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Headspace solid-phase microextraction-gas chromatography-quadrupole mass spectrometric methodology for the establishment of the volatile composition of *Passiflora* fruit species

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ABSTRACT

Dynamic headspace solid-phase microextraction (HS-SPME) followed by thermal desorption gas chromatography-quadrupole mass spectrometry analysis (GC-$_q$MS), was used to investigate the aroma profile of different species of passion fruit samples. The performance of five commercially available SPME fibres: 65 μm polydimethylsiloxane/divinylbenzene, PDMS/DVB; 100 μm polydimethylsiloxane, PDMS; 85 μm polyacrylate, PA; 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane, DVB/CAR/PDMS (StableFlex); and 75 μm carboxen/polydimethylsiloxane, CAR/PDMS; was evaluated and compared. Several extraction times and temperature conditions were also tested to achieve optimum recovery. The SPME fibre coated with 65 μm PDMS/DVB afforded the highest extraction efficiency, when the samples were extracted at 50 °C for 40 min with a constant stirring velocity of 750 rpm, after saturating the sample with NaCl (17%, w/v — 0.2 g). A comparison among different passion fruit species has been established in terms of qualitative and semi-quantitative differences in volatile composition. By using the optimal extraction conditions and GC-qMS it was possible to tentatively identify seventy one different compounds in *Passiflora* species: 51 volatiles in *Passiflora edulis* Sims (purple passion fruit), 24 in *P. edulis* Sims f. *flavicarpa* (yellow passion fruit) and 21 compounds in *Passiflora mollissima* (banana passion fruit). It was found that the ethyl esters comprise the largest class of the passion fruit volatiles, including 82.8% in *P. edulis* variety, 77.4% in *P. edulis* Sims f. *flavicarpa* variety and 39.9% in *P. mollissima*.

The semi-quantitative results were then submitted to principal component analysis (PCA) in order to establish relationships between the compounds and the different passion fruit species under investigation.

1. Introduction

The genus *Passiflora* comprising about 500 species is the largest of their family (Passifloraceae). This genus is common in places with tropical and warm climate, although it is much rarer in Asia, Australia, and Tropical Africa. From the several *Passiflora* species that grow in the tropics, the most widely found is *Passiflora edulis* Sims (passion fruit or purple granadilla) [1,2]. Madeira Island has a subtropical temperature, propitious to the development of some of these species, namely purple, yellow and, in little extension, banana passion fruit. The interest of researchers and producers by these species has been stimulated due to their good nutritional characteristics for industrialization since it presents a fresh pulp, soft peel, high sugar content and strong exotic flavor [3]. Because passion fruits are often inexpensive and extremely rich in vitamins, their popularity has increased, especially in Europe and in United States [3,4]. Volatiles directly affect the sensorial quality of fresh and processed fruit products, in which aroma is formed by a complex group of chemical substances (e.g., aldehydes, alcohols, ketones, esters, lactones, terpenes). The concentration of these volatile compounds is generally low (μg/L) and can be affected by a number of agronomic (variety, climatological conditions, ripening stage) and technological (harvest, post-harvest treatments, storage and processing conditions) factors [5].

Passiflora fruits are normally used to produce juice and are one of the most nutritious fruit juices. It has been used as traditional folk medicines in Europe and North America owing to their sedative and antihypertensive properties, and a consistent number of these species are used as drugs in the pharmacopeias of several countries [2]. They are rich in carbohydrates, flavonoids, alkaloids, ascorbic acid, carotenoids, vitamins, minerals and terpenoid compounds. In addition, it is a good source of nicotinic acid, riboflavin and a fair source of mineral matter [6,7]. Carotenoids are widely regarded as effective quenchers of singlet oxygen, triplet oxygen and peroxyl radicals. Their polarity may influence antioxidant functioning since xanthophylls should function more efficiently against polar radicals [1,8], have performed pharmacological studies on various species of passion fruits and they have noticed their significance in the treatment of central nervous system depressant effect.

Purple passion fruits (*P. edulis* Sims) are round, purple externally when ripe and filled with orange pulp. The yellow passion fruits

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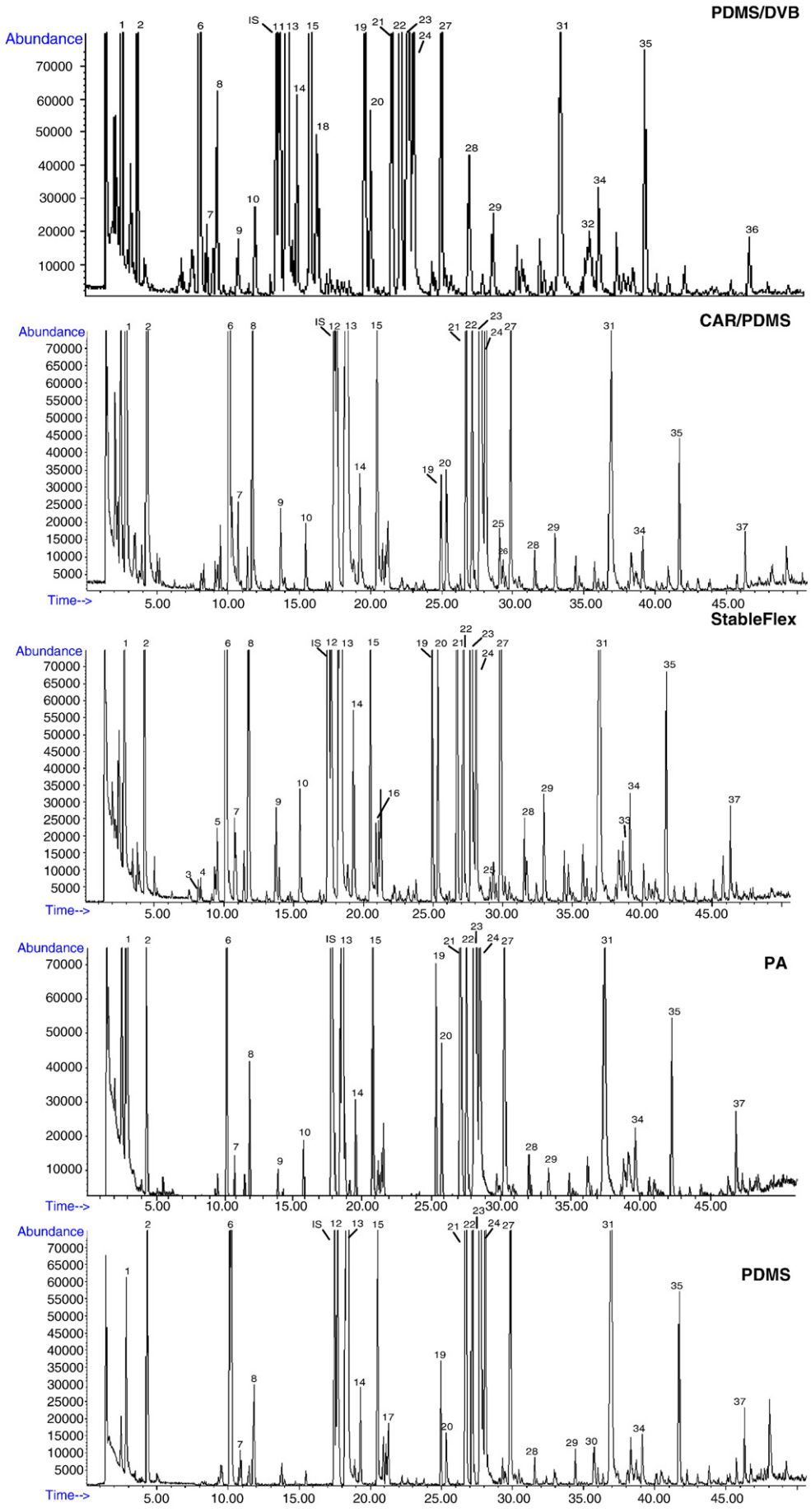
PDMS/DVB
CAR/PDMS
StableFlex
PA
PDMS
Abundance
Time-->

