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Screening of Brazilian fruit aromas using solid-phase microextraction–gas chromatography–mass spectrometry

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Abstract

Manual headspace solid-phase microextraction (SPME) coupled to gas chromatography–mass spectrometry (GC–MS) was used for the qualitative analysis of the aromas of four native Brazilian fruits: cupuassu (*Theobroma grandiflorum*, Spreng.), cajá (*Spondias lutea*, L.), siriguela (*Spondias purpurea*, L.) and graviola (*Anona reticulata*, L). Industrialized pulps of these fruits were used as samples, and extractions with SPME fibers coated with polydimethylsiloxane, polyacrylate, Carbowax and Carboxen were carried out. The analytes identified included several alcohols, esters, carbonyl compounds and terpernoids. The highest amounts extracted, evaluated from the sum of peak areas, were achieved using the Carboxen fiber.

Keywords: Fruits; Aroma compounds; Solid-phase microextraction; Extraction methods; Headspace analysis; Alcohols; Esters; Carbonyl compounds; Terpenoids; Volatile organic compounds

1. Introduction

Since its introduction by Arthur and Pawliszyn [1] in 1990 the technique of solid-phase microextraction (SPME) has been widely used for the extraction and pre-concentration of an extensive range of analytes in a variety of samples. Flavors and fragrances are samples for which SPME has been extensively used. These samples commonly have components that may undergo processes of thermal decomposition, oxidation, photolysis, etc. [2,3], and the possibility of such undesirable processes during the SPME sample processing is favorably reduced due to the simplicity of sample manipulation that is characteristic of the technique.

Chin et al. [4] used headspace SPME and gas chromatography–mass spectrometry (GC–MS) to analyze the aroma constituents in cheese samples; after the identification of compounds such as ethanol, acetone, diacetyl, acetoin, volatile fatty acids, esters and lactones, the authors have applied chemometric classification schemes for the studied samples. The application of SPME coupled to GC–MS and GC–FID (flame ionization detection) to detect and quantify aroma-related heterocyclic compounds generated by the Maillard reaction was addressed by Coleman III [5] with detection limits as low as $\mu g\ l^{-1}$. Headspace SPME was also applied to determine sulfur-containing compounds in onion [6] and wine [7] aromas. Several fruit juices and pulp aromas also have been studied with SPME coupled to GC: orange [8], tomato and strawberry [9] and mango [10], among others.

In this work, manual headspace SPME–GC–MS was employed to isolate and identify the main

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constituents of the aroma of four Brazilian tropical fruits:

(1) Cupuassu (*Theobroma grandiflorum*, Spreng.), is a native plant from Western Amazon that has a white to yellow pulp and a characteristic smell and slightly acidic taste. Its seeds are employed to prepare a chocolate-like product (''cupulate'') and the pulp is consumed as juice, ice cream and jam.

(2) Cajá (*Spondias lutea*, L.), is also an Amazonian plant. Its orange, small fruits produce a slightly bitter juice with a pleasant taste and an aroma resembling that of mango. It is used in juices, ice cream and similar preparations.

(3) Siriguela (*Spondias purpurea*, L.) is widely found in the northern part of South America. Its small and reddish fruits have an aromatic pulp of bittersweet taste and are consumed in natura or as juice and jam.

(4) Graviola (*Anona reticulata*, L.) is a typical fruit of the Brazilian Northeast states; the fruit has a green skin and an aromatic white pulp and is consumed as a juice.

2. Experimental

2.1. Fruit pulp samples

The samples consisted of frozen integral pulps, packed in polyethylene bags, of: graviola (De Marchi, Jundiaí, São Paulo, Brazil), cajá, siriguela and cupuassu (Brasfruit Frutos do Brasil, Feira de Santana, Bahia, Brazil).

2.2. SPME fibers

The following fibers (Supelco, Bellefonte, PA, USA) were used for the extraction procedures: 100 μm polydimethylsiloxane (PDMS), 85 μm polyacrylate (PA), 65 μm Carbowax–divinylbenzene (CW–DVB) and 75 μm Carboxen–PDMS (CAR–PDMS) fibers. Fibers were conditioned prior use according to supplier's prescriptions.

