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Short communication

Static headspace analysis using polyurethane phases – Application to roasted coffee volatiles characterization

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ABSTRACT

Static headspace sorptive extraction using polyurethane foams (HSSE(PU)) followed by gas chromatography coupled to mass spectrometry is proposed for volatile analysis. The application of this novel analytical approach to characterize the volatiles profile from roasted coffee samples, selected as model system, revealed remarkable advantages under convenient experimental conditions.

The comparison of HSSE(PU) with other well-established procedures, such as headspace sorptive extraction using polydimethylsiloxane (HSSE(PDMS)) and headspace solid phase microextraction using carboxen/polydimethylsiloxane fibers (HS-SPME(CAR/PDMS)), showed that the former presented much higher capacity, sensitivity and even selectivity, where larger abundance and number of roasted coffee volatile compounds (e.g. furans, pyrazines, ketones, acids and pyrroles) could be achieved, under similar experimental conditions. The data presented herein proved, for the first time, that PU foams present great performance for static headspace sorption-based procedures, showing to be an alternative polymeric phase for volatile analysis.

1. Introduction

During the last years, sorptive extraction techniques have played a very important role on trace analysis in various types of matrices [1]. So far, several sorption-based enrichment methods have been proposed prior to chromatographic analysis, such as solid phase extraction, solid phase micro-extraction (SPME) [2] and more recently, stir bar sorptive extraction (SBSE) [3], which has been successfully applied for trace analysis of priority organic pollutants in several matrices [4–8]. The use of stir bars in the headspace mode, i.e. headspace sorptive extraction (HSSE), follows the same principles as direct SBSE [9], and has already been successfully applied for the volatile analysis of several matrices, such as aromatic and medicinal plants [10], coffee [11], fungi [12], *pesto* [13] and wine vinegars [14]. SBSE presents several advantages since it is an environmentally friendly technology, easy to manipulate, with remarkable reproducibility and very good sensitivity for trace level analysis. However, it is only commercially available with the non-polar polydimethylsiloxane (PDMS) phase, which cannot retain all types of analytes, in particular the more polar ones ($\log K_{O/W} < 3$). To overcome this limitation, several authors have recently proposed other strategies, such as the dual-phase stir bar [15], as well as other polymeric phases [16,17], but without embracing the ruggedness and the wide range of applicability demonstrated by the PDMS polymer. Lately, our group has introduced the polyurethane (PU) foams as novel polymeric phases for SBSE, due to the very interesting physical and chemical properties they exhibit [18,19]. These polymers are easily produced, very versatile and have shown great capacity to retain polar compounds by SBSE [19–21]. Although PU foams demonstrated a remarkable performance in liquid phase, these polymeric sorbents were never been applied in the headspace mode for volatile analysis. A way to evaluate the performance of novel analytical approaches or polymeric phases through static HS mode can be done by testing well-known volatile systems [22]. The roasted coffee in particular, present a complex volatile profile widely studied by the food chemistry scientific community. Roasted coffee volatile fraction is constituted by several classes of compounds (e.g. pyrroles, pyrazines, furans, ketones, pyridines, alkanes, acids, etc.) having a large number of chemical precursors with very different contents, volatilities and polarities [15]. Therefore, the volatile profile of roasted coffee may be used to evaluate the performance of PU foams through static HS analysis, as well as to compare it with other well-established or reference analytical procedures, such as HSSE(PDMS) or HS-SPME using carboxen/polydimethylsiloxane (CAR/PDMS) polymeric fibers [11,22].

In this work we propose, for the first time, the application of static headspace sorptive extraction using PU foams (HSSE(PU)) for volatile analysis followed by gas chromatography–mass spectrometry (GC–MS), using roasted coffee samples as model system. The capacity, sensitivity, selectivity, advantages and analytical

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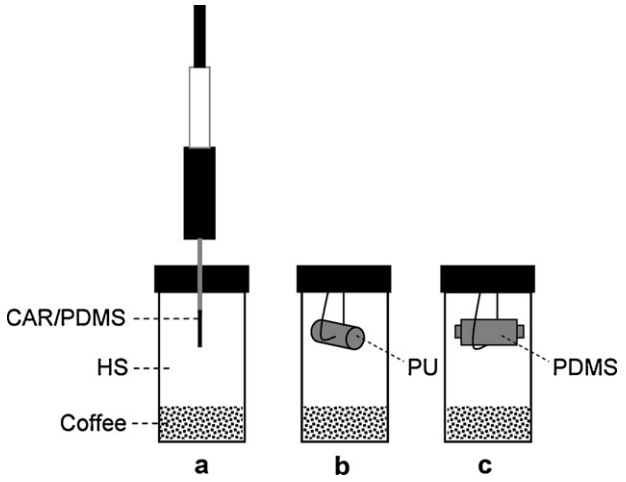

