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Studies on the aroma of cupuassu liquor by headspace solid-phase microextraction and gas chromatography

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Abstract

Headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography with ion trap mass spectrometric detection and with atomic emission detection (GC–AED) was employed to identify possible odor-impact volatile organic compounds in cupuassu (*Theobroma grandiflorum* Spreng) liquor, as well as to quantify alkylpyrazines present in these samples. SPME fibers coated with 100 μm polydimethylsiloxane (PDMS), 65 μm PDMS–divinylbenzene (DVB) and 75 μm Carboxen (CAR)–PDMS were tested, the later being chosen for the optimized extraction procedure. The principal compounds found in the sample headspace were 3-methylbutanal, dimethylsulfide, dimethyldisulfide, β-linalool and several alkylpyrazines (notably tetramethylpyrazine). The procedure for quantitation of the alkylpyrazines, using GC–AED for their separation and detection, allowed the detection of $\mu g\,g^{-1}$ levels of the analytes in the samples, with acceptable precision (R.S.D. less than 10%).

Keywords: *Theobroma grandiflorum*; Plant materials; Alkylpyrazines; Volatile organic compounds; Aroma compounds

1. Introduction

Cupuassu (*Theobroma grandiflorum* Spreng) (Sterculiaceae)—is a tree native to eastern Amazon, being a commercial culture of local importance. The fruits, which can weigh up to 1.5 kg, have a white-yellow pulp with a pleasant acidic taste and a strong fragrance, which is widely consumed as juice, ice cream and jam [1]. Its seeds can be processed in a similar fashion to its close cousin, cocoa (*Theobroma cacao* L.), to produce cupuassu chocolate (or "cupulate") [2]. The production of cupuassu chocolate is similar to that of conventional cocoa chocolate [3]: after harvesting, the seeds are separated from the fruits and left to dry, when a fermentation of sugars in the pulp covering the seeds simultaneously occurs. The dry fermented seeds are then roasted, winnowed and finely ground. The resulting solid product ("liquor") is the raw material for the chocolate and cupulate industries. Cupulate has a good market potential as an exotic replacement for chocolate, and it could be an alternative income source to the local Amazonian population. The volatile fraction of cupuassu pulp has already been well characterized using several extraction and pre-concentration techniques, always combined with gas chromatography (GC): liquid–liquid extraction [4], simultaneous distillation–extraction (SDE) [5], solid-phase extraction [6,7], a combination of the former techniques [8] or, more recently, solid-phase microextraction (SPME) [9]. Among the volatile organic compounds with potential impact on the aroma, several esters (ethyl and butyl butyrate, ethyl and butyl 2-methylbutyrate), terpenoids (β-linalool) and heterocyclic compounds (piperazine; 2,5-dihydro-2,5-dimethoxyfuran) were detected. However, no study regarding the composition of the volatile fraction of cupuassu liquor or chocolate was found in the literature.

Most of the volatile substances with substantial aroma-impact in chocolate and other foods prepared by heating are oxygenated and nitrogenated heterocyclic compounds produced by the so-called Maillard reactions (non-enzymatic browning) [10], which are a group of complex heat-induced chemical reactions between substrates such as reducing sugars, dipeptides, free aminoacids, triglycerides and other naturally-occurring substances. Among the Maillard reac-

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tion products, alkylpyrazines are the most significant in terms of aroma-impact on cocoa products [11]. In light of its resemblance to cocoa, any methodology for aroma screening of cupuassu chocolate or liquor should be able to isolate and detect alkylpyrazines. However, some characteristics of cupuassu liquor make the obtention of a reliable procedure difficult. Thus, a headspace manipulation procedure may be indicated for the isolation and pre-concentration of the target analytes [12]. However, the matrix has a typical lipid content of up to 50% (w/w) [13], and it has been demonstrated that analyte concentration in the headspace is severely reduced in samples having high lipid contents [14], causing a critical decrease in the extraction efficiency and method sensitivity. Another problem arises from the possibility of generation of additional amounts of Maillard products, especially alkylpyrazines, in isolation and pre-concentration procedures which involve sample heating: there is evidence of generation of alkylpyrazines in cocoa liquor at temperatures as low as 60 °C [15]. Therefore, use of procedures like SDE or similar sample preparation techniques should be avoided.

Both headspace SPME and procedure based on dynamic headspace manipulation are good potential choices for chemical studies of aromas of chocolate and chocolate-like products. SPME, a simple, relatively inexpensive and solventless technique–whose theoretical foundations and methodological aspects were already presented in the literature [16]–has been widely applied to aroma-related analytical problems [17–20]; its suitability and convenience for the isolation of Maillard reaction products, especially alkylpyrazines has also been demonstrated [21]. In other hand, the higher sensitivity of dynamic headspace (HS) manipulation methods (specially when compared to SPME) accounts for their extense use to these samples [22,23]. However, even considering its lower sensitivity, in studies comparing both techniques for aroma analysis, performance of SPME has been found to be adequate [24,25]. Also, dynamic headspace procedures have some drawbacks [26], such as retention of water (when using carbon-based trapping materials), longer analysis time and possibility of degradation of trapped analytes when using thermal desorption. Therefore, in this work, we adopted SPME coupled to GC–mass spectrometric (MS) and GC–atomic emission detection (AED) for the identification of volatile compounds from cupuassu liquor and for the quantitation of alkylpyrazines from these samples.

