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Analytical Methods

Dynamic headspace solid-phase microextraction combined with one-dimensional gas chromatography–mass spectrometry as a powerful tool to differentiate banana cultivars based on their volatile metabolite profile

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ABSTRACT

In this study the effect of the cultivar on the volatile profile of five different banana varieties was evaluated and determined by dynamic headspace solid-phase microextraction (dHS-SPME) combined with one-dimensional gas chromatography–mass spectrometry (1D-GC–qMS). This approach allowed the definition of a volatile metabolite profile to each banana variety and can be used as pertinent criteria of differentiation. The investigated banana varieties (Dwarf Cavendish, Prata, Maçã, Ouro and Platano) have certified botanical origin and belong to the Musaceae family, the most common genomic group cultivated in Madeira Island (Portugal). The influence of dHS-SPME experimental factors, namely, fibre coating, extraction time and extraction temperature, on the equilibrium headspace analysis was investigated and optimised using univariate optimisation design. A total of 68 volatile organic metabolites (VOMs) were tentatively identified and used to profile the volatile composition in different banana cultivars, thus emphasising the sensitivity and applicability of SPME for establishment of the volatile metabolomic pattern of plant secondary metabolites. Ethyl esters were found to comprise the largest chemical class accounting 80.9%, 86.5%, 51.2%, 90.1% and 6.1% of total peak area for Dwarf Cavendish, Prata, Ouro, Maçã and Platano volatile fraction, respectively. Gas chromatographic peak areas were submitted to multivariate statistical analysis (principal component and stepwise linear discriminant analysis) in order to visualise clusters within samples and to detect the volatile metabolites able to differentiate banana cultivars. The application of the multivariate analysis on the VOMs data set resulted in predictive abilities of 90% as evaluated by the cross-validation procedure.

1. Introduction

As aroma is one of the most appreciated fruit characteristics, volatile flavour compounds are likely to play a key role in determining the perception and acceptability of products by consumers. Identification of key volatile flavour metabolites that carry the unique character of the natural fruit is essential, as it provides the principal sensory identity and characteristic flavour of the fruit (Augusto, Valente, dos Santos Tada, & Rivellino, 2000; Cheong et al., 2010; Navarro, De Lorenzo, & Perez, 2004).

Banana (genus *Musa* of the Musaceae family) is one of the most important crops in the world. According to the Food and Agriculture Organization of the United Nations (FAO) statistics estimations (Arias, Dankers, Liu, & Pilkauskas, 2003), the 10 major banana producing countries accounted for more than 75% of total banana production. Furthermore, India, China, the Philippines, Brazil and Ecuador alone produced more than 60% of total world banana production. World exports of bananas also show a high level of concentration, with developing countries accounting for the bulk of exports. Only Latin America and the Caribbean supplied, in average, about 70% of world exports. The four leading banana exporting countries in 2006 (Ecuador, Costa Rica, Philippines and Colombia) accounted for 64% of world exports with Ecuador alone provided more than 30% of global banana exports. From a consumer perspective, the most appealing features of banana are their flavour, nutrition or health aspects and convenience for consumption (Boudhrioua, Giampaoli, & Bonazzi, 2003). Is a fruit of high nutritional value, very rich in sugar, minerals, mainly potassium (very important to regulate blood pressure), magnesium, phosphorous, calcium, sodium, vitamins (C, pyridoxine and riboflavin, mainly) and dietary fibres (helpful to regulate bowel movement). Their typical aroma is characterised by the presence of a wide range of volatile metabolites, with different volatilities and concentrations, that can vary among the different cultivars, as the initial work of Cano and collaborators with Spanish and Columbian Enana cultivars showed (Cano et al., 1997). This volatiles belong to distinct chemical classes mainly ethyl esters (Jordan, Tandon, Shaw, & Goodner, 2001) and,

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to a lesser extent carbonyl compounds, monoterpenoids, C_{13}-norisoprenoids and furan compounds. According to Tressl et al., (Tressl & Albrecht, 1985) although the acetates and butyrate esters are the most abundant in bananas, some 3-methylbutyrate esters, 2-methylpropionates and caproates are also produced in minor accounts. Acetates are of particular importance due to their high concentrations and low odour thresholds. The biosynthetic pathway for their formation in ripening climacteric fruits is well-established (Wyllie & Fellman, 2000; Yoshioka, Ueda, & Iwata, 1982). They are biosynthesized from long-chain fatty acid metabolism or formed by a complex group of chemical substances (e.g. aldehydes, alcohols, ketones, esters, lactones, terpenes), with a usually low concentration (μg/L) and can be affected by a number of agronomical (variety, climatic conditions, ripening stage of the fruit) and technological (harvest, post-harvest, treatments, storage and processing conditions) factors. However, these factors control the quantitative and qualitative composition of the ester profile, which in many cases determine the character and perceived quality of the fruits (Brat et al., 2004). Fruit esters also are formed by the reaction between alcohols and acyl CoA's derived from both fatty acid and amino acid metabolism and are catalysed by the enzyme *acyl alcohol transferase* (ATT) (Tressl & Drawert, 1973).

Boudhrioua et al. (2003) showed that 3-methylbutyl acetate, 3-methylbutyl butyrate and 3-methylbutyl-3-methyl butyrate, isoamyl acetate, pentan-2-one and isoamyl butyrate are characteristics of the geographic origin of the fruit. In addition, fatty acids, generated by the oxidative degradation of linoleic and linolenic acids, contribute to ester biosynthesis in fruit. Degradation of fatty acids results in the production of volatile aldehydes, which in turn are utilised by alcohol dehydrogenases generating alcohols, aldehydes and branched/aromatic aliphatic acids. These compounds contribute, and in some cases are determining, to the primary aroma of many fruits (Perez, Olias, Luaces, & Sanz, 2002). Moreover, some volatiles are particularly good indicators of the ripening stage of bananas. This is the case of hexanal and (*E*)-2-hexenal, characteristic of riped bananas, and isobutyl acetate and isopentyl acetate, more abundant in ripening bananas (Mayr, Märk, Lindinger, Brevard, & Yeretzian, 2003).

Increasing consumer demand for safety and nutritional excellence together with higher market competition underlines the importance of food analysis. This drives a need for analysts to develop rapid, reliable, less labour-consuming, and more accurate analytical procedures. However, this is a daunting task as food stuffs contain a broad range of components. In the fruit-producing or -processing industry, good analytical methods are crucial to the success of any quality control during storage, processing, and/or product development studies. The most typically used methods for extraction and pre-concentration are headspace techniques, purge-and-trap, liquid–liquid extraction, and simultaneous distillation and extraction. The majority of these methods present some drawbacks, such as the possibility of sample contamination, loss of analytes during the concentration step, it requires large amounts of toxic and expensive solvents that can be harmful to the operator and to the environment, are time-consuming and usually offers low recoveries.

Considering the limitations of the traditional sampling techniques, headspace solid-phase microextraction (HS-SPME) emerges as an attractive alternative (Augusto et al., 2000). It is a solvent-free sample preparation technique that integrates sampling, isolation/extraction and concentration into a single uninterrupted process, resulting in high sample throughput (Mendes, Gonçalves, & Câmara, 2012). Seems particularly appealing since it also eliminates problems associated with chemically and thermally unstable samples where generation of artefacts can be problematic. However, presents some disadvantages since (i) it works by equilibration thus gives incomplete recoveries; (ii) it only allows measurement of what partitions into the fibre phase; and (iii) it's partitioning is highly dependent on matrix. Nevertheless, its simplicity of use and fibre reusability had made SPME an attractive choice for many analytical applications (Coelho et al., 2008; Ferreira, Perestrelo, Caldeira, & Camara, 2009; Ibáñez, López-Sebastián, Ramos, Tabera, & Reglero, 1998; Perestrelo, Nogueira, & Camara, 2009). In particular in combination with GC–MS, this technique have been largely used in environmental (Ternes, 1998), food (Camara, Alves, & Marques, 2006; Cheong et al., 2010; Pereira, Pereira, & Câmara, 2011; Pozo, Pueyo, Martin-Alvarez, & Polo, 2001), forensic (Chien, Uang, Kuo, Shih, & Jen, 2000; Kumazawa, Lee, Sato, & Suzuki, 2003), pharmaceutical and biomedical (Deng, Li, & Zhang, 2004a; Deng, Zhang, & Li, 2004b; Silva, Passos, & Câmara, 2011, 2012) coffee (Risticevic, Carasek, & Pawliszyn, 2008) and biological (Moeder, Schrader, Winkler, & Popp, 2000) analysis.

