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Comparative analysis of the volatile fraction from *Annona cherimola* Mill. cultivars by solid-phase microextraction and gas chromatography–quadrupole mass spectrometry detection

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ABSTRACT

The analysis of volatile compounds in Funchal, Madeira, Mateus and Perry Vidal cultivars of *Annona cherimola* Mill. (cherimoya) was carried out by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography–quadrupole mass spectrometry detection (GC–qMSD). HS-SPME technique was optimized in terms of fibre selection, extraction time, extraction temperature and sample amount to reach the best extraction efficiency. The best result was obtained with 2 g of sample, using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre for 30 min at 30 °C under constant magnetic stirring (800 rpm).

After optimization of the extraction methodology, all the cherimoya samples were analysed with the best conditions that allowed to identify about 60 volatile compounds. The major compounds identified in the four cherimoya cultivars were methyl butanoate, butyl butanoate, 3-methylbutyl butanoate, 3-methylbutyl 3-methylbutanoate and 5-hydroxymethyl-2-furfural. These compounds represent 69.08 ± 5.22%, 56.56 ± 15.36%, 56.69 ± 9.28% and 71.82 ± 1.29% of the total volatiles for Funchal, Madeira, Mateus and Perry Vidal cultivars, respectively. This study showed that each cherimoya cultivars have 40 common compounds, corresponding to different chemical families, namely terpenes, esters, alcohols, fatty acids and carbonyl compounds and using PCA, the volatile composition in terms of average peak areas, provided a suitable tool to differentiate among the cherimoya cultivars.

1. Introduction

The family of annonaceae that includes *Annona squamosa*, *Annona muricata*, *Annona reticulata* and *Annona cherimola* contains a considerable number of plants with economic significance because of their edible fruits around the world, namely tropical America, Australia, Africa, India, Malaysia and Mediterranean Europe [1]. The edaphoclimatic conditions of the Madeira Islands are favourable for the production of tropical and subtropical fruits. *Annona cherimola* Mill. (cherimoya) production in Madeira Islands remains from its colonization and nowadays have an important role for the economic development with an annual production around 1000 Ton per year, exporting to the mainland, France, Spain and England markets.

The pulp of this fruit is creamy, very sweet and pleasantly flavoured. It is well known as a dessert fruit and has a lot of applications in ice creams and beverages [2]. The cherimoya fruit is used by the natural products industry due to the high presence of secondary metabolites that show antimicrobial activity. The cherimoya is also known as a medicinal plant. Tea made from leaves and bark is relaxing. The pulp is moderately laxative and benefits the digestion with a particular taste as result of the harmonic combination of acids and sugars.

In fruits, aroma is one of the most appreciated characteristics on their consumption [3]. The volatile compounds (e.g. esters, terpenes, alcohols, carbonyl compounds, furanic compounds, among others) that form the fruit flavour are produced through metabolic pathways during ripening, harvest, post-harvest and storage which depends on many factors related to the species, variety and type of technological treatment [4]. The main volatiles identified in tropical fruits belong to esters such as methyl and ethyl esters [5]. The ester compounds play a role in the ripe fruit, serving both as "biological bribes" for the attraction of animals and as protectants against pathogens. In the case of some fruit species like apple, pear, annona, banana and others, they are the major volatile compounds on their characteristical aroma profile [6]. Several studies report that cherimoya fruit contains about 208 volatile compounds, 23 hydrocarbons, 58 esters, 47 carbonyl compounds, terpenoids

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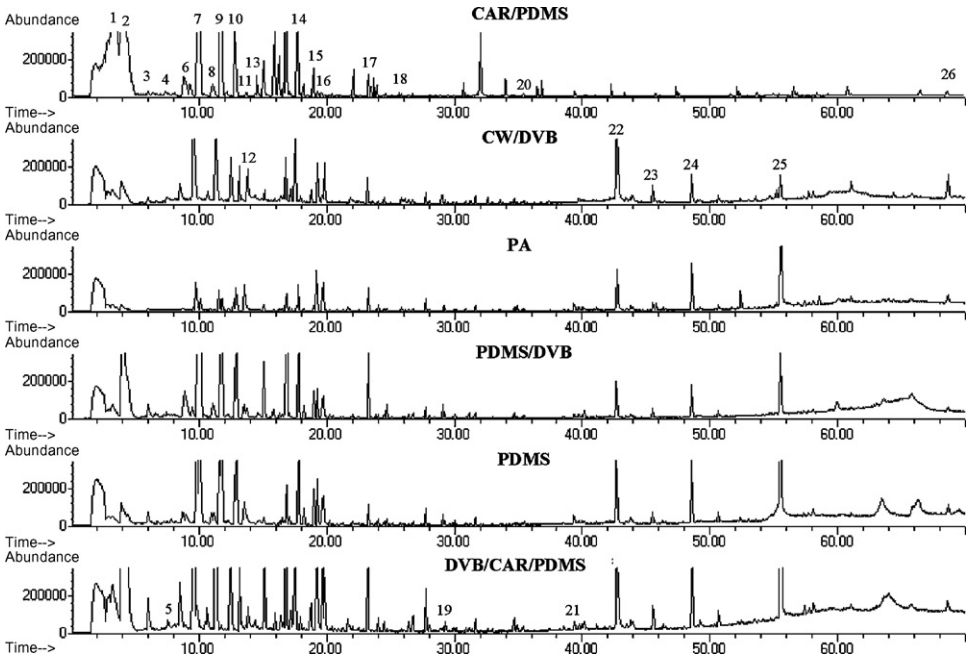

Fig. 1. TIC chromatogram obtained by HS-SPME/GC–qMSD analysis of Mateus cultivar with different fibre coatings during 30 min at 25 °C under constant magnetic stirring (800 rpm).