2. Experimental

2.1. Samples

Fresh cupuassu seeds were fermented and roasted using adequate conditions. After cooling to ambient temperature, the roasted seeds were minced, mixed with solid CO_2 (to avoid loss of volatile species) and the liquor obtained reduced to a fine powder in a blender. After sublimation of the remaining CO_2, the material was transferred to sealed glass containers and kept at −12 °C until use.

2.2. Chemicals and reagents

Stock methanolic solutions of methylpyrazine; 2,3-, 2,5- and 2,6-dimethylpyrazine; ethylpyrazine; 2,3,5-trimethylpyrazine and tetramethylpyrazine (Aldrich, St. Louis, MO, USA) were used for the quantitative studies. SPME fibers coated with 100 μm polydimethylsiloxane (PDMS) (P100), 75 μm Carboxen (CAR)–PDMS and 65 μm PDMS–divinylbenzene (DVB) and the holder for manual SPME were supplied by Supelco (Bellefonte, PA, USA). Prior to use, the fibers were conditioned according to the supplier's instructions. The extractions were performed in 4 ml or 16 ml septum-sealed glass vials.

2.3. Chromatography

The optimization of the SPME method and the qualitative studies were conducted on a G-2350A GC–AED system (Agilent Technologies, Wilmington, DE, USA) and on a Saturn 2100 GC-ion trap (IT) MS system (Varian, Walnut Creek, CA, USA). The GC–AED system was fitted with a 25 m × 0.32 mm, 0.17 μm HP-1 capillary column (Agilent) and the operational conditions were: T_{COL} = 2 min at 50 °C → 40 °C min^{-1} → 150 °C → 25 °C min^{-1} → 250 °C; T_{INJ} = 210 °C (PDMS–DVB extractions), 270 °C (P100) or 280 °C (CAR–PDMS extractions), and T (transfer line) = 270 °C. As specified by the supplier, doping gases were added to the plasma support gas (helium) depending on the elemental emission line monitored: H_2 and O_2 for the 193 nm C, 174 nm N and 181 nm S lines; H_2, O_2 and CH_4 for the 388 nm N (CN) band. A 30 m × 0.25 mm, 0.25 μm CP-Sil 8 CB low-bleed capillary column (Varian) was employed in the GC–ITMS system, which was operated under the following conditions: T_{COL} = 40 °C → 10 °C min^{-1} → 1 min at 250 °C and; T_{INJ} = 210 °C (PDMS–DVB extractions) or 280 °C (P100 and CAR–PDMS extractions), and T (transfer line) = 270 °C. The quantitative assessment of alkylpyrazines was carried out in the GC–AED system, using a 25 m × 0.32 mm, 0.3 μm HP-Wax capillary column, using T_{COL} = 50 °C → 3 °C min^{-1} → 90 °C → 15 °C min^{-1} → 190 °C; other conditions being the same as in the qualitative runs. All chromatographs were fitted with split-splitless injectors operated in the splitless mode for 2 min, and helium (1.0 ml min^{-1}) was used as carrier gas throughout the work.

2.4. Optimization of the SPME method

The same general procedure was adopted for all tests performed to choose the best SPME fiber and extracting media, sample mass, extraction temperature, sample/headspace pre-equilibration time and extraction time. A suspension of m_S grams of sample and 5.0 ml of adequate suspension

Table 1
Operational conditions for the experiments for SPME method optimization

Optimized parameter	Fiber	m_S[a] (g)	T (°C)	t_{SH} (min)	t_{ext} (min)	Media
Fiber	Varied[b]	0.700	25	5	45	Satd. NaCl
Sample mass	CAR–PDMS	Varied[c]	45	3	15	Satd. NaCl
Extraction media	CAR–PDMS	1.000	45	3	15	Varied[d]
Extraction temperature	CAR–PDMS	1.000	Varied[e]	3	15	Satd. NaCl
Pre-equilibration time	CAR–PDMS	1.000	45	Varied[f]	45	Satd. NaCl
Extraction time	CAR–PDMS	1.000	45	3	Varied[g]	Satd. NaCl

[a] m_S: sample mass, T: temperature, t_{SH}: sample/headspace equilibration time and t_{ext}: extraction time.
[b] Fibers: P100, CAR–PDMS or PDMS–DVB.
[c] $m_S = 0.700$, 1.000 or 1.200 g.
[d] Media for sample suspension = saturated aqueous NaCl solution or distilled water.
[e] $T = 25$, 35 or 45 °C.
[f] $t_{SH} = 3$–10 min.
[g] $t_{ext} = 10$–60 min.

media (distilled water or saturated aqueous NaCl) in a 16 ml septum-sealed was magnetically stirred at 1200 rpm for t_{SH} min for sample/headspace pre-equilibration. After the pre-equilibration a SPME fiber was exposed to the vial headspace for t_{ext} min. The extracted analytes were immediately desorbed and analyzed using either GC-IT–MS or GC–AED (monitoring C, S and N-specific emission lines). The operational conditions for the each series of triplicate extractions conducted to optimize the parameters are listed on Table 1. Through all the work, the fibers were kept on the GC injector for 9.5 min; no sample carry-over was observed under these conditions. Identification of the extracted analytes was done by comparison of the corresponding mass spectra in the GC-IT–MS chromatograms with the US national Institute of Standards and technology (NIST) 98 library. Confirmation of peak identity was performed after extractions of samples spiked with pure standards (when available) and/or inspection of the GC–AED chromatograms in the S and N lines.

