Talanta 83 (2011) 899–906

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Effectiveness of different solid-phase microextraction fibres for differentiation of selected Madeira island fruits based on their volatile metabolite profile—Identification of novel compounds

João Pereira [a], Jorge Pereira [a], José S. Câmara [a,b,*]

[a] Centro de Química da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal
[b] Centro de Ciência Exactas e da Engenharia, Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal

ARTICLE INFO

Article history:
Received 7 June 2010
Received in revised form 24 October 2010
Accepted 29 October 2010
Available online 10 November 2010

Keywords:
Fruits
Volatile metabolite profile
Solid-phase mixcroextraction
Fibre comparison

ABSTRACT

A headspace solid-phase microextraction (HS-SPME) procedure based on five commercialised fibres (85 μm polyacrylate – PA, 100 μm polydimethylsiloxane – PDMS, 65 μm polydimethylsiloxane/divinylbenzene – PDMS/DVB, 70 μm carbowax/divinylbenzene – CW/DVB and 85 μm carboxen/polydimethylsiloxane – CAR/PDMS) is presented for the characterization of the volatile metabolite profile of four selected Madeira island fruit species, lemon (*Citrus limon*), kiwi (*Actinidia deliciosa*), papaya (*Carica papaya* L.) and Chickasaw plum (*Prunus angustifolia*). The isolation of metabolites was followed by thermal desorption gas chromatography–quadrupole mass spectrometry (GC–qMS) methodology. The performance of the target fibres was evaluated and compared. The SPME fibre coated with CW/DVB afforded the highest extraction efficiency in kiwi and papaya pulps, while in lemon and plum the same was achieved with PMDS/DVB fibre. This procedure allowed for the identification of 80 compounds, 41 in kiwi, 24 in plums, 23 in papaya and 20 in lemon. Considering the best extraction conditions, the most abundant volatiles identified in kiwi were the intense aldehydes and ethyl esters such as (E)-2-hexenal and ethyl butyrate, while in Chicasaw plum predominate 2-hexenal, 2-methyl-4-pentenal, hexanal, (Z)-3-hexenol and cyclohexylene oxide. The major compounds identified in the papaya pulp were benzyl isothiocyanate, linalool oxide, furfural, hydroxypropanone, linalool and acetic acid. Finally, lemon was shown to be the most divergent of the four fruits, being its aroma profile composed almost exclusively by terpens, namely limonene, γ-terpinene, o-cymene and α-terpinolene. Thirty two volatiles were identified for the first time in the fruit or close related species analysed and 14 volatiles are reported as novel volatile metabolites in fruits. This includes 5 new compounds in kiwi (2-cyclohexene-1,4-dione, furyl hydroxymethyl ketone, 4-hydroxydihydro-2(3H)-furanone, 5-acetoxymethyl-2-furaldehyde and ethanedioic acid), 4 in plum (4-hydroxydihydro-2(3H)-furanone, 5-methyl-2-pyrazinylmethanol, cyclohexylene oxide and 1-methylcyclohexene), 4 in papaya (octaethyleneglycol, 1,2-cyclopentanedione, 3-methyl-1,2-cyclopentanedione and 2-furyl methyl ketone) and 2 in lemon (geranyl farnesate and safranal). It is noteworthy that among the 15 volatile metabolites identified in papaya, 3-methyl-1,2-cyclopentanedione was previously described as a novel PPARγ (peroxisome proliferator-activated receptor γ) agonist, having a potential to minimize inflammation.

1. Introduction

Aroma is one of the most important attributes that affects the flavour of a fruit and thus its acceptability and consumption. The formation of the aroma compounds is a dynamic process influenced by several factors. Fruit ripeness is one of the most relevant as typical flavour of most fruits is not present during early formation and only develops after fruit ripening. During this period, metabolism changes to catabolism and volatile metabolites are formed from major plant constituents through various biochemical pathways [1]. In many cases, there are also pharmacological effects associated to some fruits and plants. Therefore the characterization of the volatiles that compose specific plant and fruit aromas may have considerable economical and health impacts [2]. This characterization will be also the fingerprint of each plant and fruit and could contribute to certify its identity and quality [3].

Based on these evidences, the volatile metabolite composition of four fruits produced in Madeira island: lemon (*Citrus limon* (L.) Burman f.), kiwi (*Actinidia deliciosa* cv. Hayward), Chickasaw plum

---

* Corresponding author at: Centro de Ciência Exactas e da Engenharia, Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal. Tel.: +351 291705112; fax: +351 291705149.
E-mail address: jsc@uma.pt (J.S. Câmara).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.10.064

(*Prunus angustifolia* Marsh. var. angustifolia) and papaya (*Carica papaya* Linn), was investigated.

Lemon (*C. limon* (L.) Burman f., specie name according to the Tanaka system [4]) is a rich source of nutrients, including flavonoids, citric acid, vitamin C, and minerals [5]. Its specific high content in flavonoids, especially flavanone (eriocitrin and hesperidin) and flavone glycosides is unique among fruits and vegetables and is specially relevant as these compounds have been extensively shown to have several protective effects against oxidative stress damage in human cells [6]. Lemons have a pleasant aroma and there are several studies characterizing the volatile composition of several parts of its plant [7]. Limonene, several terpenes and aldehydes were the main volatiles identified in these studies [8]. However, many of the compounds that constitute lemon juice aroma remain to be identified.

The kiwi fruit is the edible fruit of *Actinidia chinensis*, a specie native from China [9]. It is rich in potassium, vitamin C, A and E and carotenoids [10]. Several studies [11–13] indicate that there are more than 70 volatile compounds involved in the composition of kiwi, mainly C6 aldehydes, alcohols and esters, being the aldehydes more prevalent in unripe fruit, and esters in the ripe fruit. Hexanal, *trans*-2-hexenal and ethyl butanoate are the compounds more often reported.

Plums belong to the *Prunus* genus of plants that also include peach (*Prunus persica*), cherry (*P. avium*), almond (P. *amygdalus*) and apricot (*P. armeniaca* L.). These fruits are a valuable source of antioxidant phytochemicals to the human diet and include L-ascorbic acid, quercetin, kaempferol, myricetin, p-coumaric acid, gallic acid, and ellagic acid [14,15].

