Liseth Ferreira
Rosa Perestrelo
Michael Caldeira
José S. Câmara

Centro de Química da Madeira, Departamento de Química, Universidade da Madeira, Campus Universitário da Penteada, Funchal, Portugal

Original Paper

Characterization of volatile substances in apples from *Rosaceae* family by headspace solid-phase microextraction followed by GC-qMS

The volatile composition of different apple varieties of *Malus domestica* Borkh. species from different geographic regions at Madeira Islands, namely Ponta do Pargo (PP), Porto Santo (PS), and Santo da Serra (SS) was established by headspace solid-phase microextraction (HS-SPME) procedure followed by GC-MS (GC-qMS) analysis. Significant parameters affecting sorption process such as fiber coating, extraction temperature, extraction time, sample amount, dilution factor, ionic strength, and desorption time, were optimized and discussed. The SPME fiber coated with 50/30 μm divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS) afforded highest extraction efficiency of volatile compounds, providing the best sensitivity for the target volatiles, particularly when the samples were extracted at 50°C for 30 min with constant magnetic stirring. A qualitative and semi-quantitative analysis between the investigated apple species has been established. It was possible to identify about 100 of volatile compounds among pulp (46, 45, and 39), peel (64, 60, and 64), and entire fruit (65, 43, and 50) in PP, PS, and SS apples, respectively. Ethyl esters, terpenes, and higher alcohols were found to be the most representative volatiles. α-Farnesene, hexan-1-ol and hexyl 2-methylbutyrate were the compounds found in the volatile profile of studied apples with the largest GC area, representing, on average, 24.71, 14.06, and 10.80% of the total volatile fraction from PP, PS, and SS apples. In PP entire apple, the most abundant compounds identified were α-farnesene (30.49%), the unknown compound *m/z* (69, 101, 157) (21.82%) and hexyl acetate (6.57%). Regarding PS entire apple the major compounds were α-farnesene (16.87%), estragole (15.43%), hexan-1-ol (10.94), and *E*-2-hexenal (10.67). α-Farnesene (30.3%), hexan-1-ol (18.90%), 2-methylbutanoic acid (4.7%), and pentan-1-ol (4.6%) were also found as SS entire apple volatiles present in a higher relative content. Principal component analysis (PCA) of the results clustered the apples into three groups according to geographic origin. Linear discriminant analysis (LDA) was performed in order to detect the volatile compounds able to differentiate the three kinds of apples investigated. The most important contributions to the differentiation of the PP, PS, and SS apples were ethyl hexanoate, hexyl 2-methylbutyrate, *E*,*E*-2,4-heptadienal, *p*-ethyl styrene, and *E*-2-hexenal.

Keywords: Apples / GC-qMS / *Malus domestica* Borkh. / Solid-phase microextraction / Volatile profile

Received: January 14, 2009; revised: March 11, 2009; accepted: March 11, 2009

DOI 10.1002/jssc.200900024

1 Introduction

From hybrid origin, the apple tree from *Malus domestica* Borkh. (family Rosaceae) is largely cultivated in Madeira Islands for its edible fruits, where its production has a significant impact on the economic activity of the region, being produced ~3300 Ton *per* year. Because its volatile composition has not yet been characterized, its aroma was subjectively defined as *sui generis*. Aroma vola-

Correspondence: Professor José S. Câmara, Centro de Química da Madeira, Departamento de Química, Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal
E-mail: jsc@uma.pt
Fax: +351-291705149

Abbreviations: CAR, carboxen; **CW**, carbowax; **DVB**, divinylbenzene; **GC-qMSD**, gas chromatography-mass spectrometry; **HS-SPME**, headspace solid-phase microextraction; **LDA**, linear discriminant analysis; **PA**, polyacrylate; **PCA**, principal component analysis; **PP**, ponta do pargo; **PS**, Porto Santo; **RPA%**, percent total peak areas; **SS**, santo da serra; **SPME**, solid-phase microextraction; **SVOCs**, semi-volatile organic compounds; **TIC**, total ion current; **VOCs**, volatile organic compounds

tile compounds are of the utmost importance on the establishment of fruit quality criteria and especially on determining consumer acceptance. Apple aroma profiles are complex since, they are constituted by a large number of volatile compounds (volatile organic compound (VOCs)) that contributed to the overall sensory quality. Over 300 VOCs have been measured in the aroma profile of apples. These compounds include carboxylic esters, alcohols, aldehydes, ketones, acids, and ethers, but just about 20 of these chemicals are character impact compounds. Some are present in very low concentrations and contribute potent aroma characteristics typical of apple flavor (*e.g.*, ethyl 2-methyl butyrate). Others contribute to the aroma intensity (*e.g.*, *E*-2-hexenal) or are related to aroma quality (alcohols). The contribution of each volatile compound depends on both its odor threshold and their respective concentration that in turn depends on the activity of related enzymes and on substrate availability [1]. Therefore, the final aroma profile of a fruit is the result of a balance between all volatile compounds emitted and any modification in this fine balance would result in changes in the fruit flavor [2]. The volatile composition of apples depends on several factors, mainly, cultivar, cultural practices, climacteric conditions, and the state of fruit maturity [3]. Although there is a great range of compounds in the volatile composition of apples, the majority are carboxylic esters and higher alcohols [4–6]. The most abundant compounds are even numbered carbon chains including combinations of ethanoic, butanoic, and hexanoic acids with ethyl, butyl, and hexyl alcohols. Ethyl esters, which are the most significant contributors to apple aroma profile, being generated by esterification of alcohols and acyl-CoA derived from fatty acids and amino acid metabolism. Lipoxygenase enzyme has also been reported to play an important role on the formation of straight-chain volatile aldehydes and alcohols through a number of different pathways. Other important aroma compounds are branched-chain volatile compounds, which are derived from branch-chained amino acids leucine, isoleucine, and valine, as well as from alanine and aspartic acid. It has also been noticed that fruits produce acetaldehyde and alcohol during their maturation and ripening [4, 6, 7]. However, only a few volatile compounds have a decisive impact on the sensory quality of apple fruits, such as ethyl acetate, ethyl butyrate, and methyl anthranilate [8]. The contribution of each volatile compound depends on both, its odor threshold and their respective concentration that in turn depends on the activity of related enzymes and on substrate availability [1]. Therefore, the final aroma profile of a fruit is the result of a balance between all volatile compounds emitted and any modification in this fine balance would result in changes in the fruit flavor [2].

The development of analytical methodologies for aroma characterization of food products has been important for the identification of volatiles and understanding their role in aroma and organoleptic quality. Several extraction techniques such as headspace, purge, and trap, liquid–liquid extraction (LLE) and SPE are described in different studies; however, these methods are based on the use of solvents and present some drawbacks such as possibility of sample contamination and the loss of some important volatiles, depending on solvent selectivity and volatility, during the concentration step. Additionally they require large amounts of sample, are laborious and time consuming methods [9, 10]. Solid-phase microextraction (SPME) is a solventless extraction technique that presents itself as an alternative to the conventional sample extraction techniques [9, 11] and moreover is faster and easier than solvent extractions and distillations, as well as being highly reproducible and sensitive. Moreover a range of fiber coating are commercially available, providing specificity for a wide range of polar, nonpolar, volatile, and semivolatile compounds. This technique has been applied to the analysis of volatile and nonvolatile compounds (in gaseous, solid, and liquid samples) present in several matrixes and also for the analysis of volatiles in a large variety of fruits, such as apples [1, 12–19], peaches [5], pears [5, 20], strawberries [9, 21], annonas [22], among others. Its suitability has also been verified in the detection of characteristic aromas, off-flavors, pesticides, and even antibiotics in various food matrices, as for example, wine [10, 23–25], whisky [26], and beer [27].

Since headspace solid-phase microextraction (HS-SPME) is an equilibrium technique, it requires a previous optimization of sampling conditions in order to obtain high recoveries of volatiles and good precision of the method, since several parameters associated with the extraction process influence the extraction efficiency of VOCs [24, 28]. Therefore, the aim of this study is to characterize the volatile (VOCs) and semi-volatile organic compounds (SVOCs) composition of *M. domestica* Borkh. apple species cultivated at different geographical regions in Madeira Islands namely Ponta do Pargo (PP), Porto Santo (PS), and Santo da Serra (SS), using HS-SPME followed by GC-MS (GC-qMS) analysis and identify possible geographic markers. A preliminary screening of six fibers commercially available with different polarities was carried out in order to select the best suited coating for volatile extraction. Other experimental parameters that might affect the HS-SPME methodology such as extraction time and temperature, ionic strength, sample amount, dilution factor, and desorption time, were also tested and evaluated. Multivariate techniques of data analysis – PCA and LDA – were employed, to establish differentiation criteria as a function of the apple variety and to detect the volatile compounds able to differenti-

ate the three apple varieties investigated. To our knowledge, there are no reports in the literature on the aroma compounds of these apples species cultivated at Madeira Island.

2 Materials and methods

2.1 Chemicals and reagents

Both, the SPME commercial fibers and the SPME holder for manual sampling were supplied by Supelco (Aldrich, Bellefonte, PA, USA). The reagents and solvents used in this study were of analytical quality and HPLC grade, higher than 98%. Sodium chloride was purchased from Panreac (Barcelone, Spain) and C_8-C_{20} *n*-alkanes series from Sigma–Aldrich (Buchs, Switzerland). Ultra pure water was obtained from a Milli-Q system (Millipore).