2.5. Quantification of alkylpyrazines in cupuassu liquor using the optimized HS-SPME–GC–AED procedure

Standard addition analytical curves were employed to assess the applicability of the HS-SPME method for the quantitation of alkylpyrazines. The extractions were carried out using the operational parameters optimized in 2.4 above: (1.000 ± 0.005) g of finely grinded cupuassu liquor was suspended in 5.0 ml of saturated NaCl at 45 °C, and after 3 min of pre-equilibration under magnetic stirring the vial headspace was extracted with a CAR–PDMS fiber for 45 min. For each sample, a single standard addition calibration curve with four to five points was constructed using triplicate extractions of the raw sample and of portions of the same spiked with up to 40 $\mu g\,g^{-1}$ of each alkylpyrazine (four to five points per curve). Separation and detection of the extracted analytes were performed by GC–AED, monitoring either the 174 nm N line or the 388 nm CN emission band.

3. Results and discussion

3.1. Qualitative analysis of volatiles from cupuassu liquor

Fig. 1 shows typical GC-IT-MS chromatograms of cupuassu liquor headspace. The most intense peak corresponds to tetramethylpyrazine (a product of the Maillard process), which was already recognized as one of the key odorants in cocoa products [11,27]. Other detected alkylpyrazines include 2,3,5-trimethylpyrazine; 3-ethyl-2,5-dimethylpyrazine; 2,3,5-trimethyl-6-ethylpyrazine; 2,5-dimethylpyrazine and 2,3-dimethylpyrazine. Tailing is observed on the tri- and tetramethylpyrazine chromatographic peaks after extractions with CAR–PDMS fibers; the peak shape for the same analytes extracted with the other tested fibers is almost symmetrical. Hence, the tailing of the peaks of these alkylpyrazines is a particularity of CAR–PDMS fibers and probably originates on the desorption step. The coating on these fibers is a dispersion of Carboxen 1006 (a synthetic porous carbon) on PDMS; most of the pores on this adsorbent pass completely through its particles [28]. Desorption of analytes from Carboxen is slow, since most of the adsorbed molecules have to diffuse through these long pores before being released. Also, adsorption involves interaction between dipoles (permanent or induced) and polar analytes such as the alkylpyrazines are expected to be very strongly adsorbed. Both factors combined probably cause slower desorption of tri- and tetramethylpyrazines from the CAR–PDMS fibers and can be accountable for the tailed peaks of these species.

Apart from tetramethylpyrazine, the most intense peak in the chromatograms was identified as 3-methylbutanal. This compound has been indicated as one of the main ones responsible for the characteristic chocolate flavor [29], especially when associated with dimethylsulfide [30] which was also detected in the cupuassu samples, along with another alkyl sulfide, dimethyldisulfide. Oxidation and esterification of 3-methylbutanal possibly accounts for some of the esters found in the