The aim of the current research study is dedicated towards the development a simple, robust, reliable and solvent-free technique, without sample derivatization, based on the dHS-SPME combined with 1D-GC–qMS, in order to allow a qualitative and semi-quantitative screening of a broad range of VOMs in banana from different cultivars: Dwarf Cavendish, Prata, Maçã, Ouro and Platano, growing at Madeira Island (Portugal), which could be used as cultivar markers. The main qualitative and semi-quantitative differences were established. In a previous step this work focuses on optimisation of the most influencing factors of the dHS-SPME. The optimised method will be then applied to the analysis of real banana samples, after which the acquired data will be submitted to PCA statistical evaluation to establish an objective comparison among banana from different cultivars. To the best of our knowledge this is the first paper reporting the volatile composition of Prata (*Musa balbisiana*), Ouro (*Musa paradisiaca*), Maçã (*Musa acuminate*) and Platano (*Musa paradisiaca*) banana varieties growing at Madeira Island, as well as the first paper reporting differentiation among banana cultivars based on their volatile metabolomic profile.

2. Material and methods

2.1. Chemicals and sample preparation

All chemicals used were analytical quality and all solvents were HPLC grade. Sodium chloride (99.5%) used to adjust the ionic strength (decrease the solubility of the VOMs which then partition more readily into the headspace improving the adsorption of analytes by coating), was supplied by Merck (Darmstadt, Germany). Deionised water was obtained from Milli-Q water purification system (Millipore, Bedford, PA, USA). The *n*-alkanes mixture containing C_8–C_{20} straight-chain alkanes in hexane, and the internal standard, octan-3-ol, were purchased from Sigma–Aldrich (Spain). Helium, ultra-pure grade (Air Liquide, Portugal) was used as carrier gas in the GC system. The SPME fibre optimisation step was carried out by testing commercially available silica SPME fibres purchased from Supelco (Bellefonte, PA, USA) and coated with the following polymers: polydimethylsiloxane (PDMS, 100 μm), polyacrylate (PA, 85 μm), divinylbenzene/carboxen on polydimethylsiloxane (DBV/CAR/PDMS; StableFlex, 50/30 μm), carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm) and polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm). Prior to their first use, all fibres were conditioned according to the manufacturer's instructions by heating in the injection port of the GC. Before the initial application, blank runs were completed before each sampling to ensure no carry-over of analytes from the previous extraction. Clear glass screw cap vials for SPME with PTFE/silica septa were purchased from Supelco.

Three sets of 250 g of mature banana fruits (*Musa acuminate* L.) from Dwarf Cavendish (DC), Prata (BP), Ouro (BO), Maçã (BM) and Platano (P) cultivars growing at Madeira Island (Portugal) were obtained from commercial sources, at green stage, and kept at

room temperature during maturation. Fruits were selected at random, carefully hand-peeled, sliced and pureed at room temperature for 2 min in a blender to obtain a homogeneous paste. A small volume (1.5 mL) of methanol was sprayed onto the paste in order to avoid subsequent enzymatic activity. The banana puree was placed into glass vials and stored at −20 °C until analysis. All experiments and analytical determinations were carried out at least in triplicate.

2.2. dHS-SPME procedure

The handling of an SPME device is simple, and the analysis of volatile metabolites could be easy. However, the process becomes complex while analyzing sample matrices of heterogeneous nature. The complexity also increases depending upon the nature of compounds to be extracted and matrix. Therefore, careful selection and optimisation of extraction parameters have to be undertaken to improve the sensitivity and the reproducibility of this method.

For this particular optimisation experiment Dwarf Cavendish banana was selected as the matrix. The analysis were carried out by placing 0.5 g portions of banana puree, previously homogenised, diluted with 1 mL ultra-pure water (Milli-Q) in a 4 mL headspace glass vial covered with a PTFE/silicone septum and containing a micro stirring bar (Supelco). As stirring of the solution 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 velocity (750 min^{-1}). After the addition of 0.3 g of NaCl, in order to improve the extraction efficiency by decreasing the solubility of hydrophilic metabolites in the aqueous phase and 1 μL of internal standard (octan-3-ol, 4.22 mg/L), the vials were immersed in a thermostat bath maintained at the temperature selected for the extraction. Afterwards the SPME fibres were manually inserted into the headspace over the samples. In search of optimised conditions the (i) nature of the fibres; (ii) the exposure time (10–80 min) of the fibre to the headspace, and (iii) the extraction temperature, were tested and evaluated.

After exposition period, the SPME fibres were withdrawn into the needle, removed from the glass vial and immediately inserted into the injection port of the gas chromatograph where the extracted metabolites were thermally desorbed for 6 min and transferred directly to the analytical column. Desorption temperatures, depending on the fibre used, 250 °C for PDMS/DVB, PDMS and StableFLex, 260 °C for PA and 300 °C for CAR/PDMS coating.

2.3. Selection of fibre coating

The polarity and volatility characteristics of the target metabolites are the primary issues addressed before selecting a fibre coating. The most commonly used fibre coatings are: 100 μm PDMS; 65 μm DVB/PDMS; 75 μm CAR/PDMS; and 85 μm PA. Polydimethylsiloxane (PDMS) is the most widely used coating. The main advantage of using this coating is its ability to withstand high temperatures, up to about 300 °C and its greater stability than CAR/PDMS and DVB/PDMS coatings. Another advantage of this fibre coating is its possibility of estimating the distribution constants for organic compounds from retention times on PDMS-coated GC columns.

In this step the extraction efficiency of five SPME fibres (Supelco, Bellefonte, USA), with different stationary phases and various film thicknesses, was evaluated and compared. The extraction procedure is described in the above section. The extraction procedures were carried out at 50 °C for 40 min of exposure fibre (on all five fibres). The analytes, being characterised by different volatilities and polarities, were selected across the GC chromatogram and the total sum of their peak areas was used to select the SPME coating having best performance characteristics. The 65 μm PDMS/DVB coating fibre was found to give the best performance for the whole range of banana VOMs. Therefore this fibre was used in all further experiments. At least four replicates were done for each coating fibre.

Different fibre coatings used influenced the chemical nature of the extracted metabolite depending on their characteristic polarity and volatility. In order to evaluate the effect of the nature of the fibre on the extraction efficiency of VOMs from bananas by dHS-SPME, and taking account the physical/chemical characteristics of the targets under consideration, a set of five coating fibres (CAR/PDMS, PDMS/DVB, PDMS, PA and StableFlex), covering a wide range of polarities and mechanisms, were used among those most routinely used for assaying volatiles. Each fibre was exposed to the headspace for the same time (40 min) at the same temperature (50 °C). Blank runs were conducted between extractions with the chosen fibre, to check for absence of carry-over which would cause memory effects and misinterpretation of results. All tests were carried out using the same banana cultivar (Dwarf Cavendish). The comparison of the SPME fibre performance was made in terms of extraction efficiency, number of identifiable metabolites in the extract and reproducibility.

2.4. Selection of extraction time and temperature

The extraction time profile was initially constructed at the extraction temperature of 50 °C with 10, 20, 40, 60 and 80 min of analysis isolation. An optimal extraction time of 60 min was selected for further studies. To select the temperature which gives the highest extraction efficiency, another experiment was carried out. A range of four different temperatures, 30, 40, 50 and 60 °C were tested using 60 min of analyses extraction. A temperature of 50 °C was selected. Desorption time was 6 min and the GC liner temperature varied according to fibre coating (Section 2.2).

2.5. 1D-GC–MS analyses

The desorbed volatile metabolites were analysed in an Agilent Technologies 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, and interfaced with an Agilent 5975 quadrupole inert mass selective detector. Helium (Helium N60, Air Liquid, Portugal) was used as carrier gas at a flow rate of 1 mL/min (column-head pressure: 89.6 kPa). The injector was operated in the splitless mode. The GC oven temperature program was set at an initial value of 40 °C (1 min hold); increased to 150 °C at 2.5 °C/min, then raised to 220 °C at 15 °C/min and hold for 5 min (total GC run time of 54 min).

For the 5975 MS system, the temperatures of the transfer line, quadrupole and ionisation source were 270, 150 and 230 °C respectively; electron impact mass spectra were recorded at 70 eV ionisation voltages and the ionisation current was 10 μA. The acquisitions were performed in Scan mode (35–300 m/z). The electron multiplier was set to the auto tune procedure. Agilent MS ChemStation Software, equipped with a NIST05 mass spectral library was employed for identification of the volatile metabolites extracted from real samples. A series of C_8–C_{20} *n*-alkanes were analysed by dHS-SPME/^{1}D-GC–qMS to establish the retention indices (RI), in order to tentatively identify the compounds by comparison with the literature. The relative amounts of individual metabolites are expressed as percent peak areas relative to the total peak area ($RPA(\%) = A_i/\Sigma A_i \times 100$), where A_i is the GC peak area of *i* metabolite.