Fig. 1. Sampling apparatus configuration applied for the characterization of roasted coffee volatile compounds by HS-SPME(CAR/PDMS) (a), HSSE(PU) (b) and HSSE(PDMS) (c) in the present study.

performance achieved for volatile analysis, as well as the comparison in between HSSE(PU), HSSE(PDMS) and HS-SPME(CAR/PDMS) methodologies are also addressed.

2. Experimental

2.1. Chemicals

HPLC-grade methanol (MeOH, 99.9%, Panreac, Spain), acetonitrile (ACN, 99.9%, LabScan, Poland), iso-propanol (99.7% Carlo Erba, Italy), pentane (99%, Riedel-de Haën, Germany), diethyl ether (99.5%, Absolve-José Manuel Gomes dos Santos, Portugal) and acetone (99.8% Panreac, Spain were used. PU synthesis and clean-up procedures were performed according to previous report [19], were a tin catalyst, silicon oil (Dow Corning, Midland, USA), methylene bisphenyl diisocyanate (Lupanat, BSF, Lemförde, Germany), ultra-pure water, glycerol propoxylate (Sigma–Aldrich) and trimethylolpropane ethoxylate (Sigma–Aldrich) were used. Ultra-pure water was obtained from Milli-Q water purification systems (Millipore, Bedford, MA, USA). Commercial stir bars (Twister; Gerstel, Müllheim a/d Ruhr, Germany) coated with PDMS (20 mm length and 1 mm film thickness; 126 μL) were pre-conditioned by treatment with ACN before use. A manual SPME device and fibers coated with CAR/PDMS (75 μm) were supplied from Supelco Inc. (Bellefonte, PA, USA).

2.2. Samples

The roasted coffee used for the optimization of the procedure as well as for the comparison of different procedures was acquired at the local market. For the performed studies 2.0 g (Mettler AE 240, Spain) of grounded coffee sample were introduced in 20 mL vials sealed with caps having PTFE-faced silicone septa (Supelco).

2.3. HS-SPME assays

For SPME assays, a CAR/PDMS fiber was inserted into the HS of each sample (Fig. 1a) during 60 min and the sample was kept in a thermostatised water-bath at 60 °C, according to several authors [11,22]. Subsequently, the fiber was introduced in the split/splitless (S/SL) injection port of the GC–MS system. The back-extraction process was performed by using thermal desorption. Blank runs using empty vials were also performed.

2.4. HSSE(PU) and HSSE(PDMS) assays

For HSSE(PU) assays, PU having cylinder configuration (1.3 cm length and 0.5 cm diameter; average volume of 32 μL) were used and for HSSE(PDMS) assays commercial stir bars were applied. Both devices were hold to the silicone septa of each cap by two wires in the middle of the HS (Fig. 1b and c). The vials were introduced in a thermostatized water-bath. Different temperatures (30, 60 and 90 °C) and extraction times (30, 60 and 120 min) were evaluated. Subsequently, the sampling vials were opened and the PU or PDMS bars were removed and inserted in 1.5 mL vials, with 1 mL of a back extraction solvent. Different back-extraction solvents (acetone, MeOH, iso-propanol, pentane, ACN and diethyl ether) and desorption times (20, 40 and 60 min) were assessed, under ultrasonic treatment, after which the PU or PDMS bars were removed and the obtained extract analyzed by LVI–GC–MS. Blank runs using empty vials were also performed.