(*P. edulis* Sims f. *flavicarpa*) are bigger than the purple ones; they are yellow externally when ripe and filled with yellow pulp. Finally, banana passion fruits (*Passiflora mollissima*) have a long pendulum form, range from dark green to yellow-orange when fully ripe contain an orange-coulored pulp. This species is sweeter than the others and has less juice. All these species have black seeds. Purple passion fruit is the most used in the industry of fruit juices production and is known to be better for eating than yellow passion fruit because it is sweeter and not quite as acidic. The tolerance to heat or cold is similar to yellow passion fruit. Concerning yellow passion fruits, they have a "*floral*", "*estery*" aroma with an exotic tropical "*sulphury*" note. The main volatile compounds identified belong to the esters, namely methyl and ethyl esters [3,9]. Volatile esters are produced by virtually all soft fruit species during ripening. They play a role in the ripe fruit, serving both as "biological bribes" for the attraction of animals and as protectors against pathogens. Currently consumers are more interested in the provenance and authenticity of their food products. Fast identification of potential markers, suitable to qualitatively differentiate the food from various countries and regions, is a crucial requirement in regulations and for consumer confidence. Since the aroma is one of the most typical features of a food, the characterization of the aromatic profile can represent a useful tool to evaluate the organoleptic quality and it could be used to guarantee its authenticity.

Different techniques have been proposed for the extraction of the volatile compounds of foods. In general, volatile fractions have been recovered by conventional sampling methodologies such as steam distillation or solvent extraction. To overcome the problems associated with these techniques related with elevation costs, time-consumption, and the use of large volumes of organics solvents, the solid-phase microextraction (SPME) emerges as an attractive alternative. In addition, the SPME procedure will more closely reflect the true flavor profile of the fruit than those that might be generated by distillation and solvent extraction processes [3,10]. Since the first SPME fibres became commercially available, the technique of SPME has been more and more used and the fields of application have been continuously growing, including a wide range of food analysis, namely the volatile composition of wines [11–17] beers [18,19], whiskeys [20,21], honeys [22,23], sausages [24] and several kinds of fruits [25–29]. The technique gained growing acceptance and increasing use in routine laboratories and industrial applications.

In the present work, HS-SPME combined with GC-qMS, was developed and applied to evaluate the volatile composition profile of different passion fruit species: purple passion fruits (*P. edulis* Sims); yellow passion fruits (*P. edulis* Sims f. *flavicarpa*) and banana passion fruits (*P. mollissima*). A preliminary screening of fibres of various polarities was carried out in order to select the best coating for the matrix. Comparison between the performance of five sorbent materials is given. Other conditions that might affect the SPME procedure, such as extraction time and temperature, were also investigated. To confirm the applicability of the SPME, a comparative study on the characteristic GC-qMS volatile passion fruit profiles was performed. The possibility of differentiation from the investigated passion fruit was evaluated.

2. Materials and methods

2.1. Chemicals and reagents

All reagents used were of analytical quality: sodium chloride (99.5%) used to obtain the adequate ionic strength was supplied by Merck (Darmstadt, Germany) and water used was obtained by a Milli-Q purification system (Millipore). The C_8-C_{20} n-alkanes series and the chemical standards were supplied by Sigma-Aldrich (Spain). The silica fibres and a SPME holder for manual sampling were obtained from Supelco (Bellefonte, PA, USA).

2.2. Materials

The SPME fibres and the fibre holders were purchased from Supelco (Bellefonte, PA, USA). Amber silanized glass vials (4.0 mL) were obtained from Agilent Technologies (Palo Alto, CA, USA). According to manufacturer's recommendation, the fibres were first conditioned in the gas chromatography (GC) injection port to remove fibre contaminants. Prior to extraction the fibre was, daily, inserted in the hot injection port for 6 min. A blank test was performed to check possible carry-over.

2.3. Passion fruit samples

The samples used in this work consisted of three passion fruit species: purple (MP), yellow (MY) and banana (MB) passion fruit, growing at Madeira Island (Portugal). All samples were obtained from local stores. Passion fruits were opened and the pulp was separated from the seeds to make juice. The juice was homogenised and stored at −28 °C in glass bottles until analysis. All analysis, were carried out four times.

2.4. SPME methodology

HS-SPME is an equilibrium technique that requires a preceding optimization of the extraction parameters which might affect extraction efficiencies, in order to obtain high recoveries of volatiles. SPME parameters include fibre type, extraction temperature and extraction time.

2.4.1. Selection of fibre coating

Successful application of SPME depends primarily on the selection of a suitable fibre for a particular analysis. The efficiency of the analyte extraction and desorption from the fibre depends on the molecular weight, boiling point, vapour pressure of analytes and polarity and functional groups of the analytes and fibres. Five fibres were used for screening the passion fruits volatile profile: dimethylsiloxane layer (PDMS, 100 μm), recommended for nonpolar volatiles; polyacrylate (PA, 85 μm) with high selectivity for polar semivolatile compounds; divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS (StableFlex), 50/30 μm) recommended for flavours (volatiles and semivolatiles); carboxen-polydimethylsiloxane (CAR/PDMS, 75 μm); polydimethylsiloxane-divinylbenzene (PDMS/DVB, 65 μm). The coating of all fibres was 1 cm long. Purple passion fruit sample was selected as the matrix for comparison of the performance of all fibres. The fibre that presented the most complete profile of purple passion fruit volatile compounds was chosen to the study. The extraction was carried out for 20 min at 50 °C (controlled temperature) and each measurement was repeated four times. The PDMS/DVB fibre revealed to be the most suitable and was subsequently used in all further experiments.

Fig. 1. Typical GC-qMS chromatograms of purple passion fruit volatiles extracted by HS-SPME (50 °C; 20 min; 0.2 g NaCl (17%, w/v); 750 rpm) using different fibres (PDMS/DVB — polydimethylsiloxane/divinylbenzene; PDMS — polydimethylsiloxane (100 μm); PA — polyacrylate (85 μm); DVB/CAR/PDMS (SbleFlex) — divinylbenzene/carboxen on polydimethylsiloxane (50/30 μm); and CAR/PDMS — carboxen/polydimethylsiloxane (75 μm)). *Peak identification* 1: ethyl acetate; 2: ethyl butanoate; 3: heptan-2-one; 4: limonene; 5: butyl butanoate; 6: ethyl hexanoate; 7: (*Z*)-β-ocimene; 8: hexyl acetate; 9: (*Z*)-3-hexen-1-yl acetate; 10: hexan-1-ol; 11: octan-3-ol (IS); 12: heptan-2-yl butanoate; 13: 1-methyl octyl butanoate; 14: phenylmethyl butanoate; 15: hexyl butanoate; 16: ethyl octanoate; 17: (*Z*)-hexen-3-yl butanoate; 18: trans-edulan I; 19: octyl acetate; 20: octyl butanoate; 21: linalool; 22: octan-1-ol; 23: 2-methylpropyl hexanoate; 24: cis-edulan I; 25: hexyl hexanoate; 26: decyl butanoate; 27: (*Z*)-hexen-3-ol hexanoate; 28: *p*-menth-1-en-8-ol; 29: phenylmethyl acetate; 30: pantolactone; 31: octyl hexanoate; 32: (*Z*)-cyclodecene; 33: (*Z*)-3-decen-1-yl acetate; 34: hexanoic acid; 35: phenylmethyl butanoate; 36: α-ionone; 37: phenylmethyl hexanoate.