2.3. GC–MS system

The extracts were analyzed with a HP-6890 GC instrument (Hewlett-Packard, Wilmington, DE, USA) equipped with a HP-5973 mass-selective detector and a HP-5MS fused-silica capillary column (30 m×0.25 mm, 0.25 μm) and a split–splitless injector, operated in the splitless mode for all chromatographic runs. Grade 5.0 helium was used as carrier gas.

2.4. Data treatment

Chromatographic data treatment and peak identification was performed using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) v. 1.01 software and the NIST Mass Spectral Search Program v. 1.6d (NIST, Washington,

Table 1
GC–MS operational conditions and AMDIS software analysis parameters employed

GC–MS operational conditions	
Injector temperature	250°C
Column oven temperature program	2 min at 40°C→ 2°C min^{-1} to 100°C→30°C min^{-1} to 250°C
MS detector source temperature	230°C
MS quadrupole temperature	150°C
Carrier gas	He at 1.0 ml min^{-1}
MS mass scan range	30 to 300 u
AMDIS data analysis software parameters	
Mass range	30 m/z to 300 m/z
Threshold	Off
Scan direction	High to low
Spectral deconvolution	Three components
Adjacent peak subtraction	2
Resolution and sensitivity	High
Column bleed detection	207 m/z

DC, USA). The operational conditions for the chromatographic separations and data treatment are enumerated in Table 1.

2.5. Pre-extraction sample manipulation

Each sample was homogenized in a bender for ca. 1 min and the resulting slurry was immediately transferred to a 50-ml glass syringe which had its tip sealed with a silicone cap. Care was taken to not defrost the sample, which completely filled the syringe. The syringe containing the slurry was kept refrigerated at 12°C when not in use. The aforementioned procedure was thoroughly tested and adopted as it minimized the loss of the most volatile compounds found in the aromas.

Table 2
Compounds identified in cajá aroma after SPME extraction

Group	Compound	Fibers			
		CW–DVB	CAR–PDMS	PA	PDMS
Alcohols	1-Butanol		x		
	Amyl alcohol		x		
	Prenol (3-methyl-2-buten-1-ol)		x		
	3-Hexen-1-ol		x		
	1-Hexanol		x		
Aldehydes	Decyl aldehyde	x		x	x
Esters	Ethyl acetate		x	x	x
	Methyl butyrate		x		
	Ethyl butyrate	x	x	x	x
	Butyl acetate		x		
	Isoamyl acetate		x		
	Isobutyl butyrate		x		x
	Butyl butyrate		x	x	x
	Ethyl caproate		x	x	x
	Hexyl acetate		x	x	x
	Isoamyl butyrate		x	x	x
	Methyl benzoate		x	x	
	Ethyl benzoate		x	x	x
	Hexyl butyrate		x	x	x
	Ethyl caprylate		x	x	x
	Octyl acetate				x
Ketones	1-Penten-3-one		x		
Terpenic compounds	α-Pinene	x	x	x	x
	Camphene		x		x
	Sabinene		x	x	x
	β-Mircene		x	x	x
	Limonene		x	x	x
	γ-Terpinene		x	x	x
	Terpinolene		x	x	x
	β-Linalool				x
	Fenchyl alcohol		x	x	x
	α-Terpineol		x	x	x
	Copaene		x	x	x
	Caryophyllene		x	x	x
Total identified compounds per fiber		3	31	21	24

2.6. Headspace extraction procedure

In each extraction (1.00±0.05) g of the pulp slurry was transferred from the syringe to a 4-ml septum-sealed glass vial containing 1 ml of aqueous saturated NaCl solution and the system was kept at 60°C under magnetic stirring, to achieve the partition equilibration of the analytes between the sample and the headspace. After 15 min a SPME fiber was exposed to the headspace during 30 min. The sample transfer to the GC column was accomplished by keeping the SPME fiber for 5 min in the heated chromatograph injector. This headspace extraction procedure was performed in duplicate for each sample using the SPME fibers listed above. Blank runs with the used fibers were conducted between extractions, to check the absence of carry over which would cause memory effects and misinterpretation of results.