(mono and sesquiterpens), 54 miscellaneous structures of alcohols as butan-1-ol, 3-methylbutan-1-ol, hexan-1-ol, linalool and 3-methyl butanoate [1,7,8]. For the analysis of the volatile compounds in the annonaceae family, some publications are available using gas chromatography–quadrupole mass spectrometry detection (GC–qMSD) followed by liquid–liquid extraction and steam distillation [1,7,8]. These techniques, however, have some disadvantages such as higher costs, extent time-consumption and larger volumes of organic solvents used [9]. Recently, the headspace solid-phase microextraction (HS-SPME) technique emerges as an attractive alternative for volatile analysis because it offers many advantages like high sensitivity and reproducibility, combines extraction and pre-concentration in a simple step without pretreatment of samples and does not require solvents. This technique is fast, inexpensive, requires low volume of sample and can be easily automated [10–12]. It is an equilibrium technique that requires a previous optimization step of the sampling conditions in order to obtain high recoveries of volatiles and a good precision of the method [13]. The analysis of headspace volatile compounds by HS-SPME is greatly influenced by the vapour pressure of flavour compounds of the matrix. Since the first HS-SPME fibres became commercially available, they have been used in several applications, including a wide range of food analysis, like volatile composition in wines [14–16], beers [17,18], whiskeys [19,20], honeys [21], medicinal plants [22,23] and several kinds of fruits [24,25]. Up to now, this technique has been widely applied in the several matrixes. At the moment, no references have been found on the use of HS-SPME to describe the volatile composition of any cherimoya species. The purpose of this study was to develop and optimize an HS-SPME methodology coupled with GC–qMSD for the analysis of the volatile composition of four different cherimoya cultivars from Madeira Island. A preliminary screening of six commercial available fibres with different polarities was carried out in order to select the best coating for the matrix and other parameters that affect the HS-SPME procedure like extraction time and temperature were also tested and evaluated and using PCA the volatile composition in terms of average peak areas, provided a suitable tool to differentiate among the cherimoya cultivars.

2. Experimental

2.1. Fruit samples

The four cherimoya cultivars were kindly provided by "Direcção Regional de Agricultura – Divisão de Fruticultura" of Madeira Islands. Each fruit pulp was separated from the seeds and bark,

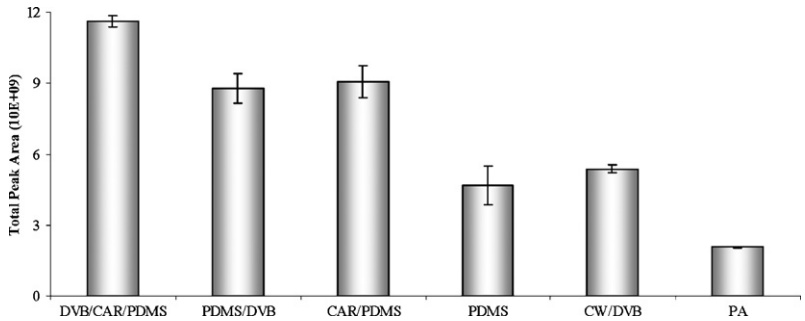

Fig. 2. Sorption capacity of different fibres for extraction of Mateus volatile compounds during dynamic HS-SPME method, expressed as total peak area (30 min at 25 °C). Error bars represent standard error of the mean ($n = 3$ for each data point).

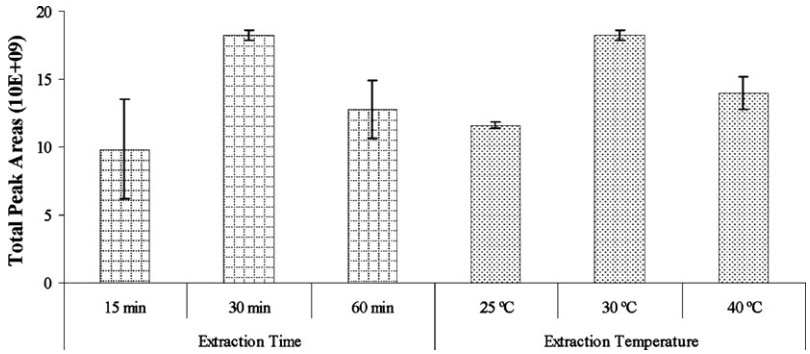

Fig. 3. Effect of the extraction time (DVB/CAR/PDMS coating at 30 °C) and extraction temperature (DVB/CAR/PDMS coating during 30 min) on the extraction efficiency of the volatile compounds from Mateus cultivars. Error bars represent standard error of the mean ($n = 3$ for each data point).

homogenised with a home blender, added with an amount of calcium chloride ($CaCl_2$) in order to inhibit the enzyme activity and finally stored in polyethylene bottles at −20 °C until analysis.

2.2. Standards and materials

All reagents used were analytical quality and all solvents were HPLC grade. Sodium chloride (99.5%) was supplied from Panreac (Spain, Barcelone). C8–C20 *n*-alkanes were run under the same chromatographic conditions as the samples to calculate the Kovats indices of the compounds were purchased from Sigma–Aldrich (Switzerland, Buchs). Water Mili-Q purification system (Milipore). SPME fibres and SPME holder for manual sampling were obtained from Supelco (Bellenfonte, PA, USA).

2.3. HS-SPME procedure

To determine the volatile compounds in cherimoya cultivars, the sample extraction is a key technique for those who are always present at very low concentrations. To obtain the optimal HS-SPME conditions, the experimental parameters including different fibre coating, extraction time, extraction temperature and sample amount, which can affect the extraction efficiency were systematically studied.

For the fibre screening, six commercially available fibres: carbowax-divinylbenzene (CW/DVB, 70 μm), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm), carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm), polyacrylate (PA, 85 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm) and polydimethysiloxane (PDMS, 100 μm) were tested and examined. All the fibres were of the same length (1 cm) and conditioned prior to use, according to the manufacturer's instructions. Before daily analysis each fibre was conditioned for 15 min at 250 °C. For each extraction, fibres were exposed to the headspace of a 4 mL septum-sealed glass vial containing 2 ± 0.001 g of sample, 0.5 mL of water, 1 μL of internal standard (3-octanol, 4.22 mg L^{-1}) and 0.10 g of NaCl for 30 min at 25 ± 1 °C under constant magnetic stirring (800 rpm). Once sampling was finished, the fibre was withdrawn into the needle and inserted into the GC system injection port at 250 °C for 6 min where the analytes are thermally desorbed from the fibre coating and transferred directly to the GC system column. Blank runs were conducted between extractions to check the absence of carry over which would cause memory effects and misinterpretation of results.