P. angustifolia cv. Marsh., commonly known as Chickasaw plum, is produced in Madeira island, but this cultivar is native from USA. Its aroma composition is poorly characterized and the data available refers to close related *P. domestica* cultivars. Benzaldehyde, nonanal, and 2-furancarboxaldehyde are indicated as the major constituents of canned plums (*P. domestica*) [16], hexanol, linalool, benzaldehyde, methyl cinnamate and γ-decalactone, in others *P. domestica* cultivars [17], and (E)-2-hexenol, benzaldehyde, hexanal and (E)-2-hexenal, in sweet cherries (*P. avium* L.) [18]. In the "Hujingmilu" peach (*P. persica* L.), the most representative volatiles switch from *trans*-2-hexenal and cis-3-hexenol in immature fruits, to γ- and δ-lactones in the ripe fruit [19].

Papaya fruit (*C. papaya* Linn) is a native fruit from the Caribbean coast of Panama and Colombia. The Brazilian 'Golden' papaya cultivar is the most appreciated by its pleasant flavour and taste qualities, such as rich pulp, sweetness, redness, and softness [20]. In several countries, papaya leaves are used to tenderize meat. This degradation effect is due to the presence of the cysteine protease papain [21]. Papaya is also very used in the traditional medicine, particularly as an antiseptic and contraceptive [22] and wound care [23]. In a recent report [24], Collard and Roy provide evidences that the wound diabetic mouse model benefits from a fermented papaya supplementation that specifically influences the response of wound-site macrophages and subsequent angiogenic mechanism. Another work [25] showed that antioxidative stress potential of papaya juice is comparable to the antioxidant compound α-tocopherol, explaining why papaya extracts seem to be helpful against age-related complications protection against oxidative stress damage in the cells [25]. Papaya does not possess a strong aromatic profile and therefore very little is known about its volatile composition. However, given its importance in human health, will be very important to characterize as much as possible the volatile composition of this fruit.

The methodology used to identify the volatile composition of a certain fruit usually involves different extraction methods, followed by gas chromatography–mass spectrometry (GC–MS). Several works showed that the use of solid-phase microextraction (SPME) to extract fruit volatiles has considerable advantages when compared with the classic methods employing organic solvents [26–28]. In fact, SPME is a simple, rapid and solvent-free method, integrating sampling, extraction, concentration and sample introduction into a single step and thus involves less laborious manipulation and sample requirements. Moreover, as does not require any concentration step prior to analysis, prevents the production of artefacts [29,30].

Hence, in this work, the characterization of the volatile metabolites of the pulp of lemon, kiwi, Chickasaw plum and papaya, all produced in Madeira island, was performed by HS-SPME/GC–qMS, using five different SPME fibres. The performance of the five SPME fibres in the extraction of volatiles was evaluated. This approach was shown to be a reliable and fast process and allowed to discriminate which is the best fibre to use with each fruit. To our knowledge, this is the first study reporting *C. papaya* and *P. angustifolia* plum pulps volatile composition using HS-SPME/GC–qMS methodology. This approach allowed for the identification of new volatiles, some reported in the aroma composition of other fruits and others identified for the first time.

2. Materials and methods

2.1. Reagents and materials

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

2.2. Fruit samples

The fruit samples (3 samples of each fruit) used in this work consisted of fresh integral pulps from kiwi (*A. deliciosa* cv. Hayward), Chickasaw plum (*P. angustifolia* cv. Marsh.), papaya (*C. papaya* Linn, cv. Golden) and lemon (*C. limon* L. Burman f. cv. Eureka). They were acquired in Madeira island local producers. Each ripped fruit pulp was separated from the seeds and skin, homogenised with a home blender, added with an amount of calcium chloride ($CaCl_2$) in order to inhibit the enzyme activity and finally stored in polyethylene bottles at −20 °C until analysis.

2.3. HS-SPME procedure

Five fibres were used for screening the aroma from fruit pulps: carbowax–divinylbenzene (CW/DVB, 70 μm), carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm), polyacrylate (PA, 85 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm) and polydimethysiloxane (PDMS, 100 μm). The coating of all fibres was 1 cm length and prior to GC–qMS analysis, each fibre was conditioned according to the instructions provided by the manufacturer.

The HS-SPME procedure was performed according to Ferreira et al. [31]. Briefly after conditioning, fibres were exposed to the headspace of a 4 mL septum-sealed glass vial containing 2 ± 0.001 g of sample, 0.5 mL of ultra-pure water, 1 μL of internal standard (3-octanol, 4.22 mg L^{-1}) and 0.10 g of NaCl. This was performed for 30 min at 30 1 °C, under constant magnetic stirring (800 rpm). Once sampling was finished, the SPME fibre was withdrawn into the needle and immediately inserted into the GC system injection port at 250 °C for 6 min, where the analytes are thermally desorbed