3. Results and discussion

3.1. Cajá

Table 2 shows that 34 compounds were detected in the cajá aroma. Fig. 1 depicts four representative total ion chromatograms (TICs) of the cajá aroma, which were obtained with, respectively, CAR–PDMS, PDMS, PA and CW–DVB SPME fibers. After inspection of Fig. 1 and Table 2 it is found that 13 of the detected peaks may be attributed to aliphatic esters, and that nine of these esters belong to a series of the type $CH_3(CH_2)_xCOO(CH_2)_yCH_3$, where x is an odd number and y is an even number (e.g., ethyl acetate: $x=0$, $y=1$; ethyl butyrate: $x=2$, $y=1$; butyl acetate: $x=0$, $y=3$, etc.). Fig. 1 also depicts that ethyl butyrate, 1-butanol, ethyl caproate and α-pinene peaks were intense in the CAR–PDMS TIC while in the PDMS TIC there are pronounced peaks of the terpenoids α-pinene, limonene, γ-terpinene, ocimene, fenchyl alcohol, copaene and caryophyllene. In a previous study by Allegroni and Barbeni [11] cajá pulps were submitted to liquid–liquid extraction and vapor stripping followed by GC–MS analysis of the obtained cajá aroma. In this study more than 100 compounds were detected including butyric acid, 1-butanol and 3-hydroxybutyric acid esters.

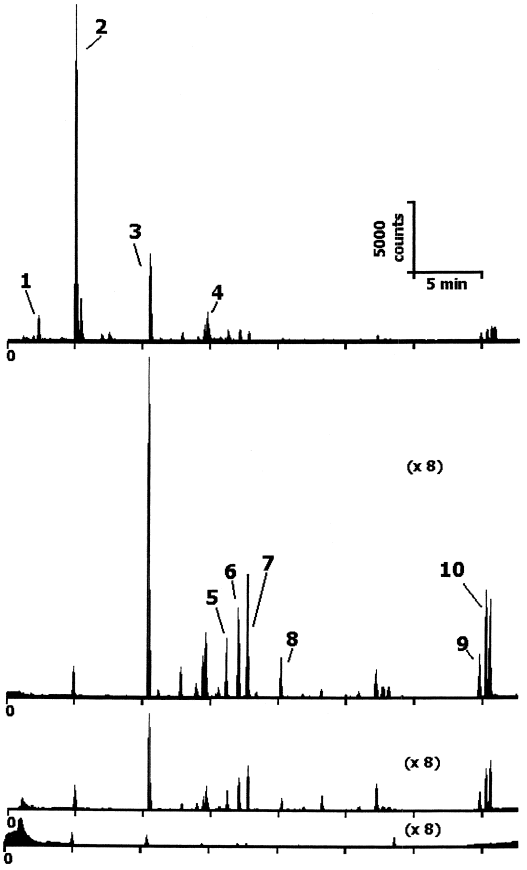

Fig. 1. From top to bottom, TICs of cajá aroma after extraction with CAR–PDMS, PDMS, PA and CW–DVB fibers. PDMS, PA and CW–DVB chromatograms magnified by 8×. Peaks: 1=*n*-butanol; 2=ethyl butyrate; 3=α-pinene; 4=ethyl caproate; 5=limonene; 6=γ-terpinene; 7=ocimene; 8=fenchyl alcohol; 9=copaene and 10=caryophyllene.

3.2. Graviola

Table 3 shows that 21 compounds were detected in the graviola aroma and that 12 of these compounds are esters. Fig. 2 shows four TICs obtained for this sample with, respectively, CAR–PDMS, PDMS, PA and CW–DVB fibers. From the data of Table 3 and of Fig. 2 it may be concluded that the prevailing compounds in the graviola aroma that were extracted by the SPME fibers are α-unsaturated methyl esters of the type R-CH=CH-$COOCH_3$ (R=