HS-SPME operating conditions were optimized with extractions at different extraction temperatures (25 °C, 30 °C and 40 °C), extraction times (15 min, 30 min and 60 min) and sample amounts (1 g, 2 g and 4 g).

2.4. Gas chromatography–quadrupole mass spectrometry detection (GC–qMSD) analysis

The analyses were carried out with an Agilent 6890 N gas chromatograph system (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5975 quadrupole inert mass selective detector. The extracted compounds were separated on a BP-20 fused silica capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness). Splitless injection was employed with helium as the carrier gas (Helium N60, *Air Liquide*, Portugal) at a flow rate of ≈1 mL min^{-1} (column head pressure 13 psi). The initial oven temperature was 40 °C, followed by a linear programmed temperature from 40 °C to 220 °C held for 10 min at a rate of 3 °C min^{-1}. The injection and ion source temperatures were 250 and 220 °C, respectively. The mass spectra of the compounds were acquired in electron-impact (EI) mode at 70 eV. The electron multiplier was set to the auto tune procedure. All data were obtained by collecting the full-scan mass spectra within the range of 30–300 *m*/*z*.

2.5. Qualitative and quantitative analysis

The volatile compounds were identified by matching mass spectra with spectra of reference compounds in National Institute of Standards and Technology (NIST05) mass spectral library. The relative amounts of individual components were expressed as percent peak areas relative to total peak areas.

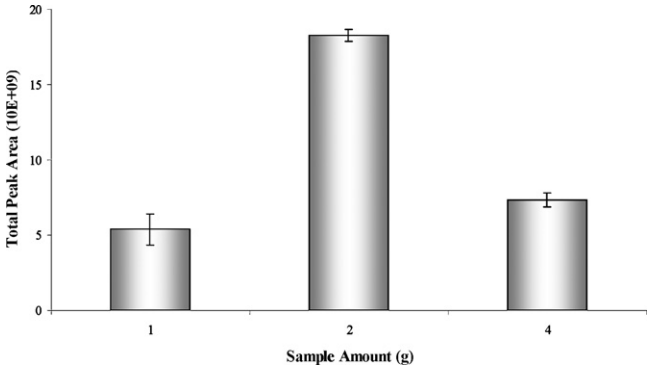

Fig. 4. Extraction efficiencies measured for different Mateus cultivar amounts at 30 °C during 30 min under constant magnetic stirring (800 rpm) with DVB/CAR/PDMS fibre. Error bars represent standard error of the mean ($n = 3$ for each data point).

Table 1
Identification of volatile compounds in Mateus cultivars by dynamic HS-SPME/GC–qMSD using different fibre coatings (extraction temperature: 25 °C, extraction time: 30, 800 rpm)

RT (min)	Peak no.	Compounds	CAR–PDMS	CW/DVB	DVB/CAR/PDMS	PA	PDMS	PDMS/DVB
3.12	1	Ethanol	x	x	x	x	x	x
3.96	2	Methyl butanoate	x	x	x	x	x	x
5.58	3	β-pinene	x	x	x	x	x	x
7.07	4	Butyl propanoate	–	–	x	–	x	x
7.65	5	Butan-1-ol	x	–	x	–	–	–
8.16		β-mircene	x	–	–	–	–	–
8.56	6	Methyl hexanoate	x	x	x	x	x	x
9.48		3-Methylbutan-1-ol	–	–	x	x	x	x
9.69	7	Butyl butanoate	x	x	x	x	x	x
10.78	8	Butyl pentanoate	x	x	x	x	x	x
11.47	9	3-Methylbutyl butanoate	x	x	x	x	x	x
12.68	10	3-Methylbutyl 3-methylbutanoate	x	x	x	x	x	x
12.90	11	1-Hydroxypropan-2-one	–	x	–	–	–	–
13.83	12	Hydroxyacetaldehyde		x				
14.78	13	Pentyl butanoate	x	–	x	x	–	x
15.05		Hexan-1-ol	x	x	x	x	x	x
15.80		Methyl 3-hydroxy-3-methylbutanoate	–	–	x	–	x	x
16.31		(*Z*)-3-hexen-1-ol	–	–	x	–	–	x
16.65		Methyl octanoate	–	–	–	–	–	x
17.68	14	Hexyl butanoate	x	x	x	x	–	x
18.22		Hexyl 2-methylbutanoate	x	x	x	x	x	x
18.96	15	Hexyl 3-methylbutanoate	x	x	x	x	x	x
19.13	16	Acetic acid	x	x	x	x	x	x
19.52		(*Z*)-3-Hexenyl butanoate	x	–	x	–	–	–
19.69		2-Furfural	x	x	x	x	x	x
20.24		2,5,5-Trimethyl-1,3,6-heptatriene	–	–	–	–	x	–
20.80		2-Ethylhexan-1-ol	–	–	x	x	–	x
21.29		1-(2-Furanyl)-ethanone	–	–	–	–	x	–
21.83		Benzaldehyde	x	–	x	–	–	–
23.24	17	Linalool	x	x	x	x	x	x
24.03	18	5-Methyl-2-furfural	–	x	x	–	x	x
24.50		2-Cyclopenten-1,4-dione	–	–	–	–	x	–
25.15		Methyl decanoate	–	–	–	–	x	–
25.76		Hexyl hexanoate	–	–	–	–	x	–
25.87		2-(2-etoxyethoxy)-ethanol	–	x	–	–	–	–
26.38	19	Butanoic acid	x	x	x	–	–	x
27.73		2-Furanmethanol	–	x	x	x	x	x
28.01		3-Methylbutanoic acid	x	x	x	x	–	–
28.38		Diethyl succinate	–	–	x	–	–	–
29.29		3-Methoxybutan-1-ol	–	x	x	–	–	–
30.92		(5H)-Furan-2-one	–	x	x	–	x	x
31.62		2-Hydroxy-2-cyclopenten-1-one	–	x	x	x	x	x
32.97		2-Cyclohexen-1-ol	–	x	x	–	x	x
34.65	20	Hexanoic acid	x	x	x	x	x	x
35.44		Phenylmethyl butanoate	x	x	x	–	x	–
36.84		2-Phenylethanol	–	–	x	x	–	x
39.37	21	2,5-Furandicarbaldehyde	–	–	x	–	x	x
40.01		Methyl 2-furoate	–	–	–	–	x	–
42.69	22	Dihydroxyacetone	–	x	x	x	–	–
45.49	23	2-Hidroxy-γ-butyrolactone	–	x	x	–	–	–
45.65		Octanoic acid	–	x	x	–	x	x
48.57	24	3-Hydroxy-2,3-dihydromaltol	–	x	x	x	x	–
55.48	25	5-Hydroxymethyl-2-furfural	–	x	x	–	x	x
58.13		DHF[a]	–	x	x	–	x	–
68.61	26	*n*-Hexadecanoic acid	x	x	x	x	–	x
Total compounds identified per fibre			25	35	44	26	35	34

–: Not detected.
[a] DHF: 5,6-Dihydro-4-ydroxy-(3H)-furan-2-one.