Table 1
Volatile compounds identified in purple passion fruit juice after dynamic headspace solid-phase microextraction using different coating (extraction temperature: 50 °C; extraction time: 20 min; 750 rpm; 17% (w/v) NaCl).

RT (min)	Compounds	SPME coatings				
		PDMS/DVB	CAR/PDMS	PDMS	Stableflex	PA
2.539	Ethyl acetate	x	x	–	x	–
3.670	Methyl butanoate	x	x	–	–	–
4.039	2-methyl methylbutanoate	x	–	–	–	–
4.540	Ethyl butanoate	x	x	x	x	x
5.294	Butyl acetate	x	x	–	–	–
5.298	Methylpropyl acetate	x	x	–	–	–
8.553	Heptan-2-one	–	–	–	x	–
8.698	Limonene	–	x	–	x	–
8.750	Methyl hexanoate	x	–	–	–	–
9.756	1-methylbutyl butanoate	x	x	–	x	–
9.942	Butyl butanoate	x	x	–	x	–
10.534	Ethyl hexanoate	x	x	x	x	x
11.187	(*Z*)-β-ocimene	x	x	x	x	–
11.865	2-heptyl acetate	x	–	–	x	–
12.260	Hexyl acetate	x	x	x	x	x
13.574	3-ethyl hexanoate	x	x	–	x	–
14.220	(*Z*)-3-hexen-1-yl acetate	x	x	x	x	x
14.437	Heptan-2-ol	x	–	–	x	–
15.287	Hexyl propanoate	x	–	–	–	–
15.925	Hexan-1-ol	x	x	–	x	x
17.362	(*Z*)-3-hexen-1-ol	x	–	–	x	
17.614	2-heptyl butanoate	–	–	–	–	x
18.248	1-methylhexyl butanoate	x	x	x	x	–
18.350	2-methylbutyl hexanoate	x	–	–	–	–
18.780	1,1-dimethylpropyl hexanoate	–	–	x	x	–
18.827	Butyl hexanoate	x	x	–	x	x
18.915	Hexyl butanoate	x	x	x	x	x
19.490	3-methylhexyl butanoate	x	–	–	–	x
19.899	Ethyl octanoate	x	x	x	x	x
21.144	(*Z*)-3-hexenyl butanoate	x	x	x	x	x
21.582	Trans-edulan i	x	x	x	x	x
21.672	Octyl acetate	x	–	–	–	–
21.752	Ethyl 4-octenoate	x	–	–	x	–
21.893	Octyl butanoate	x	–	x	–	–
23.210	2-methylbutyl propanoate	–	x	–	x	–
23.719	3-cyclohexene-1-propanol	–	–	x	–	–
25.531	Linalool	x	x	x	x	x
25.887	Octan-1-ol	x	x	–	x	x
27.371	2-methylpropyl hexanoate	–	x	–	–	x
27.881	Cis-edulan i	x	x	x	x	x
27.905	2-pentyl octanoate	–	x	x	x	–
28.420	Hexyl hexanoate	x	–	x	x	x
28.767	Decyl butanoate	x	x	x	x	x
29.080	Ethyl decanoate	–	x	–	x	–
29.293	1-ethenyl hexyl butanoate	–	–	–	–	x
29.472	(*Z*)-5-octen-1-ol	–	–	x	–	x
29.490	1,4-octadiene	–	–	–	x	–
29.502	2-methyl-bicyclo[4.1.0]heptane	–	x	–	–	–
29.983	(*E*)-3-octen-1-yl acetate	x	x	x	x	–
30.532	(*Z*)-3-hexenyl hexanoate	x	x	x	x	x
32.153	*P*-menth-1-en-8-ol	x	x	–	x	–
33.689	Phenylmethyl acetate	x	x	x	x	x
35.417	2-hydroxymethyl benzoate	x	–	–	x	–
35.735	Pantolactone	–	–	x	–	–
37.543	Hexyl hexanoate	x	–	–	–	–
37.585	Octyl hexanoate	x	–	x	x	x
38.314	4-ethyl cyclohexene	–	x	–	–	x
38.320	(*Z*)-3-decen-1-yl acetate	–	–	x	x	–
38.926	Hexanoic acid	x	–	–	x	–
39.324	Geranyl cetone	x	–	–	–	–
39.857	Phenylmethyl butanoate	–	x	x	x	x
40.922	2-phenylethanol	–	x	–	–	–
41.152	Linalyl acetate	x	–	–	–	–
42.459	Dihydro-β-ionone	x	x	x	x	x
43.021	2-methyl-2-phenylethyl butanoate	x	x	x	x	–
45.740	(*Z*)-cyclodecene	–	–	x	–	x
46.698	Phenylmethyl hexanoate	x	x	x	x	x
46.948	3-phenylethyl propanoate	–	–	x	x	–
47.114	5-hydroxymethyl furfural	x	–	–	–	–
47.627	Ethyl cinamate	x	–	–	–	–
49.385	DDMP[a]	x	–	–	–	–
Total compounds per fibre		51	39	31	45	28

–: Not detected.
[a] DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one.

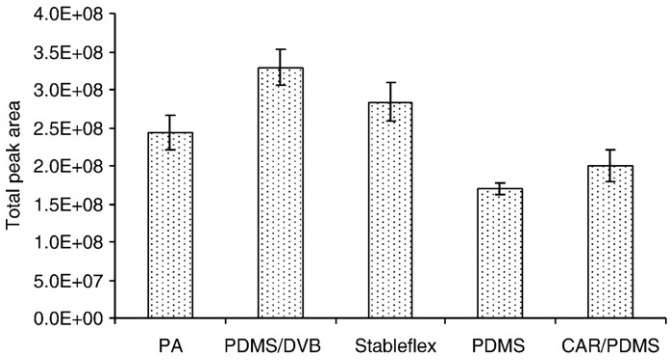

Fig. 2. Extraction efficiencies measured for different SPME coatings. Error bars represent standard error of the mean ($n=4$ for each data point).

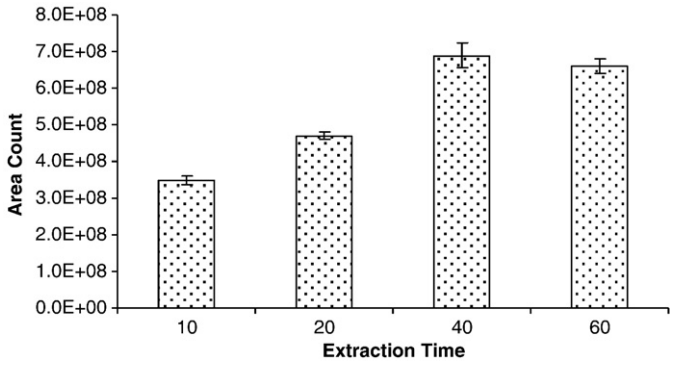

Fig. 4. Influence of extraction time on absorption of purple passion fruit flavour compounds during HS-SPME extraction using a PDMS/DVB fibre (50 °C, with 0.2 g of NaCl (17%, w/v)).