Identification of volatile metabolites was achieved by: (1) comparison of the mass spectrometric data and chromatographic retention of authentic standards, when available, (2) comparison the Kovats retention index (RI) value, which was determined according to the Van den Dool and Kratz RI equation (Van Den Dool & Kratz, 1963), and (3) similarity of mass spectrometric data. As generated by the NIST, 2005 software (Mass Spectral Search Program V.2.0d; NIST 2005, Washington, DC). For the determination of the RI, a C_8–C_{20} *n*-alkanes series was used, and the values were compared, when available, with values reported in the literature for similar chromatographic columns (Bianchi, Careri, Mangia, & Musci, 2007; Brat et al., 2004; Hognadottir & Rouseff, 2003). More than 60 VOMs distributed by different chemical groups were identified to profile the global signature of the banana cultivars under study. In order to evaluate quantitative differences in the aromatic profile of the samples investigated, GC peak areas were calculated as total ion current for all the analytes.

2.6. Statistical analysis

Significant differences among banana cultivars were determined by one-way analysis of variance (*Anova*) using a SPSS Program, version 16.0 (SPSS Inc., 2008). Principal component analysis (PCA) was performed using the same SPSS program. This technique was applied to the normalised total peak areas from different chemical classes.

Principal component analysis (PCA) is an unsupervised technique that reduces the dimensionality of the original data matrix retaining the maximum amount of variability (Forina, Armanino, Castino, & Ubigli, 1986), allowing the visualisation of the different banana cultivars in a two-dimensional space and identifying the directions in which most of the information is retained. It is therefore possible to explain the differences between several cultivars by means of factors obtained from the data sets and, at the same time, to determine which variables contributed the most for such differences.

Stepwise linear discriminant analysis (SLDA) probably the most widely applied supervised pattern recognition method, searches for directions (discriminant functions) which achieve maximum separation among categories by maximising between the class variance relative to the within-class variance. SLDA renders a number of orthogonal linear discriminant functions equal to the number of categories minus one. This method minimises the variance within categories and maximises the variance between categories (Powers & Keith, 1968). The variables included in the analyses are determined with a stepwise-LDA using a Wilk's Lambda as a selection criterion and an *F* statistic factor to establish the significance of the changes in Lambda when a new variable is tested. The prediction capacity of the discriminant models was studied by "cross-validation" in order to determine the stability of the model.

3. Results and discussion

The sequence followed in this study consisted of two steps. The first focused on obtaining the best experimental conditions to extract VOMs from banana of different cultivars by dHS-SPME procedure combined with 1D-GC–qMS. In the second step an objective comparison among the investigated banana cultivars has been established in terms of qualitative and semi-quantitative differences on volatile composition by using the optimised conditions. Different volatile patterns were observed according to banana cultivars. Among other metabolite classes, ethyl esters, acetates, isoamyl esters, alcohols and phenols, were identified.

3.1. Optimisation of SPME conditions

To optimise dHS-SPME method some parameters controlling the performance of the extraction efficiency were taken into account. This process has been conducted by considering one parameter at a time keeping all other variables constant (univariate experimental design).

3.2. Evaluation of the fibre

The nature of the fibre is an important aspect for the pre-concentration of the VOMs in Dwarf Cavendish banana since each fibre shows a different extraction profile. The volatile compounds identified using the five fibres on the same Dwarf Cavendish sample, in rigorously reproduced temperature and exposure time conditions and the relative extraction efficiencies of the tested fibres are summarised in Table 1.

Altogether 64 VOMs were extracted and identified on the basis of their mass spectra (MS) and retention indices (RI) (Table 1). These included ethyl esters, isoamyl esters, acetates, higher alcohols, ketones and phenols. The qualitative composition of banana volatiles and the number of identified metabolites using the five fibres under study is very different (Table 1).

A comparison among the fibre's performance is relative since it would have to be performed in the optimised conditions to each fibre. Nevertheless, among the five fibres, it clearly shows (Fig. 1a) that the semi-polar PDMS/DVB fibre, made of a mixture of solid DVB polymers coated with a PDMS liquid polymeric film, exhibited the highest efficiency in the extraction process of the banana VOMs, extracting around three times more than PA and PDMS, and around 1.5 times more than StableFlex and CAR/PDMS fibres. Similar amounts of volatiles (30.8–34.2%) were extracted by PDMS and PA fibres coating while 66.1–71.6% were extracted when using CAR/PDMS and StableFlex coatings. Based on the data evaluation completed within this particular optimisation experiment, PDMS/DVB fibre provided the best sensitivity in terms of total compound peak areas, number of isolated metabolites (51) and high reproducibility (lower than 7%), and therefore was utilised in all further optimisation/real analyses experiments.

Fig. 1b, illustrated that the fibres show different selectivity to volatile metabolites. PDMS/DVB coating have a larger affinity for ethyl esters (EE), furan compounds (FUR) and norisoprenoids/monoterpenoids/sesquiterpenoids (NMS) compounds. CAR/PDMS fibre shown to be relevant for the extraction of carbonyl compounds (CC) whereas StableFlex coating affords a higher extraction for higher alcohols (HA). According to Fig. 1b, independent of the employed fibre, ethyl esters (EE) showed the most intense signals which suggest that ethyl esters might be VOMs with higher concentrations.

3.3. Influence of extraction temperature and time

The extraction temperature and the exposure time of the fibre to the headspace are two important factors that control sample recovery by the fibres and consequently, must be studied in order to establish the optimum extraction conditions.

Temperature is an important parameter for the SPME extraction process. Since extraction by this methodology is an exothermic process, it controls the diffusion rate of the analytes into the fibre coating. As the temperature increases, diffusion coefficients and Henry's constants increase while partition coefficients to the extraction phase decrease, because diffusion coefficients increase, the time required to reach equilibrium decreases. Finally, due to the lower partition coefficients to the extraction phase, the equilibrium amount decrease (Lambropoulou & Albanis, 2001; Perestrelo, Caldeira, Rodrigues, & Camara, 2008). To check the effect of

Table 1
Comparison of the relative extraction efficiencies of different SPME coatings for VOMs extraction from Dwarf Cavendish cultivar.