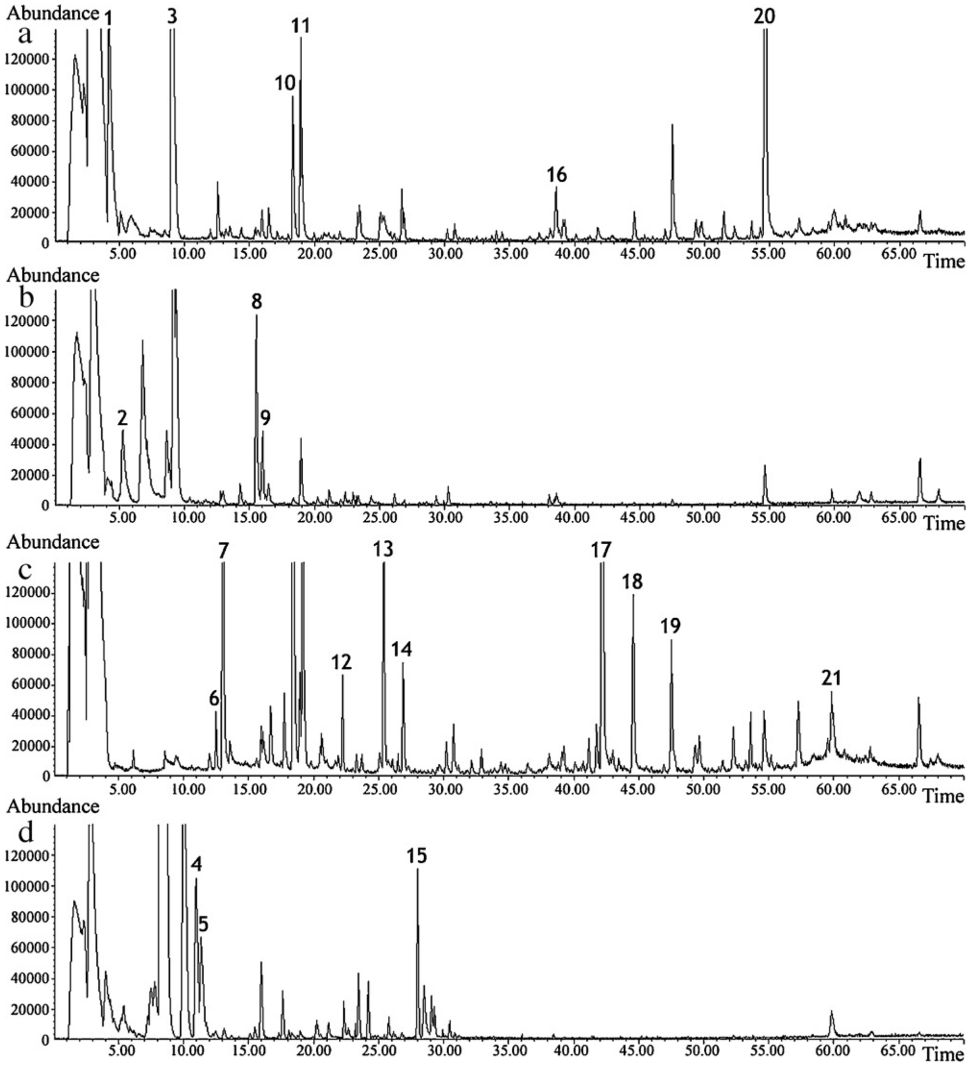

Fig. 1. GC–qMS chromatograms of fruit pulp samples: (a) kiwi CW/DVB, (b) plum PDMS/DVB, (c) papaya CW/DVB and (d) lemon PDMS/DVB. Peak identification – 1: ethyl butanoate; 2: (E)-2-hexenal; 3: 2-hexenal: 4: *p*-cymene; 5: terpinolene; 6: 3-hydroxy-2-butanone; 7: 1-hydroxy-2-propanone; 8: 3-hexenol; 9: 3-octanol (IS); 10: acetic acid; 11: furfural; 12: β-myrcene; 13: butanoic acid; 14: 2-furanmethanol; 15: α-terpineol; 16: 2,5-furandicarboxaldehyde; 17: benzyl isothiocyanate; 18: 2-hydroxy-γ-butyrolactone; 19: 3-hydroxy-2,3-dihydromaltol; 20: 5-hydroxymethylfurfural; 21: *n*-tetradecanoic acid; 22: *n*-hexadecanoic acid.

from the fibre coating and transferred directly to the GC system column. Blank runs were conducted between extractions to check the absence of carry over which would cause memory effects and misinterpretation of results. Each extraction was done in triplicate. The repeatability of the HS-analysis, expressed as coefficient of variation (CV %), was found to be between 4.8 and 18.3%.

2.4. GC–qMS analysis

The volatile metabolites extracted by HS-SPME procedure from fruit pulps were separated in an Agilent Technologies 6890N Network gas chromatograph system (Agilent Technologies, 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 constant flow rate of 1 mL min^{-1} (column-head pressure: 13 psi). The injector was operated in the splitless mode. The initial oven temperature was 40 °C, followed by a linear programmed temperature from 40 °C to 220 °C held for 10 min at a rate of 3 °C min^{-1}. The injection and ion source temperatures were 250 and 220 °C, respectively. For the 5975 MS system, the temperatures of the transfer line, quadrupole and ionization source were 270, 150 and 230 °C respectively; electron impact mass spectra were recorded at 70 eV ionization voltages and the ionization current was 10 μA. The acquisitions were performed in scan mode in the range 35–300 *m*/*z*.

2.5. Qualitative and semi-quantitative analysis

The volatile metabolites were identified on the basis of their fragmentation patterns using the NIST Mass Spectral Search Program (NIST, Washington, DC). In addition, the compounds were tentatively identified by comparing the experimental Retention Indices with the theoretical ones, which were obtained from literature. The relative amounts of individual components were expressed as percent peak areas relative to total peak areas.

3. Results and discussion

3.1. Comparison of the fibre efficiency

The characterization of the volatile composition of four ripped selected fruits, kiwi, plum, papaya and lemon, produced in 2009

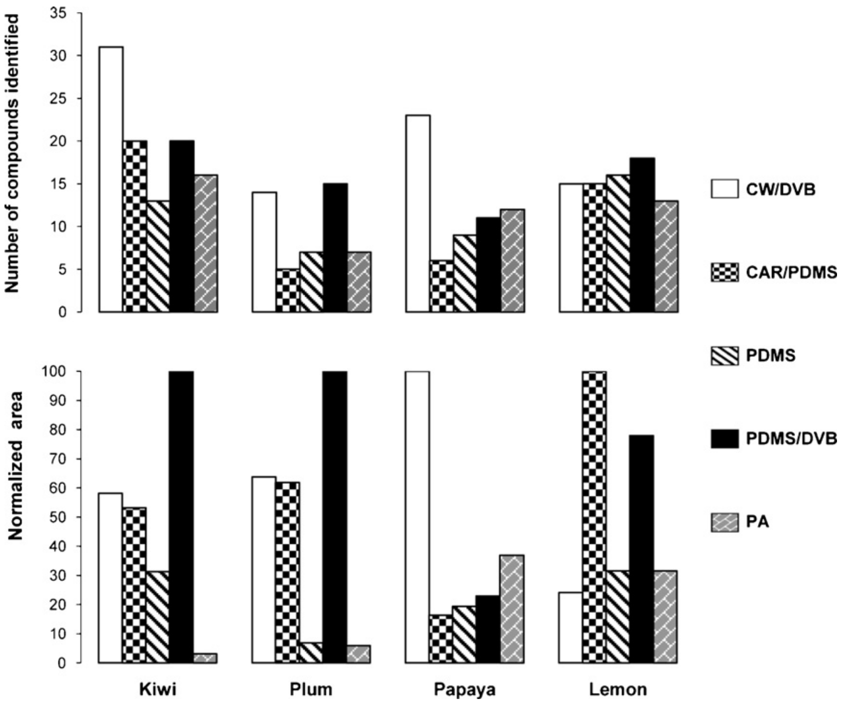

Fig. 2. Normalized extraction efficiencies and number of volatile metabolites extracted with different fibres measured for the selected fruits.