Table 3
Compounds identified in graviola aroma after SPME extraction

Group	Compound	Fibers			
		CW–DVB	CAR–PDMS	PA	PDMS
Alcohols	1-Butanol		x		
	3-Hexen-1-ol		x		
Aldehydes	Nonyl aldehyde	x		x	x
	Decyl aldehyde	x		x	x
Esters	Ethyl acetate		x		
	Methyl butyrate		x		x
	Methyl crotonate	x	x	x	x
	Ethyl butyrate		x		x
	Butyl acetate		x	x	x
	Ethyl crotonate		x		
	Methyl caproate	x	x	x	x
	Methyl 2-hexenoate	x	x	x	x
	Ethyl caproate				x
	Ethyl 2-hexenoate				x
	Methyl caprylate				x
	Methyl 2-octenoate	x		x	x
Ketones	1-Phenyl-1-penten-3-one				x
Terpenic compounds	Limonene				x
	β-Linalool				x
Others	2,5-Dihydro-2,5-dimethoxyfuran		x		x
	Palmitic acid	x		x	
Total identified compounds per fiber		7	11	8	16

ethyl, butyl or hexyl), as methyl crotonate (R= ethyl), methyl 2-hexenoate (R=butyl) and methyl 2-octenoate (R=hexyl), as well as aliphatic esters of butyric and caproic acids and ethanol. Earlier studies of the volatile fraction of the graviola pulp [12,13] did not mention identification of the extracted compounds.

3.3. Cupuassu

Table 4 and Fig. 3 show that 37 compounds were identified in the cupuassu aroma. As shown in Fig. 3, there are intense peaks in CAR–PDMS and PDMS chromatograms corresponding to ethyl esters (peaks 1, 3, 4 and 6); a large peak was identified as β-linalool in the PA extract (peak 7). Peak 2 was attributed to 2,4,5-trimethyl-1,3-dioxolane, a heterocyclic compound previously reported in the aroma of fermented beverages [14,15]. Prior studies report several components of the cupuassu aroma. Alves and Jennings [16], after steam distillation of the cooked pulp and separation of the extract with GC–MS, identified ethyl butyrate as the main constituent of the volatile fraction of cupuassu and among other compounds these authors have also detected ethyl acetate, ethyl 2-methylbutyrate, butyl butyrate and butyl 2-methylbutyrate. Velho et al. [17] reported that piperazine, palmitic acid and 9-octadecanoic acid as the main volatile components found in the pulp. Fischer et al. [18] found methyl esters such as butyrate, crotonate, *trans*-2-hexenoate and 3-hydroxy-3-methylbutyrate as the main constituents of the aroma of the cupuassu pulp and of the aroma of cupuassu juice.

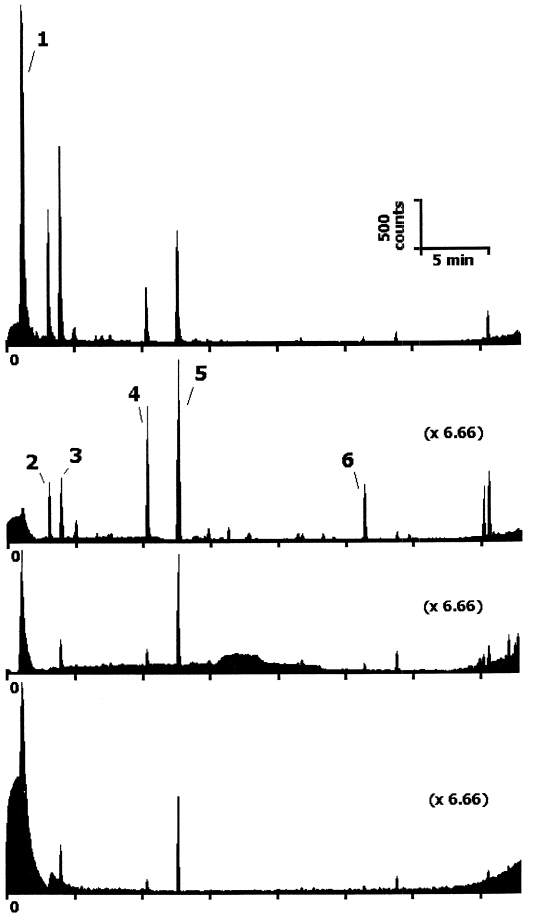

Fig. 2. From top to bottom, TICs of graviola aroma after extraction with CAR–PDMS, PDMS, PA and CW–DVB fibers. PDMS, PA and CW–DVB chromatograms magnified by 6.66×. Peaks: 1=ethanol; 2=methyl crotonate; 3=methyl 2-butenoate; 4=methyl caproate; 5=methyl 2-hexenoate and 6=methyl 2-octenoate.