2.2 Fruit samples

The apple samples from PP, PS, and SS used in this study were harvested at commercial maturity during the 2007 season and purchased from traditional local stores. The apples of each location were cleaned, deseeded, and the bulbs and talks were removed. In order to homogenize the apple samples (pulp, peel, and entire fruit) each piece of fruit was cut into small pieces and immediately transferred into a domestic blender. An amount (3%, w/v[a]) of calcium chloride ($CaCl_2$) was added to inhibit the enzyme activity. The mixture was stored in glass vials at −20°C until analysis. All analyses were carried out in triplicate.

2.3 HS-SPME optimization

The HS-SPME experimental parameters such as fiber coating, extraction temperature and time, sample amount, dilution factor (water volume), ionic strength, and desorption time were systematically evaluated. To assess the effects of these experimental factors on the extraction efficiency of VOCS, the number of the tentatively identified compounds and the total peak areas were used as parameters to optimize the methodology. The optimization of the dynamic headspace method of extraction was done with PP apple pulp.

Six fibers were used to evaluate the effect of different coatings on the extraction efficiency of VOCs and SVOCs from apple samples: carbowax-divinylbenzene (CW/DVB, 70 μm), DVB/carboxen (CAR)/PDMS (50:30 μm), CAR/PDMS (75 μm), polyacrylate (PA, 85 μm), PDMS/DVB (65 μm), and PDMS (100 μm). Prior to use, fibers were conditioned according to the manufactures' instructions. Then, each fiber was exposed to the headspace of a 4 mL septum-sealed glass vial containing 0.50 g of sample, 0.50 mL of water, and 0.10 g of NaCl at the same temperature and time (30°C and 30 min, respectively) under constant magnetic stirring of 800 rpm. Before sampling, each fiber was reconditioned for 15 min in the GC injection port at 250°C to eliminate possible remains on the coating. The optimization of the other experimental parameters was performed with extractions at different temperatures (30, 40, and 50°C), times (15, 30, 60, and 75 min), sample amounts (0.50, 0.75, and 1.00 g), water volumes (0.50, 1.00, and 1.50 mL), NaCl amounts (0.10, 0.20, and 0.30 g) and desorption times (3, 6, and 9 min).

2.3.1 HS-SPME procedure

After the selection of the best HS-SPME sampling conditions, the following procedures were carried out using 0.75 g of apple sample in a 4 mL septum-sealed glass vial containing 1 mL of water and 0.10 g of NaCl. The system was placed in a thermostated bath adjusted to 50°C under constant magnetic stirring (800 rpm). The DVB/CAR/PDMS fiber was then exposed to the headspace for 30 min to promote the compounds transfer from the sample to the headspace. Following the sampling procedure, the SPME fiber was retracted prior removal from the sample container and immediately inserted into the GC system injection port at 250°C for 6 min, where the analytes were thermally desorbed and transferred directly to the GC system column. Before daily analysis, the fiber was preconditioned for 15 min in the GC system injection port at 250°C. At least three replicates were done for each sample. Considering that the polymer phase of fibers can absorb/adsorb aroma chemicals from air and produce a high background in the chromatogram, blank runs were conducted between extractions to verify the absence of any carry over which would cause memory effects and result discrepancy [24, 25].

2.4 GC-qMS analysis

The fiber containing the volatile compounds was introduced into the GC injector at 250°C and kept for 6 min for thermal desorption. The desorbed volatile compounds extracted were separated and tentatively identified using an Agilent 6890N gas chromatograph system (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5975 quadrupole inert mass selective detector. A BP-20 fused silica capillary column (30 m × 0.25 mm id × 0.25 μm film thickness) was used for the GC separation. Splitless injection was employed using helium as the carrier gas (Helium N60, *Air liquide*, Portugal) at a flow rate of about 1 mL/min (column head pressure 13 psi). The initial oven temperature was 40°C, followed by a linear programmed temperature from 40 to 220°C at rate of 3°C/min and held for 10 min at the end. The ion

[a] According to editor and reviewers suggestion

source and transfer line temperatures were 220°C. Quadrupole mass detector was operated at 180°C in the 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*. For the determination of Kovat's index (KI), a C_8 – C_{20} *n*-alkanes series was used.

The volatile compounds were identified by matching mass spectra with spectra of reference compounds in the National Institute of Standards and Technology (NIST05) Mass Spectral Search Program. In addition, the compounds were tentatively identified by comparing the experimental retention indices with the theoretical ones obtained from literature. The relative amounts of the individual components are expressed as percentage relative to the total area (RPA, %).

2.5 Statistical analysis

PCA was used to examine the relationship among the composition and the wine variety. It is an unsupervised technique that reduces the dimensionality of the original data matrix retaining the maximum amount of variance. LDA is a supervised technique method used for classification purposes. Both methods were carried out using the SPSS Program, version 11.0 (SPSS Headquarters, Chicago, IL, USA) and were applied to the normalized areas of the volatiles identified by HS-SPME$_{DVB/CAR/PDMS}$/GC-qMS.

3 Results and discussion

3.1 HS-SPME optimization

According to the proposed methodology, the volatile compounds were extracted from apple samples using the HS-SPME technique that is very sensitive to experimental conditions. Since it is an equilibrium technique, any modification of an experimental parameter will change the distribution coefficient and absorption rate, influencing the amount absorbed/adsorbed by the SPME fiber and its corresponding reproducibility [24]. Therefore, the experimental parameters that influence the extraction efficiency such as fiber coating, extraction temperature and time, sample amount, dilution factor (water volume), ionic strength (NaCl amount), and desorption time were systematically studied.

3.1.1 SPME Fiber coating

The extraction efficiency of volatile compounds by the HS-SPME technique strongly depends on their polarity and, consequently, on their affinity to the fiber-coated phase [9, 29]. Therefore, six commercial types of fibers (70 μm CW/DVB, 50/30 μm DVB/CAR/PDMS, 75 μm CAR/PDMS, 85 μm PA, 65 μm PDMS/DVB, and 100 μm PDMS) were used to evaluate the effect of different coatings on the extraction efficiency of volatile compounds in apple samples. The total ion current (TIC) chromatograms of volatile compounds identified in PP apple pulp extracts isolated by HS-SPME using different fiber coatings are shown in Fig. 1. The comparison among each TIC shows different GC profiles. The results illustrated in Fig. 2 indicate that the DVB/CAR/PDMS and PDMS/DVB coatings had higher extraction efficiency and a clear pattern of volatile compounds relatively to the other fibers. Despite the PDMS/DVB fiber coating showed a similar extraction performance comparatively to DVB/CAR/PDMS, the former fiber coating showed a higher RSD% value than the latter (Fig. 2). As reported in Table 1, the differences of extraction efficiency of volatile compounds between each fiber using the same extraction conditions are noticeable. It can be observed that the fibers displayed different selectivity to different groups of compounds, where ethyl esters accounted for the largest chemical class identified (Table 1). It is also clear that the DVB/CAR/PDMS fiber showed a better qualitative and quantitative behavior for apple volatiles than the remaining five studied fibers with a total of 58 volatile compounds, while PDMS/DVB fiber extracted 53 volatile compounds. The remaining fibers PA, CAR/PDMS, CW/DVB, and PDMS extracted less volatile compounds, accounting with 44, 43, 36, and 24 VOCs, respectively. Under the same conditions, the DVB/CAR/PDMS fiber proved to be the most universal and efficient for the isolation of PP apple pulp volatile compounds with different physic-chemical properties, while the nonpolar PDMS fiber showed the lowest adsorptive capacity, but with a good selectivity for middle to high-molecular weight analytes.

3.1.2 Extraction temperature and time

Extraction temperature and time are significant parameters in HS-SPME, since both have an effect on the equilibrium during extraction of volatile compounds [29]. The extraction temperature has a significant influence because the distribution coefficient between the sample and the headspace and between the headspace and the fiber is influenced by this specific parameter [28]. Therefore, different extraction temperatures (30, 40, and 50°C) were studied using the two most efficient fibers (DVB/CAR/PDMS and PDMS/DVB) in order to determine the best fiber coating for the extraction of VOCs from apple samples. The results summarized in Fig. 3 illustrate that for both fibers 50°C presents an increased sensitivity, hence a better extraction efficiency and a lower SD. Consequently, the heating of apple samples improved the release of analytes to the headspace and facilitated the HS-SPME process. Thus, with the temperature increase, the volatiles recovery was enhanced [24, 29]. According the results, 50°C was chosen as the extraction temperature in further experiments. As it can be observed from