2.5. GC–MS analysis

For HSSE(PU) and HSSE(PDMS) assays, a programmed temperature vaporization injector (PTV) with a septumless sampling head having a baffled liner (SLH; Gerstel, Mülheim a/d Ruhr, Germany) was used. For large volume injection (LVI) the solvent vent mode was performed with liquid nitrogen as inlet cooling (vent time: 0.30 min; flow rate: 10 mL/min; pressure: 0 psi; purge: 150 mL/min at 2 min); the inlet temperature was programmed from 45 °C (0.35 min) to 280 °C at a rate of 600 °C/min and, subsequently, decreased to 200 °C (held until end) at a rate of 50 °C/min. The injection volume and speed were 5 μL and 100 μL/min, respectively. For HS-SPME assays, a split/splitless (S/SL) injector was used, operating in the SL mode. The SPME device was introduced in the injector port (270 °C) for GC–MS analysis and was allowed to remain in the inlet for 10 min. GC–MS analyses were performed on an Agilent 6890 series gas chromatograph equipped with an Agilent 7683 automatic liquid sampler tray (Agilent 7683, Agilent Technologies, Little Falls, DE, USA) and interfaced to an Agilent 5973*N* mass selective detector (Agilent Technologies, Little Falls, DE, USA). GC analysis was performed on a TRB-5MS (30 m × 0.25 mm i.d., 0.25 mm film thickness; 5% diphenyl, 95% dimethylpolysiloxane; Teknokroma, Spain) capillary column. Helium as carrier gas was maintained in the constant pressure mode and the inlet pressure was 9.52 psi with a flow rate of 1.3 mL/min. The oven temperature was programmed from 45 °C (1 min) at 5 °C/min to 200 °C, then at 20 °C/min to 250 °C (5 min) in a 39.50 min running time. The transfer line, ion source, and quadrupole analyzer temperatures were maintained at 280, 230, and 150 °C, respectively. For HSSE(PU) and HSSE(PDMS) assays a solvent delay of 3 min was selected. In the full-scan mode, electron ionization mass spectra in the range 40–400 Da was recorded at 70 eV electron energy. The acquisition data and instrument control were performed through the MSD ChemStation software (G1701CA; version C.00.00; Agilent Technologies, Santa Clara, CA, USA). The identity of each compound was assigned by comparison of their retention index (RI), relative to a standard mixture of *n*-alkanes (C_{10}–C_{24}; [23]), as well as by comparison with the mass spectra characteristic features obtained with the Wiley's library spectral data bank (G1035B; Rev D.02.00; Agilent Technologies, Santa Clara, CA, USA). For semi-quantification purposes, the average abundances ($n = 6$) of each identified compound was used.

2.6. Statistic analysis

Kruskal–Wallis one-way analysis of variance was performed through the online version of the test from VassarStats (Vassar College, USA).

3. Results and discussion

3.1. Optimization of the experimental conditions

The PU foams had already been proposed as alternative polymeric phases for enrichment purposes with excellent results in what relates to the microextraction of the more polar compounds in aqueous media [17,19]. However, this polymeric phase had never been applied for volatile analysis through the static HS mode (HSSE(PU)). In a first approach, we chose to evaluate the performance of the HSSE(PU) by testing its response to a well-known volatile system [11,22]. Thereby, the performance of this novel sorbent phase was assessed to characterize the volatiles profile of the complex roasted coffee matrices, constituted mainly by pyrroles, pyrazines, furans, ketones, pyridines, pyranones, acids, phenolics, etc. [15]. Recently, HS-SPME(CAR/PDMS) methodology had been proposed, as reference procedure, for the characterization of roasted coffee aroma and, several reports in literature [11,22], indicate the best experimental conditions to this type of application. Therefore, to propose and compare different analytical approaches, it is peremptory to optimize the most convenient sampling parameters for microextraction assays. During our study, GC–MS analysis operating in the full scan mode acquisition was used, since it is the best choice to identify volatile compounds. However, for back-extraction purposes, we have decided to use liquid desorption (LD) instead of thermal desorption in order to avoid the formation of possible artefacts or polymeric degradation compounds, since PU foams just hold temperatures as high as 260 °C [19]. All advantages of the LD step had already been discussed in detail in previous reports [4–8]. Furthermore, to enhance higher sensitivity, LVI were adopted for GC–MS analysis, using an injection of 5 μL, once larger volumes could lead to an increment of solvent background, which decreases the signal-to-noise ratio.