Peak number	RT[a] (min)	RI[b]	Metabolite	SPME fibre type				
				PDMS/DVB[c]	PDMS	PA	StableFlex	CAR/PDMS
2	2.408	906	Ethyl acetate	25	8	–	38	100
	2.656	928	3-Methyl-butanal		100			
3	3.333	979	Pentan-2-one	83	15	23	73	100
4	3.882	1013	Isobutyl acetate	100	20	20	44	31
5	4.287	1035	Ethyl butyrate	100	18		51	84
	4.997	1070	Butyl acetate	100	34	22	67	81
8	5.206	1079	Hexanal	85	32	21	100	–
9	5.399	1087	2-Methyl-2-methylpropyl propanoate	97	–	–	100	–
	5.677	1098	2-Methyl propan-1-ol	100	–	–	88	–
10	6.258	1120	Isoamyl acetate	100	35	25	58	42
11	6.569	1131	Pentan-2-ol	–	–	–	–	100
	6.971	1144	Butyl isobutyrate	100	–	–	–	–
	7.062	1147	Butan-1-ol	68	–	–	–	100
12	7.386	1157	2-Methylpropyl butyrate	100	20	22	57	38
	7.925	1173	2-Methyl-2-methylpropyl butyrate	100	–	–	51	46
	8.135	1179	Heptan-2-one	100	–	–	66	86
14	8.481	1188	Isoamyl butyrate	100	–	–	44	28
16	8.582	1191	2-Methyl-3-methylbutyl propanoate	100	–	–	–	–
	8.635	1192	4-Methyl heptane	–	–	–	100	–
	8.716	1194	2,3-Dimethyl hexane	–	–	100	–	–
18	9.142	1204	3-Methylbutan-1-ol	–	21	–	100	–
19	9.334	1206	1-Methylbutyl butyrate	100	11	–	–	–
21	9.494	1209	Butyl butyrate	37	10	10	28	100
	10.017	1216	2-Methylpentyl butyrate	100	–	–	–	–
	10.720	1226	Isoamyl butyrate	5	100	–	2	2
	10.841	1228	5-Hepen-2-one					
	11.385	1235	2-Methyl-3-methylbutyl propanoate	–	–	50	–	100
25	11.394	1236	3-Methylbutyl butyrate	100	–	–	79	–
26	11.746	1239	Hexyl acetate	100	33	21	66	79
28	11.920	1241	2-Methyl-3-methylbutyl butyrate	100	39	34	87	66
30	12.730	1251	3-Methyl-3-methylbutyl butyrate	100	43	24	83	51
	13.335	1258	(*Z*)-4-hexen-1-yl acetate	–	100	–	–	–
31	13.544	1260	3-Hepten-1-yl acetate	100	–	–	–	–
	14.459	1269	(*E*)-3-methyl-1,3-pentadiene	–	–	–	100	–
32	14.865	1273	(*E*)-4-hexen-1-yl acetate	100	–	–	62	12
33	15.466	1279	Hexan-1-ol	100	54	38	97	68
	15.469	1279	4-Methyl pentan-1-ol	100	–	–	–	–
	15.731	1281	3-Methylbutyl pentanoate	100	–	–	99	–
	17.999	1402	(*E*)-2-hexen-1-ol	100	–	–	154	–
36	18.343	1410	Hexyl butyrate	100	38	–	87	82
	18.890	1424	(*Z*)-4-hexen-1-ol	–	100	–	–	–
	18.986	1424	(*E*)-3-hexen-1-ol	93	–	–	100	71
	19.791	1442	3-Methylhexyl butyrate	100	47	33	86	81
37	19.987	1446	(*Z*)-2-Methyl cyclohexyl butyrate	100	47	–	83	82
38	20.428	1455	Isopentyl hexanoate	100	50	–	90	55
	20.621	1459	Acetic acid	–	–	–	–	100
	20.627	1459	Furfural	100	–	–	–	–
40	21.791	1482	4-Hexen-1-yl butyrate	100	36	33	79	68
41	22.794	1500	Ethenyl cyclohexane	100	33	35	68	77
	23.504	1516	Heptan-4-one	14	–	23	100	48
	23.729	1521	2-Methylbutyl isobutyrate	100	–	–	77	65
45	25.553	1559	Cycloocta-1,3-diene	100	–	–	–	53
	25.592	1560	3-Ethenyl cyclohexene	–	100	–	–	–
46	27.312	1594	3-Methyl bicyclo[4.1.0]heptane	100	–	–	–	–
	27.763	1603	Hexyl hexanoate	–	100	–	–	–
47	28.439	1618	2-Methyl-1-methyl butyl propanoate	100	–	–	–	39
48	32.414	1702	2-Methyl ethyl propanoate	100	–	–	–	–
	34.890	1759	2-Methyl-1-nonen-3-one	100	–	–	–	–
	46.312	2028	Octanoic acid	100	–	–	–	–
50	46.430	2030	5-Hydroxymethylfurfural	100	–	–	–	–
51	46.717	2037	1,3-Dihydroxypropan-2-one	100	–	–	–	–
	47.907	2063	Eugenol	100	–	–	–	15
52	48.695	2080	Elemicin	100	44	85	48	48
53	49.156	2090	DDMP[d]	100	–	81	–	–
Total compounds per fibre				51	28	19	36	34

[a] Retention time.
[b] Experimentally determined retention indices on the BP-20 column, relative to C_8–C_{20} hydrocarbons.
[c] The extraction efficiency of the PDMS/DVB fibre was set as 100%.
[d] DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

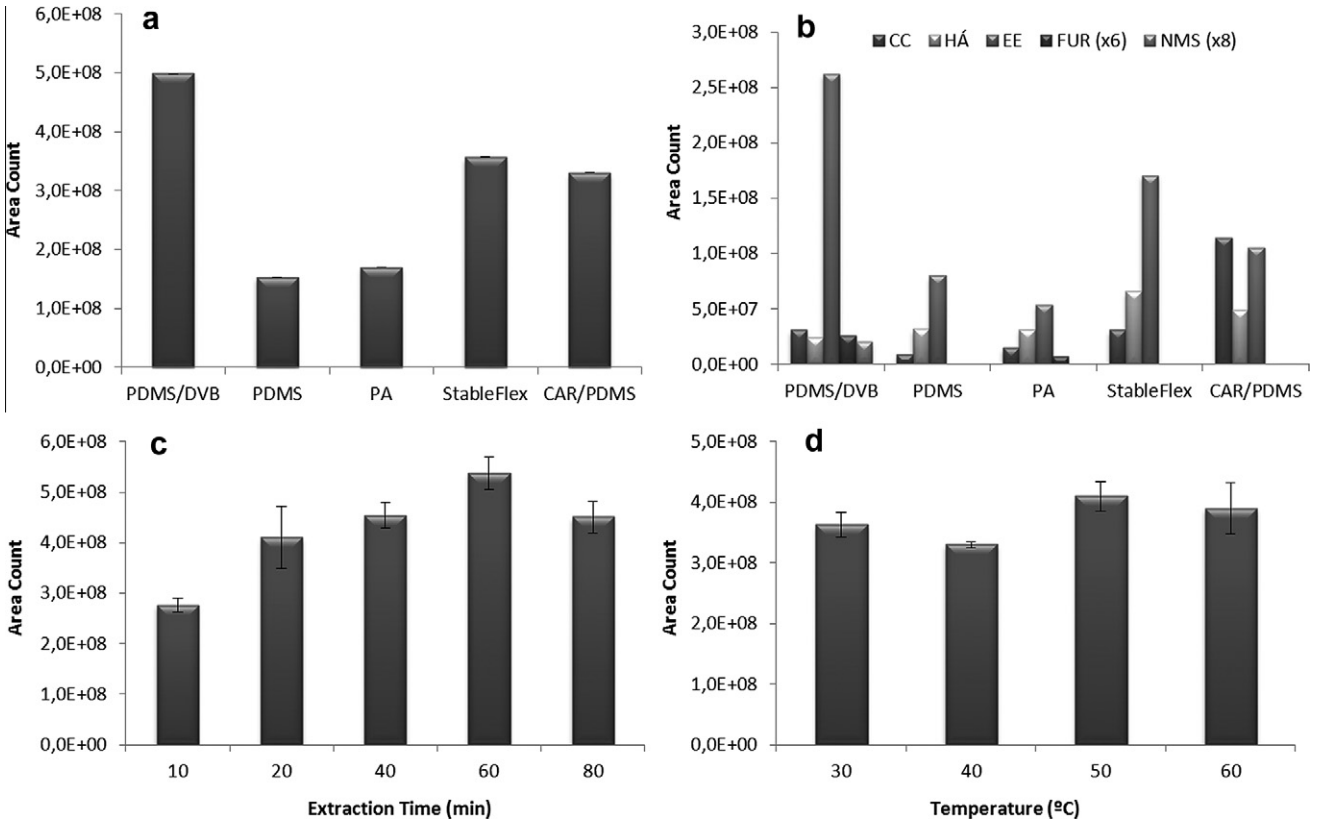

Fig. 1. Optimisation of the extraction-influencing factors which affect the efficiency of dHS-SPME: (a) effect of SPME fibre coating; (b) performance of SPME coatings on the extraction efficiency of higher alcohols (HA), carbonyl compounds (CC), ethyl esters (EE), furan compounds (FUR), and C_{13}-norisoprenoids/monoterpenes/sesquiterpenes (NMS); (c) effect of the extraction temperature (PDMS/DVB coating during 60 min); and (d) influence of the extraction time (PDMS/DVB coating at 50 °C), on the extraction efficiency of VOMs from Dwarf Cavendish bananas. Error bars represent standard error of the mean ($n = 4$ for each data point).

extraction temperature on SPME extraction efficiency, PDMS/DVB fibre was exposed to the headspace at four different temperatures 30, 40, 50 and 60 °C, for 60 min. The results of triplicate experiments are summarised in Fig. 1c. In general, as the temperature increases, the recovery of volatile compounds improves, since heating of solid samples help to release analytes into the headspace and facilitate the SPME process. The best recovery of volatiles was obtained at 50 °C.

The time that the fibre was in contact with the headspace of the sample, had a significant effect on the chromatographic peak areas of the extracted metabolites, as is apparent from Fig. 1d. The adsorption time was evaluated by exposing the fibre into the headspace between 10 and 80 min (10, 20, 40, 60 and 80 min) at 50 °C. The influence of the fibre exposure time on the yield of microextraction is displayed in Fig. 1d.