3.4. Siriguela

In the siriguela aroma (Table 5 and Fig. 4) 27 substances were identified. The most intense peak (4) in the CAR–PDMS TIC of Fig. 4 corresponds to 3-hexen-1-ol, an unsaturated alcohol biosynthesized from linoleic acid [19]; this substance was also detected in the PDMS and PA extracts. As shown in Table 5 alcohols, low-chain esters, caproic aldehyde, nonyl aldehyde and decyl aldehyde were also detected. In a previous study, after simultaneous steam distillation and solvent extraction of the siriguela skin and of the siriguela pulp Koziol and Macia [20] have reported the major constituents of their volatile fractions as 2-hexenylic aldehyde, caproic aldehyde, palmitic acid and tetradecanoic acid.

3.5. Additives and contaminants

Some of the identified substances in the studied pulps could not be attributed to the natural fruit aromas, being possibly introduced in the samples during their industrial processing. A food anti-oxi-

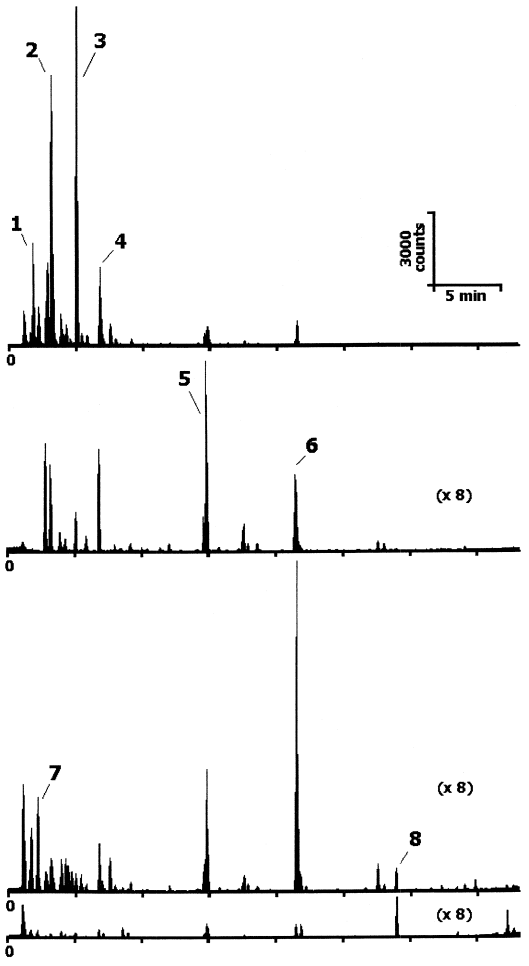

Fig. 3. From top to bottom, TICs of cupuassu aroma after extraction with CAR–PDMS, PDMS, PA and CW–DVB fibers. PDMS, PA and CW–DVB chromatograms magnified by 8×. Peaks: 1=ethyl acetate; 2=2,4,5-trimethyl-1,3-dioxolane; 3=ethyl butyrate; 4=ethyl 2-methylbutyrate; 5=*n*-butanol; 6=ethyl caproate; 7=β-linalool and 8=decyl aldehyde.