3. Results and discussion

3.1. HS-SPME optimization

The extraction time, extraction temperature and sample amount are important variables influencing the vapour pressure and equilibrium of the aroma compounds in the headspace, therefore they were chosen and optimized in this study [3,26]. The optimization method evaluated the effect of one variable at a time, keeping all other variables constant during the assays. Before carrying out the optimization of the HS-SPME conditions for the analysis of the volatile compounds of cherimoya cultivars, fibre screening was carried out. The Mateus cultivar was selected as the matrix for the optimization of this methodology. The results were expressed in terms of the total peak areas obtained by GC–qMSD analysis using HS-SPME technique.

3.1.1. SPME fibre

The selection of the most appropriate SPME fibre depends on the compounds targeted and therefore on the plant material under

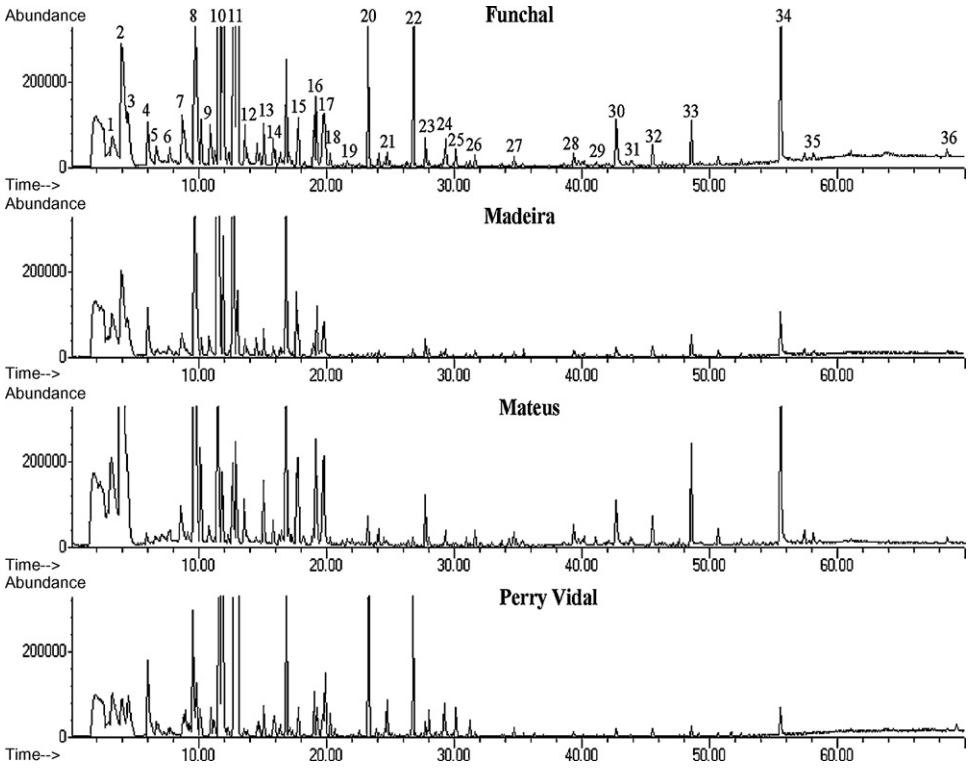

Fig. 5. TIC chromatogram obtained for Funchal, Madeira, Mateus and Perry Vidal cultivars using HS-SPME$_{DVB/CAR/PDMS}$/GC–qMSD methodology at 30 °C during 30 min under constant magnetic stirring (800 rpm).

study [22]. So, the six fibres (CW/DVB, DVB/CAR/PDMS, CAR/PDMS, PDMS/DVB and PDMS) were tested and compared individually to evaluate the effect of different fibre coatings on the extraction of volatile compounds in Mateus samples. Fig. 1 shows the typical total ion chromatograms (TIC) obtained for 2 g of Mateus cultivar using different fibre coatings with 30 min of extraction at 25 °C under constant magnetic stirring (800 rpm). The comparison among the six types of fibre coatings used in this study showed different GC responses, their performances were determined based on the intensity of the response observed including extraction efficiency, number of identifiable compounds in the extract and reproducibility (Table 1). Each extraction was done in triplicate and the repeatability (RSD%) was lower than 20%. The results in terms of total peak areas are illustrated in Fig. 2. According to the results, CAR/PDMS and DVB/CAR/PDMS fibre coatings had much better extraction efficiencies than the others, however, CAR/PDMS and PDMS/DVB fibre presented similar extraction efficiency. Of these three fibres, the retention ability of DVB/CAR/PDMS fibre for the volatile compounds in the Mateus samples is much stronger than the rest of the other fibres. Given to the better profile shown by this coating, this fibre was selected for the extraction of the volatile compounds of cherimoya cultivars [10,11].

3.1.2. Extraction time

Time affects the mass transfer of the analytes between the three system phases in HS-SPME technique. The optimal time for extraction should be the time of equilibrium since this methodology is based on the equilibrium between analyte and the fibre coating [10]. Assays focusing on the dynamics of Mateus volatiles were conducted with 15, 30 and 60 min of exposure time of the fibres into the headspace. The results are illustrated in Fig. 3. The best extraction efficiency was obtained at 30 min. The decline of the total peak after 30 min probably resulted from a partial desorption of some high volatile compounds from the fibre coating, the same behaviour was observed by Zhang et al. [11], due to competition phenomenon. The high values of % RSD that were observed at 15 min are due to the fact of the system may have not reached the equilibrium. According to these results, the time of extraction selected was 30 min.