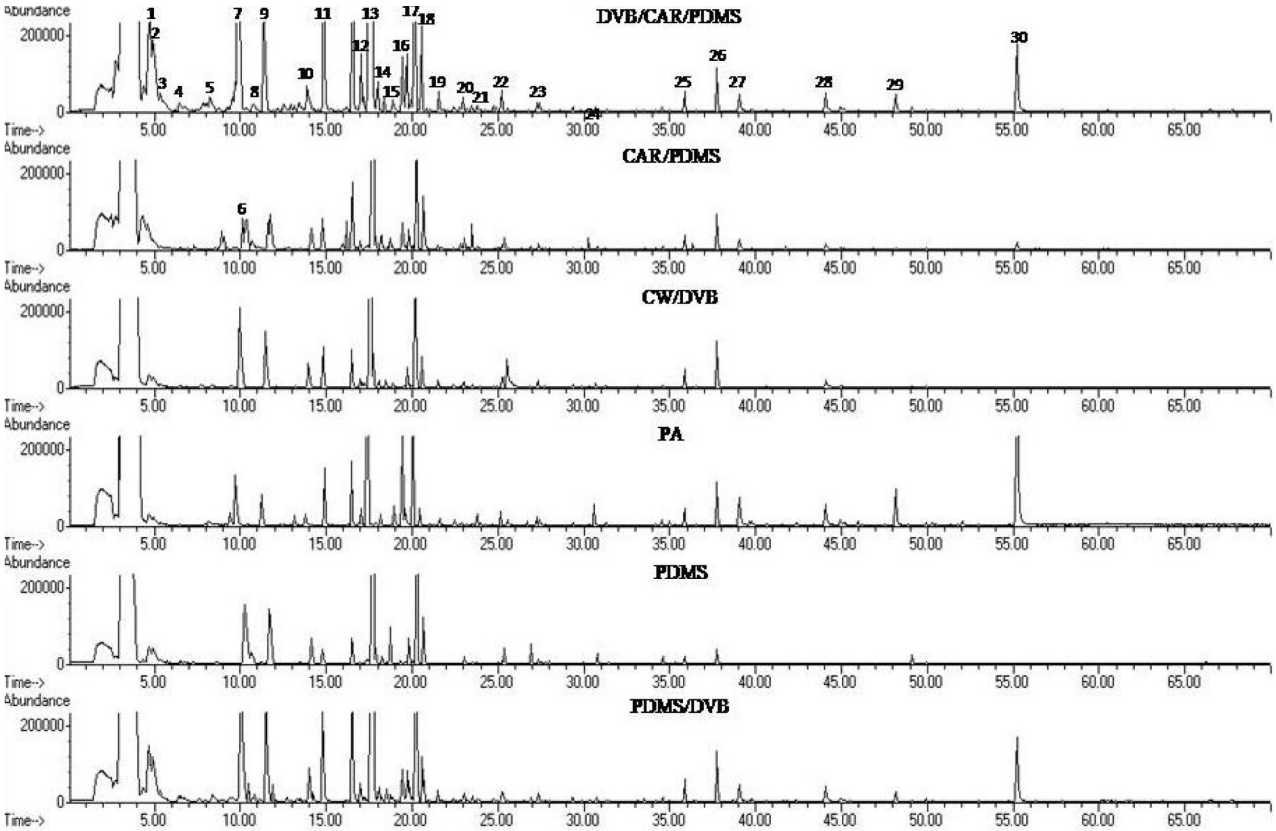

Figure 1. Typical HS-SPMS/GC-qMS chromatograms (TIC) of the volatile composition of pulp from PP apple using different fiber coatings. Peak identification: 1, ethyl butyrate; 2, ethyl 2-methylbutyrate; 3, butyl acetate; 4, 2-methylbutyl acetate; 5, methyl hexanoate; 6, *E*-hexenal; 7, ethyl hexanoate; 8, styrene; 9, hexyl acetate; 10, *E* -2-hexen-1-ol acetate; 11, hexan-1-ol; 12, *E*-2-hexen-1-ol; 13, N. I. *m*/*z* (69, 105, 157); 14, ethyl octanoate; 15, furfural; 16, *E*-2-hexenyl butyrate; 17, 3,4,5-trimethyl-4-heptanol; 18, 2,4-dimethyl-4-octanol; 19, ethyl 3-hydroxybutyrate; 20, ethyl 3-(methylthio)-propionate; 21, 4-carvomenthenol; 22, hexyl hexanoate; 23, ethylbenzoate; 24, methionol; 25, ethyl 3-hydroxydodecanoate; 26, diethyl malate; 27, 2,5-furandicarboxaldehyde; 28, 1,3-octanediol; 29, 2-ethylhexyl salicylate; 30, 5-hydroxymethyl-2-furfural.

Fig. 3, the DVB/CAR/PDMS fiber coating showed a better extraction efficiency than PDMS/DVB. Based on these results this coating was chosen to perform the extraction of VOCs and SVOCs from *M. domestica* Borkh. apple species.

The effect of extraction time was studied by increase in the exposure time of the DVB/CAR/PDMS fiber to the headspace, from 15 to 75 min, maintaining the remaining experimental parameters strictly the same during sampling. The influence of this parameter is displayed in Fig. 3. As can be seen the best extraction efficiency of VOCs from apple samples was obtained with 75 min. It was also observed a typical extraction profile that consists of an initial portioning followed by a "steady-state" equilibrium between the fiber and the vapor phase of analytes. For practical purposes, the extraction time of 30 min was adopted because the volatile profile and the total number of obtained compounds are identical comparatively to the volatile profile obtained with an extraction time of 75 min.

3.1.3 Sample amount

The sample amount was found to be volume dependent, even when 1.0 g of sample was placed inside the 4.0 mL vial instead of 0.75 or 0.5 g. However, larger sample amounts do not mean better results [29]. As seen in Fig. 4, the best extraction efficiency was obtained with 0.5 g of sample associated with a high SD, whereas 0.75 g of sample also showed good extraction efficiency but with a lower deviation. Finally, the lowest total peak area was obtained using 1.0 g of sample and a high SD was observed. Considering the results, 0.75 g was selected as a sample amount to perform the volatiles extraction.

3.1.4 Dilution factor

Another procedure that can be adopted in order to improve the transfer of volatile compounds from the solid phase to the gaseous-headspace phase is the addition of water to the sample matrix. Therefore, the homogenization of apple samples was performed in

Table 1. Volatile compounds identified in PP apple pulp by HS-SPME/GC-qMS using different fiber coatings (extraction temperature, 30°C; extraction time, 30 min, 800 rpm)

RT (min)	Compounds	Identification[a)]	SPME coating					
			DVB/CAR/PDMS	PDMS/DVB	CAR/PDMS	PDMS	CW/DVB	PA
4.68	Ethyl butyrate	A, B, C	x	x	x	x	x	x
4.97	Ethyl 2-methylbutyrate	A, C	x	x	x	x	x	x
5.36	Butyl acetate	A, B, C	x	x	x	x	x	x
5.63	Hexanal	A, B, C	x	–	–	–	–	–
6.45	2-Methylbutyl acetate	A, C	x	x	–	–	–	–
7.77	Butan-1-ol	A, B, C	x	x	x	x	x	x
8.35	Methyl hexanoate	A, C	x	x	x	x	x	–
9.16	Butyl butyrate	A, C	x	x	–	–	x	–
9.59	*E*-2-Hexenal	A, C	x	x	x	–	–	–
9.93	Ethyl hexanoate	A, B, C	x	x	x	x	x	x
10.72	Styrene	A, B, C	x	x	x	–	–	x
11.43	Hexyl acetate	A, B, C	x	x	x	x	x	x
13.14	Propyl hexanoate	A, C	x	x	–	–	x	x
13.44	Z-2-Heptenal	A, C	x	–	–	–	–	x
13.79	Ethyl heptanoate	A, C	x	x	–	–	–	–
13.93	*E*-2-Hexenyl acetate	A, C	x	x	x	x	x	x
14.08	6-Methyl-5-hepten-2-one	A, C	x	–	–	–	–	–
14.80	Hexan-1-ol	A, B, C	x	x	x	x	x	x
16.22	Nonanal	A, C	x	x	–	–	–	–
16.99	*E*-2-Hexen-1-ol	A, B, C	x	x	x	x	x	x
17.18	Butyl hexanoate	A, C	x	x	x	x	x	x
17.54	NI (69,101,157)	A, C	x	x	x	x	x	x
17.90	Hexyl 2-methylbutyrate	A, C	x	x	x	x	x	–
17.89	*p*-Ethylstyrene	A, C	x	–	x	–	x	–
18.25	Ethyl octanoate	A, B, C	–	x	–	x	–	x
18.96	Acetic acid	A, B, C	x	x	x	x	x	x
19.50	Furfural	A, B, C	x	x	x	–	x	x
19.70	*E*-2-Hexenyl butyrate	A, C	x	x	x	x	–	x
20.21	3,4,5-Trimethyl-4-heptanol	A, C	x	x	x	x	x	x
20.58	2,4-Dimethyl-4-octanol	A, C	x	x	x	x	x	x
20.86	*Z*-3-Octenyl acetate	A, C	x	–	–	–	–	–
20.98	1-(2-Furanyl)-ethanone	A, C	x	–	–	–	–	x
21.52	Ethyl 3-hydroxybutyrate	A, C	x	x	x	–	x	x
22.40	R,S-2,3-Butanediol	A, B, C	x	x	–	–	x	x
23.18	Octan-1-ol	A, B, C	x	x	x	–	–	x
23.47	Ethyl 3-(methylthio)-propionate	A, C	x	x	–	–	–	–
23.81	5-Methyl-2-furfural	A, B, C	x	x	x	–	–	x
24.72	4-Carvomenthenol	A, C	x	x	x	x	x	x
25.26	Hexyl hexanoate	A, C	x	x	x	x	x	x
25.30	Methyl benzoate	A, C	–	–	x	–	–	–
25.54	*Z*-5-Octen-1-ol	A, C	–	x	–	–	–	–
27.20	3-Methylbutyl octanoate	A, C	–	–	–	–	–	x
27.36	Ethyl benzoate	A, B, C	x	x	x	x	x	x
27.41	2-Furanmethanol	A, C	x	–	–	–	–	x
27.44	Z-β-Farnesene	A, C	–	–	–	–	–	x
27.60	*E*-2-Hexenyl hexanoate	A, C	x	x	x	–	–	–
27.97	Ethyl 3-hydroxyhexanoate	A, B, C	x	x	x	–	–	–
28.58	5-Ethyl-dihydro-2-(3H)-furanone	A, C	x	–	–	–	–	–
29.38	Methionol	A, B, C	x	x	x	–	x	x
29.85	Azulene	A, C	x	x	x	–	x	x
30.35	Ethyl 3-(methylthio)-*E*-2-propenoate	A, C	–	x	–	–	x	x
30.77	α-Farnesene	A, C	x	x	x	x	x	x
33.07	α-Damascenone	A, B, C	x	x	x	–	–	–
33.51	Isoestragole	A, C	x	x	x	–	–	x
34.22	Hexanoic acid	A, B, C	x	x	x	–	x	x
34.54	Geranyl acetone	A, B, C	x	x	x	x	x	x
35.88	Ethyl 3-hydroxydodecanoate	A, C	x	x	x	x	x	x
36.49	2-Phenylethanol	A, B, C	x	x	x	–	x	x