2.4.2. Selection of extraction time and extraction temperature

In order to obtain the maximum extraction yield for each volatile constituent, exposure times and temperatures were adjusted. With other conditions invariable, the extraction time profile was constructed at the extraction temperatures of 50 °C with 10, 20, 40 and 60 min of the analytes isolation. An optimal extraction time of 40 min was selected. Keeping the extraction time constant, the extraction temperature was carried out from 40 to 80 °C. Desorption time was 6 min and the temperature in the GC liner was 260 °C.

2.4.3. HS-SPME procedure

The volatile compounds were monitored by sampling the headspace of the passion fruits by using the headspace SPME technique. For each extraction, an aliquot of 0.2 mL of juice sample diluted with 1 mL deionised water (Milli-Q), was introduced in a 2 mL glass vial hermetically sealed and equilibrated during 20 min in a thermostatic bath on a stirrer. The extractions were carried out without pH adjustment. The ionic strength was increased using NaCl (17% (w/v) — 0.2 g), analytical grade) to improve the extraction efficiency by decreasing the solubility of hydrophilic compounds in the aqueous phase. The SPME fibre was then exposed to the headspace of the sample and kept for 20 min at 50 °C. As stirring usually improves the extraction, because the static layer resistant to mass transfer is destroyed (facilitate mass transport between the bulk of the aqueous sample and the fibre), all the experiments were performed under constant stirring speed of 750 rpm.

After sampling, the SPME fibre was withdrawn into the needle, removed from the vial and inserted into the hot injector port (260 °C) of the GC-qMS system for 6 min where the extracted analytes were thermally desorbed and transferred directly to the analytical column.

2.5. Gas chromatography-quadrupole mass spectrometry analysis (GC-qMS)

The volatiles extracted by dynamic HS-SPME from passion fruit samples, were separated and identified in an Agilent 6890N Network gas chromatograph system (Palo Alto, CA, USA) equipped with a 30 m × 0.25 mm i.d., with a 0.25 μm film thickness, BP-20 fused silica capillary column (polar) from Scientific Glass Engineering (Milton Keynes, UK), coupled to an Agilent 5975 quadrupole inert mass selective detector. Helium (Helium N60, Air Liquid, Portugal) was used as the carrier gas at a flow rate of about 1 mL/min (column-head pressure: 13 psi). The liner injection port was lined with a 0.75 mm (I. D.) splitless glass liner. The injections were performed in the splitless mode. The initial oven temperature, 40 °C, was held for 1 min and then increased in two steps: 40 °C to 150 °C, at 2.5 °C/min and 150 to 220 °C at 15 °C/min. The manifold, GC-qMS interface and quadrupole temperatures were held at 180 °C, 220 °C and 180 °C, respectively. The detection was performed by a 5975 mass spectrometer in the electronic impact mode (EI at 70 eV). The electron multiplier was set to the auto tune procedure. The mass acquisition range, made in a full scan mode, was 30–300 m/z; 1.9 spectra/s.

Volatile compounds were identified by comparing the MS fragmentation pattern with those of the pure standards and mass spectrum of the unknown peaks with those stored in the NIST05 library, retention times of the pure standards obtained under the same

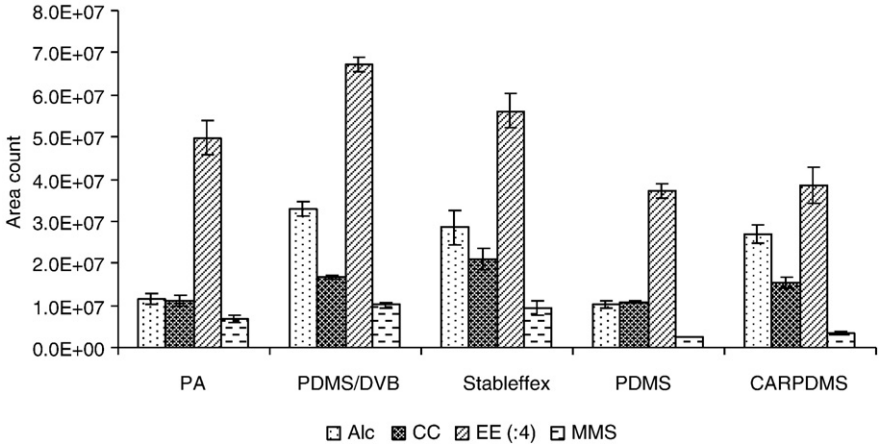

Fig. 3. Comparison of the performance of different SPME coatings on the higher alcohols (Alc), carbonyl compounds (CC), ethyl esters (EE) and C_{13}-norisoprenoids/monoterpenes/sesquiterpenes (NMS).

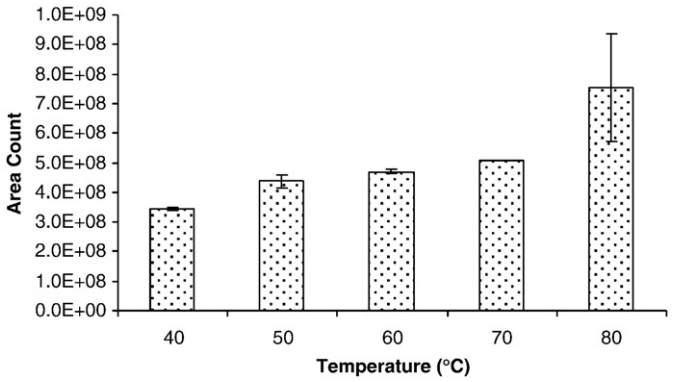

Fig. 5. Effect of extraction temperature on SPME of passion fruit volatile organic compounds with the PDMS/DVB fibre (extraction time: 20 min, headspace sampling with 17% (w/v) of NaCl).

conditions, and Kováts retention indices (KI). For the determination of the KI a C_8-C_{20} n-alkanes series was used. The relative amounts of individual components are expressed as percent peak areas relative to the total peak area (Relative Peak Area, RPA%).

2.6. Statistical analysis

Significant differences among the passion fruit samples were determined by one-way analysis of variance (Anova) using SPSS Program, version 14.0 (SPSS Inc., 2006). Principal component analysis (PCA) was applied using the same SPSS program. This technique was applied to the normalized peak areas from different chemical classes.

3. Results and discussion

Headspace SPME was used as a substitute of direct sampling mode since for volatile analytes, in the former mode, the equilibrium times are shorter compared to direct extraction. The headspace also protects the fibre from adverse effects caused by non-volatile, high molecular weight substances present in the sample matrix, and allows matrix modifications. A few key experimental factors which might influence the HS-SPME extraction yield, namely time required for the target analytes to reach equilibrium and extraction temperature were optimised. Temperature has a significant effect on the extraction kinetics since it determines the vapour pressure of the analytes, and for this reason their influence in the extraction process was also investigated.