3.1.3. Extraction temperature

The extraction temperature has a significant role in the extraction of the analytes because it can influence the distribution coefficients of the compounds between the sample and the headspace and between the headspace and the fibre [26]. Therefore, it is an important parameter because it controls the diffusion rate of the analytes into the coating. In order to determine the best temperature for the extraction of volatile compounds of Mateus cultivar, the effect of this parameter in the extraction of the analytes was checked. Fig. 3 reports the results of the three temperatures tested using the DVB/CAR/PDMS fibre during 30 min of extraction and 2 g of sample under constant magnetic stirring (800 rpm). As can be

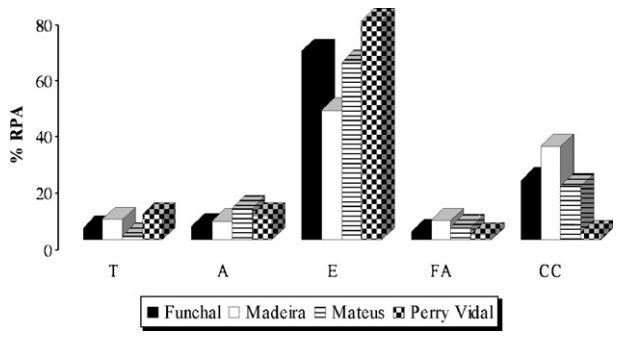

Fig. 6. Distribution of chemical families identified for the cherimoya cultivars (T: terpenes and sesquiterpenes; A: alcohols; E: esters; FA: fatty acids; CC: carbonyl compounds).

Table 2
Chemical components in the HS-SPME$_{DVB/CAR/PDMS}$ volatile compounds detected in Funchal, Madeira, Mateus and Perry Vidal cherimoya cultivars

KI	Compounds	Peak no.	Odor description [28–30]	Molecular weight	Funchal		Madeira		Mateus		Perry Vidal	
					Content	Similarity	Content	Similarity	Content	Similarity	Content	Similarity
966	Ethanol	1	Sweet	46.07	2.04	78	4.66	83	7.66	82	4.24	90
1018	Methyl butanoate	2	Ether, fruity, sweet	102.13	5.98	90	6.80	91	20.52	90	2.40	91
1042	α-Pinene	3	Pine, turpentine	136.23	1.99	90	2.60	96	–	–	2.30	95
1105	β-pinene	4	Pine, resin, turpentine	136.23	0.79	94	3.60	95	0.91	91	2.66	91
1128	Isoamyl acetate	5	Banana	130.18	0.63	74	0.48	72	0.29	72	0.71	90
1143	Butyl propanoate	6	Sweet, fruity	130.18	–	–	–	–	0.32	72	–	–
1159	Butan-1-ol		Medicine, fruit	74.12	–	–	–	–	0.87	70	0.45	83
1182	Methyl hexanoate	7	Fruity, fresh, sweet	130.18	2.24	73	2.00	70	2.97	80	1.76	78
1205	3-Methylbutan-1-ol		Alcohol, malt, fusel	88.15	–	–	–	–	–	–	1.61	90
1212	Butyl butanoate	8	Fruity, apple	144.21	5.15	83	7.27	90	16.83	78	2.22	91
1244	Butyl pentanoate	9	Sweet, fruity, green	158.24	1.04	83	0.82	80	1.03	83	1.16	90
1262	3-Methylbutyl butanoate	10	Fruity, green	158.24	21.10	83	18.61	90	9.74	78	25.14	83
1292	3-Methylbutyl 3-methylbutanoate	11	Sweet, fruity, green	172.26	27.80	90	7.05	91	4.06	91	41.28	90
1297	1-Hydroxypropan-2-one		–	74.08	0.92	77	–	–	2.22	74	–	–
1313	Hydroxyacetaldehyde	12	–	60.05	0.77	78	0.41	78	0.7	76	0.24	87
1319	(*Z*)-2-Penten-1-ol		Green, plastic, rubber	86.13	0.24	74	0.62	86	–	–	0.15	76
1342	Pentyl butanoate		Banana	158.24	0.55	70	0.62	70	0.39	77	0.58	72
1348	Hexan-1-ol	13	Resin, flower, green	102.17	0.59	78	0.60	83	1.64	78	0.53	83
1364	Methyl 3-hydroxy-3-methylbutanoate		–	132.16	0.51	83	0.28	73	0.41	74	0.68	78
1374	(*Z*)-3-hexen-1-ol	14	Grass	100.16	0.22	70	0.21	79	0.53	76	0.22	83
1402	Hexyl butanoate	15	Apple peel	172.26	0.85	91	1.00	91	4.57	83	0.72	91
1417	Hexyl 2-methylbutanoate		Strawberry	186.29	0.09	76	–	–	0.5	79	0.09	73
1418	5-Methyl-(3H)-furan-2-one		–	98.10	0.11	70	0.13	73	–	–	–	–
1436	Hexyl 3-methylbutanoate	16	Sweet, green, fruity	186.29	0.78	80	0.37	80	0.85	83	0.95	80
1441	Acetic acid		Acid, spicy	60.05	1.23	91	1.66	91	3	91	0.52	91
1451	(*Z*)-3-Hexenyl butanoate		Wine, green	170.25	0.21	79	–	–	0.13	74	0.22	72
1455	2-Furfural	17	Bread, almond, sweet	96.08	1.71	95	1.89	94	1.58	95	0.35	81
1471	Methyl 3-hydroxybutanoate	18	–	118.13	0.06	74	–	–	0.19	90	–	–
1494	1-(2-Furanyl)-ethanone		Balsamic	110.11	0.11	72	0.14	86	0.16	86	0.04	80
1521	DMDF[a]		–	144.12	0.09	74	0.10	72	0.25	73	–	–
1530	Propanoic acid	19	Pungent, rancid, soy	74.08	0.04	76	0.20	90	0.11	74	0.07	74
1540	Linalool	20	Flower, fresh	154.25	0.95	86	0.18	80	0.59	80	3.28	93
1558	5-Methyl-2-furfural		Almond, caramel, burnt sugar	110.11	0.37	95	0.73	94	0.38	95	0.07	90
1568	2-Cyclopenten-1,4-dione		–	96.08	0.11	72	0.39	83	0.21	87	–	–
1574	Caryophyllene	21	Wood, spice	204.35	0.24	80	0.32	90	0.23	72	0.18	83
1611	Butanoic acid		Rancid, cheesy, sweet	88.11	0.09	75	0.28	70	0.14	83	0.03	74
1622	4,4-Dimethylhexan-3-ol	22	–	130.23	1.70	70	0.16	75	0.24	72	1.54	75
1650	2-Furanmethanol	23	Burnt	98.10	0.52	96	0.68	96	0.97	96	0.25	96
1657	3-Methylbutanoic acid		Cheesy, spicy, rancid	102.13	0.07	74	0.37	78	0.19	78	0.34	78
1683	α-Terpineol		Pine, teil, íris	154.25	0.04	79	0.10	90	–	–	0.05	90
1689	D-Germacrene		Wood, spice	204.35	0.11	95	0.35	97	–	–	0.22	97
1692	3-Methoxybutan-1-ol	24	–	104.15	0.09	70	0.34	76	0.29	73	0.17	70
1715	γ-Elemene	25	–	204.35	0.14	76	0.17	78	–	–	0.23	93
1740	(5H)-furan-2-one		–	84.07	0.10	90	0.29	91	0.15	90	–	–
1748	γ-Cadinene		Thyme, wood	204.35	0.07	77	0.21	99	0.14	73	0.13	98
1760	2-Hydroxy-2-cyclopenten-1-one	26	–	98.10	0.22	90	0.25	80	0.31	86	0.08	83
1798	2-Cyclohexen-1-ol		–	98.14	0.05	70	0.17	70	0.09	73	–	–
1847	Hexanoic acid	27	Sweet, fatty, cheesy	116.16	0.11	73	0.24	74	0.24	74	0.08	70
1854	Dihydromaltol		–	128.13	0.15	79	0.86	78	–	–	–	–
1869	Phenylmethyl butanoate		Plum	178.23	0.04	83	0.20	91	0.27	74	0.04	91
1873	Maltol		Caramel	128.13	0.17	72	0.38	80	0.16	86	–	–
1973	2,5-Furandicarbaldehyde	28	–	124.09	0.53	91	1.48	87	0.61	87	0.12	90
1989	Methyl 2-furoate		Fruity, mushroom, sweet	126.11	0.21	72	0.52	88	0.19	80	–	–
2122	Furaneol	29	Caramel, strawberry	128.13	0.17	74	0.42	75	0.2	91	–	–