Table 1
Volatile compounds identified in kiwi (K), plum (C), papaya (P) and lemon (L) fruit pulp after dynamic headspace solid-phase microextraction using different fiber coating.

	RT (min)	RI	Compounds	SPME fibers				
				CW/DVB	CAR/PDMS	PA	PDMS	PDMS/DVB
			Aldehydes					
ACEGH	5.28	1111	Hexanal	K	KC	K	K	KC
AJ	6.92	1167	2-Methyl-4-pentenal		K			KC
ACGH	9.15	1228	(E)-2-hexenal	KC	KC	KC	KC	KC
IJ	16.43	1377	(E,E)-2,4-hexadienal		K	K		
CI	17.19	1390	(E)-2-octenal					K
BCDEI	20.21	1447	Decanal					CPL
BCDEI	21.04	1462	Benzaldehyde		KP	P	P	KP
CDHI	21.64	1472	(E)-2-nonenal					K
AJ	28.37	1588	3-Ethylbenzaldehyde		K			
BCHI	57.27	2261	Acetaldehyde	P				
			Alcohols					
HI	5.69	1127	Isobutanol	K				
ABCEI	14.32	1339	1-Hexanol	K	KC	C		KC
ABCEF	15.51	1361	(Z)-3-hexenol	KC	C	C	C	C
ABI	16.55	1379	(Z)-2-hexenol	K			K	K
HI	23.49	1503	2,3-Butanediol			K		K
CIJ	32.15	1645	Cyclo-2-hexenol	P				
BI	34.74	1804	Benzyl alcohol					P
K	38.61	1879	5-Methyl-2-pyrazinylmethanol				C	C
K	59.97	2318	Octaethyleneglycol	P				
			Esters					
ABEFH	4.40	1072	Ethyl butanoate	K	K	K	K	K
BEFI	12.80	1451	(Z)-3-hexenylbutanoate					C
I	19.16	1427	Methyl pyruvate	P				
ABHI	26.83	1563	Ethyl benzoate	K	K	K	K	K
B	42.21	1953	Benzyl isothiocyanate	P	P	P	P	P
			Ketones					
ABCD	12.50	1301	3-Hydroxy-2-butanone	C				
C	13.01	1312	Hydroxyacetone	KCP				
BD	13.74	1327	6-methyl-5-heptene-2-one	K	K			P
BCI	26.19	1552	Acetophenone	K	K			KCP
	29.59	1607	2-Cyclohexene-1,4-dione		K			
K	30.75	1625	1,2-Cyclopentanedione	P				
F	30.76	1626	2-Hydroxy-2-cyclopenten-1-one	K				
K	32.92	1656	3-Methyl-1,2-cyclopentanedione	P				
F	41.77	1934	Dihydroxyacetone	KP				
IJ	47.53	2053	3-Hydroxy-2,3-dihydromaltol	KCP		KP	KC	KC

Table 1 (*Continued*)

	RT (min)	RI	Compounds	SPME fibers				
				CW/DVB	CAR/PDMS	PA	PDMS	PDMS/DVB
BHI	53.63	2181	Benzophenone	KP		KP	KP	KP
			Furan compounds					
BCFGHIJ	18.94	1421	Furfural	KCPL	K	KCPL	KCPL	KCPL
K	20.50	1452	2-Furyl methyl ketone	P		P		
ACFHJ	23.35	1500	5-Methylfurfural	KC	K	C	K	C
HI	25.32	1537	Ethyl 2-furoate	K	K	K		K
EF	26.87	1564	2-Furanmethanol	P				
HJ	26.85	1565	3-Furanmethanol	KC				
BCIJ	28.09	1584	γ-Hexalactone		K			
I	30.19	1617	2(5H)-furanone (γ-crotonolactone)	KCP				
F	38.57	1878	2,5-Frandicarboxaldehyde	K	K	K	K	K
K	39.14	1889	Furyl hydroxymethyl ketone	K				
F	44.59	1995	2-Hydroxy-γ-butyrolactone	P				
K	45.40	2011	5-Acetoxymethyl-2-furaldehyde	K				
FGHJ	54.64	2202	5-Hdroxymethylfurfural	KCP	K	KCP	KCP	KP
K	57.25	2260	4-Hdroxydihydro-2(3H)-furanone	KC				
			Acids					
ABCDEFH	18.49	1414	Acetic acid	KCPL	KCP	KCP	KCPL	KCPL
BCFI	25.34	1537	Butanoic acid	P	P	P	P	P
J	51.51	2134	Ethanedioic acid	K				
FJ	52.28	2151	Benzoic acid	KP				
HJ	59.83	2315	n-Tetradecanoic acid	KP		K		K
CFJ	66.60	2451	n-Hexadecanoic acid	KCP		K		C
			Terpenic compounds					
ABDEFG	5.48	1119	β-Pinene	L	L	L	L	L
DF	7.16	1174	β-Myrcene	L	L	L	L	L
ABCDI	8.16	1201	D-Limonene	L	L	L	L	L
BCDI	9.78	1243	γ-Terpinene	L	L	L	L	L
DH	10.77	1266	o-Cymene	L	L	L	L	L
DI	11.35	1278	α-Terpinolene	L			L	L
CD	17.64	1397	*p*-Cymenene	L	L		L	L
BDJ	17.71	1399	Cis-linalool oxide		P	P	P	P
ABDEFI	22.24	1482	Linalool	P	P		P	P
D	22.34	1484	Bergamol	L	L	L	L	L
DH	23.47	1502	Bergamotene	L	L	L	L	L
ABDI	24.21	1517	Terpinen-4-ol	L	L	L	L	L
J	25.76	1545	Safranal					L
ABDE	28.01	1583	α-Terpineol	L	L	L	L	L
D	28.43	1589	γ-Selinene		L	L	L	L
DH	29.08	1599	β-Bisabolene	L	L	L	L	L
D	29.43	1605	Geranial		L			
BDE	30.29	1618	3-Carene	L	L			
J	30.47	1621	Geranyl valerate		L	L	L	L
EHI	34.01	1670	*trans*-geranylacetone	K		K		
			Others					
J	8.62	1214	Cyclohexylene oxide		C			C
	16.47	1378	1-Methylcyclohexene		C	C	C	C
IJ	24.37	1520	Ethyl-cyclohexane		K			
BCJ	29.37	1604	Naphthalene	C				C