3.1. Fibre selection

The fibre coating is very important to define the optimum extraction conditions from the headspace. To evaluate the extraction yields of the passion fruit volatile components by HS-SPME, and taking account their physico/chemical characteristics, five types of fibres (CAR/PDMS, PDMS/DVB, PDMS, PA and StableFlex) with a range of polarities and mechanisms, among those used routinely for assaying volatiles, were tested. Each fibre was exposed to the headspace at the same temperature (50 °C) during the same extraction time (20 min). All tests were carried out with the same passion fruit sample (purple passion fruit). The comparison of the SPME fibre performance was made in terms of extraction efficiency, estimated by the number of identifiable compounds in the extract and reproducibility. The chromatographic profiles, obtained for a purple passion fruit sample by using different coatings in the same experimental conditions, are presented in Fig. 1 indicating that PDMS/DVB fibre provided higher extraction efficiency to the volatiles. The results obtained using the five fibres on the same purple passion sample, in rigorously reproduced temperature and exposure time conditions, are reported in Table 1. A total of seventy one volatile compounds, belonging to the carbonyl compounds, ethyl esters, higher alcohols, fatty acids and monoterpenoids, were tentatively identified by matching mass spectra with spectra of reference compounds in NIST mass spectral libraries with a resemblance percentage above 80%. The Kováts retention indices (KI) were calculated for each peak and, when available, compared with the literature in order to certify the compound identification. The qualitative composition of passion fruit volatiles and the number of identified compounds using the five fibres under study are very different. As can be seen in Table 1 with PDMS/DVB fibre a total of 51 compounds were identified whilst with the StableFlex, CAR/PDMS, PDMS and PA coatings, were detected only, 45, 39, 31 and 28 compounds, respectively.

As shown in Fig. 2, where the total amounts obtained for each fibre are plotted, the semi-polar PDMS/DVB fibre allowed the best efficiency of extraction for volatile compounds, extracting around 1.1 times more than PA and StableFlex fibres and 1.3 times more than PDMS fibre. This fibre provided the best sensitivity in terms of total compound peak areas, highest number of detected compounds and high reproducibility, hence was chosen for screening the volatile compounds in passion fruits. CAR/PDMS fibre coating extracted 60.6% of PDMS/DVB while PDMS coating extracted the lowest amount (about 51.6%). Similar amounts of volatiles (73.8–88.1%) were extracted when using either, PA or StableFlex coatings. Fig. 3 showed that fibres exhibited different selectivity to different target compounds. Ethyl esters (EE) have a larger affinity for PDMS/DVB fibre. This coating also presents a good sensitivity for higher alcohols (Alc) and C13-norisoprenoids/monoterpenes/sesquiterpenes (NMS). StableFlex was the fibre with most affinity for carbonyl compounds (CC).

Some characteristic passion fruit compounds were isolated by the five fibres: ethyl butanoate, ethyl hexanoate, hexyl acetate, (*Z*)-3-hexenyl acetate, hexyl butanoate, ethyl octanoate, (*Z*)-3-hexenyl butanoate, cis- and trans-edulan I, linalool, β-ionone, (Z)-3-hexenyl hexanoate, benzyl acetate, phenylmethyl butanoate and phenylmethyl hexanoate.

3.2. Extraction time and temperature

Extraction time and temperature are important factors in SPME processes. Since time affects the mass transfer of the analytes onto the fibre, optimum time is required for the fibre to reach equilibrium with headspace. To study the effects of extraction time, purple passion fruit juice solutions were extracted for various periods ranging from 10 to 60 min at of 50 °C. The results are shown in Fig. 4. It indicated that the peak areas increase with increasing extraction time in the range of 10–40 min and reach their maximum value in 40–60 min. When the time

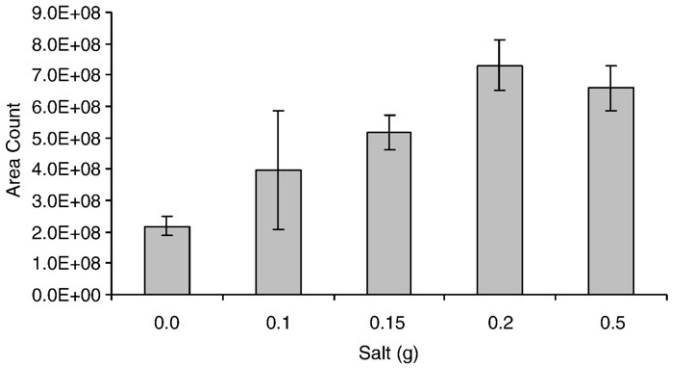

Fig. 6. Effect of salt addition (0.1, 0.15, 0.2 and 0.5 g NaCl, 1.2 ml of sample) on the HS-SPME extraction efficiency of purple passion fruit, using a PDMS/DVB fibre (40 min extraction time, 50 °C extraction temperature).