2178	Dihydroxyacetone	30	–	90.08	2.03	74	1.28	83	1.46	72	0.19	73
2204	γ-Ethylbutyrolactone	31	Coumarin, sweet	114.14	0.19	70	0.18	78	0.17	78	–	–
2269	2-Hidroxy-γ-butyrolactone	32	–	102.09	0.36	78	0.93	74	0.77	72	–	–
2294	5-Acetoxymethyl-2-furfural		–	168.15	0.23	74	0.70	88	0.1	80	–	–
2311	3-Hydroxy-2,3-dihydromaltol	33	–	144.13	2.16	94	3.59	91	2.49	87	0.24	87
2314	5-Hydroxymaltol		–	142.11	0.25	78	0.85	91	0.28	74	–	–
2334	Benzoic acid		Urine	122.12	0.18	73	0.97	87	0.15	91	0.08	79
2335	Furancarboxylic acid		–	112.08	0.24	80	0.27	72	0.21	74	0.07	70
2343	5-Hydroxymethyl-2-furfural	34	Cardboard	126.11	9.05	95	16.65	95	5.54	94	0.78	94
2355	DHF[b]	35	–	102.01	0.65	74	0.81	74	0.5	73	–	–
2395	*n*-Hexadecanoic acid	36	Sightly fatty, waxy	256.42	0.57	94	2.58	98	0.3	76	0.55	78

–: Not detected.
[a] DMDF: 2,5-Dimethyl-2,4-dihydroxy-(2H)-furan-3-one.
[b] DHF: 5,6-Dihydro-4-hydroxy-(3H)-furan-2-one.

observed, the higher temperature does not contribute positively for the extraction efficiency, due to thermodynamic reasons; decrease of partition coefficients, and to the decrease of the extraction by the fibre coating. Thus, the highest temperature used in this study was 40 °C, to avoid the possible loss of thermally unstable analytes and thermal degradation of some compounds present in matrix [13,21]. All the extractions were carried out in triplicate. From these results, the temperature of 30 °C was selected for further experiments.

3.1.4. Sample amount

For a given volume vial, sample amount has a positive effect on the peak areas of the compounds. But this does not mean that the larger the sample amount, the better the results [11]. In this assay, the sample amounts tested were 1, 2 and 4 g and the results of the effect of sample amount on the total peak areas are shown in Fig. 4. Considering the results, the sample amount of 2 g was chosen for the present study. Between 2 and 4 g of sample amount it was observed that a decrease in total peak area may be due to the saturation of the fibre coating.

3.2. Analysis and comparison of the volatile components in Funchal, Madeira, Mateus and Perry Vidal cultivars

The TIC chromatograms of the HS-SPME/GC–qMSD methodology with the optimized sampling conditions for Funchal, Madeira, Mateus and Perry Vidal cultivars are shown in Fig. 5. Each sample was analysed in triplicate. A total of 66 volatile compounds, among them 23 carbonyl compounds, 17 esters, 9 alcohols, 9 fatty acids and 8 terpenes, were tentatively identified by matching mass spectra with spectra of reference compounds in NIST mass spectral libraries with a resemblance percentage above 70%. The Kováts retention indices were calculated, under the same chromatographic conditions as the samples, for each compound and compared with the literature in order to certify the compound identification. In general, Funchal, Madeira and Mateus cultivars showed a similar aroma complexity profile comparatively to Perry Vidal. The relative amount percent of the individual components was expressed as percent peak areas relative to total peak areas (RPA%) and are listed in Table 2. The qualitative data analysis showed that the presence of the volatile compounds tentatively identified in this study are characteristic for the annonaceae family [1,27]. The RPA% values obtained for the different chemical families in Funchal, Madeira, Mateus and Perry Vidal are shown in Fig. 6. Esters, carbonyl compounds, alcohols, terpenes and fatty acids represent, in average $63.62 \pm 7.89\%$, $18.92 \pm 7.58\%$, $7.98 \pm 1.57\%$, $5.47 \pm 1.21\%$ and $4.02 \pm 1.70\%$ for the total volatile profile, respectively.