Table 1. Continued

RT (min)	Compounds	Identification[a)]	SPME coating					
			DVB/CAR/PDMS	PDMS/DVB	CAR/PDMS	PDMS	CW/DVB	PA
37.74	Diethyl malate	A, B, C	x	x	x	x	x	x
39.07	2,5-Furandicarboxaldehyde	A, B, C	x	x	x	–	–	x
44.09	1,3-Octanediol	A, C	x	x	x	–	x	x
48.15	DDMP	A, C	x	x	x	–	–	x
49.15	2-Ethylhexyl salicylate	A, C	x	x	x	–	x	–
52.93	Benzenecarboxylic acid	A, C	x	x	–	–	x	–
55.18	5-Hydroxymethyl-2-furfural	A, B, C	x	x	x	–	–	x
Chemical classes								
Acids			3	3	2	1	3	2
Higher alcohols			11	11	9	5	9	11
Carbonyl compounds			12	7	6	1	2	7
Esters			23	24	17	14	17	16
Hydrocarbons			2	1	2	0	1	1
Terpenes			7	7	7	3	4	7
Total compounds			58	53	43	24	36	44

a) The reliability of the identification or structural proposal is indicated by the following: A, structural proposals given on the basis of mass spectral data (NIST05); B, mass spectrum and retention time consistent with those of an authentic standard; C, mass spectrum consistent with spectra found in the literature.

– Not detected. DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

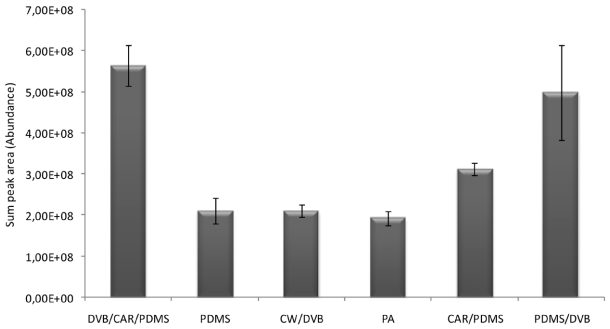

Figure 2. Effect of different fiber coatings on the extraction efficiency of all volatile compounds obtained from PP apple pulp. Error bars represent S.E.M. ($n = 3$ for each data point).

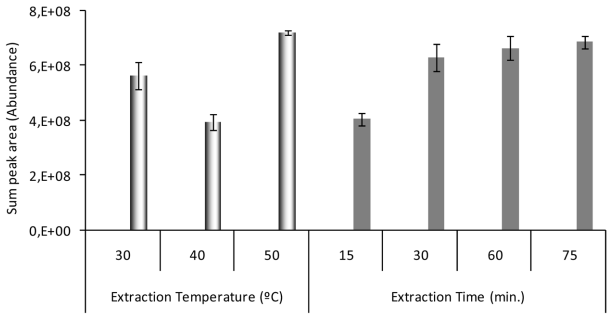

Figure 3. Effect of extraction temperature and time on the total peak areas of volatile compounds from PP apple pulp extract. Error bars represent S.E.M. ($n = 3$ for each data point).

order to produce a matrix to assure the most representative extraction, since the analytes are not distributed uniformly throughout the fruit pulp. Thus, the homogenized samples represent more closely the volatile profile than the entire or even cut apple. As observed in Fig. 4, the increase in water content in the sample matrix resulted in the decrease in extracted volatile compounds. Hence, in order to facilitate the sample agitation and consequent volatile release to the headspace, the addition of 1.0 mL of water was considered for further analysis.

3.1.5 Ionic strength

The dissolution of salt (NaCl) into the sample matrix enhances the partition coefficient of analytes between the headspace and the sample phase and therefore to the fiber [9, 24, 29]. To analyze the salt effect on the sample matrix, three different NaCl amounts (0.1, 0.2, and 0.3 g) were dissolved in order to saturate the sample matrix. Figure 4 shows the variation of the extracted amount of volatile compounds from the matrix with the amount of salt added. From these results, the amount of 0.1 g of NaCl was added to all samples in the remaining assays.

3.1.6 Desorption time

Desorption time was also investigated for 3, 6, and 9 min. The purpose of optimizing desorption time is to eliminate any carryover and improve peak shape [9, 24]. The effects produced by desorption time are illustrated in Fig. 4 as total peak areas. Desorption time of 6 min proved to be enough to have the most complete desorp-

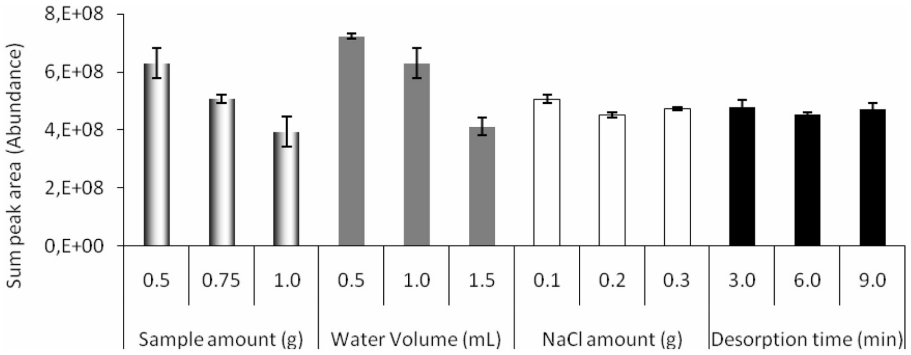

Figure 4. Sample amount, water addition (dilution factor), ionic strength, and desorption time effect on extraction efficiency of VOCs and SVOCs from PP pulp apples by HS-SPME. Error bars represent S.E.M. ($n = 3$ for each data point).

tion of the analytes, since a longer desorption time may damage the fiber, shortening its life time [29]. The higher number of extracted compounds and lower deviation were also factors taken into consideration.

3.2 Characterization of volatiles in apples (pulp, peel, and entire fruit)

Characterization of VOCs and SVOCs compounds was done with the best HS-SPME methodology conditions on commercialized fruits from different geographical origins of Madeira Islands, bought in 2007. About 100 different compounds present in pulp, peel, and entire apple samples from PP, PS, and SS *M. domestica* Borkh. species (19 of which were common to PP, PS, and SS entire fruit, 32 to peel and 27 to pulp (see Table 2)), were extracted followed by GC-qMS analysis and afterward, tentatively identified by matching to US NIST (resemblance percentage above 80%), by matching calculated RI values to literature values, or by injection of authentic standards. Kováts retention indices were calculated for each peak and compared with the literature in order to certify the compound identification. Typical chromatograms (TIC) obtained from pulp, peel, and entire fruit of PP apples using the HS-SPME/GC-qMS methodology with the optimized sampling conditions are shown in Fig. 5. Table 2 lists the VOCs and SVOCs tentatively identified, in the three apple varieties, comprising 39 esters, 20 alcohols, 15 carbonyl compounds, 15 terpenoids, and 5 acids (Table 2). The peels of all three apple varieties presented the higher number of identified compounds, 64, 60, and 64, in PP, PS, and SS, respectively, while the pulp presented the lowest. The number and nature of VOCs and SVOCs varied according apple geographical origin and apple fraction (pulp, peel, and entire fruit). The relative amount percent of the individual components are expressed as percent peak areas relative to total peak areas (RPA%) and are listed in Table 2.

Through a comparative analysis of results shown in Fig. 6 and Table 2, it could be found that the chemical classes that contribute to the total volatile profile of the studied apple samples are terpenes, ethyl esters, and higher alcohols. α-Farnesene was on average the most abundant compound in all three *M. domestica* Borkh. apple varieties studied. Among ethyl esters which are responsible for a fruity, estery aroma, hexyl 2-methylbutanoate (10.80%), hexyl acetate (4.02%), and ethyl hexanoate (3.52%) contribute to apple aroma characteristics as well as to aroma intensity [30]. Higher alcohols and carbonyls of six carbon atoms, namely hexan-1-ol, *E*-2-hexenal, and *E*-2-hexen-1-ol, responsible for the herbaceous odor of several fruits, were also significantly abundant in all apple varieties analyzed. The amount of these compounds was, on average, 14.06, 3.35, and 2.76%, respectively.

