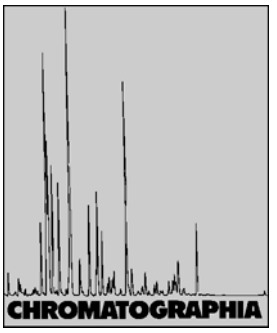
Comparison of Two SPME Fibers for Differentiation of Coffee by Analysis of Volatile Compounds



2001, *54*, 647–652

A. M. Costa Freitas[1]* / C. Parreira[2] / L. Vilas-Boas[3]

[1] Centro de Química Fina e Biotecnologia, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825 Monte da Caparica, Portugal; E-Mail: ana.freitas@dg.fct.unl.pt
[2] Dias de Sousa Lda. Praceta Aníbal Faustino, 6 B 2625 Póvoa de St. Iria, Portugal
[3] Instituto Superior Técnico, Av. Rovisco Pais 1096 Lisboa Codex, Portugal

Key Words

Gas chromatography
Solid-phase microextraction
Fiber selection
Coffee aroma compounds

Summary

Solid-phase microextraction (SPME) is based on the affinity of different headspace compounds for the coating on a fiber. In the work described in this paper we evaluated and compared two different coatings, a medium polarity coating, Carbowax divinylbenzene (CW-DVB), and an apolar coating, polydimethylsiloxane (PDMS), for the analysis and classification of coffee. PCA (principal-component analysis) was used to evaluate the results obtained by use of the two fibers and, at the same time, to determine their suitability for use for the classification and differentiation of coffee. When PCA is used as a method of classification, using the 30 major peaks as variables, the results obtained with the CW-DVB fiber enable classification according to geographic origin; those obtained with the PDMS fiber enable classification based on variety (*Arabica* or *Robusta*).

Introduction

Solid-phase microextraction was developed in 1989 by Pawliszyn and marketed in 1993 by Supelco [1]. The field of application has since been expanded. The method is of great relevance for application to different types of solid, liquid, or gaseous matrix [2–11]. When compared with other extraction techniques SPME has the advantages of being solvent-free; this avoids the need for the tedious and error-prone elimination of the solvent which is characteristic of liquid-liquid and/or solid-liquid extraction [12]. In SPME, analytes are either *ab*sorbed or *ad*sorbed by the fiber coating, i. e. the solutes either dissolve or become partitioned in the bulk of the fiber coating material in the first mechanism or become bound to the fiber surface in the second mechanism. Two conditions are combined when using the headspace (HS) technique coupled with SPME sampling and analysis – headspace temperature and exposure time (the time the fiber is in contact with the headspace) [13]. The key component of SPME techniques is the piece of fused silica fiber (ca 1-cm long) coated with a polymeric stationary phase the characteristics of which influence the extraction [14, 15]. Analysis of volatile compounds by different techniques can yield different quantitative and even qualitative results, depending on the experimental design and the conditions being used [16]; SPME analysis performed with different fibers reflects the same phenomena [14, 17].

Coffee classification has been achieved by use of HS-SPME-GC for roasted coffee and LS (liquid sampling)-SPME-GC (on a polydimethylsiloxane, or PDMS, fiber) for coffee beverages [11]. SPME with a PDMS coating and PCA analysis was the method used. In this work we compared two fibers a medium polarity coating, Carbowax-divinylbenzene (CW-DVB), 65 μm, and an apolar coating, PDMS, 100 μm. CW-DVB is a porous solid, which thus extracts analytes by adsorption. PDMS is an apolar viscous liquid coating, extracting analytes mainly by absorption rather then adsorption [15, 18]. Absorption is a much weaker process then adsorption [18]. The purpose of this study was to evaluate how the different extraction mechanisms of the different fibers can influence the classification results. Results were evaluated by PCA analysis.