Table 4
Compounds identified in cupuassu aroma after SPME extraction

Group	Compound	Fibers			
		CW–DVB	CAR–PDMS	PA	PDMS
Alcohols	Ethanol	x	x	x	
	1-Butanol	x	x	x	
	Isoamyl alcohol			x	
	Prenol		x	x	x
	2,3-Butanediol		x	x	
	3-Hexen-1-ol			x	
	1-Hexanol		x	x	
Aldehydes	Nonyl aldehyde	x		x	x
	Decyl aldehyde	x		x	x
Esters	Ethyl acetate	x			x
	Ethyl propionate		x		
	Ethyl isobutyrate		x	x	x
	Ethyl butyrate	x	x	x	x
	Ethyl 2-methylbutyrate	x	x	x	x
	Isoamyl acetate		x	x	x
	Methyl caproate			x	x
	Butyl isobutyrate			x	x
	Butyl butyrate		x	x	x
	Ethyl caproate	x	x	x	x
	Hexyl acetate			x	x
	Butyl 2-methylbutyrate		x	x	x
	Isoamyl butyrate			x	x
	Butyl caproate			x	x
Ketones	1-Phenyl-2-pentanone			x	
Terpenic compounds	Camphene				x
	β-Mircene				x
	Limonene				x
	Ocimene			x	x
	β-Linalool	x	x	x	x
	α-Terpineol			x	x
	Geraniol			x	x
Others	Diacetyl		x	x	
	Acetic acid	x		x	
	2,5-Dihydro-2,5-dimethoxyfuran		x	x	x
	2,4,5-Trimethyl-1,3-dioxolane		x	x	x
	γ-Octalactone		x	x	x
	Palmitic acid	x			
Total identified compounds per fiber		11	18	31	26

dizer, butylated hydroxytoluene, was detected in samples of the cajá fruit and of the graviola fruit – in spite of the statement of ‘‘exempt of chemical additives’’ on the product’s labels. Toluene was detected as a possible contaminant in extracts of cajá, cupuassu and siriguela. As this analyte was not found in blank runs, the possibility of contamination during the analytical processing was excluded.

Table 5
Compounds identified in siriguela aroma after SPME extraction

Group	Compound	Fibers			
		CW–DVB	CAR–PDMS	PA	PDMS
Alcohols	Ethanol			x	
	1-Butanol		x		
	Isoamyl alcohol		x		
	Amyl alcohol		x		
	2,3-butanediol		x		
	3-Hexen-1-ol	x	x	x	x
	2-Hexen-1-ol		x	x	
	1-Hexanol		x	x	x
Aldehydes	Caproic aldehyde		x	x	x
	Nonyl aldehyde		x	x	x
	Decyl aldehyde			x	x
Esters	Ethyl acetate		x		
	Ethyl propionate		x		
	Ethyl crotonate		x		
	Ethyl 2-methylbutyrate		x		
	Isoamyl acetate		x		
	Methyl caproate		x		
	Methyl 2-hexenoate		x	x	x
	Ethyl caproate		x	x	x
	Isobutyl 2-methylcrotonate			x	x
	Ethyl benzoate			x	
Ketones	1-Penten-3-one		x		
Terpenic compounds	Limonene			x	x
	Copaene			x	x
Others	Diacetyl		x		
	Acetic acid			x	
	Palmitic acid			x	x
Total identified compounds per fiber		1	19	15	11

3.6. Inter-fiber comparison

The comparison of the SPME fiber performance can be made both in terms of extraction efficiency and number of identifiable compounds in the extracts. Therefore, the normalized extraction efficiency $N_{i,X}$ was defined as:

$$N = 100 \cdot \frac{\sum A_{i,X}}{\sum A_{i,\mathrm{CAR-PDMS}}}$$

where $\Sigma A_{i,X}$ is the sum of the areas of the detected peaks in the TIC of the extract of fruit pulp i with fiber X and $\Sigma A_{i,\mathrm{CAR-PDMS}}$ is the corresponding sum obtained after extraction of the same pulp aroma i with the CAR–PDMS fiber. Fig. 5A to D show the results (average of duplicate extractions) for the studied samples. The variation between duplicated ranged from 0.7 to 6.3%. From these figures, it can be seen that the amounts extracted with the CAR–PDMS fiber are considerably larger than the amounts extracted with the other fibers. An inspection of the chromatograms shown in Figs. 1–4 also reveals that as compared to the other fibers, the CAR–PDMS fiber has a higher efficiency especially for the less