For most metabolites the extraction efficiency increased with the extraction time, during the first 60 min of the fibre exposure decreasing thereafter (Fig. 1d). An exposure time of 60 min was chosen because fibre exposure times longer than 60 min were too long and the differences in response between 60 and 80 min were less than 10%. Taking these results into account the best results were obtained using an extraction time of 60 min at 50 °C.

3.4. Analyses of volatile metabolites in different banana cultivars

The optimised technique was applied to different banana cultivars taken from commercial sources. Each banana sample was analysed four times using the optimised extraction conditions described above (PDMS/DVB fibre; 50 °C; 60 min; salt addition with continuous stirring). A typical total ion current (TIC) chromatogram of the volatile constituents from different banana varieties extracted by dHS-SPME is shown in Fig. 2. The technique used made it possible to identify 68 VOMs in the investigated banana cultivars.

The identified volatile metabolites are shown in Table 2 where are listed in order of their elution on the BP-20 column together with their retention indices and percentage of relative area. Most of the volatile metabolites were tentatively identified by a library search NIST05. In some cases a comparison with authentic compounds was performed. The retention indices were calculated for each peak and compared with the literature in order to ensure the correct identification of the metabolites. Table 2 summarises the average ($n = 4$) relative composition observed in the headspace of bananas from different cultivars studied. As previously reported to the common export banana cultivar Gran Enano, there is a great diversity in the aroma composition of bananas in different ripening stages and even in bananas in the same ripening stage treated or not with ethylene (Vermeir, Hertog, Vankerschaver, Swennen, Nicolaï, & Lammertyn, 2009).

The relative composition of each flavour 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 (RPC,%) for each banana cultivar. Although comparison of GC profiles showed qualitative differences, the quantitative relationship among different compounds in any flavour fraction may have a drastic effect on final aroma. In this sense, the headspace composition of cv. DC showed a richer aroma profile, with a higher number of metabolites contributing to the overall aroma intensity.

According to their chemical structure, the compounds can be classified as monoterpenoids/C_{13}-norisoprenoids (NMS), higher alcohols (HA), ethyl esters (EE), carbonyl compounds (CC) and furan compounds (FUR).

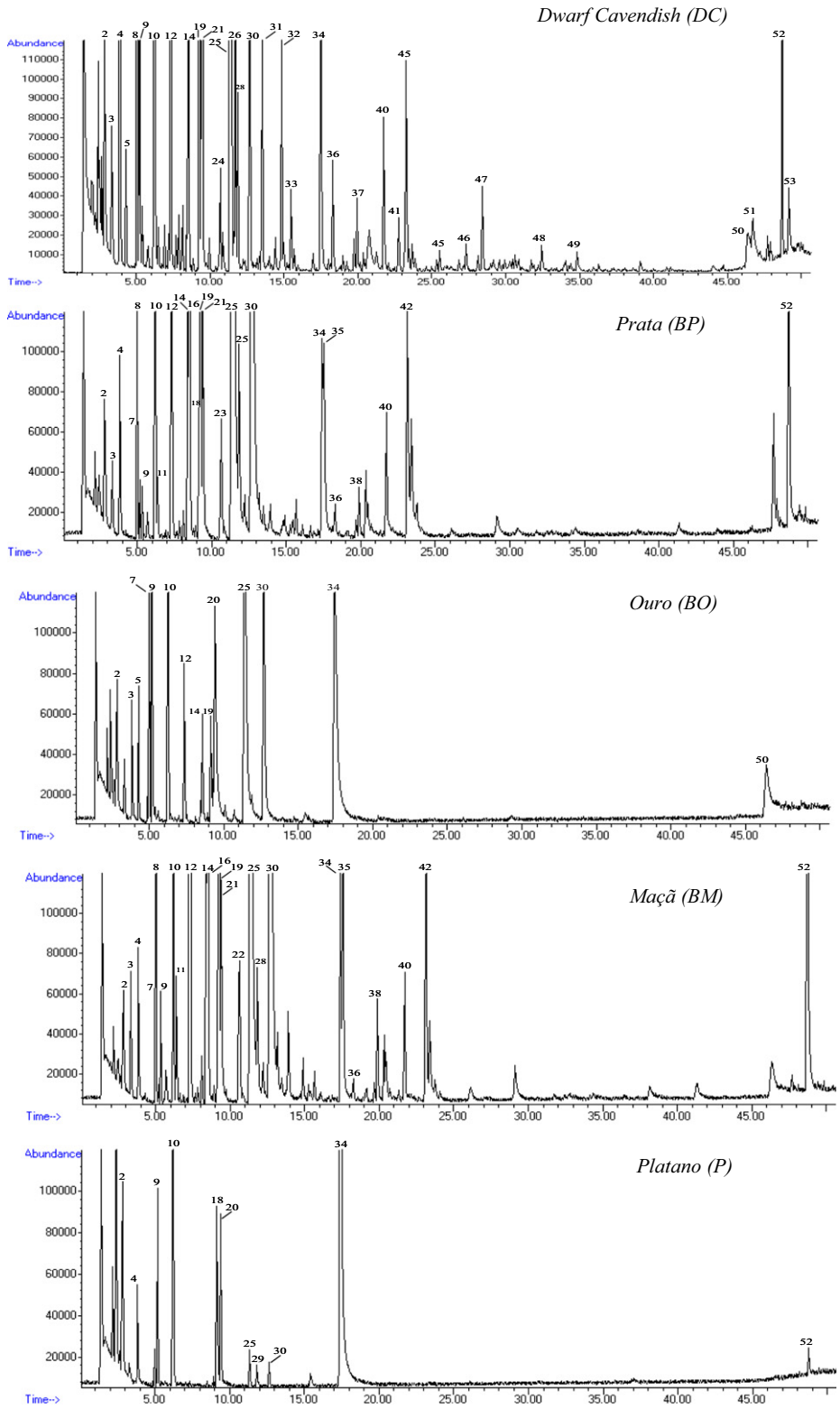

Fig. 2. Representative chromatograms (fingerprint signals) obtained from the dHS-SPME$_{PDMS/DVB}$/^{1}D-GC–qMS analysis of five samples corresponding to different banana cultivars ($T_{extraction}$: 50 °C; $t_{extraction}$: 60 min; NaCl 0.3 g; 750 min^{-1}). Peak Identification: ***2:*** ethyl acetate; ***3:*** pentan-2-one; ***4:*** isobutyl acetate; ***5:*** ethyl butyrate; ***7:*** pentyl acetate; ***8:*** hexanal; ***9:*** 2-methyl-2-methylpropyl propanoate; ***10:*** isoamyl acetate; ***11:*** pentan-2-ol; ***12:*** 2-methylpropyl butyrate; ***14:*** isoamyl butyrate; ***16:*** 2-methyl-3-methylbutyl propanoate; ***18:*** 3-methyl butan-1-ol; ***19:*** 1-methylbutyl butyrate; ***20:*** (*E*)-hexen-2-al; ***21:*** butyl butyrate; ***22:*** 2-pentyl pentanoate; ***23:*** butyl pentanoate; ***24:*** hexyl valerate; ***25:*** 3-methylbutyl butyrate; ***26:*** hexyl acetate; ***28:*** 2-methyl-3-methylbutyl butyrate; ***29:*** 2-methyl pyrrolidine; ***30:*** 3-methyl-3-methylbutyl butyrate; ***31:*** 3-hepten-1-yl acetate; ***32:*** 4-hexen-1-yl acetate; ***33:*** hexan-1-ol; ***34:*** octan-3-ol (Internal Standard); ***35:*** 1-methylhexyl butyrate; ***36:*** hexyl butyrate; ***37:*** 2-methylcyclohexyl butyrate; ***38:*** isopentyl hexanoate; ***40:*** 4-hexen-1-yl butyrate; ***41:*** ethenyl cyclohexane; ***45:*** cycloocta-1,3-diene; ***46:*** 3-methyl bicycle[4.1.0] heptane; ***47:*** ethane-1,1-diol dibutyrate; ***48:*** ethyl isobutyrate; ***49:*** 2-methyl-1-nonene-3-yene; ***50:*** 5-hydroxymethylfurfural; ***51:*** 1,3-dihydroxypropan-2-one; ***52:*** elemicin; ***53:*** 2,3-dihydro-3,5-dihydroxy-6-methyl-4-pyrone.

Table 2
Volatile metabolites identified in banana fruits from different cultivars after dynamic dHS-SPMS/[1]D-GC-qMS using PDMS/DVB coating (extraction temperature: 50 °C; extraction time: 60 min; 750 min^{-1}; 0.3 g NaCl).