Experimental

Sample Preparation

Roasted *Arabica* and *Robusta* coffee beans were used in our analyses. *Arabica* coffees were produced in Brazil, Colombia, Guatemala (America), and Kenya (A

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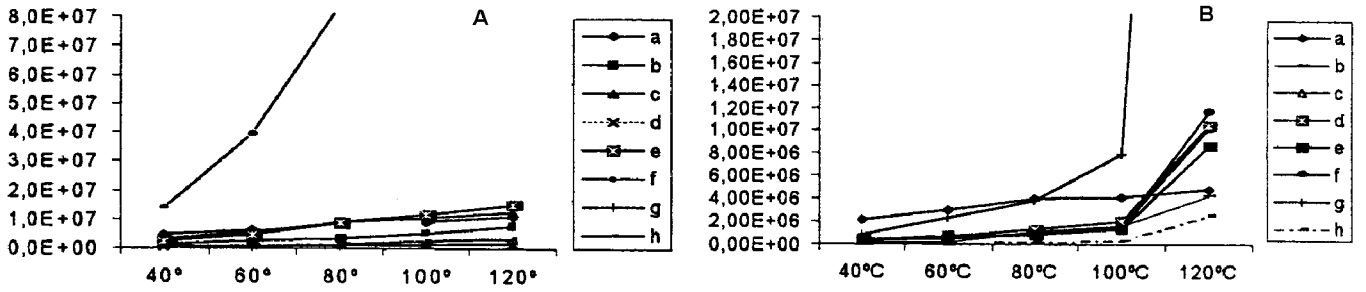

Figure 1. Plot of compound peak area against headspace development temperature for the key compounds used for optimization of SPME conditions. **A**. CW-DVB fiber; **B**. PDMS fiber. Peak identification: **a** = pyridine (variable 1); **b** = methylpyrimidine (variable 4); **c** = ethylmethylpyrazine (variable 10); **d** = 3-ethyl-2,5-dimethylpyrazine (variable 10); **e** = ethyl butyrate (variable 16); **f** = furanomethanol acetate; **g** = 2-furfurylthiol (variable 31); **h** = 5-methyl-2-furancarboxaldehyde.

and B; Africa). *Robusta* coffees came from Angola, Ivory Coast, Uganda, and Zaire (Africa). Samples were delivered in polypropylene-aluminum-polypropylene packages hermetically sealed under vacuum. Immediately after sampling the bags were again sealed under the same conditions.

The samples were ground for 3 min (medium grade) immediately before analysis using a coffee mill. The coffee (2.5 g) was placed in a 20-mL vial capped with a Teflon-lined septum and a screw cap. Three replicate analyses by HS-SPME-GC and HS-SPME-GC-MS were performed on each sample, so the total number of samples analyzed was 27.

Headspace Conditions for Solid-Phase Microextraction (SPME)

Headspace analysis was performed for each fiber at 40, 60, 80, 100, and 120 °C. Sampling was performed for 1, 3, 5, 7, 10, and 20 min. A GC oven was used for headspace thermostatting. Compounds were thermally desorbed at 250 °C in a Carlo Erba (CE) Instruments split/splitless injection port.

Gas Chromatography

Gas chromatography was performed with a Carlo Erba Vega series instrument equipped with an FID and a split/splitless injector. Splitless injection was performed with a 1-mL liner; the split vent was closed for 60 s. Compounds were separated on a 30 m × 0.25 mm i. d., 0.25 µm film thickness, DBwax capillary column. Helium was used as carrier gas, at a pressure of 90 kPa. The oven temperature was held at 60 °C for 5 min after injection then programmed linearly at 3° min^{-1} to 110 °C, which was held for 3 min, and then linearly at 5° min^{-1} to 200 °C. Detector and injector temperatures were 250 °C.

Gas Chromatography – Mass Spectrometry

GC-MS was performed under the same conditions with a Fisons MD800 instrument also equipped with split/splitless injector. Interface and ion-source temperatures were 200 °C. Ionization was by electron-impact (EI) at an electron energy of 70 eV. The scan time was 1 s.

