taste in apple: the role of sorbitol, individual sugars, organic acids and volatile compounds, *Sci. Rep.* 7 (2017) 44950.
- [30] I. Yajima, T. Yanai, M. Nakamura, H. Sakakibara, K.J.A. Hayashi, B. Chemistry, Volatile flavor components of Kogyoku apples, *Agric. Biol. Chem.* 48 (1984) 849–855.
- [31] R.A. Flath, D.R. Black, R.R. Forrey, G.M. McDonald, T.R. Mon, R. Teranishi, Volatiles in Gravenstein apple essence identified by GC-mass spectrometry, *J. Chromatogr. Sci.* 7 (1969) 508–512.
- [32] Y. Liu, N. Chen, Z. Ma, F. Che, J. Mao, B. Chen, The changes in color, soluble sugars, organic acids, anthocyanins and aroma components in “Starkrimson” during the ripening period in China, *Molecules* 21 (2016) 812.
- [33] D. Melucci, A. Bendini, F. Tesini, S. Barbieri, A. Zappi, S. Vichi, L. Conte, T. Gallina Toschi, Rapid direct analysis to discriminate geographic origin of extra virgin olive oils by flash gas chromatography electronic nose and chemometrics, *Food Chem.* 204 (2016) 263–273.
- [34] C. Xiong, Y. Zheng, Y. Xing, S. Chen, Y. Zeng, G. Ruan, Discrimination of two kinds of geographical origin protected Chinese vinegars using the characteristics of aroma compounds and multivariate statistical analysis, *Food Anal. Methods* 9 (2016) 768–776.
- [35] A. Villière, G. Arvisenet, R. Bauduin, J.M. Le Quééré, T. Sérot, Influence of cider-making process parameters on the odourant volatile composition of hard ciders, *J. I. Brewing* 121 (2015) 95–105.
- [36] T.E. Coldea, C. Socaciu, Z. Moldovan, E. Mudura, Minor volatile compounds in traditional homemade fruit brandies from Transylvania-Romania, as determined by GC-MS analysis, *Not. Bot. Horti Agrobot. Cluj-Napoca.* 42 (2014) 530–537.
- [37] D. Fraternali, D. Ricci, G. Flamini, G. Giomaro, Volatiles profile of red apple from Marche region (Italy), *Rec. Nat. Prod.* 5 (2011) 202.
- [38] S. Risticvic, J.R. DeEll, J. Pawliszyn, Solid phase microextraction coupled with comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry for high-resolution metabolite profiling in apples: implementation of structured separations for optimization of sample preparation procedure in complex samples, *J. Chromatogr. A* 1251 (2012) 208–218.
- [39] O. Bocharova, S. Reshta, V. Eshtokin, Toluene and benzyl alcohol formation in fruit juices containing benzoates, *J. Food Process. Preserv.* 41 (2017) e13054.
- [40] Guo, T. Yue, Y. Yuan, Y. Wang, Chemometric classification of apple juices according to variety and geographical origin based on polyphenolic profiles, *J. Agric. Food Chem.* 61 (2013) 6949–6963.
- [41] A. Agila, S.A. Barringer, Volatile profile of cashews (*Anacardium occidentale* L.) from different geographical origins during roasting, *J. Food Sci.* 76 (2011) C768–C774.
- [42] D. Knysak, Volatile compounds profiles in unroasted *Coffea arabica* and *Coffea canephora* beans from different countries, *Food Sci. Technol.* 37 (2017) 444–448.
- [43] A. Heiden, K. Kobel, M. Komenda, R. Koppmann, M. Shao, J.J.G.R.L. Wildt, Toluene emissions from plants, *Geophys. Res. Lett.* 26 (1999) 1283–1286.
- [44] N. Bendimerad, S.A.T. Bendiab, K. Breme, X.J.J.o.E.O.R. Fernandez, Essential oil composition of aerial parts of *Sinapis arvensis* L. from Algeria, *J. Essent. Oil Res.* 19 (2007) 206–208.
- [45] J. Pino, R. Marbot, C. Vazquez, Volatile constituents of genipap (*Genipa americana* L.) fruit from Cuba, *Flavour Fragr. J.* 20 (2005) 583–586.
- [46] M. Ligor, B. Buszewski, Study of VOC distribution in citrus fruits by chromatographic analysis, *Anal. Bioanal. Chem.* 376 (2003) 668–672.
- [47] S.L. Cruz, M.T. Rivera-García, J.J. Woodward, Review of toluene action: clinical evidence, animal studies and molecular targets, *J. Drug Alcohol Res.* 3 (2014) 235840.