Tropical fruits can be classified into two broad categories based on whether esters and terpenoids predominate in the volatiles. Flavour volatiles of cherimoya are reported to be composed of esters, especially butanoate and 3-methyl butyl esters [1], therefore this specie is included in the first category, the same results were obtained by Idstein [7]. According to the results, the volatile compounds of all cultivars were also predominantly shared by esters, which contribute for total volatiles with 46.02%, 63.26%, 67.24% and 77.25% for Madeira, Mateus, Funchal and Perry Vidal, respectively. Methyl butanoate ($8.93 \pm 1.80\%$), butyl butanoate ($7.87 \pm 2.06\%$), 3-methylbutyl butanoate ($18.65 \pm 1.51\%$) and 3-methylbutyl 3-methylbutanoate ($20.05 \pm 1.25\%$) were the main compounds identified in this chemical family. These compounds contribute to the fruity and floral odors for the cherimoya cultivars. These compounds make a positive contribution to the general quality of cherimoya cultivars, being responsible for their fruity and flowers sensory proprieties (Table 2).

The carbonyl compounds were the main chemical family identified (23 compounds), on the other hand quantitatively this chemical

Table 3
Percentage of variance and percentage of cumulative variance explained by the two first principal components

Component	Total variance explained					
	Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Raw						
1	27.113	51.156	51.156	27.110	51.150	51.150
2	17.866	33.710	84.866	17.869	33.715	84.866
Rescaled						
1	27.113	51.156	51.156	27.110	51.150	51.150
2	17.866	33.710	84.866	17.869	33.715	84.866

Extraction method: Principal component analysis.

family has the second major contribution to the total volatile profile. In average, the major carbonyl compounds detected in the analysed cultivars were 1-hydroxypropan-2-one (1.57 ± 0.19%), 2-furfural (1.38 ± 0.42%), 1,3-dihydroxypropan-2-one (1.24 ± 0.47%) and 5-hydroxymethyl-2-furfural (8.01 ± 3.67%). In Perry Vidal, 2-furfural and 1,3-dihydroxypropan-2-one were found in less amount compared with the other cultivars that reported the similar contents. The same behaviour was observed for 5-hydroxymethyl-2-furfural, but its content in Madeira cultivars (16.65%) was higher than Funchal (9.05%), Mateus (5.54%) and Perry Vidal (0.78%).

Alcohols are formed as enzymatic degradation products of unsaturated fatty acids [1]. This chemical family contributes with 4.93%, 6.76%, 8.91% and 11.32% for total volatiles profile in Funchal, Madeira, Perry Vidal and Mateus, respectively. 3-Methylbutan-1-ol (1.61%) was only detected in Perry Vidal and represents the second major alcohol content. Butan-1-ol was only detected in Mateus and Perry Vidal, still (*Z*)-2-penten-1-ol and 2-cyclohexen-1-ol were not detected in Mateus and Perry Vidal, respectively.

The dominating terpenoids found in the analysed cultivars were α-pinene, β-pinene and linalool. In Mateus, α-pinene was not detected and the amount between the others cultivars were similar (not significantly different at the 95% level). The contribution of linalool for the total volatile profile was more significant in Perry Vidal (3.28%) than Funchal, Madeira and Mateus which had a contribution lower than 1%. This chemical family contributes with pine, flowers odors to the cherimoya cultivars studied.

Another group of aroma compounds that have been studied were the fatty acids. Within this family the acetic acid and *n*-hexadecanoic acid were notable for their higher contributions. Acetic acid or its biosynthetic equivalent, acetyl CoA contributes significantly to the synthesis of fatty acids and also to aromatic compounds [1]. The Madeira cultivar, presents the highest contribution for the total volatile profile (6.79%). The fatty acids contribution in Funchal and Perry Vidal cultivars was not significantly different at the 95% level. The odors of fatty acid are described as being cheesy, fatty and rancid (Table 2).

3.3. Multivariate analysis

The proposed HS-SPME/GC–qMSD methodology was applied to Funchal, Madeira, Mateus and Perry Vidal cultivars. Their differentiation was possibly due to the different total peak areas of each volatile compound determined in the four cherimoya cultivars. Data analysis multivariate techniques represent a powerful statistical tool that explains this differentiation. The 66 analytical variables used for statistical purposes were included into five different chemical families, such as terpenes, esters, alcohols, fatty acids and carbonyl compounds. When principal component analysis (PCA) was applied to the total peak area of the different chemical families, two factors were extracted and 74.97% of the total variance was explained. The redundant variables (13) not contributing to the explanation of total variance (coefficients magnitude <0.7)