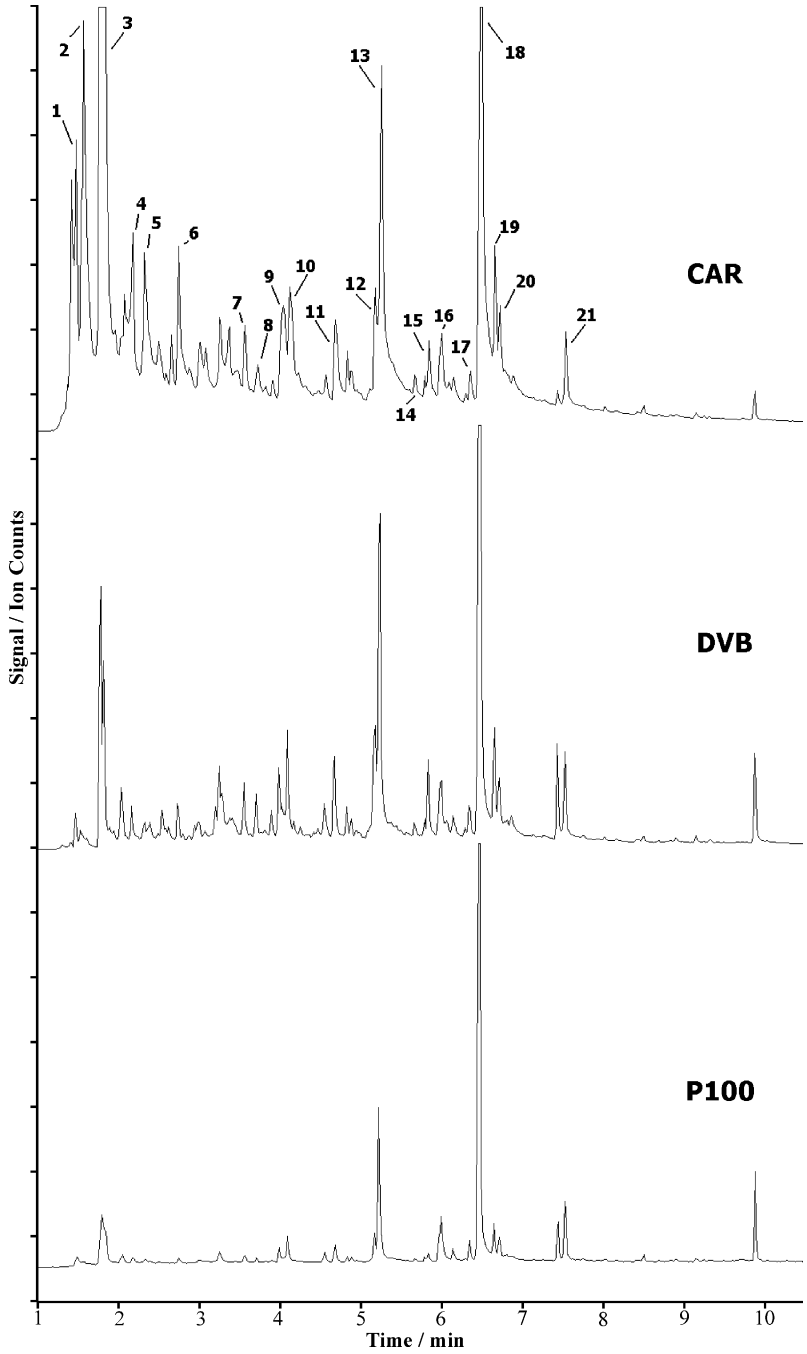

Fig. 1. GC-IT–MS chromatograms of volatiles from cupuassu liquor after extraction with CAR–PDMS, PDMS–DVB and P100 fibers. Peak identification: (1) dimethylsulfide; (2) acetic acid; (3) 3-methylbutanal; (4) 2,4,5-trimethyl-1,3-dioxolane; (5) dimethyldisulfide; (6) ethyl butyrate; (7) 3,5-dimethyl-3-hexanol; (8) 3-methyl-2-butanone; (9) 2,5-dimethylpyrazine; (10) 2,3-dimethylpyrazine; (11) benzaldehyde; (12) ethyl hexanoate; (13) trimethylpyrazine; (14) limonene; (15) butyl 3-methylbutyrate; (16) benzeneacetaldehyde; (17) 3-ethyl-2,5-dimethylpyrazine; (18) tetramethylpyrazine; (19) β-linalool; (20) 3-methylbutyl-3-methylbutyrate; (21) 2,3,5-trimethyl-6-ethylpyrazine.

volatile cupuassu fraction, such as butyl 3-methylbutyrate and 3-methylbutyl 3-methylbutyrate. The other detected aldeheydes—benzaldehyde and benzeneacetaldehyde—already have been identified as odorants in chocolate [27], as well as in other food flavors [31,32]. Additionally, benzeneacetaldehyde has already been found as the prevalent volatile compound in raw, unroasted cupuassu seeds [4]. Another identified compound with possible importance to cupuassu liquor flavor is β-linalool, which was already shown to be an important flavor marker in roasted cocoa [33]. Limonene, as well as the oxygenated heterocycle 2,4,5-trimethyl-1,3-dioxolane, also found on these samples, has already been reported in the aroma of cupuassu pulp [9].

3.2. Optimization of the SPME procedure

The chromatograms on Fig. 1 also show that the overall extraction efficiency of P100 fibers is significantly lower than that of CAR–PDMS and PDMS–DVB. Therefore, this coating was excluded from the next assays. To further assess the suitability of CAR–PDMS and PDMS–DVB fibers for the further experiments, the extraction efficiencies of these fibers for five potentially important odor-impact compounds (3-methylbutanal; 2,3,5-trimethylpyrazine; tetramethylpyrazine; dimethylsulfide and dimethyldisulfide) were compared. The results are shown on Table 2. As it can be seen CAR–PDMS fiber provided higher extraction efficiencies for the selected compounds and, therefore, it was selected for the remaining experiments. Table 2 also shows that the use of saturated aqueous NaCl instead of pure water to suspend the sample during the extraction resulted in an substantial improvement in the peak areas measured for the alkylpyrazines and dimethyldisulfide; for dimethylsulfide and 3-methylbutanal the increment on the area is less pronounced. Addition of NaCl to the extraction media usually augments the extraction efficiency, since for most analytes it causes an increase on their activity coefficients and shift the extraction equilibrium [16].

As for the optimization of sample mass (Fig. 2) and except for 3-methylbutanal, masses greater than 1.000 g do not have a significant effect on the extraction efficiency. Since the quantitation of samples where matrix effects are pronounced need to be performed using standard addition (which requires processment of several portions of each sample to be analysed), use of the minimum possible amount of sample is desirable. Therefore, 1.000 g was selected as sample mass. As for the temperature effect (Fig. 3), an increase of the extraction temperature from ambient (25 °C) to 45 °C resulted in an increment on the peak areas for the alkylpyrazines, 3-methylbutanal and dimethylsulfide without prejudice to the other analytes; therefore, 45 °C was selected as the operational temperature. Typically, increasing the extraction temperature also increases the concentration of volatile analytes on the headspace, but at the same time decreases the fiber coating/headspace distribution constant [16]. Considering the opposite nature of such effects, and specially for a complex matrix as cupuassu liquor, the optimization of temperature is an essentially empirical operation. Specifically for such samples, higher extraction temperatures were avoided due to the possibility of generation of Maillard re-

Table 2
Peak areas, in 10^3 area counts, of 3-methylbutanal (3MeB), trimethylpyrazine (Tri), tetramethylpyrazine (Tetra), dimethylsulfide (DMS) and dimethyldisulfide (DMDS) obtained in the comparison between fibers (DVB and CAR) and extracting media (pure water or saturated aqueous NaCl solution)

	Analyte				
	3MeB	Tri	Tetra	DMS	DMDS
Fiber					
PDMS–DVB	15.0	17.8	35.1	2.3	2.5
CAR–PDMS	36.1	25.8	62.3	9.4	37.9
Media					
Pure water	36.3	12.8	17.1	9.8	3.5
Satd. NaCl	40.2	30.8	41.4	12.1	7.4