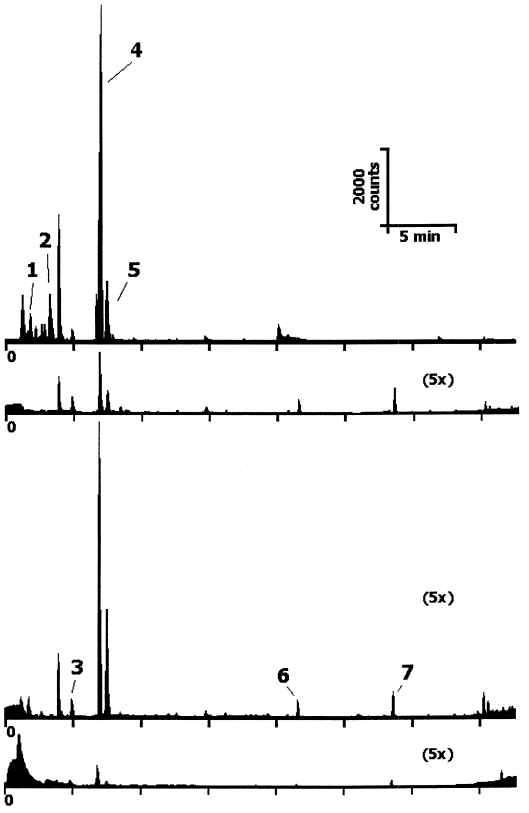

Fig. 4. From top to bottom, TICs of siriguela aroma after extraction with CAR–PDMS, PDMS, PA and CW–DVB fibers. PDMS, PA and CW–DVB chromatograms magnified by 5×. Peaks: 1=ethyl acetate; 2=isoamyl alcohol; 3=caproic aldehyde; 4=3-hexen-1-ol; 5=*n*-hexanol; 6=nonyl aldehyde and 7=decyl aldehyde.

retained compounds. It was also observed that the CW–DVB fiber showed the lowest extraction efficiency. As to the PDMS fiber (an apolar coating) and the PA fiber (moderately polar), they showed intermediate extraction efficiencies that varied with the particular sample composition. For the cajá aroma, that has an expressive amount of terpenic hydrocarbons (see Fig. 1 and Table 2), the extraction efficiency of the PDMS fiber was superior to that of the PA fiber. For the cupuassu aroma, that has a large amount of the terpenic alcohol β-linalool (Fig. 3 and Table 4) the extraction efficiency of the PA fiber is higher than that of the PDMS fiber.

In spite of the lower extraction efficiencies of PDMS and PA fibers, as compared to CAR–PDMS, a remarkable number of compounds was detected. For graviola pulp (Table 3) 16 substances were identified in the PDMS extract, which favorably compares to the 11 identifiable peaks in the corresponding CAR–PDMS extract. Similarly, in Table 4 it can be seen that 31 positive matches were observed in the PA extract, 26 in PDMS extract and 18 in CAR–PDMS extract. The normalized extraction efficiency used depends on the total mass extracted by a given fiber and it is not necessarily related to the number of identifiable substances in a chromatogram. In the graviola and cupuassu extracts mentioned, small chromatographic peaks of late-eluting compounds were found (see Figs. 3 and 4). For these more retained compounds, PDMS and PA fibers seems to have a better performance than that of CAR–PDMS fiber. This accounts for the larger number of identified compounds in the PDMS and PA TICs. On the other hand the peak sizes shown in the mentioned figures indicate that the amounts of the more retained compounds are smaller than those of the early-eluting compounds. This explains why the detection of the late-eluting peaks does not increase significantly the extraction efficiency of the corresponding fibers. As to the larger amounts extracted by the CAR–PDMS fiber, they are due to the better extraction efficiency of this fiber towards the early-eluting compounds, which show larger peak areas than the late-eluting compounds.

4. Conclusions

SPME has proved to be a useful tool for the qualitative analysis of the fruit pulp aromas examined in this work, allowing the identification of the main constituents of these samples. The chromatographic profiles obtained after extractions with CAR–PDMS, PDMS and PA fibers are complimentary, suggesting that CAR–PDMS was the most efficient for the extraction of lighter compounds. Nevertheless, the extractions made with PDMS and PA fibers provided valuable information about the higher-molecular-mass portion of the aromas. Thus, it may be pointed out that procedures combining the data obtained with the three types of fibers are important to maximize the amount of information about the sample composition.