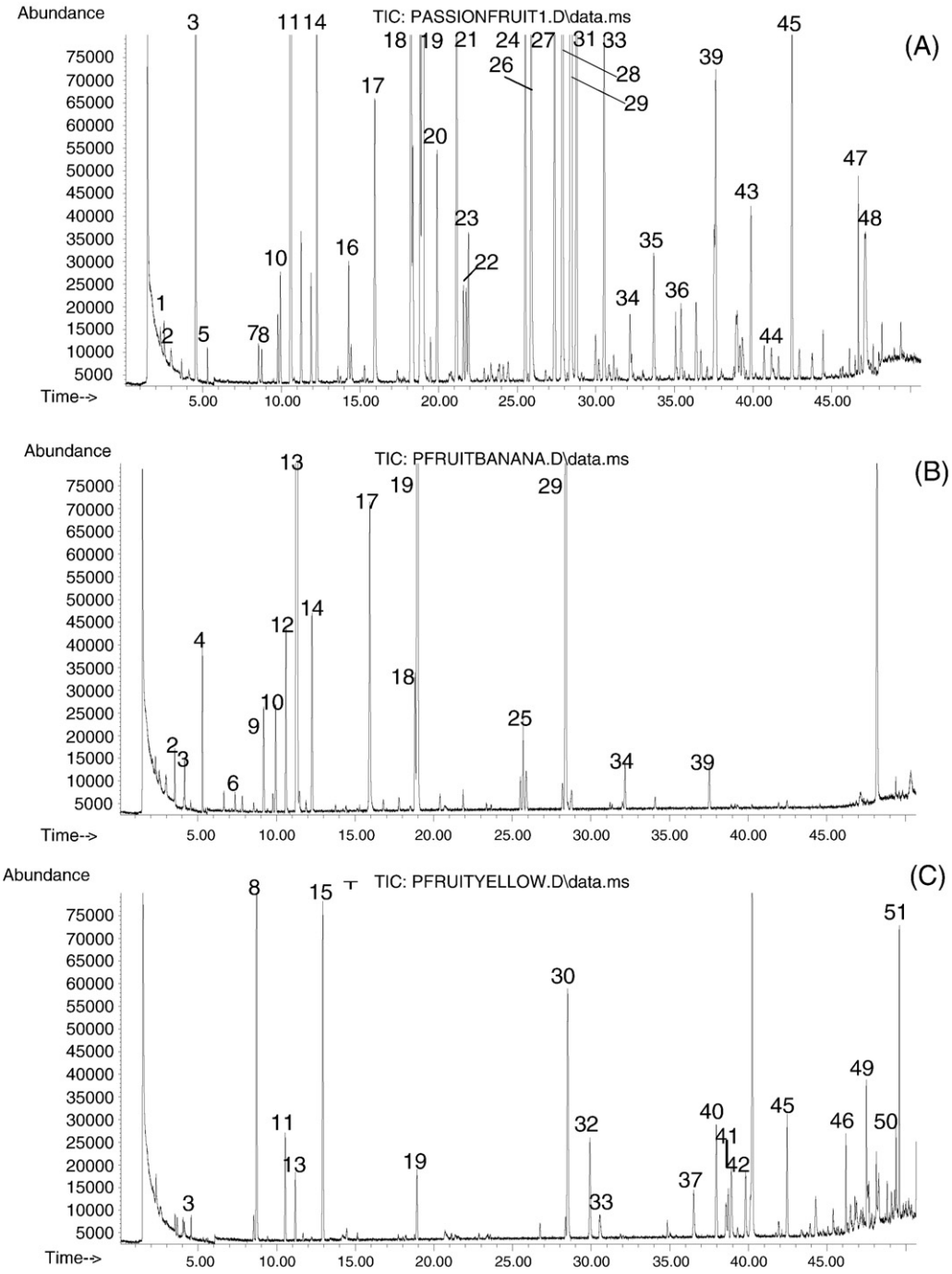

Fig. 7. Typical HS-SPME-GC-qMSD chromatograms of volatile compounds from different species of *Passiflora* fruits [(A) — *Passiflora edulis* Sims; (B) — *Passiflora mollissima*; and (C) — *Passiflora edulis* Sims f. *flavicarpa*] using: PDMS/DVB fibre at 50 °C, 40 min, with 17% NaCl (w/v) and 750 rpm (for peak identification see Table 2).

was further increased, no significant increase in the response was observed. For shorter analysis time 40 min of extraction time was selected.

Temperature is one of the most important experimental factors in SPME technique since it controls the diffusion rate of the analytes into the fibre. The extraction temperature was also determined and the results are shown in Fig. 5. Keeping the extraction time, the extraction temperature was carried out at five different temperatures from 40 to 80 °C (40, 50, 60, 70 and 80 °C) with a constant extraction time of 20 min. On the basis of Fig. 5, it can be found that the extracted amount increases with the extraction temperature. A temperature increase improves the mobility of volatile compounds through liquid and gas phases leading to an increase in extraction amounts. Despite the increasing extraction efficiency noted at high temperatures, 50 °C was chosen for further experiments. Temperatures as high as 80 °C were discarded since high temperatures might cause thermal degradation of some compounds. So, the maximum extraction efficiency was obtained at 50 °C for extraction temperature and an exposure time of 40 min keeping the sample under constant agitation.

The effect of salt added to the samples has also been tested. For some analytes, unsalted solution is not significantly different from the same solution but with salt added. Fig. 6 showed that adding NaCl increased the extraction efficiency of the volatiles from passion fruit samples.

Table 2
Volatile compounds identified in different types of passion fruit (MY — yellow, MB — banana, MP — purple) after dynamic headspace solid-phase microextraction using PDMS/DVB coating (extraction temperature: 50 °C; extraction time: 40 min; 750 rpm; 17% NaCl, w/v).

RT (min)	Peak No.	KI[a]	KI[b]	Compounds	RPA (%)			RPC (%)		
					MY	MB	MP	MY	MB	MP
2.539	1	907	907	Ethyl acetate	–	–	0.10	–	–	100
3.536	2	984	991	Pentan-2-one	0.38	0.32	–	100	84	–
3.670		990	1001	Methyl butanoate	0.41	–	0.06	100	–	15
4.039			1022	2-methyl methylbutanoate	0.40	0.05	0.30	100	12	75
4.540	3	1043	1048	Ethyl butanoate	0.46	–	3.26	14	–	100
5.294	4	1075	1077	Butyl acetate	–	0.93	–	–	100	–
5.298	5		1082	1-methylpropyl acetate	–	–	0.08	–	–	100
7.380		1143	1151	Butan-1-ol	–	0.12	–	–	100	–
7.828	6	1145	1163	B-myrcene	–	0.09	–	–	100	–
8.553	7	1170	1182	Heptan-2-one	–	–	0.12	–	–	100
8.750	8	1188	1187	Methyl hexanoate	32.87	–	0.11	100	–	0.3
9.181	9	1197	1196	Eucalyptol	–	0.83	–	–	100	–
9.756			1209	1-methylbutyl butanoate	–	0.13	0.22	–	59	100
9.942	10		1219	Butyl butanoate	–	0.78	0.37	–	100	47
10.534	11	1238	1236	Ethyl hexanoate	3.19	–	9.31	34	–	100
10.609	12	1242	1238	(*E*)-β-ocimene	–	1.40	–	–	100	–
11.187	13	1245	1254	(*Z*)-β-ocimene	1.90	56.57	0.53	3	100	0.9
11.787			1269	3-carene	–	0.09	–	–	100	–
11.865			1271	2-heptyl acetate	–	–	0.39	–	–	100
12.260	14	1274	1282	Hexyl acetate	–	0.56	1.92	–	29	100
12.943	15		1297	(*E*)-methyl-2-hexenoate	11.66	–	–	100	–	–
13.574			1313	Ethyl 4-hexenoate	–	–	0.06	–	–	100
14.220	16	1327	1328	(*Z*)-3-hexen-1-yl acetate	–	–	0.49	–	–	100
14.437		1320	1333	Heptan-2-ol	–	–	0.13	–	–	100
15.287			1352	Hexyl propanoate	–	–	0.06	–	–	100
15.925	17	1360	1366	Hexan-1-ol	–	3.05	1.31	–	43	100
17.362		1386	1395	(*Z*)-3-hexen-1-ol	–	–	0.04	–	–	100
18.248			1417	1-methylhexyl butanoate	–	–	3.89	–	–	100
18.350			1420	2-methylbutyl hexanoate	–	–	0.54	–	–	100
18.827	18	1540	1432	Butyl hexanoate	0.11	1.00	1.87	6	54	100
18.915	19	1533	1435	Hexyl butanoate	2.49	16.83	23.19	11	73	100
19.490			1450	3–methylhexyl butanoate	–	–	0.17	–	–	100
19.899	20	1439	1460	Ethyl octanoate	–	–	0.99	–	–	100
20.382			1472	3-methyl hexylbutanoate	–	0.11	–	–	100	–
21.144	21	1526	1491	(*Z*)-3-hexenyl butanoate	–	–	3.03	–	–	100
21.582	22		1501	Trans-edulan i	–	–	0.42	–	–	100
21.752			1505	Ethyl 4-octenoate	–	–	0.34	–	–	100
21.893	23	1497	1508	Octyl butanoate	–	–	0.64	–	–	100
25.531	24	1551	1590	Linalool	–	–	1.93	–	–	100
25.681	25		1593	Hexyl-2-butenoate	–	0.89	–	–	100	–
25.887	26	1553	1597	Octan-1-ol	–	0.33	2.74	–	12	100
27.371	27		1639	2-methyl propylhexanoate	–	–	2.81	–	–	100
27.881	28		1653	Cis-edulan i	–	–	5.58	–	–	100
28.420	29	1727	1668	Hexyl hexanoate	0.62	13.95	18.42	3	7	100
28.495	30	1620	1670	Methyl benzoate	11.25	–	–	100	–	–
28.767	31		1677	Decyl butanoate	–	–	2.98	–	–	100
29.927	32		1709	3-hydroxymethyl hexanoate	4.04	–	–	100	–	–
29.983			1711	(*E*)-3-octen-1-yl acetate	–	–	0.19	–	–	100
30.532	33	1729	1728	(*Z*)-3-hexenyl hexanoate	–	–	3.06	–	–	100
32.153	34		1775	P-menth-1-en-8-ol	–	0.39	0.24	–	100	62
33.689	35	1829	1819	Phenylmethyl acetate	–	–	0.70	–	–	100
35.417	36		1868	2-hydroxymethyl benzoate	–	–	0.39	–	–	100
36.490	37		1898	2-(2-butoxyethoxy)-ethanol	1.59	–	–	100	–	–
37.543	38		1926	Hexyl hexanoate	–	–	0.72	–	–	100
37.585	39	1806	1927	Octyl octanoate	–	0.38	1.53	–	25	100
37.978	40	1952	1937	α-Ionone	4.47	–	–	100	–	–
38.713			1956	Methyl dodecanoate	1.50	–	–	100	–	–
38.926	41	2060	1962	Hexanoic acid	3.12	–	0.28	100	–	9
39.324		1840	1972	Geranyl acetone	–	–	0.13	–	–	100
39.821	42		1984	MHTP[c]	2.71	–	–	100	–	–
39.857	43		1985	Phenylmethyl butanoate	–	–	0.86	–	–	100
41.152	44		2125	Linalyl acetate	–	–	0.14	–	–	100
42.459	45		2170	Dihydro β-ionone	4.89	–	1.73	100	–	35
46.182	46		2289	Isopropyl myristate	2.14		–	100	–	–
46.698	47		2301	Phenylmethyl hexanoate	–	–	0.51	–	–	100
47.114	48		2303	5-hydroxymethyl furfural	–	–	1.16	–	–	100
47.627		2097	2306	Ethyl cinamate	–	–	0.07	–	–	100
48.130	49	2202	2308	Nonanoic acid	1.06	–	–	100	–	–
48.816			2312	Methyl hexadecanoate	0.64	–	–	100	–	–
49.385	50		2314	DDMP[d]	1.96	–	0.13	100	–	7
49.591	51		2315	Methyl dihydrojasmonate	6.17	–	–	100	–	–
Total compounds identified by passion fruit								24	21	51
Total area					2.35E + 07	9.45E + 07	2.04E + 08			
% RSD ($n = 4$)					0.17	0.51	5.58			