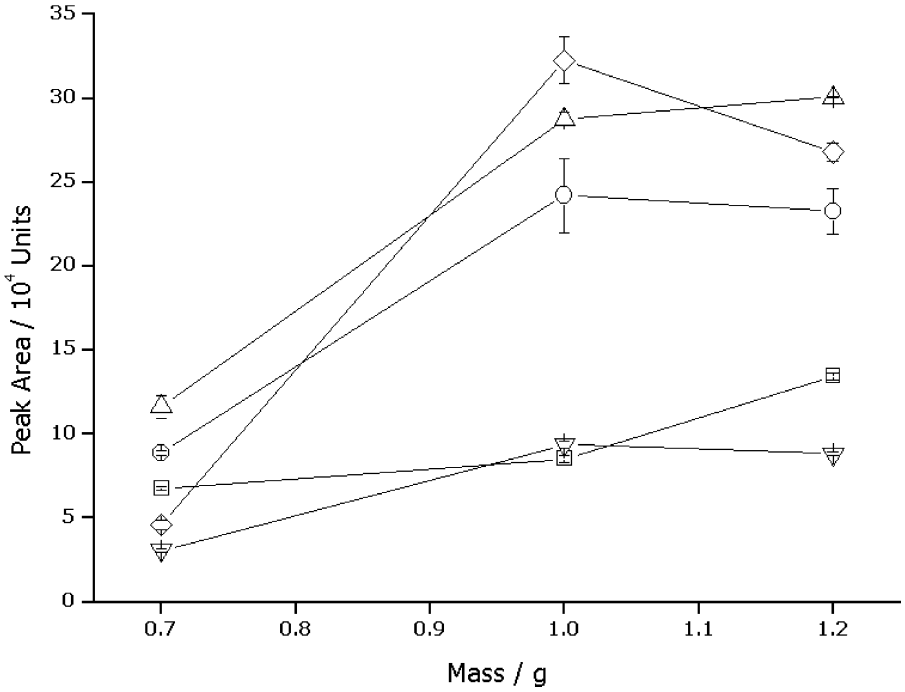

Fig. 2. Effect of sample mass on the extraction efficiency for 3-methylbutanal (□), trimethylpyrazine (○), tetramethylpyrazine (△), dimethylsulfide (▽) and dimethyldisulfide (◇). Average of triplicate measurements.

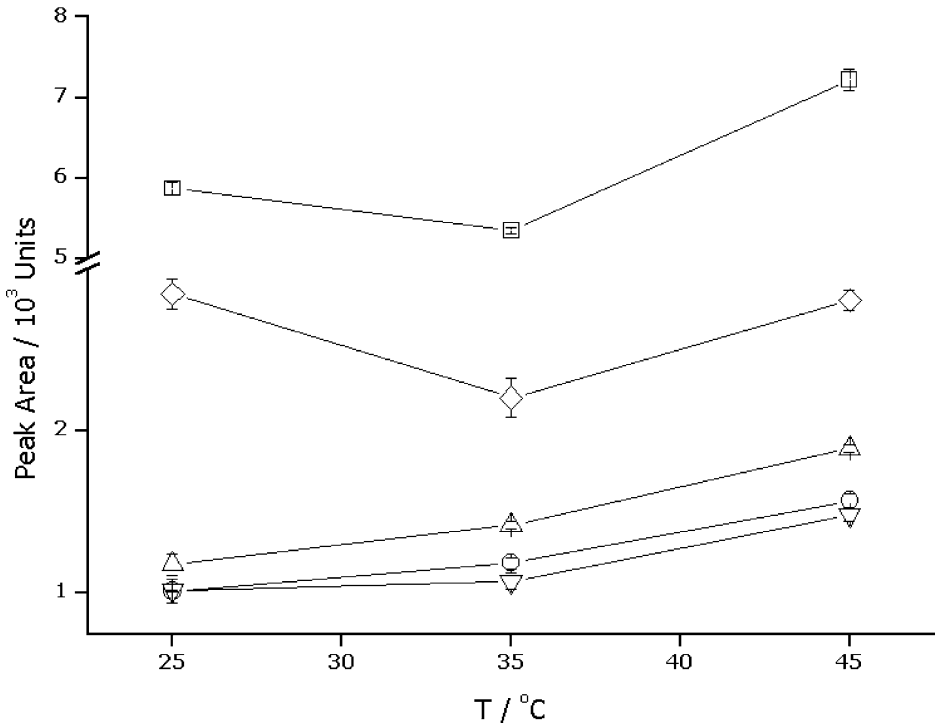

Fig. 3. Effect of temperature on the extraction efficiency for 3-methylbutanal (□), trimethylpyrazine (○), tetramethylpyrazine (△), dimethylsulfide (▽) and dimethyldisulfide (◇). Average of triplicate measurements.

action products, such as the alkylpyrazines (as observed for cocoa liquor in a previous work [15]), resulting in overestimated values for the concentrations of these compounds.