The optimization started with the evaluation of the best LD solvent. For this purpose, standard microextraction conditions, i.e. 60 min of equilibrium time under a termostatized water bath at 60 °C, were used. After extraction, the PU bars were introduced in 1.5 mL of different organic solvents (acetone, MeOH, iso-propanol, ACN, pentane and diethyl ether) followed by 60 min of ultrasonic treatment. From the data obtained, MeOH proved to be the best stripping solvent, particularly for the case of ketones, pyrazines, furans, acids and pyridines (data not shown), according to previous studies [18–20]. The LD time (20, 40 and 60 min) was also evaluated and 60 min was chosen for further HSSE(PU) assays, corresponding to the minimum period that allowed the complete back-extraction of all chemical precursors involved. Several authors [11,22], had already proposed the best equilibration temperature for HS-SPME analysis of roasted coffee volatiles aroma, which was the starting point for our study. For this purpose, sampling temperatures of 30, 60 and 90 °C were tested, in which the latter (90 °C) allowed the extraction of volatiles in the highest amount, especially for the case of furans, which were extracted two and ten times higher in relation to 30 and 60 °C, respectively. At 30 °C, ketones and phenolics were not extracted, whereas the temperature of 60 °C allowed the extraction of ketones, phenolics, alcohols and pyrazinamides, as well as higher amounts of pyridines, pyrrols, pyrazines, furans and acids. Since temperature of 60 °C is easier to control from the experimental point of view, it was selected for further studies. Subsequently, three periods of equilibrium time (30, 60 and 120 min) were also tested for HS microextraction. From the data obtained, an increase of the efficiency yields from 30 to 60 min was observed for the majority of the chemical classes; the increase of the equilibrium time to 120 min did not show a significative increase on the recovery efficiency. Therefore, the subsequent studies were carried out with an equilibrium time of 60 min. In short, the optimized experimental conditions allowed to establish a convenient HSSE(PU)–LD/LVI–GC–MS procedure (extraction time: 60 min (60 °C); back-extraction: MeOH (1.5 mL), 60 min under sonification).

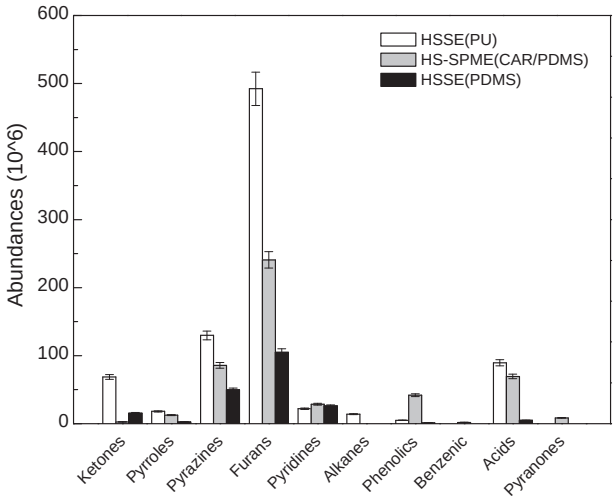

Fig. 2. Comparison of the average total abundances per chemical class from the volatile profile of roasted coffees samples obtained by HSSE(PU), HS-SPME(CAR/PDMS) and HSSE(PDMS) procedures followed GC–MS analysis, under convenient experimental conditions.

3.2. Performance and comparison of different procedures

Even though HS-SPME(CAR/PDMS) uses a different approach (thermal desorption, S/SL inlet, lower polymeric volume, as well as different theoretical principles), a comparison of this reference technique with HSSE(PU) and HSSE(PDMS) procedures was carried out. The sampling apparatus configuration applied for the characterization of roasted coffee volatiles by HS-SPME(CAR/PDMS), HSSE(PU) and HSSE(PDMS) is depicted in Fig. 1. The intention of this comparison is the assessment of the differences observed when applying a well-established technique (HS-SPME) for study the volatiles of roasted coffee samples and other approaches using different polymeric phases. Likewise, HSSE is an emerging technique on this area of study, which has already showed good performance on liquid phase [9–14] and the scientific community has also interest to test its capability for volatile analysis. Table 1 summarizes the average abundances determined using the three procedures, under similar experimental conditions. The volatiles in the HS profile of the analyzed roasted coffee blends had already been reported by several authors [11], mainly originated from the Maillard reactions in particular due to Strecker's degradations [24]. As expected, the chemical classes with higher abundance and number of constituents are furans, pyrazines, acids, ketones and pyrroles, depending on the procedure involved. When using HSSE(PU), the most abundant compounds are 2-furanmethanol, acetic acid, 2-furanmethanol acetate, 2-methylpyrazine and 2,5-dimethylpyrazine, which are known to have a remarkable impact in the coffee aroma, although the flavour evaluation is out of the scope of the present study. Fig. 2 plots the extraction capacity (in terms of total abundances per chemical class) achieved for all three procedures. As it can be seen, HSSE(PU) showed the highest capacity and recovery yields for the majority of the chemical classes, such as furans, pyrazines, ketones, acids, pyrroles and alkanes (Table 1; Fig. 2). However, alkanes were exclusively extracted by PU polymeric phase, whereas phenolic compounds present much higher affinity towards CAR/PDMS fiber (Table 1; Fig. 2). Similar