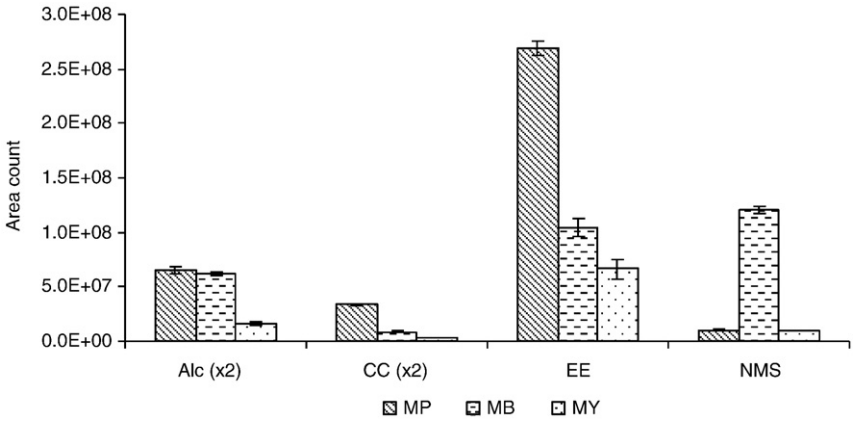

Fig. 8. Distribution of compounds classes by passion fruit samples: MP — purple passion fruit (*Passiflora edulis* Sims), MB — banana passion fruit (*Passiflora mollissima*) and MY — yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa*); CC: carbonyl compounds; EE: ethyl esters; Alc: higher alcohols; NMS: C_{13}-norisoprenoids, monoterpenes and sesquiterpenes.

3.3. Analysis of volatile compounds in passion fruit samples

The proposed HS-SPME method, previously optimised and validated, was applied to investigate the volatile profile in different species of passion fruits. Each passion fruit sample was analysed four times using the best sampling conditions described above (i.e.: PDMS/DVB fibre; 50 °C extraction temperature; 40 min extraction time; agitation at 750 rpm, salt addition: NaCl, (17%, (w/v) — 0.2 g)). This procedure allowed the detection of about seventy volatile compounds. A characteristic GC-qMS profile of each passion fruit species obtained with a PDMS/DVB fibre using the experimental optimised conditions is shown in Fig. 7. The identified compounds, described in Table 2, were organized in different groups according to their chemical structure, such as, monoterpenes/C_{13}-norisoprenoids (NMS), higher alcohols (Alc), ethyl esters (EE) and carbonyl compounds (CC). In average, ethyl esters, higher alcohols, carbonyl compounds and in less extension the monoterpenes/C_{13}-norisoprenoids, constituted a main part of the flavour of the studied passion fruit and they probably play a significant role in their organoleptic profile.

The volatile compounds were tentatively identified by matching mass spectra with spectra of reference compounds in NIST mass spectral library. In some cases a comparison with authentic compounds was performed. The Kováts retention indices were calculated for each peak and compared with the literature in order to ensure the correct identification of the compounds. Table 2 summarises the average ($n = 4$) relative composition in the studied passion fruits. The relative composition of each compound was calculated as the percent ratio of the respective peak area relative to the total peak area (RPA, %) and relative to the compound with highest peak area in each passion fruit variety (RPC, %).

Among the identified compounds, only 5 volatiles were simultaneously identified in all three samples: 2-methyl methylbutanoate, (*Z*)-β-ocimene, butyl hexanoate, hexyl butanoate and hexyl hexanoate (Table 2) with different relative contents. Some compounds identified in the different passion fruits like limonene, β-myrcene, β-ionone, 3-carene and others, have been reported as common components of various passion fruits [3,5].

The different species of passion fruit showed a typical composition (Fig. 7A; Table 2). Purple passion fruit (Fig. 7A) is characterised by a great number of identified compounds (51). The dominant compounds found in this variety of *Passiflora* fruits were hexyl butanoate (23.2%), hexyl hexanoate (18.4%), ethyl hexanoate (9.3%), cis-edulan I (5.6%), 1-methylhexyl butanoate (3.9%), ethyl butanoate (3.3%) and (*Z*)-3-hexenyl butanoate (3.0%).

In banana passion fruit aroma, 22 volatile compounds were identified (Fig. 7B; Table 2). The biggest peaks correspond to (*Z*)-β-ocimene (56.6%), hexyl butanoate (16.8%), hexyl hexanoate (13.9%) and hexanol (3.1%). Eight compounds — butyl acetate, butan-1-ol, β-myrcene, eucalyptol, (*E*)-β-ocimene, 3-carene, 3-methylhexyl butanoate, and hexyl-2-butenoate, have been detected only in this species with the same experimental conditions.

In yellow passion fruit (Fig. 7C; Table 2) 24 volatile compounds were detected and tentatively identified. The major compounds found in this variety were methyl hexanoate (32.9%) followed by (*E*)-methyl-2-hexenoate (11.7%), methyl benzoate (11.3%), methyl dihydrojasmonate (6.2%), and dihydro β-ionone (4.9%). This species presents fewer compounds than purple passion fruit. Some of the identified volatiles were found only in this species of passion fruit such as (*E*)-methyl-2-hexenoate, methyl benzoate, 3-hydroxymethyl hexanoate, 2-(2-butoxyethoxy)ethanol, β-ionone, methyl dodecanoate, isopropyl miristate, ethyl cinnamate, isopropyl miristate, nonanoic acid, methyl hexadecanoate and methyl dihydrojasmonate. Fig. 8 presents the distribution of compounds classes by passion fruit sample.