Peak number	RT (min)	RI	Metabolite	ID[a]	% of relative areas achieved in different samples[b]					RPC (%)				
					DC	BP	BO	BM	P	DC	BP	BO	BM	P
2	2.408	906	Ethyl acetate	S, MS	0.82	–	4.64	–	19.59	36	–	45	–	100
	3.193	969	n-Propyl acetate	S, MS	–	–	–	–	1.87	–	–	–	–	100
3	3.333	979	Pentan-2-one	S, MS	0.93	0.34	1.98	0.36	–	100	61	47	67	–
4	3.882	1013	Isobutyl acetate	S, MS	4.73	0.57	–	0.31	3.46	100	20	–	12	8
5	4.287	1035	Ethyl butyrate	S, MS	0.54	–	2.47	–	–	99	–	100	–	–
	4.883	1064	3-Methylethyl butyrate	S, MS	–	–	0.68	–	–	–	–	100	–	–
	4.915	1066	Butyl acetate	S, MS	5.34	–	–	–	0.36	100	–	–	–	2
7	4.971	1068	2-Pentyl acetate	S, MS	–	0.73	1.72	0.56	–	–	100	31	79	–
8	5.173	1079	Hexanal	S, MS	1.70	0.18	5.91	0.03	8.63	100	18	76	3	58
9	5.399	1087	MMPP[c]	MS	0.26	0.12	–	0.21	–	70	55	–	100	–
	5.677	1098	2-Methyl propan-1-ol	S, MS	0.22	0.15	–	0.15	–	84	97	–	100	–
10	6.258	1120	Isoamyl acetate	S, MS	19.34	2.52	1.77	0.21	27.61	100	22	2	2	16
11	6.360	1124	Pentan-2-ol	S, MS	–	0.22	–	0.39	–	–	55	–	100	
	6.971	1144	Butyl isobutyrate	MS	0.18	–	–	–	–	100	–	–	–	–
	7.062	1147	Butan-1-ol	S, MS	0.18	–	–	–	–	100	–	–	–	–
12	7.386	1157	2-Methylpropyl butyrate	MS	5.66	3.40	3.15	2.88	–	99	100	12	88	–
	7.925	1173	2M2MPB[d]	MS	0.22	0.07	–	0.05	–	100	51	–	37	–
	8.135	1179	Heptan-2-one	S, MS	0.29	0.12	–	0.14	–	100	70	–	84	–
14	8.481	1188	Isoamyl butyrate	MS	2.71	2.55	2.78	0.88	–	7	100	2	36	–
16	8.582	1191	MMPB[e]	MS	1.43	0.34	2.44	2.53	0.86	32	36	12	100	2
18	9.142	1203	3-Methyl butan-1-ol	S, MS	–	2.55	3.56	–	11.41	–	100	18	–	28
19	9.334	1206	1-Methylbutyl butyrate	MS	3.95	3.48	1.40	8.16	–	28	41	2	100	
20	9.406	1207	(*E*)-hexen-2-al	S, MS	–	–	11.81	–	11.48	–	–	100	–	51
21	9.494	1209	*n*-Butyl butyrate	S, MS	7.41	1.09	–	0.90	–	100	25	–	21	–
	10.017	1216	2-Methylpentyl butyrate	S, MS	0.16	–	–	–	–	100	–	–	–	–
22	10.560	1224	2-Pentyl pentanoate	S, MS	–	–	–	0.33	–	–	–	–	100	–
23	10.635	1225	*n*-Butyl pentanoate	S, MS	–	0.59	–	–	–	–	100	–	–	–
24	10.639	1225	Hexyl *n*-valerate	S, MS	0.33	–	–	–	–	–	–	–	100	–
25	11.394	1235	3-Methylbutyl butyrate	S, MS	28.30	37.85	50.01	34.35	–	1	100	17	55	–
26	11.746	1239	Hexyl acetate	S, MS	1.53	–	–	–	–	100	–	–	–	–
	11.832	1240	DMEHP[f]	MS	–	0.92	–	–	–	–	100	–	–	–
28	11.920	1241	MMBB[g]	MS	0.93	–	–	0.46	–	100	–	–	87	–
29	11.932	1240	2-Methyl pyrrolidine	MS	–	–	–	–	0.59	–	–	–	–	100
30	12.730	1251	3M3MBB[h]	MS	2.75	32.02	4.57	34.23	0.88	5	90	2	100	5
	13.152	1256	2-Methylbutyl butyrate	S, MS	–	–	–	0.32	–	–	–	–	100	–
31	13.544	1260	3-Hepten-1-yl acetate	S, MS	1.45	–	–	–	–	100	–	–	–	–
	13.907	1264	Heptan-2-ol	S, MS	–	0.27	–	0.34	–	–	76	–	100	–
32	14.865	1273	4-Hexen-1-yl acetate	S, MS	1.38	–	–	–	–	100	–	–	–	–
	15.26	1277	Butyl hexanoate	S, MS	–	–	–	0.05	–	–	–	–	100	–
33	15.466	1279	Hexan-1-ol	S, MS	0.49	–	–	–	–	100	–	–	–	–
	15.643	1281	3M2MBB[i]	MS	–	0.19	–	–	–	–	100	–	–	–
	15.731	1281	3-Methylbutyl pentanoate	MS	0.13	–	–	0.10	–	72	–	–	100	–
35	17.526	1297	1-Methylhexyl butyrate	MS	–	1.27	–	1.97	–	–	62	–	100	–
	17.999	1402	(*E*)-2-hexen-1-ol	S, MS	0.08	–	–	–	–	100	–	–	–	–
36	18.343	1410	Hexyl butyrate	S, MS	0.60	0.22	–	0.12	–	100	63	–	34	–
	18.986	1424	(*E*)-3-hexen-1-ol	S, MS	0.09	–	–	–	–	100	–	–	–	–
	19.791	1442	3-Methylhexyl butyrate	MS	0.19	0.08	–	0.09	–	100	72	–	89	–
	19.879	1443	3-Methyl cyclohexanol	S, MS	–	0.22	–	0.46	–	–	45	–	100	–
37	19.987	1446	MCHB[j] butyrate	MS	0.42	–	–	–	–	100	–	–	–	–
38	20.428	1455	Isopentyl hexanoate	S, MS	0.11	0.29	–	0.33	–	20	85	–	100	–
	20.627	1459	Furfural	S, MS	0.36	–	–	–	–	100	–	–	–	–
40	21.791	1482	4-Hexen-1-yl butyrate	S, MS	0.98	0.66	–	0.66	–	85	97	–	100	–
41	22.794	1500	Ethenyl cyclohexane	MS	0.33	–	–	–	–	100	–	–	–	–
	23.118	1508	4-Hexen-1-yl pentanoate	MS	–	1.24	–	1.61	–	–	74	–	100	–
42	23.383	1514	Linalool	S, MS	–	0.79	–	0.45	–	–	100	–	59	–
	23.504	1517	Heptan-4-one	S, MS	0.19	–	–	0.26	–	42	–	–	100	–
	23.629	1524	Cyclooctene	MS	0.18	–	–	–	–	100	–	–	–	–
	23.736	1531	(*Z*)-2-hexenyl butyrate	MS	–	0.26	–	0.36	–	–	70	–	100	–
45	25.553	1559	Cycloocta-1,3-diene	S, MS	0.13	–	–	–	–	100	–	–	–	–
46	27.312	1594	MBCH[l]	MS	0.22	–	–	–	–	100	–	–	–	–
47	28.439	1618	2M1MBP[m]	S, MS	0.60	–	–	–	–	100	–	–	–	–
48	32.414	1702	MEP[n]	S, MS	0.22	–	–	–	–	100	–	–	–	–
	46.312	2028	Octanoic acid	S, MS	0.28	–	–	–	–	100	–	–	–	–
50	46.43	2030	5-Hydroxymethylfurfural	S, MS	0.67	–	3.54	–	11.74	49	–	58	–	100
51	47.725	2059	DHP[o]	MS	–	0.66	–	–	–	–	100	–	–	–
	47.907	2063	Eugenol	S, MS	0.64	0.13	–	–	–	100	34	–	–	–
52	48.695	2080	Elemicin	MS	1.61	3.27	–	5.41	1.73	17	58	–	100	2
53	49.156	2090	DDMP[p]	MS	0.09	–	–	–	–	100	–	–	–	–

Table 2 (*continued*)