The RPA (%) values obtained for the different chemical classes in PP, PS, and SS pulp, peel, and entire fruit samples are illustrated in Fig. 6. Higher alcohols, ethyl esters, carbonyl compounds, and acids represent 45.57, 38.28, 12.59, 7.64, and 1.18%, respectively, of the total volatile fraction in pulp apples. As for peel, the significant contribution for the total volatile profile arises from terpenes (50.94%) followed by ethyl esters (23.71%), higher alcohols (12.94%), carbonyl compounds (7.76%), and acids (1.18%). Finally, for entire fruit samples terpenes, higher alcohols, ethyl esters, carbonyl compounds, and acids accounts for 34.61, 23.27, 19.16, 10.85, and 3.10%, respectively, of the total GC peak area of the chromatograms.

PP apple reports the higher content of VOCs and SVOCs compounds, having the most representative volatile profile in all matrices relatively to PS and SS samples. The total amounts of PP apple compounds were 65.14 and 5.79% higher than those of the PS and SS apples, respectively. Results reported in Table 2 demonstrate that the most prominent constituents found in PP apple pulp were hexyl 2-methylbutyrate (49.05%), 3,4,5-trimethyl-4-heptanol (15.44%), ethyl hexanoate (5.81%), hexyl acetate (5.1%), and hexan-1-ol (4.57%). Relatively to PP apple peel the VOCs and SVOCs with major contribution to their volatile profile are α-farnesene (45.39%), hexyl acetate (12.66%), ethyl hexanoate (6.14%), and *E*-2-hexen-1-ol acetate (4.88%). Concerning PP apple entire fruit these are α-farnesene (30.49%), 3,4,5-trimethyl-4-hexyl acetate (6.57%), heptanol (6.41%), and ethyl hexanoate (3.43%). As for PS apple, the major volatile compounds deter-

Table 2. Relative percent amount (RPA%) of volatile compounds of pulp, peel, and entire fruit from PP, PS, and SS *M. domestica* Borkh. apples obtained by HS-SPME$_{DVB/CAR/PDMS}$ at the optimal sampling conditions (extraction temperature, 50°C; extraction time, 30 min, 800 rpm)

KI	Compounds	Pulp			Peel			Entire fruit		
		PP	PS	SS	PP	PS	SS	PP	PS	SS
Acids										
1437	Acetic acid	0.03	0.73	0.80	0.04	–	0.42	0.18	0.46	0.55
1647	2-Methylbutanoic acid	–	–	1.81	–	–	1.19	–	–	4.66
1835	Hexanoic acid	–	0.17	–	–	0.26	0.11	0.39	0.27	0.33
2332	Benzenecarboxylic acid	–	–	–	–	0.07	0.05	0.03	0.14	–
2392	Hexadecanoic acid	–	–	–	–	0.59	0.81	0.21	1.11	0.97
Alcohols										
1162	Butan-1-ol	0.26	0.88	1.12	0.01	0.40	0.14	0.23	0.66	4.05
1202	2-Methylbutan-1-ol	0.31	11.52	6.00	–	–	–	–	1.94	4.60
1244	Pentan-1-ol	–	0.13	0.24	–	–	0.08	–	0.60	0.82
1342	Hexan-1-ol	4.57	29.77	40.20	2.84	12.32	4.08	2.89	10.94	18.97
1372	*Z*-3-Hexen-1-ol	–	0.23	0.39	–	–	1.48	–	–	–
1388	*E*-2-Hexen-1-ol	0.87	2.28	9.68	0.93	2.60	1.98	0.09	6.12	0.33
1426	*Z*-2-Hexen-1-ol	–	–	–	–	0.33	–	–	–	–
1433	1-Octen-3-ol	–	0.34	–	–	7.27	–	–	–	–
1438	Heptan-1-ol	–	–	–	–	–	–	–	0.48	0.64
1468	3,4,5-Trimethyl-4-heptanol	15.44	2.30	2.29	1.50	0.48	0.78	6.41	–	2.38
1473	2-Ethyl hexan-1-ol	–	0.39	0.43	–	–	–	–	0.43	0.23
1477	2,4-Dimethyl-4-octanol	1.88	–	–	0.27	–	–	1.19	–	–
1539	Octan-1-ol	0.28	0.60	0.63	0.08	0.32	0.10	0.32	0.78	1.11
1591	*Z*-5-Octen-1-ol	0.08	0.11	0.32	0.07	–	0.02	–	–	0.46
1640	2-Furanmethanol	–	0.44	–	–	–	–	–	–	–
1694	Methionol	0.15	0.05	–	–	–	–	0.09	–	0.17
1748	Dodecan-1-ol	0.21	0.34	0.57	0.03	–	0.05	0.10	0.55	0.44
1777	1-(2-Butoxyethoxy)-ethanol	0.03	0.13	–	–	–	0.35	0.09	–	–
1898	2-Phenylethanol	0.05	0.09	–	0.10	–	0.04	0.10	–	0.22
2224	1,3-Octanediol	1.10	–	–	0.49	–	–	0.71	–	0.68
Carbonyls										
1095	Hexanal	0.12	3.66	1.81	0.29	2.14	0.55	0.18	5.14	1.41
1209	*E*-2-Hexenal	5.81	4.74	–	0.25	2.74	1.26	0.87	10.67	2.90
1310	*E*-2-Heptenal	0.05	0.62	0.67	0.03	1.11	0.20	0.10	0.36	0.99
1325	6-Methyl-5-hepten-2-one	–	4.14	1.26	2.16	5.77	1.67	–	1.32	1.27
1373	Nonanal	0.07	0.68	0.84	0.03	0.74	0.17	0.18	0.14	0.78
1450	2-Furfural	0.12	0.79	1.31	–	0.11	0.67	0.09	0.66	0.35
1472	*E*,*E*-2,4-Heptadienal	–	–	–	–	0.31	0.31	–	–	–
1482	Decanal	–	0.73	0.68	–	0.18	0.07	–	1.28	0.85
1501	Benzaldehyde	–	–	–	–	0.24	0.27	–	0.71	0.37
1515	*E*-2-Nonenal	–	–	–	–	0.35	0.08	–	0.31	0.14
1553	5-Methyl-2-furfural	–	–	–	–	0.03	0.11	0.02	–	–
1613	*E*-2-Decenal	–	–	–	–	0.55	0.29	–	0.53	0.37
1670	5-Ethyl-dihydro-2-(3H)-furanone	0.04	–	–	–	–	–	–	–	–
1966	2,5-Furandicarboxaldehyde	–	–	0.60	–	–	0.03	0.03	0.33	–
2342	5-Hydroxymethyl-2-furfural	–	–	–	–	0.05	0.51	0.04	0.17	–
Esters										
1055	Ethyl butyrate	1.29	1.67	2.38	0.28	0.24	0.25	0.56	–	1.73
1067	Ethyl 2-methylbutyrate	0.51	0.87	1.37	0.44	0.68	0.22	0.29	1.98	0.42
1084	Butyl acetate	0.13	–	–	0.07	–	–	0.28	0.17	–
1123	2-Methylbutyl acetate	0.17	0.72	–	0.13	0.14	–	0.17	0.61	–
1177	Methyl hexanoate	0.21	1.46	0.85	0.27	0.37	0.08	0.24	–	–
1196	Butyl butyrate	–	0.84	8.13	0.03	0.39	0.07	–	–	–
1219	Ethyl hexanoate	–	–	3.51	6.14	1.14	1.06	3.43	–	–
1261	Hexyl acetate	5.10	1.06	–	12.66	1.13	0.76	6.57	0.86	–
1287	Ethyl 3-hexenoate	–	–	–	0.30	–	0.06	0.06	–	–
1294	Z-3-Hexenyl acetate	–	–	–	0.13	–	0.07	0.10	–	–
1302	Propyl hexanoate	0.06	0.72	0.94	0.10	0.15	0.06	0.05	0.20	0.14
1319	Ethyl heptanoate	–	–	–	0.08	0.03	0.02	0.03	–	–
1322	*E*-2-Hexenyl acetate	2.43	–	–	4.88	0.32	0.11	3.94	–	–
1368	Methyl octanoate	–	–	–	0.08	–	–	–	–	–
1380	*E*-2-Hexenyl propionate	–	–	–	0.03	0.92	–	–	–	–