3.4. Principal component analysis

The principal component analysis (PCA) was performed to study the main sources of variability between the passion fruit samples and detect the potential relationships/variables responsible for differentiation. The PCA led to the extraction of two principal components (eigenvalues >1) which contributed to 100.0% of the total variance of the data set (Table 3). The first principal component (PC1) identified as a linear combination of hexan-1-ol (1.000), butyl butanoate (0.997) and p-menth-1-en-8-ol (0.971) accounted for 64.41% of the variance. The second principal component (PC2) was strongly characterized by octan-1-ol (1.000), penten-2-one (0.999), octyl octanoate (0.991) and hexyl acetate (0.984), and this factor contributed to 35.59% of the total

Notes to Table 2:
–: not detected.
[a] Kovàts retention indices from the literature.
[b] Experimentally determined Kovàts indices on the BP-20 column, relative to C_8–C_{20} hydrocarbons.
[c] MHTP: 2-methyl-3-hydroxy-2,4,4-trimethylpentyl propanoate.
[d] DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one.

Table 3
Percentage of variance and percentage of cumulative variance explained by the four extracted principal components.

Total variance explained									
Component	Initial Eigenvalues			Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	13.527	64.412	64.412	13.527	64.412	64.412	10.832	51.580	51.580
2	7.473	35.588	64.412	7.473	35.588	100.00	10.168	48.420	100.00

Table 4
Loadings of volatiles in the first two principal components (1-PC1 and 2-PC2; rotation method: Varimax with Kaiser normalization; CC: carbonyl compounds; EE: ethyl esters; Alc: higher alcohols; NMS: norisoprenoids/monoterpenoids/sesquiterpenoids).

Rotated component matrix[a]		
	Component	
	1	2
Zscore(c6oh)	1.000	.028
Zscore(c4c4)	.997	.079
Zscore(mc4cl)	−.991	.133
Zscore(menthene)	.971	.239
Zscore(ionone)	−.962	−.273
Zscore(Eocymene)	.915	−.403
Zscore(Zocymene)	.906	−.422
Zscore(mc4c)	−.879	−.477
Zscore(c6ooh)	−.852	−.524
Zscore(ddmp)	−.840	−.542
Zscore(mc6)	−.808	−.589
Zscore(c8oh)	.002	1.000
Zscore(pent2one)	−.038	−.999
Zscore(c8c8)	.131	.991
Zscore(c6acetate)	.178	.984
Zscore(c4c2)	−.237	.971
Zscore(c4c6)	.409	.913
Zscore(c2c6)	−.438	.899
Zscore(mbc4)	.496	.868
Zscore(c6c4)	.592	.806
Zscore(c6c6)	.640	.768

Extraction method: principal component analysis.
Rotation method: Varimax with Kaiser normalization.
[a] Rotation converged in 3 iterations.

variance. The scores scatter plot of PCA scores (Table 4) illustrated in Fig. 9A and B, demonstrate a clear separation between *P. edulis* Sims *flavicarpa* (MB), *P. edulis* Sims (MP) and *P. mollissima* (MY). This shows that the variables selected are able to explain the differences observed between groups.

4. Conclusions

The headspace solid-phase microextraction technique coupled with GC-qMS provides an appropriate and selective way to characterize the volatile compounds in different passion fruit species.

The chromatographic profiles obtained after extraction with PDMS, PA, CAR/PDMS, StableFlex and PDMS/DVB coatings suggest that the latter was the most suitable for the SPME analysis of passion fruit volatile organic compounds. The main volatile compounds identified in *Passiflora* fruits belong to the esters. The SPME procedure allowed the identification of 51 compounds in the *P. edulis* Sims, the prevailing compounds being: hexyl butanoate (23.2%), hexyl hexanoate (18.4%), ethyl hexanoate (9.3%) followed by cis-edulan I (5.6%). Only 24 volatile compounds were identified in the yellow passion fruit aroma, the most intense being methyl hexanoate (32.9%) followed by (*E*)-methyl-2-hexenoate (11.7%) and methyl benzoate (11.3%). In *P. mollissima* aroma the predominant volatiles were (*Z*)-α-ocimene (56.6%), hexyl butanoate (16.8%) and hexyl hexanoate (13.9%).

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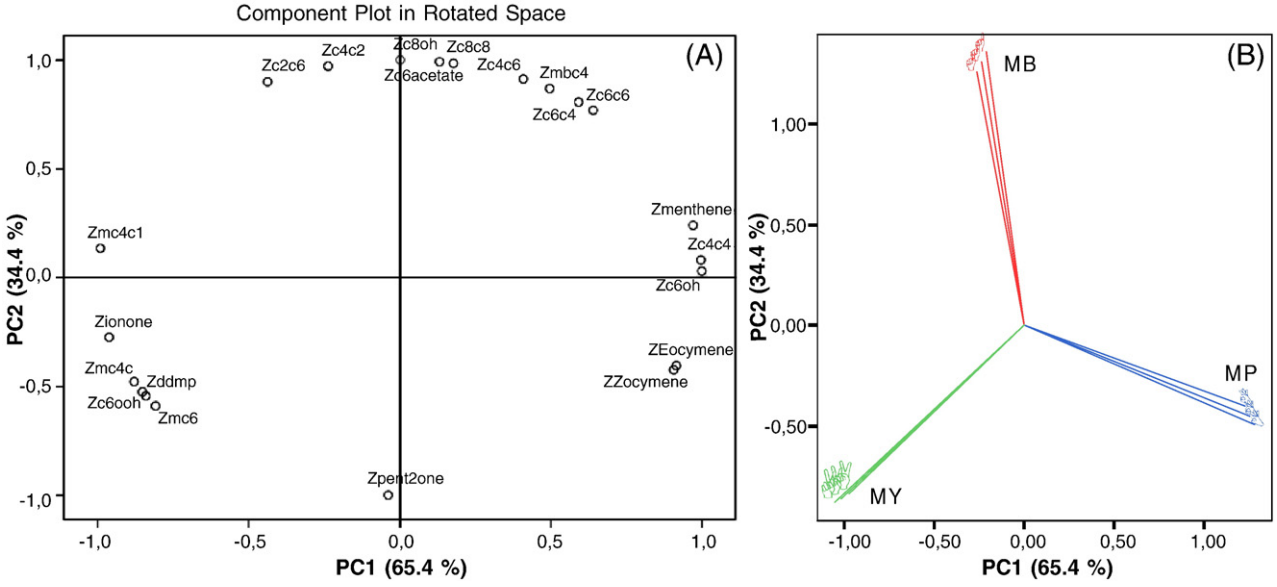

Fig. 9. PC1 and PC2 scatter plot of the main sources of variability between passion fruit samples. (A) Relation between the chemical classes (loadings); (B) Distinction between the samples (scores); Variables identification: pent2one: pentan-2-one; mc4c: methyl butanoate; mc4c1: 2-methyl methylbutanoate; c4c2: ethyl butanoate; mc6: methyl hexanoate; mbc4: 1-methylbutyl butanoate; c4c4: butyl butanoate; c2c6: ethyl hexanoate; Eocymene: (E)-β-ocimene; Zocymene: (Z)-β-ocimene; c6acetate: hexyl acetate; c6oh: hexan-1-ol; c4c6: butyl hexanoate; c6c4: hexyl butanoate; c8oh: octan-1-ol; c6c6: hexyl hexanoate; menthene: p-menth-1-en-8-ol; c8c8: octyl octanoate; c6ooh: hexanoic acid; ionone: dihydro β-ionone; ddmp: DDMP.

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