Table 1
Average abundances for the volatiles founded in roasted coffee samples obtained by HS-SPME(CAR/PDMS), HSSE(PU) and HSSE(PDMS) procedures followed by GC–MS, under similar experimental conditions.

Compound	Abundance ($\times 10^{-5}$)[b]			
	RI[a]	HS-SPME(CAR/PDMS)	HSSE(PU)	HSSE(PDMS)
Ketones				
2-Hydroxy-3-methyl-2-cyclopenten-1-one	763	26.1		
3-Hydroxy-2-butanone	787		59.2	
1-Hydroxy-2-propanone	784		139.2	
1-(Acetyloxy)-2-propanone	824		477.6	152.3
Total Ketones		26.1	676.0	152.3
Pyrroles				
1H-pyrrole-2-carboxaldehyde	863	40.9		
1H-pyrrole-2-carboxaldehyde, 1-methyl-	879		116.6	
1-(1H-pyrrol-2-yl)-ethanone	893	83.5		
1-(1-Methyl-1H-pyrrol-2-yl) ethanone	915		60.9	24.4
Total Pyrroles		124.4	177.5	24.4
Pyrazines				
2-Methylpyrazine	808	177.5	342.8	209.2
2,3-Dimethylpyrazine	840		58.3	61.2
2,5-Dimethylpyrazine	834	453.3	324.5	
(1-Methylethenyl)-pyrazine	837	20.0		
Ethylpyrazine	829		268.0	161.0
2-Acetyl-3-methylpyrazine	867		50.5	
2-Ethyl-6-methyl-pyrazine	876		80.0	
2-Ethyl-5-methyl-pyrazine	878		86.0	
2-Ethyl-3,5-dimethyl-pyrazine	931		67.0	
3-Ethyl-5-dimethyl-pyrazine	917	75.7		
3-Ethyl-2,5-dimethylpyrazine	922			57.4
Total Pyrazines		726.5	1277.1	488.8
Furans				
2-*n*-Butyl furan	802		41.8	
Furfural	804	638.6	276.7	98.3
2-Furanmethanol	817	818.7	3326.9	565.5
5-Methylfurfural	821	539.2	353.2	94.0
2-Furanmethanol, acetate	871	325.4	481.1	200.1
2-Furanmethanol, propanoate	861		32.2	
Furfuryl formate	836		81.8	
Dihydro-2-methyl-3(2H)-furanone	847		165.3	
2,5-Dimethyl-3(2H)-furanone	850		50.1	
2,2′-[Oxybis(methylene)]bis-furan	1021	47.0		
2,2′-Methylene furan	1006		38.3	
Total Furans		2368.9	4847.4	957.9
Pyridines				
Pyridine	772	281.0	159.2	257.5
Total Pyridines		281.0	159.2	257.5
Alkanes				
Dodecane	1186		69.6	
Tetradecane	1278		67.9	
Total Alkanes			137.5	
Phenolics				
2-Methoxyphenol (guaiacol)	1063	79.2	49.6	11.3
4-Ethyl-2-methoxy-phenol	1264	72.2		
2-Methoxy-4-vinylphenol	1300	241.0		
Total Phenolics		392.4	49.6	11.3
Benzenic				
3,4-Dimethoxy styrene	1324	18.3		
Total Benzenic		18.3		
Acids				
Acetic acid	744	683.8	881.3	48.7
Total Acids		683.8	881.3	48.7
Pyranones				
3-Hydroxy-2-methyl-4H-pyran-4-one (maltol)	1094	83.5		
Total Pyranones		83.5		