Table 2. Continued

KI	Compounds	Pulp			Peel			Entire fruit		
		PP	PS	SS	PP	PS	SS	PP	PS	SS
1392	Butyl hexanoate	0.06	6.31	1.17	0.16	2.76	1.34	0.64	0.70	1.65
1406	Hexyl 2-methylbutyrate	49.05	4.79	1.53	–	8.51	1.37	–	9.39	0.97
1418	Ethyl octanoate	0.17	–	–	2.25	0.33	0.30	1.10	–	–
1436	Isopentyl hexanoate	–	–	–	–	0.32	–	–	–	–
1455	*E*-2-Hexenyl butyrate	1.31	0.59	0.47	0.60	0.69	0.23	0.56	0.36	0.37
1462	*E*-2-Hexenyl pentanoate	–	–	–	0.06	1.59	0.37	0.84	–	–
1496	Pentyl hexanoate	–	0.12	–	–	0.56	0.17	–	0.38	0.12
1499	Ethyl 3-hydroxybutyrate	0.34	–	–	0.23	–	–	0.22	–	–
1506	Butyl 2-methylbutyrate	–	–	–	–	0.12	–	–	–	–
1530	Ethyl *E*-2-octenoate	–	–	–	0.10	0.10	–	0.08	–	–
1546	Ethyl 3-(methylthio)-propionate	0.23	–	–	–	–	–	0.11	–	–
1585	Hexyl hexanoate	0.61	0.97	0.20	0.64	3.47	1.95	1.00	2.51	1.51
1586	Methyl benzoate	–	–	–	–	–	–	0.19	–	–
1587	Butyl octanoate	–	–	–	0.04	0.80	0.09	0.02	–	–
1610	Ethyl decanoate	–	–	–	0.25	–	–	0.19	–	–
1635	3-Methylbutyl octanoate	–	–	–	–	0.42	–	–	–	–
1639	Ethyl benzoate	0.12	–	–	0.16	–	0.10	0.38	–	–
1646	*E*-2-Hexenyl hexanoate	–	–	–	0.14	1.09	–	0.27	–	–
1656	Ethyl 3-hydroxyhexanoate	0.09	–	–	0.04	–	–	–	–	–
1689	Octyl heptanoate	–	–	–	–	0.24	–	–	–	–
1722	Ethyl *E*-3-(methylthio)-2-propenoate	0.07	–	–	0.16	–	–	0.39	–	–
1800	2-Phenylethyl acetate	–	–	–	0.07	–	–	0.12	–	–
1801	Hexyl octanoate	–	–	–	–	0.31	0.09	–	–	–
1831	Ethyl *E,Z*-2,4-decadienoate	–	–	–	0.62	–	–	0.51	–	–
1881	Ethyl 3-hydroxydodecanoate	1.87	–	–	0.71	–	–	2.20	–	0.28
1932	Diethyl malate	4.27	0.14	0.29	2.62	–	0.39	6.30	–	1.22
2308	Ethyl hexadecanoate	–	–	–	0.03	0.10	0.05	0.20	0.24	0.62
2314	2-Ethylhexyl salicylate	–	–	–	0.05	0.22	0.24	–	–	–
Terpenes										
1148	β-Myrcene	–	–	–	0.07	–	–	–	–	–
1170	Limonene	–	–	0.30	0.34	–	–	–	0.44	0.44
1531	Linalool	–	–	0.28	–	–	–	–	–	1.22
1573	4-Carvomenthenol	0.18	–	–	0.09	–	–	0.17	–	–
1641	*Z*-β-Farnesene	–	–	–	0.45	–	–	0.23	–	0.18
1645	Estragole	–	–	–	–	0.68	0.18	–	15.43	–
1673	β-Ocimene	–	–	–	0.43	–	–	–	–	–
1711	*Z,E*-α-Farnesene	–	–	–	1.91	0.79	1.21	0.33	–	0.75
1735	α-Farnesene	0.05	1.21	0.24	45.39	30.57	67.33	30.49	16.87	30.26
1773	α-Himachalene	–	–	–	0.79	–	–	0.06	–	–
1802	β-Damascenone	0.06	0.12	0.26	–	–	–	–	–	0.19
1814	Isoestragole	–	0.07	–	–	0.03	0.07	0.12	0.28	–
1844	Geranyl acetone	0.08	0.49	0.24	0.08	0.28	0.13	0.32	1.77	1.56
2309	Farnesal	–	–	–	–	–	0.30	–	–	–
2323	Farnesol	–	–	–	0.06	0.49	1.16	0.19	1.37	1.17
Others										
1223	Styrene	0.08	1.57	0.36	0.03	0.10	0.09	0.34	–	–
1399	N.I. *m/z* (69, 101, 157)	–	9.60	5.23	4.59	0.69	1.13	21.82	–	3.67
1413	*p*-Ethylstyrene	0.17	0.83	0.60	–	–	–	–	0.31	0.30
1810	N.I. *m/z* (135, 107)	–	–	–	0.18	–	–	–	–	–
1901	N.I. *m/z* (135, 107)	–	–	–	1.93	0.25	1.09	0.36	–	0.18
Number of identified compounds		46	45	39	64	60	64	65	43	50
RSD (%)		5.14	3.77	8.89	17.69	16.85	5.01	7.51	18.30	8.71

– Not detected. N.I., detected but not identified compound.

mined in pulp extracts are hexan-1-ol (29.77%), 2-methylbutan-1-ol (11.52%), and butyl hexanoate (6.31%), in peel α-farnese (30.57%), hexan-1-ol (12.32%), hexyl 2-methylbutyrate (8.51%), and 1-octen-3-ol (7.27%); and then, for juice extracts α-farnesene (16.87%), estragole (15.43%), hexan-1-ol (10.94%), *E*-2-hexenal (10.67%), and hexyl 2-methylbutyrate (9.39%).

Finally in SS apples volatile extracts, 59 compounds were identified, among these 39 were detected in the pulp, 64 in the peel and 50 in the entire fruit. Some of

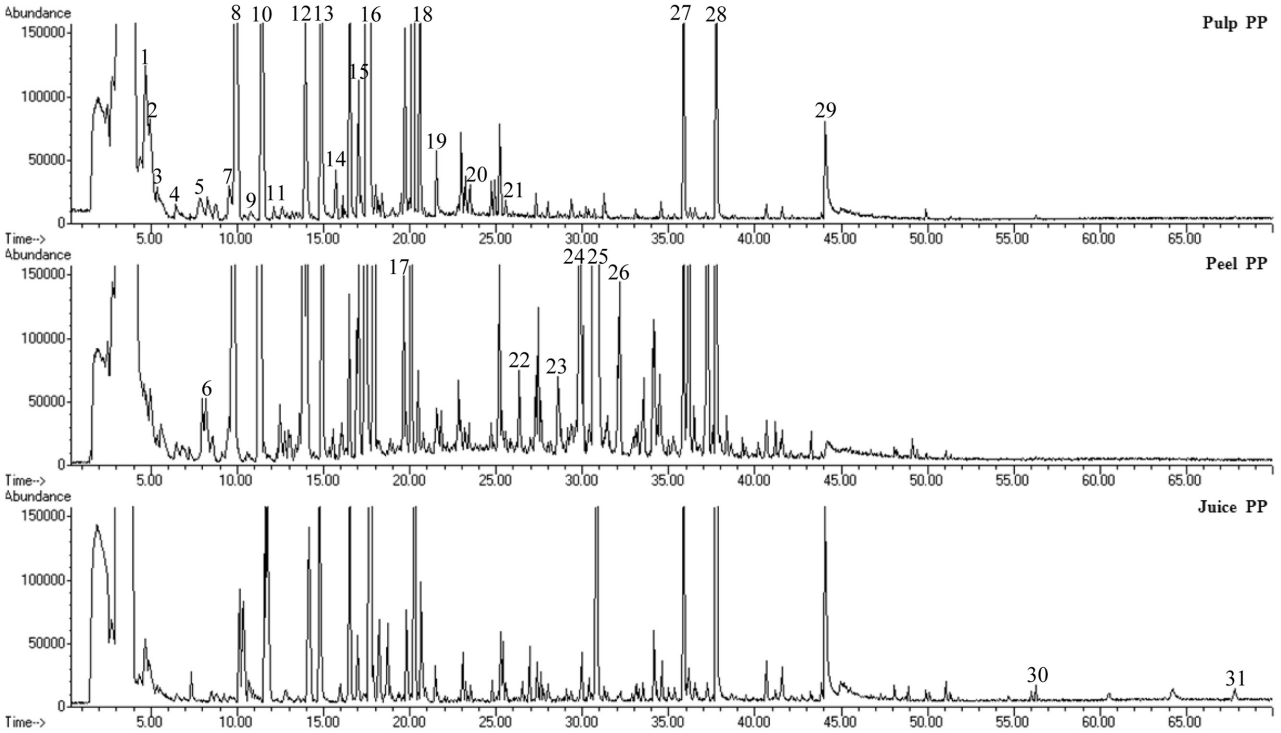

Figure 5. Total ion chromatogram of obtained from pulp, peel, and entire fruit extracts from PP apples obtained by HS-SPME$_{DVB/CAR/PDMS}$ at the optimal sampling conditions (50°C for 30 min under constant magnetic stirring (800 rpm), 0.1 g of NaCl). Time scale in minutes on *x*-axis; ion abundance (mV) on *y*-axis. Peak identification: 1, ethyl butyrate; 2, ethyl 2-methylbutyrate; 3, butyl acetate; 4, 2-methylbutyl acetate; 5, butan-1-ol; 6, methyl hexanoate; 7, 2-methylbutan-1-ol; 8, ethyl hexanoate; 9, styrene; 10, hexyl acetate; 11, ethyl 3-hexenoate; 12, *E*-2-hexen-1-ol acetate; 13, hexan-1-ol; 14, nonanal; 15, *E*-2-hexen-1-ol; 16, N.I. *m/z* (69, 101, 157); 17, *E*-2-hexenyl butyrate; 18, 3,4,5-trimethyl-4-heptanol; 19, 2,4-dimethyl-4-octanol; 20, ethyl 3-hydroxy butyrate; 21, hexyl hexanoate; 22, ethyl dodecanoate; 23, β-ocimene; 24, *Z,E*-α-farnesene; 25, α-farnesene; 26, α-himachalene; 27, ethyl 3-hydroxydodecanoate; 28, diethyl malate; 29, 1,3-octanediol; 30, 5-hydroxymethyl-2-furfural; 31, hexadecanoic acid.