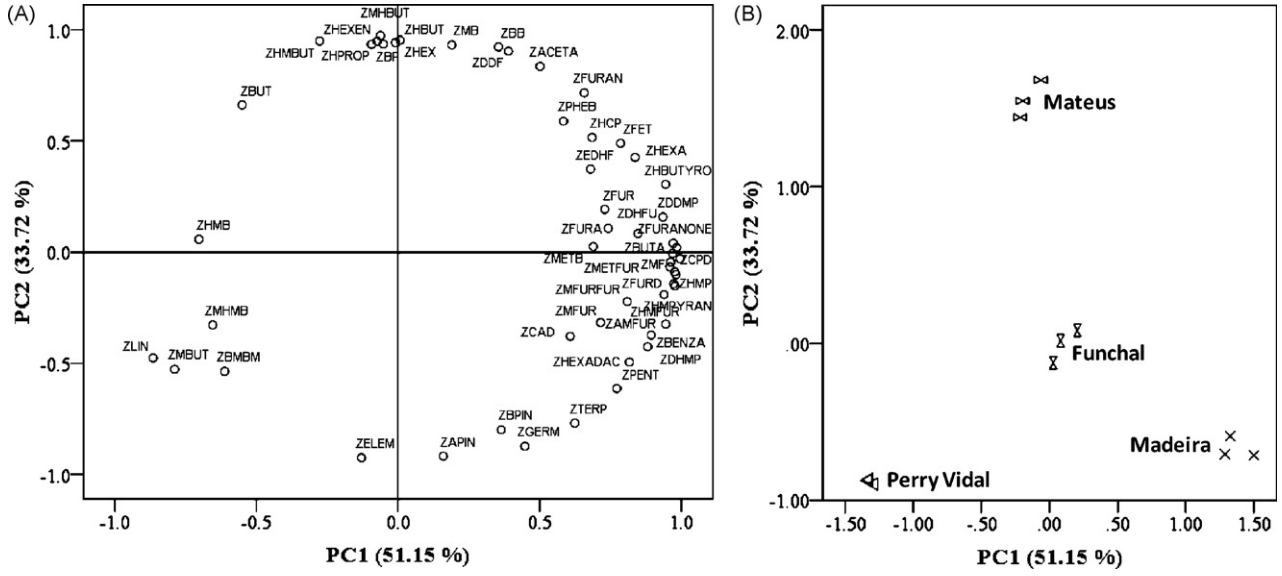

Fig. 7. PC1 and PC2 scatter plot of the main sources of variability between cherimoya cultivars. (A) relationships between the chemical compounds (loadings); (B) Distinction between the samples (scores).

Table 4
Prediction abilities for the different cultivars, using stepwise discriminant analysis (Anonacul: Anona cultivars)

	Anonnacul	Classification results[a,b]				
		Predicted group membership				Total
		Funchal	Madeira	Mateus	Perry Vidal	
Original						
Count	Funchal	3	0	0	0	3
	Madeira	0	3	0	0	3
	Mateus	0	0	3	0	3
	Perry Vidal	0	0	0	3	3
%	Funchal	100.0	.0	.0	.0	100.0
	Madeira	.0	100.0	.0	.0	100.0
	Mateus	.0	.0	100.0	.0	100.0
	Perry Vidal	.0	.0	.0	100.0	100.0
Cross-validated[c]						
Count	Funchal	0	0	1	2	3
	Madeira	0	1	2	0	3
	Mateus	0	2	1	0	3
	Perry Vidal	0	0	0	3	3
%	Funchal	.0	.0	33.3	66.7	100.0
	Madeira	.0	33.3	66.7	.0	100.0
	Mateus	.0	33.3	66.7	.0	100.0
	Perry Vidal	.0	.0	.0	100.0	100.0

[a] 100.0% of original grouped cases correctly classified.
[b] 41.7% of cross-validated grouped cases correctly classified.
[c] Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

were removed with the purpose to maximize the total variance from the data set. So, the first two components explain 84.87% of the total variance of the initial data set (Table 3). Furaneol (0.99), 2-cyclopenten-1,4-dione (0.98) and methyl 2-furoate (0.98) are the variables with the highest contribution on the first component (51.15%); 33.72% of total variance, corresponding to second component, was correlated with methyl 3-hydroxybutanoate (0.98), hexyl 2-methylbutanoate (0.96) and hexan-1-ol (0.94).

Fig. 7 reports PC1 and PC2 scatter plot of the main sources of variability between cherimoya cultivars. Madeira and Funchal cultivars (four quadrant) are essentially characterized by α-terpeneol, furaneol, benzoic acid, methyl 2-furoate, 3-methoxybutan-1-ol, 5-methyl-(3H)-furan-2-one and (*Z*)-2-penten-1-ol. The Perry Vidal (third quadrant) is described by linalool, 3-methylbutyl 3-methylbutanoate, 3-methylbutan-1-ol and methyl 3-hydroxy-3-methylbutanoate. The Mateus cultivar localized in the second quadrant is characterized by butyl propanoate (*Z*)-3-hexen-1-ol and methyl-3-hydroxybutanoate.

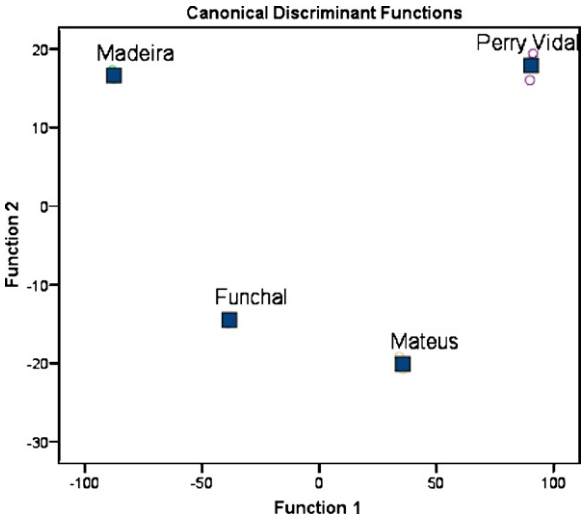

Fig. 8. Differentiation between *Annona Cherimola* Mill. cultivars by applying LDA.

A linear discriminant analysis (LDA) was run, using the above-mentioned variables, in order to obtain suitable classification rules. Fig. 8 shows a projection of the investigated cultivars of cherimoya in two-dimensional space, generated by the two first discriminate functions that explain 99.9% of the total variance. Four groups representing Funchal, Madeira, Mateus and Perry Vidal cultivars, were clearly observed. The good agreement achieved indicates that very acceptable classification functions can be deduced. The leave-one-out classification method was used as cross-validation procedure to evaluate the classification performance (Table 4). From the results it can be concluded that headspace SPME coupled to GC–qMSD and chemometrics is a very appropriate sampling technique to distinguish the different cherimoya cultivars growing in Madeira Islands studied based on their volatile profile.

4. Conclusions

A simple, rapid and solvent-free method to extract and determine the volatile compounds in cherimoya cultivars with the HS-SPME/GC–qMSD was developed. The volatile compounds play an important role in assessing the classification of this fruit. The qualitative profile of the volatile compounds of Funchal, Madeira and Mateus was similar, but their relative abundance showed several differences. The esters, alcohols, fatty acids and terpenes constitute important aroma group compounds which contribute with fruity, cheese/fatty and flowers notes to cherimoya cultivars sensory properties. Using PCA, the volatile composition in terms of average peak areas, provided a suitable tool to differentiate among the cherimoya cultivars.

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