Data Treatment

Multivariate analysis of GC data was accomplished with Unscrambler V5.03 for Windows, from Trondheim, Norway, on an IBM 486 personal computer (PC). All peaks in the GC chromatogram with reproducible, measurable areas were used to perform PCA analysis. Routine statistical analysis was performed to ensure reproducibly measurable areas for all the samples under investigation. Peak areas were processed on a Shimadzu CR3A computing integrator and introduced manually to the PC for statistical analysis. Data had a medium *RSD* of approximately 6.8%. Principal-component analysis (PCA) was used for data evaluation.

Sensory Analysis

Sensory tests were performed at NovaDelta by a certified panel according to the Portuguese Rules NP 4258 (1993), NP 4263 (1994), and ISO 6658 (1985). Coffee was prepared by lightly roasting until a "kraft" color was achieved. Grinding was rough. The coffee was tasted in transparent glass cups (in accordance with NP) placed on a rotating table. Ten replicates are tasted. All tasters used the same cup. Approximately 10 g coffee was placed in each cup and boiling water was added. Tasting was performed after settling of the powder. A small spoon was used for tasting (in accordance with NP) and after aroma evaluation the infusion was swallowed from the spoon. The spoon was kept hot by immersion in hot water. Room humidity and temperature were kept at 60% and 22 °C, respectively.

Results and Discussion

To optimize the extraction process, factors which might influence the solid and the headspace between the analytes in the sample and their extraction by the fiber were taken into account. Extraction is affected by time and temperature. Agitation was not considered, because it is always less important when solid samples are used. Always using the same degree of coffee grounding minimized matrix effects.

Because two different extraction mechanisms were under evaluation for SPME optimization, eight peaks belonging to seven different chemical families were randomly chosen from the GC chromatogram.

For fiber evaluation the 30 major peaks obtained were used as variables. We always used area percentage values and not absolute areas, because *RSD* was higher if absolute areas were used, probably because of operator errors, particularly manual injection. Although we were able to observe this problem mainly with the PDMS fiber, it does not seem to be a consequence of SPME analysis, because others have already reached similar conclusions using different sampling methods [16].

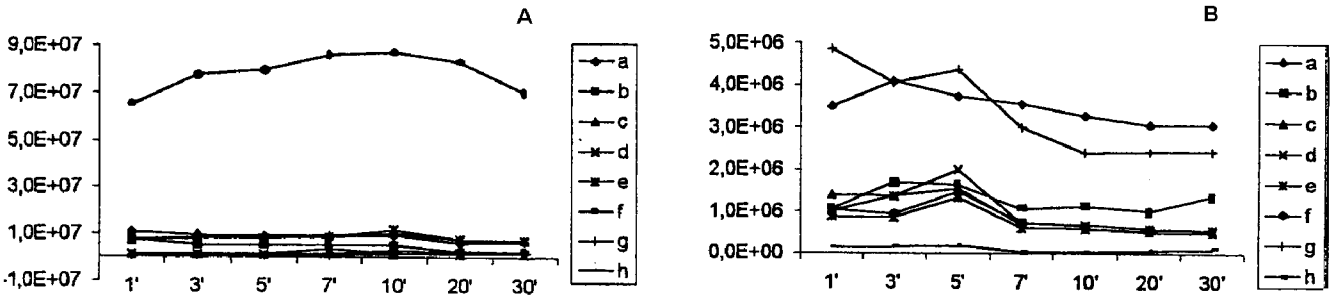

Figure 2. Plot of compound peak area against sampling time for the key compounds used for optimization of SPME conditions. **A**. CW-DVB fiber; **B**. PDMS fiber. Peak identification: **a** = pyridine (variable 1); **b** = methylpyrimidine (variable 4); **c** = ethylmethylpyrazine (variable 10); **d** = 3-ethyl-2,5 dimethylpyrazine (variable 10); **e** = ethyl butyrate (variable 16); **f** = furanomethanol acetate; **g** = 2-furfurylthiol (variable 31); **h** = 5-methyl-2-furancarboxaldehyde.

Study of Extraction Conditions

To optimize the extraction conditions eight peaks from seven different chemical families were chosen (Figures 1 and 2).