Different volatiles identified in: A – Kiwi species [38,41], B – Papaya species ([42-46]), C – Prunus species [38], D – Citrus species [38], E – Passion fruit species [34], F – Annona fruit species [32]; G – Banana cultivars (our unpublished results), H – Rosaceae family apples [33,47], I – other fruits (see detailed references in Supplementary Table 1); J – other plant species [48]; K – other sources (see detailed references in Supplementary Table 1).

in Madeira island, was done with the best HS-SPME methodology conditions previously reported [31–33]. In those reports, we have also shown that the selection of the appropriate fibre depends on the target compounds and the matrix studied. Therefore, five types of fibres (CAR/PDMS, PDMS/DVB, PDMS, PA and CW/DVB) with a range of polarities and mechanisms, among those used routinely for assaying volatiles, were tested and compared to evaluate the effect of different coatings on the extraction of volatile metabolites among each fruit. Each fibre was exposed to the headspace at the same temperature (30 °C), during the same extraction time (30 min) and under constant magnetic stirring (800 rpm).

As an example, Fig. 1 shows the typical total ion chromatograms (TIC) obtained for 2 g of fruit pulp using the best fibre coating for each fruit.

The comparison of the SPME fibre performance was made in terms of extraction efficiency, estimated by the number of identifiable compounds in each fruit extract, total GC–qMS peak area and reproducibility. The results obtained are illustrated in Fig. 2.

According to them, the fibre coating that has the best efficiency is not the same to all fruits. While in plum and lemon, the semi-polar PDMS/DVB fibre afforded the best extraction efficiency, in kiwi and papaya this was achieved by the CW/DVB fibre. It is noteworthy that in kiwi the extraction efficiency of the PDMS/DVB fibre, considered only in terms of the total peak areas, is almost the double of CW/DVB. However, the number of compounds identified in the first fibre is only two thirds of the ones identified in the CW/DVB fibre. Notably, in the only report we found about the use of SPME in the characterization of kiwi aroma profile, CW/DVB was the fibre used to extract kiwi volatiles [34].

Fig. 2 shows that all the fibres used in this study allowed for the identification of more than 65% of the volatiles that are present in lemon. Moreover, the most represented compounds (D-limonene and γ-terpinene, as we will show below) are equally represented

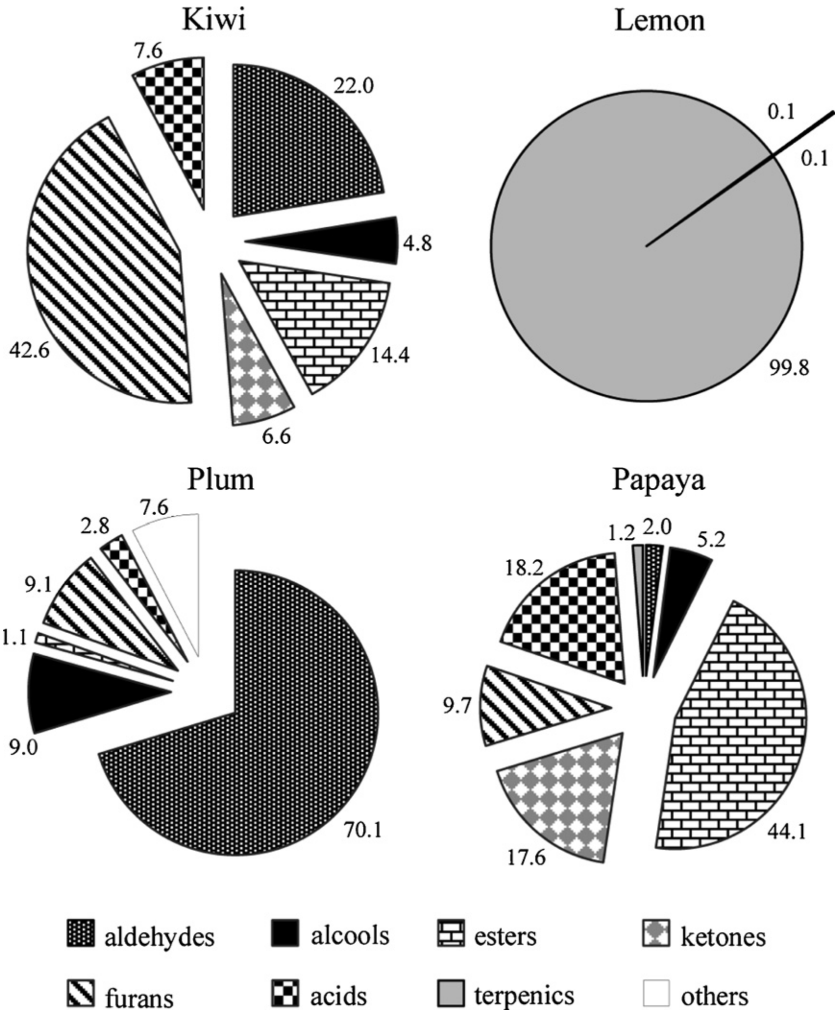

Fig. 3. Distribution of fruit volatiles by chemical families. The values indicate the relative peak area (in percentage) of each chemical family.

in all fibres (data not shown). Therefore, the fibre that afforded the best extraction in lemon was PDMS/DVB because the number of volatiles identified with this fibre was higher, although the peak areas were slightly lower than the obtained with the CAR/PDMS fibre (see Fig. 2).

In the whole, CW/DVB can be considered the more flexible fibre to use with different fruits as it allowed for the characterization of more than 67% of the volatiles identified in any of the four fruits here studied. The same result was previously obtained in the characterization of annona [31] and apples [32] volatiles.

3.2. Characterization of the volatile metabolites profile

The volatile metabolites identified in the selected fruits and using different fibres are listed in Table 1. A total of 80 different compounds, 41 in kiwi, 24 in plums, 23 in papaya and 20 in lemon, were tentatively identified by matching mass spectra with spectra of reference compounds in NIST mass spectral libraries with a resemblance percentage above 80%. The Retention Indices (RIs, see Table 1) were calculated for each peak and, when available, compared with the literature in order to certify the compound identification. These compounds were organized in different groups according to their chemical structure as terpenes (20), furans (14), ketones (12), aldehydes (10), alcohols (9), acids (6) and esters (5). As can be observed in Table 1, the qualitative composition of each fruit volatiles and the number of identified compounds using the five fibres under study are very different. The volatiles identified were grouped by chemical family and the relative peak area of each one in the four fruits is represented in Fig. 3.