Figs. 4 and 5 show sample/headspace and headspace/fiber time profiles found for some of the alkylpyrazines detectable in the cupuassu liquor (2,3- and 2,5-dimethylpyrazine; 2,3,5-trimethylpyrazine and tetramethylpyrazine), using GC–AED and monitoring the 388 nm CN emission band. To improve the readability of these figures, the raw peak areas were normalized in order to have values for 5 min

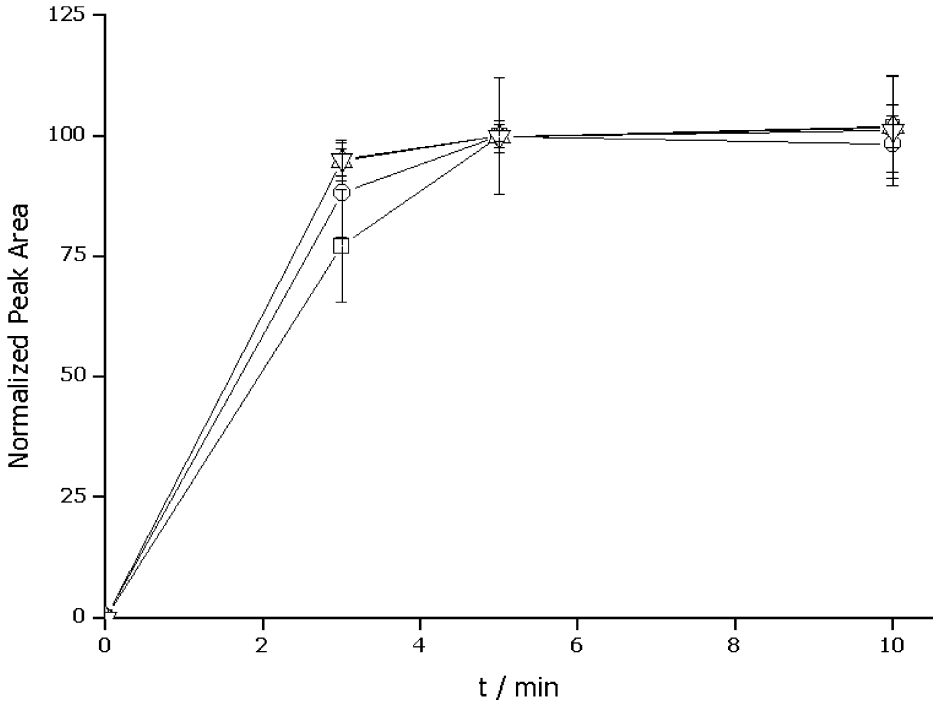

Fig. 4. Sample/headspace equilibration profiles for 2,3-dimethylpyrazine (□); 2,5-dimethylpyrazine (○); trimethylpyrazine (△) and tetramethylpyrazine (▽).

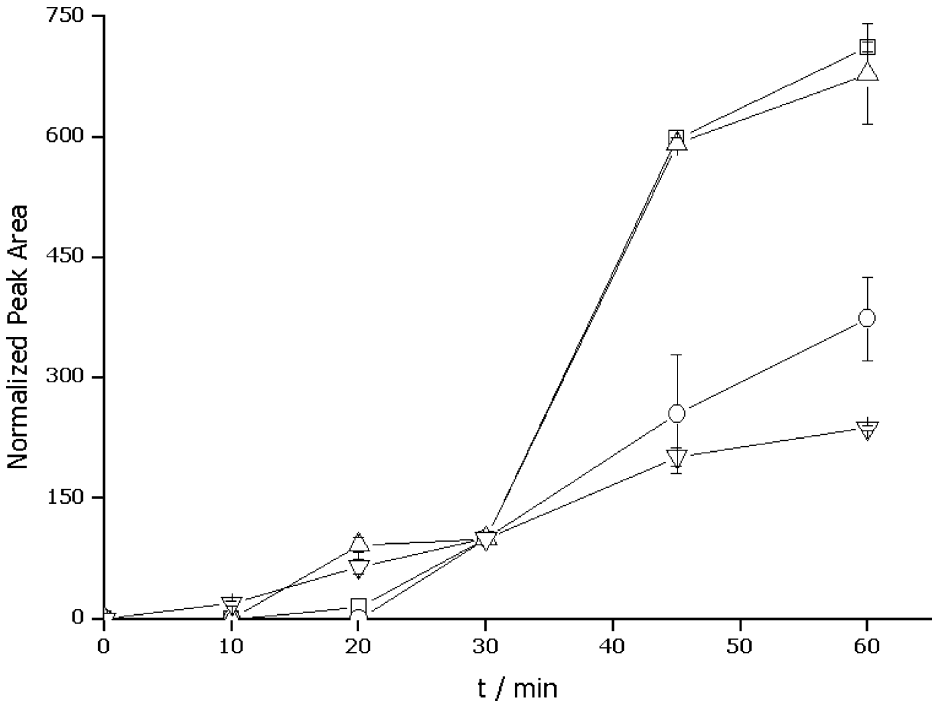

Fig. 5. Headspace/CAR–PDMS fiber equilibration profiles for 2,3-dimethylpyrazine (□); 2,5-dimethylpyrazine (○); trimethylpyrazine (△) and tetramethylpyrazine (▽).

sample/headspace or 30 min headspace/fiber equilibration times equal to 100 units. As it can be seen in Fig. 4, considering the uncertainties and except for 2,3-dimethylpyrazine, after a 3 min sample/headspace equilibration, the peak areas remain approximately constant; therefore, this value was adopted as the pre-equilibration time for the final methodology. The same does not occur with the extraction profiles (Fig. 5): no plateau was observed for extraction profiles up to 60 min extraction. Since except for 2,5-dimethylpyrazine the increment in the peak area for extraction times longer than 45 min is relatively less pronounced and larger extraction times would not be feasible for practical reasons, this time was employed in the final quantitative procedure. Large equilibration times such as those observed here are usual when using CAR fibers [34]; the same for this are the same as above discussed for the slower desorption characteristics of this fiber [16].