Peak number	RT (min)	RI	Metabolite	ID[a]	% of relative areas achieved in different samples[b]					RPC (%)				
					DC	BP	BO	BM	P	DC	BP	BO	BM	P
	Total compounds identified in banana fruit									51	34	16	35	13
	Sum of total area		3.93E + 08		3.93E+08	6.61E+08	8.62E+07	6.88E+08	4.52E+07					
	% RSD (n = 4)		5.25		5.25	1.64	2.66	5.19	0.32					

[a] ID: reliability of identification: S-standard, MS-mass spectrum.
[b] DC-Dwarf Cavendish; BP-Prata; BO-Ouro;BM-Maçã;P-Plátano.
[c] MMPP: 2-methyl-2-methylpropyl propanoate.
[d] 2M2MPB: 2-methyl-2-methylpropyl butyrate.
[e] MMBP: 2-methyl-3-methylbutyl propanoate.
[f] DMEHP: 2,2-dimethyl-2-ethylhexyl propanoate.
[g] MMBB: 2-methyl-3-methylbutyl butyrate.
[h] 3M3MBB: 3-methyl-3-methylbutyl butyrate.
[i] 3M2MBB: 3-methyl-2-methylbutyl butyrate.
[j] MCHB: *cis*-2-methylcyclohexyl butyrate.
[l] MBCH: 3-methyl-bicyclo[4.1.0]heptane.
[m] 2M1MBP: 2-methyl-1-methylbutyl propanoate.
[n] MEP: 2-methylethyl propanoate.
[o] DHP[o] 1,3-dihydroxy propan-2-one.
[p] DDMP: 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

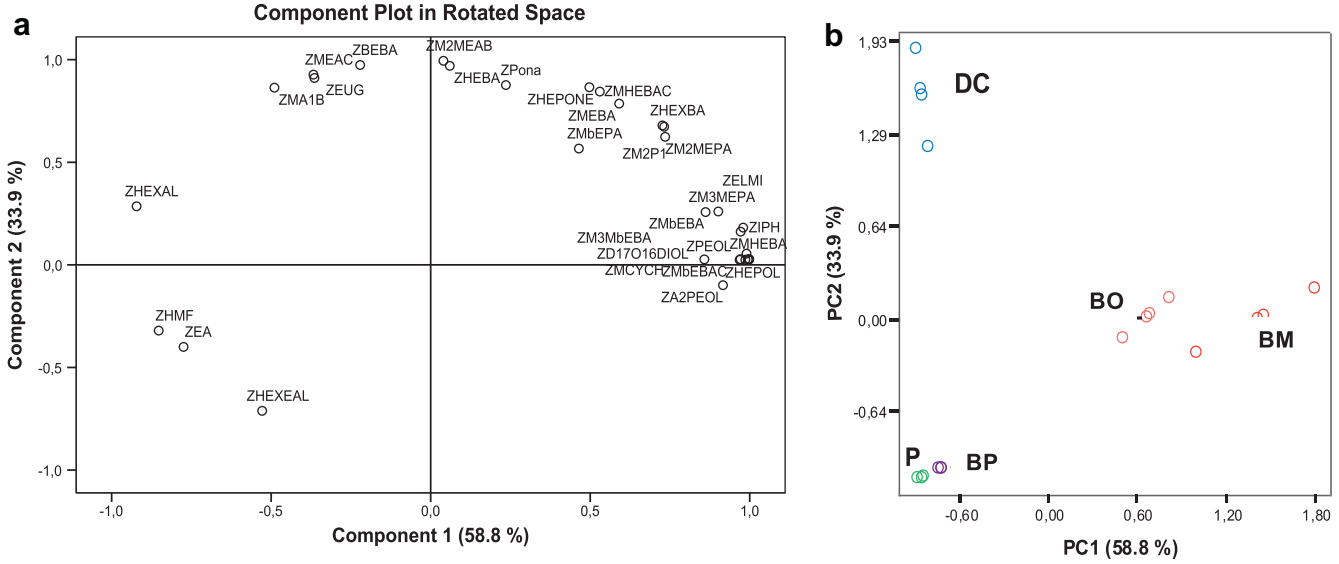

Fig. 3. Results from PCA analysis. (a) Distribution of variables (loadings); and (b) projection of the samples (scores). Variable identification: *EA*: ethyl acetate; *Pona*: pentan-2-one; *MEAC*: isobutyl acetate; *A2PEOL*: 2-pentyl acetate; *HEXAL*: hexanal; *M2MEPA*: 2-methyl-2-methylpropyl propanoate; *M2P1*: 2-methyl propan-1-ol; *MA1B*: isoamyl acetate; *PEOL*: pentan-2-ol; *MEBA*: 2-methylpropyl butyrate; *M2MEAB*: 2-methyl-2-methylpropyl butyrate; *HEPONE*: heptan-2-one; *M3MEPA*: 2-methyl-3-methylbutyl propanoate; *M1B*: 3-methyl butan-1-ol; *MbEBA*: 1-methylbutyl butyrate; *HEXEAL*: hexen-2-al; *BEBA*: *n*-butyl butyrate; *MbEBAC*: 3-methylbutyl butyrate; *M3MbEBA:* 3-methyl-3-methylbutyl butyrate; *HEPOL*: heptan-2-ol; *MbEPA*: 3-methylbutyl pentanoate; *MHEBA*: 1-methylhexyl butyrate; *MHEBAC*: 3-methylhexyl butyrate; *MCYCH*: 3-methyl cyclohexanol; *IPH*: isopentyl hexanoate; *HEXBA*: (*Z*)-2-hexenyl butyrate; *D17O16DIOL*; linalool; *HMF*: 5-hydroxymethylfurfural; *EUG*: eugenol; *ELMI*: elemicin.

The highest amount of volatile metabolites was obtained in bananas from Prata and Maçã cultivars, followed by Dwarf Cavendish and Ouro cultivars. Contrarily bananas from Platano cultivar was found to had the lowest content of volatiles. Only four volatile metabolites among the total of 68 identified were found to be common to all studied cultivars: hexanal, isoamyl acetate, 2-methyl-3-methylbutyl propanoate and 3-methyl-3-methylbutyl butyrate (Table 2). Isoamyl acetate, considered the character impact metabolite in banana flavour (Nogueira, Fernandes, & Nascimento, 2003), is one of the few metabolites present in a higher amount in banana, mainly from Dwarf Cavendish and Platano cultivars, while other short esters such as butyl acetate, with a fruity–estery odour description, are present only at low levels. With exception of bananas from Platano cultivar, characterised by high levels of higher alcohols (54.6%), all other banana cultivars were largely dominated by ethyl esters which accounted for 90.1%, 86.5%, 80.9% and 51.2% in Maçã, Prata, Dwarf Cavendish and Ouro total GC peak areas, respectively. Carbonyl compounds followed by higher alcohols were found to be the second and third most abundant chemical groups. These impart green-woody notes that also contributed to banana flavour.

Among volatile metabolites identified in bananas from Dwarf Cavendish cultivar, the most dominant were found to be 3-methylbutyl butyrate, isoamyl acetate, butyl butyrate, 2-methylpropyl butyrate, butyl acetate, isobutyl acetate and 1-methylbutyl butyrate, constituting 72.6% of the total volatile composition. Banana

Table 3
Percentage of cumulative variance explained by the two first principal components and percentage of correctly classified samples corresponding to the SLDA model developed for the five-class approach analysed.