As can be observed, the volatile composition of lemon aroma is the least heterogenic. Lemon volatile metabolites are almost exclusively terpenics, being decanal, furfural and acetic acid the only non-terpenic volatile metabolites identified in this fruit. Among the 20 compounds identified in this fruit, these three non-terpenic compounds represent less than 0.2% of the total peak area obtained. Moreover, decanal presents a citrus-like flavour.

In opposition, kiwi, plum and papaya volatile composition is much more heterogeneous. The chemical classes that contribute to the aroma profile of these fruits are aldehydes, esters, alcohols, ketones, acids and furans in different proportions and this plays a significant role in their organoleptic profile differences. Esters compounds, for instance, are important in the fruit ripening and their relative contribution to the fruit aroma increases with the ripeness. Different reports show that in some fruit species like apple, pear, annona and banana [35], as well as other tropical fruit [36], esters are the major volatile compounds on their characteristic aroma profile. Our previous work confirms this feature in annona [31], apples [32] and banana (our unpublished work). In this study we show that papaya and kiwi belong to this group of fruits with high content of esters in their aroma profile, but not plum. The ester content obtained in papaya was almost half of the volatile composition and in kiwi about 15%, but in plum, esters represent only 1.1% of its

Table 2
Most abundant volatiles identified in each fruit and the corresponding relative peak areas.

	Fruit			
Volatiles	Kiwi	Plum	Papaya	Lemon
5-Hydroxymethylfurfural	28.7	[a]	4.5	
2-Hexenal	21.0	38.1		
Ethyl butanoate	13.4			
furfural	8.5	4.6	2.2	0.1
2-Methyl-4-pentenal	[a]	22.5		
hexanal	1.0	9.3		
3-Hexenol	0.2	7.3		
cyclohexylene oxide		6.1		
Benzyl isothiocyanate			32.0	
Methyl pyruvate			12.1	
Acetic acid	3.1	0.4	11.2	0.1
Hydroxyacetone	1.1	[a]	7.3	
D-limonene				75.9
γ-Terpinene				10.2

[a] Compounds identified in the respective fruit using a fibre other than that afforded the best extraction.

Table 3
Volatiles identified for the first time in the respective fruit.

Volatile	Fruit[a]	Volatile	Fruit[a]
(E,E)-2,4-hexadienal	K	Benzoic acid	KP
(E)-2-octenal	K	n-Tetradecanoic acid	KP
Benzaldehyde	K	n-Hexadecanoic acid	KP
(E)-2-nonenal	K	Hydroxyacetone	KP
Isobutanol	K	Dihydroxyacetone	KP
2,3-Butanediol	K	3-Hydroxy-2-butanone	C
6-Methyl-5-heptene-2-one	K	(E)-2-hexenal	C
Acetophenone	K	2-Methyl-4-pentenal	C
2-Hydroxy-2-cyclopenten-1-one	K	5-Methyl-2-pyrazinylmethanol[b]	C
Benzophenone	K	Cyclohexylene oxide[b]	C
γ-Hexalactone	K	1-Methylcyclohexene[b]	C
Furfural	K	2-Cyclohexenol	P
Ethyl 2-furoate	K	Methyl pyruvate	P
2,5-Furandicarboxaldehyde	K	2-Hydroxy-γ-butyrolactone	P
trans-geranylacetone	K	2-Furanmethanol	P
Ethyl-cyclohexane	K	Octaethyleneglycol[b]	P
2-Cyclohexene-1,4-dione[b]	K	1,2-Cyclopentanedione[b]	P
Furyl hydroxymethyl ketone[b]	K	3-Methyl-1,2-cyclopentanedione[b]	P
Ethanedioic acid[b]	K	2-Furyl methyl ketone[b]	P
5-Acetoxymethyl-2-Furaldehyde[b]	K	Safranal[b]	L
4-Hydroxydihydro-2(3H)-furanone[b]	KC	Geranyl valerate[b]	L
3-Furanmethanol	KC		
5-Hydroxymethylfurfural	KCP		
2(5H)-furanone	KCP		
3-Hydroxy-2,3-dihydromaltol	KCP		

[a] K – kiwi; C – plum; P – papaya; L – lemon.
[b] Volatiles identified for the first time in fruits (see Supplementary Table 1 for references details).

aroma composition. Notably, the degree of ripeness associated to the consumption of papaya, kiwi and plum is in the same order as it is the relative contribution of esters to the final fruit aroma.

Aldehydes were found to comprise the largest chemical class in plum, accounting for 70% of its volatile composition, but only 22% in kiwi and 2% in papaya. Furans are the most important chemical family in kiwi, with almost half of its volatile composition, while in plum and papaya represent about 10%. Acids and ketones have a significant contribution to the volatile profile of papaya (almost 20% each group), being lower in kiwi (about 7% each group). In plums, the content in acids volatiles are very low (2.8%) and ketones are present at trace levels.

Concerning to the most abundant volatiles in each fruit, lemon continues to be the most homogeneous. As Table 2 indicates, D-limonene composes more than 75% of the volatile composition of this fruit, being the main responsible for the citrus aroma of lemon, followed by about 10% of γ-terpinene.

In kiwi, furans 5-hydroxymethylfurfural and furfural, the aldehyde 2-hexenal and the ester ethyl butanoate are the most abundant volatiles, representing more than 70% of the aroma composition of this fruit. 5-Hydroxymethylfurfural and furfural are usually associated to the sweet flavour of fruits and have been identified in several other fruits aroma, including annona [31], apples [32] and banana (our unpublished results), as well as different *Prunus* species [37]. The volatiles composition of different kiwi species has been evaluated by different methods, but to our knowledge, only the report from Wan et al. [34] used SPME to characterize kiwi aroma profile in the same *A. deliciosa* specie. The number, but not the volatiles identified, was the same. In that report, hexanal, 2-hexenal, ethyl butanoate and acetic acid were identified but that was not the case of volatiles that we identified as the most representative in kiwi aroma, such as 5-hydroxymethylfurfural and furfural as well as hydroxyacetone and 3-hexenol. Except for hydroxyacetone, all these representative volatiles identified in kiwi, were also reported in other species, as indicated in Table 1. Moreover, as far as we know, 30 volatile metabolites were identified for the first time in kiwi fruit (Table 3). Taking together, these observations suggest that the differences obtained in the volatile composition of kiwi aroma could be a hallmark of the region where they are produced.