The results reported here are not comparable to

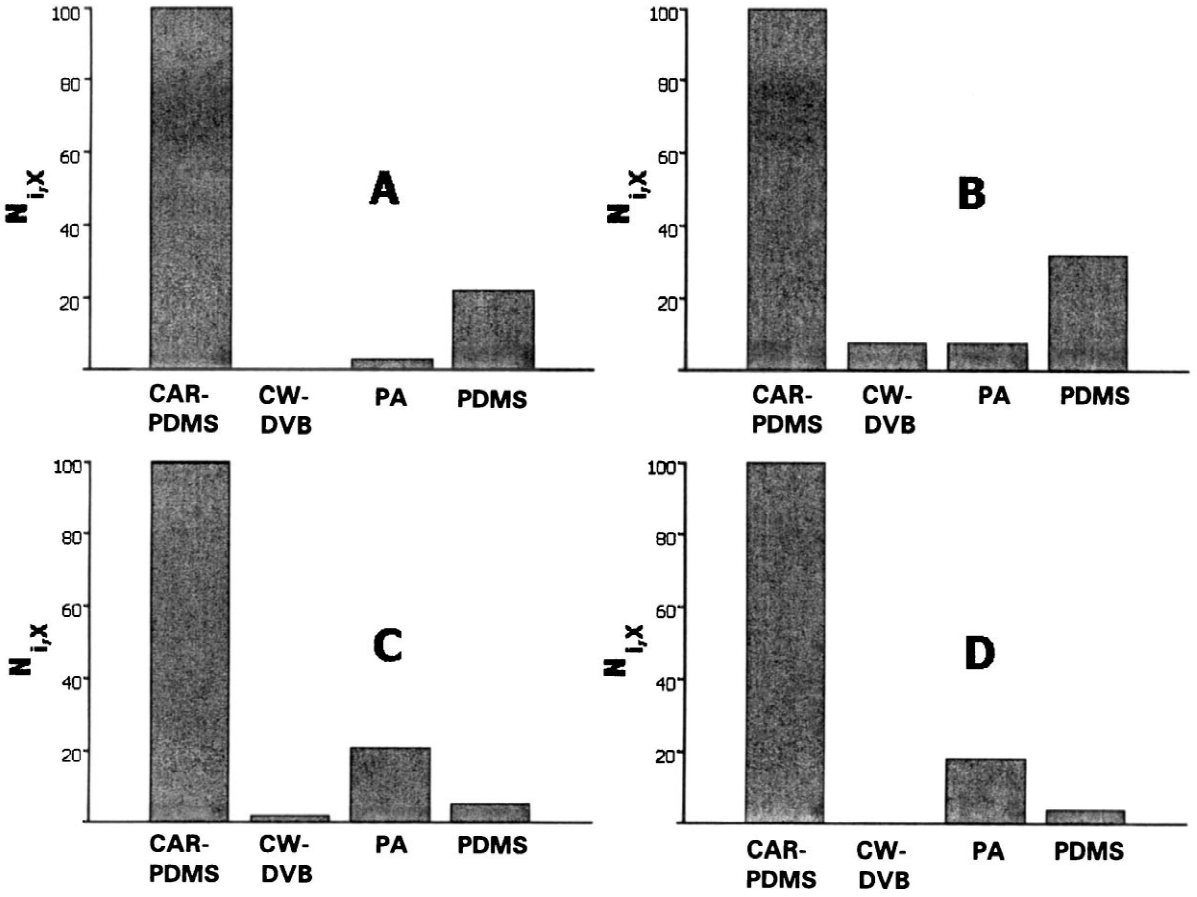

Fig. 5. Normalized extraction efficiencies $N_{i,x}$ measured for extractions with CAR–PDMS, CW–DVB, PA and PDMS fibers. Samples: A = cajá; B = graviola; C = cupuassu and D = siriguela.

previous reports on the literature. The composition of the samples should be expected to be subject to several factors, ranging from seasonal effects on the vegetable species to the manipulation of the fruit pulps prior to the analysis (storage, packing, etc.). A systematic comparison between extraction techniques applied to natural aromas should be performed using the same samples, and was beyond the scope of this work.

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