Component	Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
Total variance explained						
1	20,094	62,795	62,795	18,615	58,173	58,173
2	9,366	29,269	92,064	10,845	33,891	92,064

		Cultivar	Predicted group membership					Total
			DC	BP	BO	BM	P	
Classification results[b,c]								
Original	Count	DC	4	0	0	0	0	4
		BP	0	4	0	0	0	4
		BO	0	0	4	0	0	4
		BM	0	0	0	4	0	4
		P	0	0	0	0	4	4
	%	DC	100.0	.0	.0	.0	.0	100.0
		BP	.0	100.0	.0	.0	.0	100.0
		BO	.0	.0	100.0	.0	.0	100.0
		BM	.0	.0	.0	100.0	.0	100.0
		P	.0	.0	.0	.0	100.0	100.0
Cross-validated[a]	Count	DC	4	0	0	0	0	4
		BP	0	4	0	0	0	4
		BO	0	0	3	1	0	4
		BM	0	0	0	4	0	4
		P	0	0	0	1	3	4
	%	DC	100.0	.0	.0	.0	.0	100.0
		BP	.0	100.0	.0	.0	.0	100.0
		BO	.0	.0	75.0	25.0	.0	100.0
		BM	.0	.0	.0	100.0	.0	100.0
		P	.0	.0	.0	25.0	75,0	100.0

Extraction method: principal component analysis.
[a] Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
[b] 100.0% of original grouped cases correctly classified.
[c] 90.0% of cross-validated grouped cases correctly classified.

samples from Prata cultivar contained 3-methylbutyl butyrate, 3-methyl-3-methylbutyl butyrate, 1-methylbutyl butyrate, 2-methylpropyl butyrate, elemicin, 3-methyl butan-1-ol, isoamyl butyrate and isoamyl acetate, as the main volatile metabolites, whereas bananas from Ouro cultivars are largely dominated by 3-methylbutyl butyrate and hexen-2-al, accounting for 54.4% of the total volatile fraction. Other compounds, such as hexanal, 3-methyl-3-methylbutyl butyrate, ethyl acetate, isoamyl acetate and 3-methyl butan-1-ol, were also present at high levels. It is important to emphasise the lack of elemicin in this banana variety. Table 2 illustrates that 35 volatile metabolites were identified in banana samples from Maçã cultivar, from which 24 tentatively identified, being a large number of them esters and carbonyl compounds. 3-Methylbutyl butyrate, 3-methyl-3-methylbutyl butyrate, and 1-methylbutyl butyrate, were the most abundant metabolites identified in these samples. Bananas from this cultivar are very rich in elemicin, in comparison to all other studied cultivars. In banana samples from Platano cultivar only 13 VOMs were identified being the higher alcohols and carbonyl compounds the most dominant groups. Isoamyl acetate and ethyl acetate were the major components. Other representative metabolites of this banana variety were identified as 5-hydroxymethylfurfural, (*E*)-hexen-2-al, 3-methylbutan-1-ol, and hexanal.

Some metabolites identified in the investigated banana varieties, like butyl acetate, hexan-1-ol, 3-methylbutyl butyrate and hexyl butyrate (Nogueira et al., 2003), 1-methylhexyl butyrate, 3-methylbutyl butyrate and octanoic acid (Wang, Li, Chen, Bao, & Yang, 2007), 3-methylbutan-1-ol and eugenol (Miranda, Nogueira, Pontes, & Rezende, 2001; Nogueira et al., 2003), butan-1-ol and hexanal (Mui, Durance, & Scaman, 2002; Nogueira et al., 2003), isobutyl acetate and pentan-2-one (Salmon, Martin, Remaud, & Fourel, 1996), isoamyl acetate (Mui et al., 2002; Salmon et al., 1996) and isoamyl butyrate (Boudhrioua et al., 2003; Brat et al., 2004; Mui et al., 2002; Salmon et al., 1996; Wang et al., 2007), have been reported as common components of several banana varieties. According to some authors, the banana aroma was particularly associated with methyl, 3-methylbutyl, 2-methylpropyl and isoamyl esters of acetic, propanoic and butanoic acids. However, the volatile fraction produced by bananas from Dwarf Cavendish, Prata, Ouro and Maçã cultivars, is dominated by 3-methylbutyl butyrate which account for 28.3%, 37.8%, 50.0% and 34.5%, respectively, of total GC peak area, whereas isoamyl acetate (27.6%) present the highest level in bananas from Platano cultivar. In addition, Maçã and Prata banana varieties are richer in ethyl esters, carbonyl compounds and monoterpenoids, than Ouro, Dwarf Cavendish and Platano bananas (Table 2).

In comparison to banana cultivars from other geographical origins some compositional differences could be observed. Pentan-2-one was reported as one of major constituents in several banana cultivars from Spain and Philippines (Salmon et al., 1996; Shiota, 1993), but not in the cultivars investigated in this work. Another interesting difference is the substantial content of eugenol reported in bananas from Spain, Philippines and Latin America (Salmon et al., 1996; Shiota, 1993), which was only identified in banana from Dwarf Cavendish and Prata cultivars.

3.5. Multivariate analysis

To summarise graphically the changes in the volatile content in the different cultivars, a principal component analysis (PCA) was successfully applied to the final data set in order to study the main sources of variability between the banana samples from various cultivars and detect the potential relationships/variables responsible for differentiation. The PCA analysis led to the extraction of two principal components (PCs) having the initial eigenvalues > 1 which contributed to 92.1% of the total variance of the data set.

The first principal component (PC1) identified as a linear combination of 3-methylhexyl butyrate (MHEBAC), heptan-2-ol (HEPOL:), isopentyl hexanoate (IPH:) and 2-pentyl acetate (A2PEOL) accounted for 58.2% of the variance. PC2 explained 33.9% of the variance and was mainly represented by the linear combination of the following variables: 2-methyl-2-methylpropyl butyrate (M2MEAB), (*Z*)-2-hexenyl butyrate (HEXBA) and *n*-butyl butyrate (BEBA) (Fig. 3a). The scatter plot of PCA scores corresponding to this discrimination is illustrated in Fig. 3b.

The first axis (58.2% of the variance explained), mainly discriminate bananas from Ouro and Maçã cultivars from the others, whereas the second axis (33.9% of the variance explained) mainly discriminate Dwarf Cavendish from Platano and Prata cultivars. Bananas from Ouro and Maçã cultivars, positively located on the first axis, are particularly characterised by heptan-2-ol, 3-methylbutyl butyrate, 1-methylhexyl butyrate and pentan-2-ol. Dwarf Cavendish positively located on the second axis (PC2) is mainly discriminated among bananas from Prata and Platano cultivars by a greater content of 2-methyl-2-methylpropyl butyrate, butyl butyrate, isobutyl acetate, isoamyl acetate and pentan-2-one. These results clearly demonstrate a clear classification of banana samples according to the cultivars based on volatile metabolites identified in the free fraction. The eigenvalues and explained and accumulated variance of the first five principal components (PCs) are listed in Table 3.

After PCA, stepwise linear discriminant analysis was performed on peak area data in order to highlight the most significant variables in the differentiation between banana cultivars and to find discriminant functions to predict the group to which future samples belonged. The projection of the banana cultivars in two-dimensional space, generated by the two first discriminant functions, explain 77.8% of the total variance.

Four clusters (Dwarf Cavendish and Prata cases are grouped, low Mahlanodis distance) representing each cultivar were observed. The good agreement achieved indicates that very acceptable classification functions can be deduced. The prediction ability of the SLDA model developed was evaluated by leave-one-out cross-validation. Table 3 shows the classification and prediction rates corresponding to the SLDA model developed on the basis of the subset of variables with the largest discriminant power between the five categories selected by the stepwise procedure.

From the obtained results, we can ascertain that dHS-SPME/1D-GC–qMS together with a PCA represent a new solution toward fruit cultivar classification through the analysis of their volatile fraction.

4. Conclusions

This research consisted in a feasibility study to examine the use of dHS-SPME/1D-GC–qMS in combination with pattern recognition techniques as a simple, fast and reliable classification methodology to objectively discriminate between bananas from different cultivars on the basis of their volatile distinctive profiles. dHS-SPME in combination with gas chromatography–mass spectrometry provides a suitable and selective mean to establish the global volatile signature in different banana cultivars. The optimisation of the significant parameters affecting sorption process such as coating, extraction time and temperature, was done by using univariate experimental design. The chromatographic profile obtained, the total 1D-GC–qMS peak area and the number of identified metabolites, suggest that the PDMS/DVB coating is the most suitable for isolation the VOMs from bananas using the dHS-SPME technique. The optimum set of conditions for the influential parameters was 60 min and 50 °C for extraction time and extraction temperature, respectively.

Ethyl esters were found to comprise the largest chemical class in the bananas from studied cultivars, accounting 80.9%, 86.5%, 51.2%, 90.1% and 6.1% for the volatile fraction in Dwarf Cavendish, Prata, Ouro, Maçã and Platano varieties, respectively. As found for bananas from Ouro cultivar (50.0%), 3-methylbutyl butyrate was found to be the most abundant volatile metabolite in bananas from Dwarf Cavendish (28.3%), Prata (37.8%) and Maçã (34.4%) cultivars, while isoamyl acetate (27.6%) occur as the major volatile metabolite in bananas from Platano cultivar.

Finally the acquired data set was submitted to PCA and the corresponding bananas discrimination according to cultivars was successfully established. The application of the SLDA method on the volatile metabolites data set characterised by dHS-SPME/1D-GC–qMS resulted in predictive abilities of 90% as evaluated by the cross-validation procedure. The method could be extended to other fruit species permitting the development of a new tool useful in food quality control.

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