Further studies using kiwi samples from the same specie but produced in different regions are necessary to access this hypothesis. 2-Hexenal was identified both in kiwi and plum, being the most abundant volatile (38.1%) in the latter fruit. This aldehyde enhances the aroma intensity and this could explain why we did not detect this compound in papaya, as this fruit it is not very aromatic. Remain representative compounds in plum are 2-methyl-4-pentenal (22.5%), hexanal, 3-hexenol and cyclohexylene oxide (these three with less than 10% each) and furfural (4.6%). As already referred, this is the first report of *P. angustifolia* plum volatile composition and therefore we compared the volatiles identified in this study with the ones associated to the aroma of other *Prunus* species. In fact, several compounds, namely acetic acid, hexanal and 3-hexenol have been previously described, but many others are described for the first time in the *Prunus* family, including the majority compound 2-methyl-4-pentenal (see Tables 1 and 3).

The strong aroma of lemon is due to its high and almost exlusive (99.8% of the volatile composition) terpenic content, mainly D-limonene that was also identified in this study as the main component (75.9% of lemon volatile composition), and γ-terpinene (10.2% of lemon volatile composition). From the 20 volatiles identified in lemon aroma, this is the first time that the geranyl valerate and the safranal are reported as components of the volatile composition of a fruit and particularly a *Citrus* specie.

Papaya volatile composition is dominated by two esters, benzyl isothiocyanate (32.1%) and methyl pyruvate (12.1%). Acetic acid (11.2%) and hydroxyacetone (7.3%) are also abundant in the volatile composition of this fruit. The approach used in this study was proven to be powerful in the characterization of papaya volatile metabolites as it allowed for the identification of 15 new volatiles (see Table 3 and Supplementary Table 1 for details). Two of this new volatiles, 1,2-cyclopentanedione and its derivative 3-methyl-1,2-cyclopentanedione, identified exclusively in papaya are particularly interesting. They were previously identified in coffee extract and 3-methyl-1,2-cyclopentanedione was shown to be a novel PPARγ agonist, having a potential to minimize inflammation [38]. This could explain some health benefits associated to papaya extracts, such as non-traditional wound care [23] that are not yet fully understood [24].

Furfural and acetic acid were identified in all four fruits. However, their contribution for the aroma composition of each fruit is very different. Acetic acid is only found in representative levels in

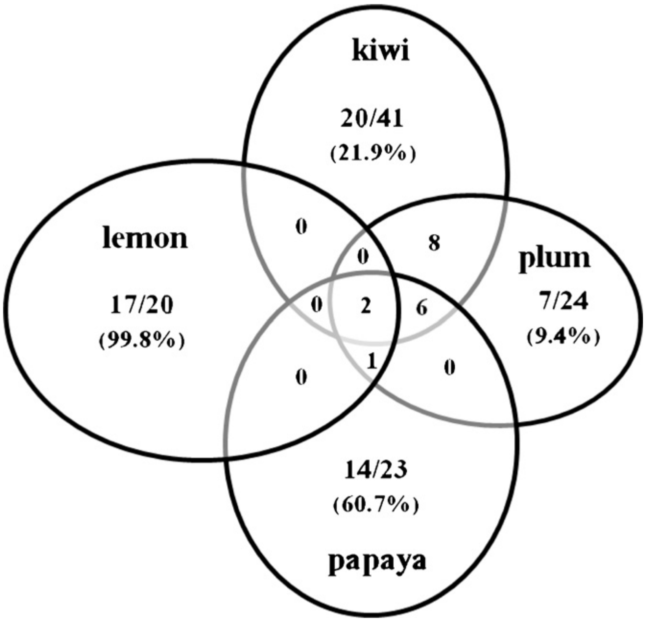

Fig. 4. Venn diagram corresponding to the analysis of the volatiles and respective relative peak areas (indicated between brackets) identified in the four fruits.

papaya and furfural in kiwi. Both volatiles are found in trace levels in lemon, as well as acetic acid in plum (0.4%).

A deeper analysis of the relative peak area obtained for the volatiles identified in each fruit, which is indicated in Table 2 only for the most pertinent volatiles, suggest that it is possible to distinguish different fruits based on the presence and abundance of certain volatiles. Some volatiles were identified only in one of the fruits, such as the terpens D-limonene and γ-terpinene in lemon, ethyl butanoate in kiwi and benzyl isothiocyanate and methyl pyruvate in papaya. Others were identified in different fruits but with completely different abundances. This is the case of 5-hydroximethylfurfural that was identified in kiwi and papaya, but it is five time more abundant in the first fruit; hexanal and 3-hexenol that represents almost 10% of the volatile composition of plum, but are poorly represented in kiwi; hydroxyacetone, also representative in papaya (7.3%), but present in low levels in kiwi; furfural, that was identified in all the fruits, but it is present in low amounts in papaya (2.2%) and in trace levels in lemon; and acetic acid, also identified in the four fruits, but in very low levels in plum and in trace levels in lemon. This analysis was extended to the 79 compounds identified and the results are presented in the Venn diagram of Fig. 4.

As can be observed, lemon is the most divergent fruit because 15 of the 20 volatiles identified were only identified in this fruit, representing 99.8% of the total peak area. In papaya, although only 11 of the 30 volatiles were exclusively identified, they represent more than 60% of the total peak area. Plum was the fruit less divergent with only 25% of the volatiles identified being specific for this fruit and representing less that 10% of the total peak area. All the volatiles identified in this study were searched in available databases and reports for their confidences as volatiles and fruits in which they were already identified. We found reliable data for more than 60% of the volatiles as indicated in Table 1.

4. Concluding remarks

HS-SPME was shown to be a fast and reliable extraction process and a valuable alternative to traditional methods for the establishment of the volatile pattern of four selected fruits produced in Madeira island, kiwi, plum, papaya and lemon, using HS-SPME/GC–qMS methodology. The chromatographic profiles obtained after extraction with PDMS, PA, CAR/PDMS, PDMS/DVB and CW/DVB coatings suggest that the latter was the most suitable for the SPME analysis of kiwi and papaya while PDMS/DVB was the most indicated to study the volatile metabolites found in plum and lemon.