3.3. Quantification of alkylpyrazines in cupuassu liquor using the optimized HS-SPME–GC–AED procedure

To assess the sensitivity and detection limits for the HS-SPME procedure developed, standard addition analytical curves for some of the detected alkylpyrazines—2,3- and 2,5-dimethylpyrazine; 2,3,5-trimethylpyrazine and tetramethylpyrazine—were determined. Considering the similarity of the composition of the volatile fraction of cupuassu liquor with cocoa, three other alkylpyrazines, usually found on the former samples (methylpyrazine; 2,6-dimethylpirazine and ethylpyrazine) were also included on these quantitative studies. Also, as reliable pure standards of 3-ethyl-2,5-dimethylpyrazine and 2,3,5-trimethyl-6-ethylpyrazine (detected in the preliminary samples) were not available, it was not possible to include these compounds. GC–AED was employed as the separation and detection tool for the quantitative assays; both available N analytical wavelengths—the 174 atomic N emission line and the 388 CN molecular emission band—were compared. The sensitivity and detection limits obtained from the standard addition analytical curves (calculated according to [35]) are shown in Table 3; Fig. 6 shows typical GC–AED chromatograms. For these runs, a more polar chromatographic column (HP-Wax) had to be used instead of the less polar columns (HP-1, methylsilicone and CP-Sil 8 CB, methylphenylsilicone) employed in the method optimization and in the qualitative experiments. The resolution of the alkylpyrazines—specially of the dimethylpyrazines and ethylpyrazine) with the last columns was poor, and their adequate separation demanded a stationary phase more selective towards polar analytes. Also, comparing these chromatograms with those on Fig. 1, it can be seen that in this case the peaks of tri- and tetramethylpyrazines are more symmetrical. The stronger retention of these polar species on this polar column (polyglycol), combined to the cooler initial oven temperature (40 °C), apparently focus the analytes in the tip of the column, eliminating the peak deformation caused by their slow desorption from the CAR–PDMS fiber.

For both nitrogen analytical wavelengths, fair to good precision (correlation coefficients ranging from 0.984 to 0.995

Table 3
Sensibilities, *S*, in $\mu g\,g^{-1}$ (as defined in [24]), correlation coefficients *r* and detection limits LD in $\mu g\,g^{-1}$ for selected alkylpyrazines in cupuassu liquor using HS-SPME coupled to GC–AED and monitoring the 174 nm N emission line or 388 nm CN emission band

	Analytes[a]						
	MP	5DP	6DP	3DP	EP	Tri	Tetra
174 nm							
S	1.4	2.3	1.4	0.81	0.30	0.91	0.39
r	0.999	0.989	0.992	0.989	0.994	0.980	0.974
LD	1.8	4.1	2.4	5.6	1.1	7.4	3.2
388 nm							
S	1.0	0.85	0.90	0.56	0.22	2.2	0.13
r	0.998	0.999	0.999	0.999	1.000	0.996	0.980
LD	2.3	1.3	0.73	1.3	0.31	3.4	0.73

[a] MP: methylpyrazine; 3DP: 2,3-dimethylpyrazine; 5DP: 2,5-dimethylpyrazine; 6DP: 2,6-dimethylpyrazine; EP: ethylpyrazine; Tri: 2,3,5-trimethylpyrazine and Tetra: tetramethylpyrazine.

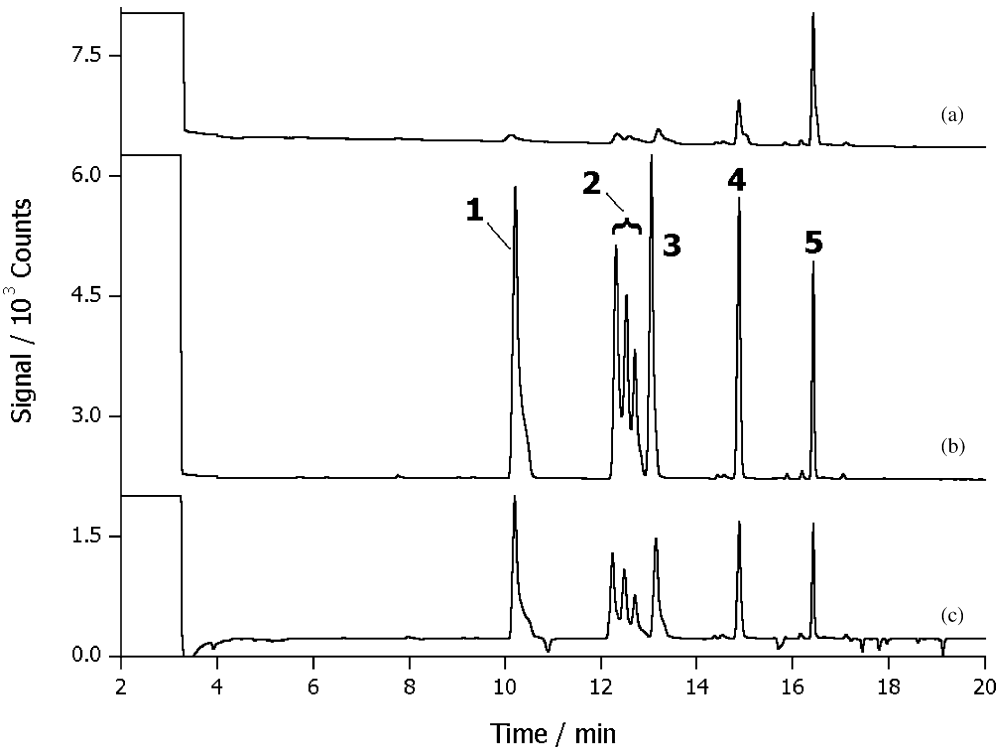

Fig. 6. Typical HS-SPME–GC–AED chromatograms of cupuassu liquor: (a) 388 nm CN emission band, raw sample; (b) same sample spiked with ~20 $\mu g\,g^{-1}$ of each alkylpyrazine and (c) same as (b) but in the 174 nm N emission line. Peak identification: (1) methylpyrazine; (2) dimethylpyrazines; (3) ethylpyrazine; (4) 2,3,5-trimethylpyrazine and (5) tetramethylpyrazine. Signal scale is same for the three chromatograms.