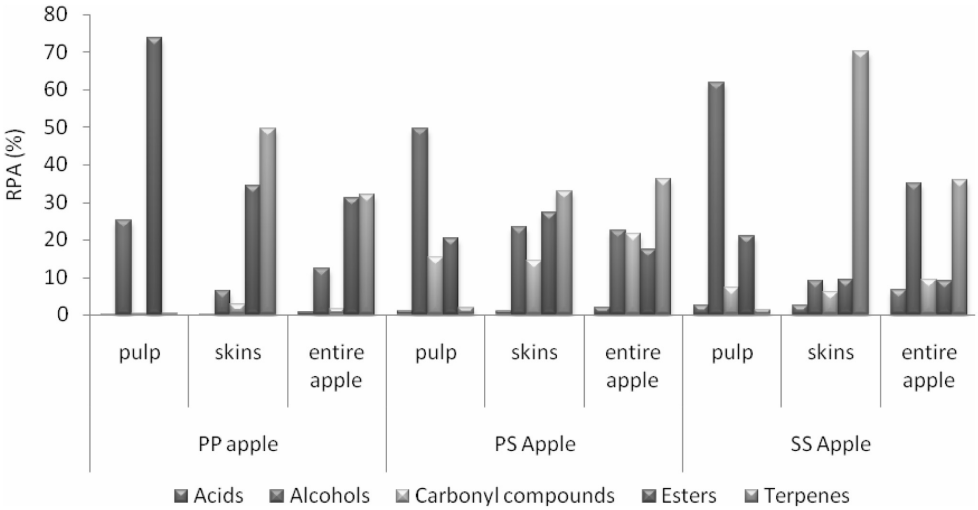

Figure 6. Distribution of the different chemical classes identified in pulp, peel, and entire fruit extracts of PP, PS, and SS *M. domestica* Borkh. apples.

the previous compounds were also identified as major compounds, such as hexan-1-ol (40.2%), *E*-2-hexen-1-ol (9.68%), and butyl butyrate (8.13%) in pulp samples; α-farnesene (67.33%) and hexan-1-ol (4.08%) in peel and, finally, α-farnesene (30.26%) and hexan-1-ol (18.97%) in entire fruit samples.

The comparison among the different extracts (pulp, peel, and entire fruit) from PP, PS, and SS apples, leads to the finding that peel extracts have the highest content of volatiles, where α-farnesene (sweet-wood odor) is the major compound accounting with 45.39, 30.57, and 67.33% for the total volatile fraction of PP, PS, and SS

Table 3. Percentage of variance and percentage of cumulative variance explained by the two first extracted PCs

Total variance explained Component	Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	45.122	67.346	67.346	45.115	67.336	67.336
2	15.140	22.597	89.944	15.147	22.607	89.944

Extraction method, PCA.

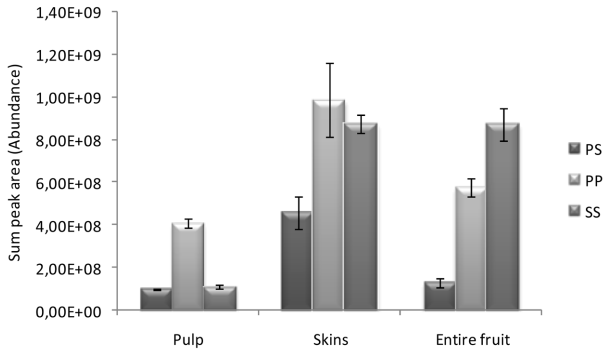

Figure 7. Distribution of VOCs and SVOCs from PP, PS, and SS *M. domestica* Borkh. apples between pulp, peels, and entire fruit.

apples, as well as in entire fruit extracts with 16.87, 30.26, and 30.49% for PS, SS, and PP, respectively (Table 2). Studies report that its presence on superficial cell layers of apples undergoes peroxidation through a, so far, unknown mechanism, where the peroxidized products somehow cause tissue damage and browning on these fruits [31, 32].

The distribution of VOCs and SVOCs between different fractions (pulp, peels, and entire fruit) of PP, PS, and SS apples shows a highly uneven distribution (Fig. 7). Hexan-1-ol, ethyl butyrate and hexyl 2-methylbutyrate, *e.g.*, were associated primarily with the pulp of the studied apples, whereas α-farnesene, hexyl acetate, ethyl octanoate and α-farnesene, hexanal, ethyl hexadecanoate were found in higher content in peels and entire fruit, respectively. The total PS apples volatile fraction (66.66 and 14.55%) occurred in the peel and pulp, whereas 49.91 and 20.69% of the total PP volatile free fractions were found in the peel and pulp, respectively. For SS apples, 47.11% of the total volatile fraction occurred in peels while only 5.92% was determined in the pulp (Fig. 7).

3.3 Multivariate analysis

By the application of PCA to the normalized relative amounts of all analytical variables (VOCs and SVOCs) and nine objects (apples), were extracted two factors that explain 81.5% of the total variance of initial dataset. The observation of the loading scores suggests that 13 variables, having coefficients magnitude <0.8, can be removed from the data matrix as they do not contribute to the explanation of data variability. The new variables set (data matrix 80×9) account for 89.9% of the total variance. The first principal component (PC)1 explains 67.3% of the variance in the initial dataset and the second PC2 explain 22.6%. Table 3 present the eigenvalues, the percentage of variance and the cumulative percentage explained by the two first PCs.

The projections of the samples along the directions identified by the first two PC's, is reported in Fig. 8 where the first PC1 of apples are plotted against the second PC2. The separations among different varieties of apples from this PC1 – PC2 scatter point plot are obvious (Fig. 8b). These figure shows that apples PS and SS were separated by the second PC, while PP apples are most influenced by the variables related with the first PC. The coefficient that defines the weight of the original variable in the PCs can be investigated to understand which chemical compounds are responsible for the ranking of wines. Methyl hexanoate (0.999), 3,4,5-trimethyl-4-heptanol (0.998), N.I. *m/z* (69, 101, 157) (0.998), ethyl hexanoate (0.998), and ethyl octanoate (0.998) were highly loaded on the first PC, while 2,5-furandicarboxaldehyde (0.972), ethyl 2-methylbutyrate (0.918), and butan-1-ol (0.887) were loaded on the second PC explaining most of the variability (Fig. 8a). PP apples (first quadrant) are essentially characterized by ethyl hexanoate, ethyl heptanoate, styrene, ethyl benzoate, and *E*-2-hexenyl acetate. The SS apples (second quadrant) are strongly associated with hexyl 2-methylbutyrate, *E*,*E*-2,4-heptadienal, *Z*-β-farnesene, and butan-1-ol, while the PS apples (third quadrant) are characterized by *p*-ethylstyrene, *E*-2-hexenal, estragole, butyl hexanoate, and hexanal.

After PCA, an LDA was applied in order to select an operative classification role for discriminating the three subspecies of apples obtained from different local in Madeira Island. Figure 9 reports a projection of apple species in 2D space, obtained by the two first discriminate functions that explain 100% of the total variance. Three groups representing each apple species, PP, PS, and SS were clearly observed. According to these results it can

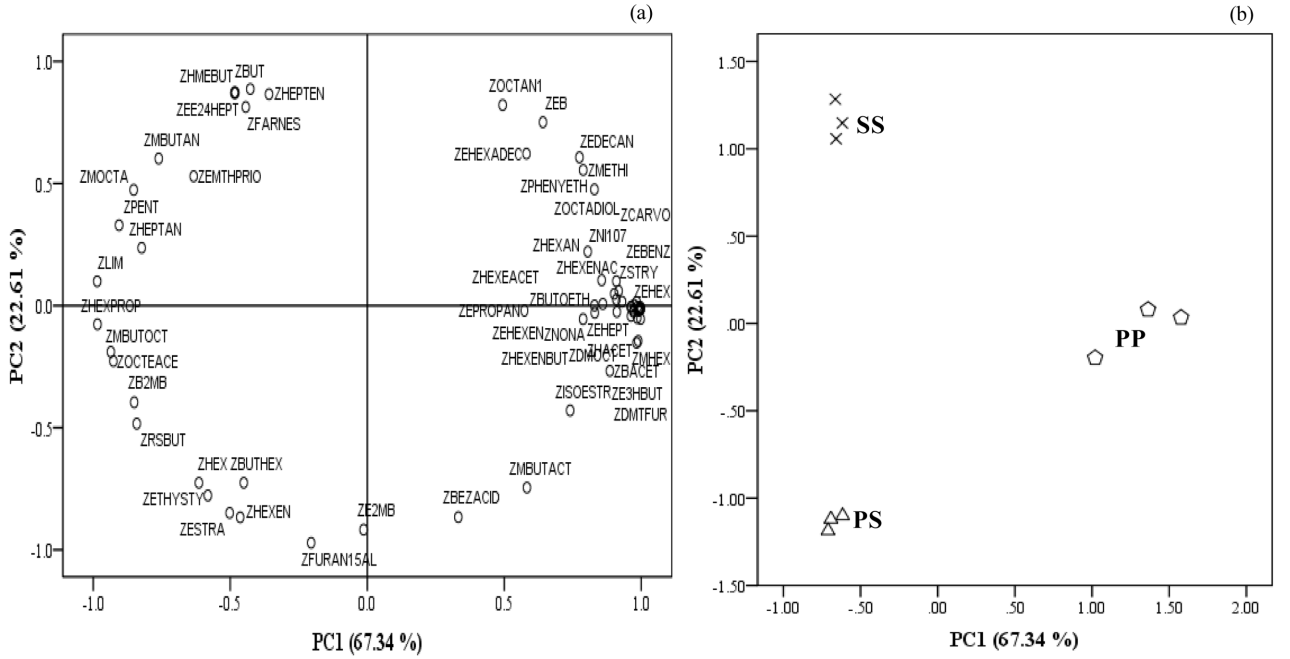

Figure 8. PC1 and PC2 scatter plot of the main sources of variability between apple fruit samples. (a) Relation between the chemical classes (loadings); (b) distinction between the samples (scores).