[a] Retention index calculated in relation to a C_{10}–C_{24} *n*-alkanes mixture in TRB-5MS capillary column.
[b] For experimental conditions see Section 2.

behaviours were observed for benzenic and pyranone compounds, which were only extracted by the CAR/PDMS fiber, but at very low level (Table 1; Fig. 2). In the case of furans, pyrazines, ketones and pyridines a higher extraction efficiency was achieved not only due to the great amounts of extracted compounds (Fig. 2) but also to the large number of different compounds (Table 1) revealed by the HSSE(PU) device in relation to the other two procedures. Kruskal–Wallis ANOVA relative to the type of polymeric phase used, as a grouping factor, demonstrates that the differences observed in the abundances of the chemical classes of ketones, pyrazines, furans, phenolics and acids present a great significance ($p < 0.05$). This evidence proves that HSSE(PU) approach seems to present large capacity, enough selectivity and sensitivity for the characterization of volatile profile from roasted coffee samples, allowing simultaneously the extraction of a wide range of different chemical precursors. Fig. 3 compares total ion chromatograms from

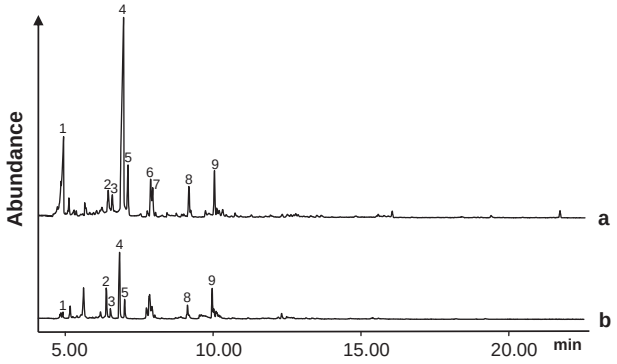

Fig. 3. Total ion chromatogram profiles from the volatile fraction of roasted coffee samples obtained by HSSE(PU) (a) and HSSE(PDMS) (b) following LD/LVI–GC–MS analysis, under similar experimental conditions. Legend: acetic acid (1); 2-methylpyrazine (2); furfural (3); 2-furanmethanol (4); 1-acetyloxy-2-propanone (5); 2,5-dimethylpyrazine (6); dihydroxy-2-methyl-3(2H)-furanone (7); 5-methylfurfural (8); 2-furanmethanol, acetate (9).

volatiles profile of roasted coffee samples obtained by HSSE(PU) and HSSE(PDMS), under similar experimental conditions. It is notice that a higher abundance is achieved by the HSSE(PU) procedure, besides the polymeric volume involved is much lower (32 μL of PU against 126 μL of PDMS). The data herein obtained emphasize the great sorptive properties exhibited by the PU phases, which may be influenced not only by the amount of polymeric phase involved but also through the residual O–H bonds of the foam matrix as previously reported [19]. From the data obtained, the PDMS polymeric phase shows an enrichment limitation due that exhibits strong non-polar characteristics, not suitable for the several chemical classes usually achieved in the roasted coffee volatiles. Furthermore, when comparing HS-SPME(CAR/PDMS) with HSSE(PU), the latter seems to present greater performance and a promising alternative for coffee aroma characterization. In fact, a much higher capacity and selectivity are definitely attained in particular for compounds having a wide range of polarity. On the other hand, the PU phases present also a remarkable regeneration since for the work carried out here were used the same set of PU cylinders, without loss of efficiency or degradation, in agreement with previous studies [19]. In short, the procedure proposed in the present work (HSSE(PU)) demonstrates great advantages and an effective alternative for volatile analysis, is quite affordable and easy to handle, allowing analytical sensitivity, while extracting compounds that are not or less recovered by other sorption-based techniques.

4. Conclusion

In the present work, PU foams have been successfully applied, for the first time, as polymeric phases for static headspace analysis. The use of HSSE(PU) procedure in the characterization of the volatiles profile of roasted coffee samples, used as model system, revealed remarkable performance, under convenient experimental conditions. The comparison between HSSE(PU), HSSE(PDMS) and HS-SPME(CAR/PDMS) analytical procedures showed that the former presents higher capacity, much better selectivity and sensitivity for roasted coffee volatiles characterization, under similar experimental conditions. For general applications, the HSSE(PU) procedure is a suitable alternative for volatile analysis.

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