when monitoring 174 nm and 0.989 to 0.998 for 388 nm) and adequate sensitivity and detectability were achieved. However, the line at 388 nm was chosen for quantitative applications due to its improved detectability (e.g. for tetramethylpyrazine the detection limits are 0.73 and 3.2 $\mu g\,g^{-1}$ at 388 and 174 nm, respectively) (Table 3). The HS-SPME procedure was applied to quantify the alkylpyrazines in cupuassu liquor samples, roasted in either conventional electric or microwave ovens (Table 4). The detected concentrations of alkylpyrazines are in the range of several $\mu g\,g^{-1}$, e.g. 6.91–33 $\mu g\,g^{-1}$ for tetramethylpyrazine, with good precision—the R.S.D. ranged from 0.2 to 10%. It should be mentioned that, since fiber-to-fiber variations can be a source of inaccuracy and imprecision, the same fiber was used to collect the data for each standard addition calibration curve.

The detected concentrations of alkylpyrazines in cupuassu liquor are larger than the figures usually reported for chocolate and other cocoa derivatives. For example, Bonvehí and Coll [11] found, for natural and alkalinized cocoa powder, 0.5–2.0 $\mu g\,g^{-1}$ tetramethylpyrazine. Counet et al. [27] found up to 6.6 $\mu g\,g^{-1}$ of this analyte in chocolate; finally, for roasted cocoa beans, Sanagi et al. [36] detected up to 0.9 $\mu g\,g^{-1}$.

Table 4
HS-SPME–GC–AED determination of alkylpyrazines in cupuassu liquor samples (concentrations expressed in $\mu g\,g^{-1}$)

Sample[a]	5DP[b]	3DP	Tri	Tetra
M1	n.d.[c]	1.9 ± 0.2[d]	7.5 ± 0.5	14.1 ± 0.9
M2	n.d.	2.0 ± 0.1	7.1 ± 0.02	16 ± 2
M3	n.d.	n.d.	n.d.	n.d.
M4	3.7 ± 0.1	5.7 ± 0.03	20 ± 1	12 ± 1
M5	2.4 ± 0.09	3.4 ± 0.1	11.5 ± 0.5	8.1 ± 0.7
M6	2.3 ± 0.09	2.9 ± 0.01	11.6 ± 0.1	33 ± 2
M7	1.8 ± 0.02	2.4 ± 0.06	8.52 ± 0.8	15 ± 1
M8	3.6 ± 0.1	5.3 ± 0.2	15 ± 1	14.3 ± 0.6
M9	2.5 ± 0.1	3.9 ± 0.2	11.6 ± 0.6	13.0 ± 0.6
M10	n.d.	2.2 ± 0.2	6.1 ± 0.3	9.3 ± 0.9
CO1	2.5 ± 0.02	5.3 ± 0.4	16 ± 1	10 ± 1
CO2	2.7 ± 0.2	5.9 ± 0.2	17.8 ± 0.5	6.9 ± 0.06

[a] Samples named according to roasting process (M: roasted in a microwave oven and CO: conventional roasting).
[b] 3DP: 2,3-dimethylpyrazine; 5DP: 2,5-dimethylpyrazine; Tri: 2,3,5-trimethylpyrazine and Tetra: tetramethylpyrazine.
[c] n.d.: not detected.
[d] Precision expressed as R.S.D. and calculated from the standard addition regression data according to [35].

4. Conclusions

The composition of the volatile fraction of cupuassu liquor is similar to that reported for cocoa and its derivatives. The principal compounds found (tri- and tetramethylpyrazine, 3-methylbutanal, dimethyl sulfide, dimethyl disulfide, β-linalool) have already been indicated in the literature as key odor-impact components of cocoa. The most remarkable difference between the composition of cupuassu headspace and the literature data on the volatiles from cocoa and its derivatives seems to be the higher concentration on alkylpyrazines—which can be extremely interesting to the acceptance of cupuassu as replacement for cocoa, considering the favorable flavor impact of these substances. However, a more systematic approach, with inspection of more samples, would be necessary to have a conclusive comparative study on this subject.

Regarding the HS-SPME methodology, it was found to be suitable for the detection and quantitation of the principal alkylpyrazines found in cupuassu liquor headspace. The detection limits are compatible with the amounts found in the analyzed samples, and the precision is adequate. The procedure is simpler and faster than alternates typically employed to process such samples, such as SDE or liquid–liquid extraction—not to mention that it is potentially more reliable than those techniques, especially SDE (considering the temperature-induced production of Maillard products such as the alkylpyrazines). It is possible that use of dynamic headspace procedures could be advantageous compared to HS-SPME, specially in terms of sensitivity; however, a specific comparative study would be necessary to demonstrate this conjecture. The use of GC–AED also proved to be advantageous to this methodology. The target analytes could be easily detected on the N-specific chromatograms generated at both analytical wavelengths accessible for this element.

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