This methodology allowed the identification of 80 different volatiles 41 in kiwi, 24 in plums, 23 in papaya and 20 in lemon, being many of them exclusively identified in the selected fruits. Moreover, 14 of these volatiles were identified as novel volatiles in fruits and 32 were associated to the volatile composition of the studied fruits for the first time.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.10.064.

References

[1] A. Perez, J. Rios, C. Sanz, J. Olias, J. Agric. Food Chem. 40 (1992) 2232–2235.
[2] F. Pellati, S. Benvenuti, F. Yoshizaki, D. Bertelli, M.C. Rossi, J. Chromatogr. A 1087 (2005) 265–273.
[3] C. Aubert, C. Chanforan, J. Agric. Food Chem. 55 (2007) 3074–3082.
[4] C.T. Federici, D.Q. Fang, R.W. Scora, M.L. Roose, Theor. Appl. Genet. 96 (1998) 812–822.
[5] J.A. Vinson, X. Su, L. Zubik, P. Bose, J. Agric. Food Chem. 49 (2001) 5315–5321.
[6] A. Gil-Izquierdo, M.T. Riquelme, I. Porras, F. Ferreres, J. Agric. Food Chem. 52 (2004) 324–331.
[7] G. Flamini, M. Tebano, P.L. Cioni, Anal. Chim. Acta 589 (2007) 120–124.
[8] R.L. Rouseff, P.R. Perez-Cacho, F. Jabalpurwala, J. Agric. Food Chem. 57 (2009) 8115–8124.
[9] J.P. Bartley, A.M. Schwede, J. Agric. Food Chem. 37 (1989) 1023–1025.
[10] I. Nishiyama, Adv. Food Nutr. Res. 52 (2007) 293–324.
[11] E.N. Friel, M. Wang, A.J. Taylor, E.A. Macrae, J. Agric. Food Chem. 55 (2007) 6664–6673.
[12] M.J. Jordan, C.A. Margaria, P.E. Shaw, K.L. Goodner, J. Agric. Food Chem. 50 (2002) 5386–5390.
[13] H. Young, V. Paterson, D. Burns, J. Sci. Food Agric. 34 (2006) 81–85.
[14] S. Kayano, H. Kikuzaki, N.F. Yamada, A. Aoki, K. Kasamatsu, Y. Yamasaki, T. Ikami, T. Suzuki, T. Mitani, N. Nakatani, Biofactors 21 (2004) 309–313.
[15] V. Shulaev, S.S. Korban, B. Sosinski, A.G. Abbott, H.S. Aldwinckle, K.M. Folta, A. Iezzoni, D. Main, P. Arus, A.M. Dandekar, K. Lewers, S.K. Brown, T.M. Davis, S.E. Gardiner, D. Potter, R.E. Veilleux, Plant Physiol. 147 (2008) 985–1003.
[16] H.M.M. Ismail, G.A. Brown, O.G. Tucknott, P.J. Holloway, A.A. Williams, Phytochemistry 16 (1977).
[17] H.M.M. Ismail, A.A. Williams, O.G. Tucknott, J. Sci. Food Agric. 32 (2006) 498–502.
[18] B. Girard, T.G. Kopp, J. Agric. Food Chem. 46 (1998) 471–476.
[19] X.M. Zhang, H.J. Jia, J. Plant Physiol. Mol. Biol. 31 (2005) 41–46.
[20] J.P. Fabi, B.R. Cordenunsi, G.P. de Mattos-Barreto, A.Z. Mercadante, F.M. Lajolo, J.R. Oliveira do Nascimento, J. Agric. Food Chem. 55 (2007) 6118–6123.
[21] F. Buhling, A. Fengler, W. Brandt, T. Welte, S. Ansorge, D.K. Nagler, Adv. Exp. Med. Biol. 477 (2000) 241–254.
[22] S. Mehdipour, N. Yasa, G. Dehghan, R. Khorasani, A. Mohammadirad, R. Rahimi, M. Abdollahi, Phytother. Res. 20 (2006) 591–594.
[23] B. Pieper, M.H. Caliri, J. Wound, Ostomy Continence Nurs. 30 (2003) 175–183.
[24] E. Collard, S. Roy, Antioxidant. Redox Signal 13 (2010) 599–606.
[25] Y. Noda, S. Murakami, M. Mankura, A. Mori, J. Clin. Biochem. Nutr. 43 (2008) 185–190.
[26] M. Chen, X. Chen, X. Wang, Z. Ci, X. Liu, T. He, L. Zhang, Agric. Sci. China 5 (2006) 879–884.
[27] N.S. Kim, D.S. Lee, J. Chromatogr. A 982 (2002) 31–47.
[28] S.N. Lee, N.S. Kim, D.S. Lee, Anal. Bioanal. Chem. 377 (2003) 749–756.
[29] F.M. Musteata, J. Pawliszyn, Trends Anal. Chem. 26 (2007) 36–45.
[30] S. Risticevic, V.H. Niri, D. Vuckovic, J. Pawliszyn, Anal. Bioanal. Chem. 393 (2009) 781–795.
[31] L. Ferreira, R. Perestrelo, J.S. Camara, Talanta 77 (2009) 1087–1096.
[32] L. Ferreira, R. Perestrelo, M. Caldeira, J.S. Camara, J. Sep. Sci. 32 (2009) 1875–1888.
[33] M. Pontes, J.C. Marques, J.S. Câmara, Microchem. J. 93 (2009) 1–11.
[34] X.M. Wan, R.J. Stevenson, X.D. Chen, L.D. Melton, Food Res. Int. 32 (1999) 175–183.
[35] J. Beekwilder, M. Alvarez-Huerta, E. Neef, F.W.A. Verstappen, H.J. Bouwmeester, A. Aharoni, Plant Physiol. 135 (2004) 1865.
[36] N. Narain, J.N. Almeida, M.S. Galvão, Cienc. Technol. Aliment. 24 (2004) 212.
[37] M. Dunkel, U. Schmidt, S. Struck, L. Berger, B. Gruening, J. Hossbach, I.S. Jaeger, U. Effmert, B. Piechulla, R. Eriksson, J. Knudsen, R. Preissner, Nucleic Acids Res. 37 (2009) D291–D294.
[38] S.Y. Choi, J.H. Chung, D.H. Kim, S.W. Chung, J.Y. Kim, B.P. Yu, H.Y. Chung, Biochim. Biophys. Acta 1770 (2007) 1612–1619.