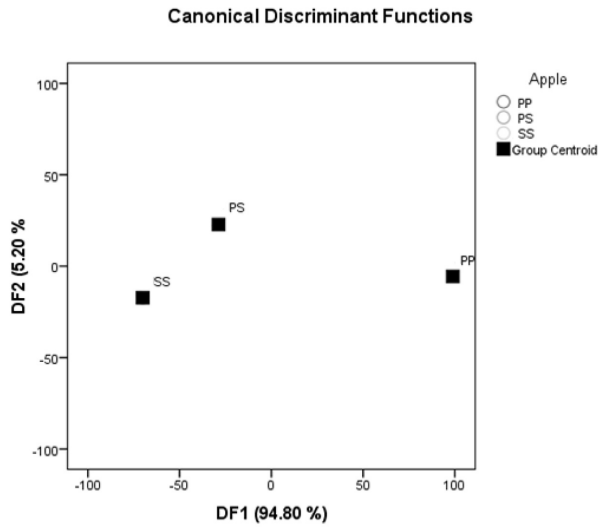

Figure 9. Scores plot on canonical variables of PP, PS, and SS *M. domestica* Borkh. apples after performing SLDA.

be concluded that the proposed HS-SPME/GC-qMS methodology is a useful sample technique to distinguish these *M. domestica* Borkh. apples from different geographic regions based on their volatile profile.

4 Concluding remarks

This study is the first investigation on VOCs and SVOCs present in different apple varieties – PP, PS, and SS, from *M. domestica* Borkh. species. The optimization of SPME parameters shows that the use of mixed phase DVB/CAR/PDMS coating fiber, demonstrated best performance characteristics for a wide range of analytes having different physic-chemical characteristics, when compared to the other five tested fibers, particularly when the samples were extracted at 50°C for 30 min under constant magnetic stirring. The applicability of the SPME technique was evaluated using three apple varieties from Madeira Islands. The optimized HS-SPME/GC-qMS method allows to identify in pulp, peel, and entire fruit of PP, PS, and SS apples *ca.* 100 VOCs and SVOCs from different chemical families, comprising esters, terpenoids, alcohols, carbonyl compounds, and fatty acids. The number and type of compounds varied according to apple variety and with the constituent parts of the apple. The peels presented the higher number of identified compounds, 64, 60, and 64, for PP, PS, and SS apples while pulp apples presented the lowest, 46, 45, and 39, respectively. The families of compounds with a great contribution to the total chromatographic area were the higher alcohols for the PP apples, esters for the PS apples and terpenoids for the PS apples. The obtained datasets were submitted to PCA and the corresponding varieties discriminations of PP, PS, and SS apples were successfully established. The most important contributions to the differentiation of the three apple varieties were ethyl hexanoate, hexyl 2-methylbutyrate, *E*,*E*-2,4-heptadienal, *p*-ethyl styrene, and *E*-2-hexenal. Prediction ability of the calculated model was estimated to be 100% by the "leave-one-out" cross-validation.

The apples samples were kindly provided by "Direcção Regional de Agricultura – Divisão de Fruticultura" of Madeira Islands. This research work is also financially supported by Instituto Regional do Emprego (IRE).

The authors declared no conflict of interest

5 References

[1] Mehinagic, E., Prost, C., Demaimay, M., *J. Agric. Food Chem.* 2004, *52*, 5175 – 5182.

[2] Lara, I., Graell, J., López, M. L., Echeverría, G., *Postharvest Biol. Technol.* 2006, *39*, 19 – 28.

[3] Dixon, J., Hewett, E. W., *J. Crop Hortic. Sci.* 2000, *28*, 155 – 173.

[4] Dixon, J., Hewett, E. W., *Postharvest Biol. Technol.* 2001, *22*, 71 – 83.

[5] Riu-Aumatell, M., Castellari, M., López-Tamames, E., Galassi, S., Buxaderas, S., *Food Chem.* 2004, *87*, 627 – 637.

[6] Holland, D., Larkov, O., Bar-Ya'akov, I., Bar, E., Zax, A., Brandeis, E., Ravid, U., Lewinsohn, E., *J. Agric. Food Chem.* 2005, *53*, 7198 – 7203.

[7] Echeverría, G., Graell, J., López, M., Lara, I., *Postharvest Biol. Technol.* 2004, *31*, 217 – 227.

[8] Krokida, M. K., Philippopoulos, C., *J. Food Eng.* 2006, *73*, 135 – 141.

[9] Ong, B., Nazimah, S., Tan, C., Mirhosseini, H., Osman, A., Hashim, D., Rusul, G., *J. Food Compos. Anal.* 2008, *21*, 416 – 422.

[10] Coelho, E., Perestrelo, R., Neng, N., Câmara, J. S., Coimbra, M., Nogueira, J., Rocha, S. M., *Anal. Chim. Acta* 2008, *864*, 79 – 89.

[11] Kataokaa, H., Lord, L. H., Pawliszyn, J., *J. Chromatogr., A* 2000, *880*, 35 – 62.

[12] Mu, R., Wang, X., Liu, S., Yuan, X., Wang, S., Fan, Z., *Chromatographia* 2007, *65*, 463 – 467.

[13] Róth, E., Berna, A. Z., Beullens, K., Yarramraju, S., Lammertyn, J., Schenk, A., Nicolaï, B. M., *Postharvest Biol. Technol.* 2007, *45*, 11 – 19.

[14] Reis, S. F. A. R., Rocha, S. M., Barros, A. S., Delgadillo, I., Coimbra, M., *Anal. Chim. Acta* 2009, *113*, 513 – 521.

[15] Young, J. C., Chu, C. L. G., Lu, X., Zhu, H., *J. Agric. Food Chem.* 2004, *52*, 8086 – 8093.

[16] Saevels, S., Lammertyn, J., Berna, A. Z., Veraverbeke, E. A., Di Natale, C., Nokolaï, B. M., *Postharvest Biol. Technol.* 2004, *31*, 9 – 19.

[17] Hern, A., Dorn, S., *Phytochem. Anal.* 2003, *14*, 232 – 240.

[18] Song, J., Gardner, B. D., Holland, J. F., Beaudry, H. M., *J. Agric. Food Chem.* 1997, *45*, 1801 – 1807.

[19] Matich, A. J., Rowan, D. D., Banks, N. H., *Anal. Chem.* 1996, *68*, 4114 – 4118.

[20] Komes, D., Lovric, T., Ganic, K. K., *LWT* 2007, *40*, 1578 – 1586.

[21] Ibañez, E., López-Sebastián, S., Ramos, E., Tabera, J., Reglero, G., *Food Chem.* 1998, *63*, 281 – 286.

[22] Ferreira, L., Perestrelo, R., Câmara, J. S., *Talanta* 2009, *77*, 1087 – 1096.

[23] Paixão, N., Pereira, V., Marques, J. C., Câmara, J. S., *J. Sep. Sci.* 2008, *31*, 2189 – 2198.

[24] Câmara, J. S., Alves, M., Marques, J. C., *Anal. Chim. Acta* 2006, *555*, 191 – 200.

[25] Perestrelo, R., Caldeira, M., Rodrigues, F., Câmara, J. S., *J. Sep. Sci.* 2008, *31*, 1841 – 1850.

[26] Caldeira, M., Rodrigues, F., Perestrelo, R., Marques, J. C., Câmara, J. S., *Talanta* 2007, *74*, 78 – 90.

[27] Rodrigues, F., Caldeira, M., Câmara, J. S., *Anal. Chim. Acta* 2008, *609*, 82 – 104.

[28] Sánchez-Palomo, E., Díaz-Maroto, C., Coelho-Pérez, M., *Talanta* 2005, *66*, 1152 – 1157.

[29] Zhang, C., Qi, M., Shao, Q., Zhou, S., Fu, R., *J. Pharm. Biomed. Anal.* 2007, *44*, 464 – 470.

[30] Dixon, J., Hewett, E. W., *N. Z. J. Crop Hortic. Sci.* 2000, *28*, 155 – 173.

[31] Paliyath, G., Whiting, M., Stasiak, M., Mum, D., Clegg, B., *Food Res. Int.* 1997, *30*, 95 – 103.

[32] Rupasinghe, H., Paliyath, G., Murr, D., *J. Am. Soc. Hortic. Sci.* 1998, *123*, 882 – 886.