The first parameter optimized was temperature. The release of aroma compounds from the matrix is substantially affected by temperature. Because water release was not a problem (roasted coffee contains approximately 6% water) 120 °C was the limit, because it is known that above 130 °C coffee starts to turn brown and above 150 °C roasting starts again [19], thus changing the composition of the headspace. Five temperatures were evaluated – 40, 60, 80, 100, and 120 °C (Figure 1). Because of these considerations, and knowing that, in general, the highest temperature yielding satisfactory sensitivity should be used [20], the temperature was fixed at 100 °C. Better recovery with the CW-DVB fiber was expected, because of the polar character of the eight molecules used for optimization.

After selection of headspace temperature exposure time was optimized. The same compounds were used and extraction time was varied from 1 to 30 min. Figure 2 shows a plot of area against compound during time optimization; exposure time was fixed at 5 min. Although smaller times have been used [11] with the PDMS fiber we observed that after equilibrium was reached the amount extracted decreased; this phenomenon has already been reported by others [21]. Although this behavior has not been clearly explained, it has been suggested that the compounds were released by the fiber as it became hot and the fiber-headspace partition coefficient was reduced [21]. The headspace volume was minimized, because only 1/3 of the vial was empty. If only the CW-DVB fiber was considered, 1 min exposure time was sufficient. Longer times do not, however, seem to affect the amount extracted when this fiber is used; this avoids changing sampling conditions when both fibers are compared.

Fiber Evaluation

Fixing the best operating conditions, does not mean we were extracting the ideal compounds for coffee differentiation and/or evaluation [22, 23]. Different compounds have been used, and different extraction conditions. HS compounds mainly characterize the aroma; taste impressions can be added by studying different fractions [11]. The same might be applied to different extracts of the same headspace but obtained with different fibers.

Figure 3 shows typical gas chromatograms obtained from coffee aroma by use of both fibers. The GC patterns obtained from the fibers indicate that the main differences were quantitative rather then qualitative.

It has already been shown that the PDMS fiber can be used to separate and differentiate between coffee samples [11]. Different classification was obtained when HS and/or LS (liquid sampling) were used. The CW-DVB fiber has not yet been used with this sample.

Our intention was to determine which changes relevant to differentiation occurred (or even if any differentiation occurred) when an extraction procedure with a different mechanism was used. With PDMS fibers absorption is affected mainly by molecular size, polarity, and pH [14]. Because absorption is the relevant process, analytes are not retained on the active surface but partitioned [18] whereas with the CW-DVB fiber, for which the mechanism is adsorption, molecules with greater affinity tend to displace those with lower affinity, especially when the concentrations of the latter are low [15]. Thus absorption is, by definition, a non-competitive process whereas adsorption is a competitive process in which molecules 'fight' for the active sites of the surface [24].

To choose between the two fibers for extracting the 'best' compounds for coffee differentiation and/or evaluation, the results from chromatographic analysis of the fiber extract were treated by PCA analysis. PCA is a well established tool for interpretation of chemical data, and several examples of its applications and basic features are available in the literature [23, 25, 26].The chromatograms obtained by use of both fibers revealed, as expected, a multitude of peaks (more than 100). From this set of data 45 peaks account for more then 90% of the total area. Of these 45 peaks the 30 major peaks with reproducible measurable areas were used as variables. The areas of these 30 peaks, expressed as a relative percentage of the 45 integrated peaks, were used as variable vectors for multivariate analysis of the data gathered to establish similarities and differences between samples.

The first stage of PCA is choice of the similarity measure. The similarity measure chosen was the covariance matrix. The values obtained are a measure of the amount of association between variables. The values were normalized using the z-transform.

The next stage in PCA is extraction of eigenvectors from the matrix to obtain the so-called principal components (uncorrelated variables). Principal components are linear combinations of the original variables and expand the maximum variance in the variables. The PC loadings are coefficients of the correlation between the variable vectors and the principal components. The PC scores are the coordinates of the samples points on the PC. When

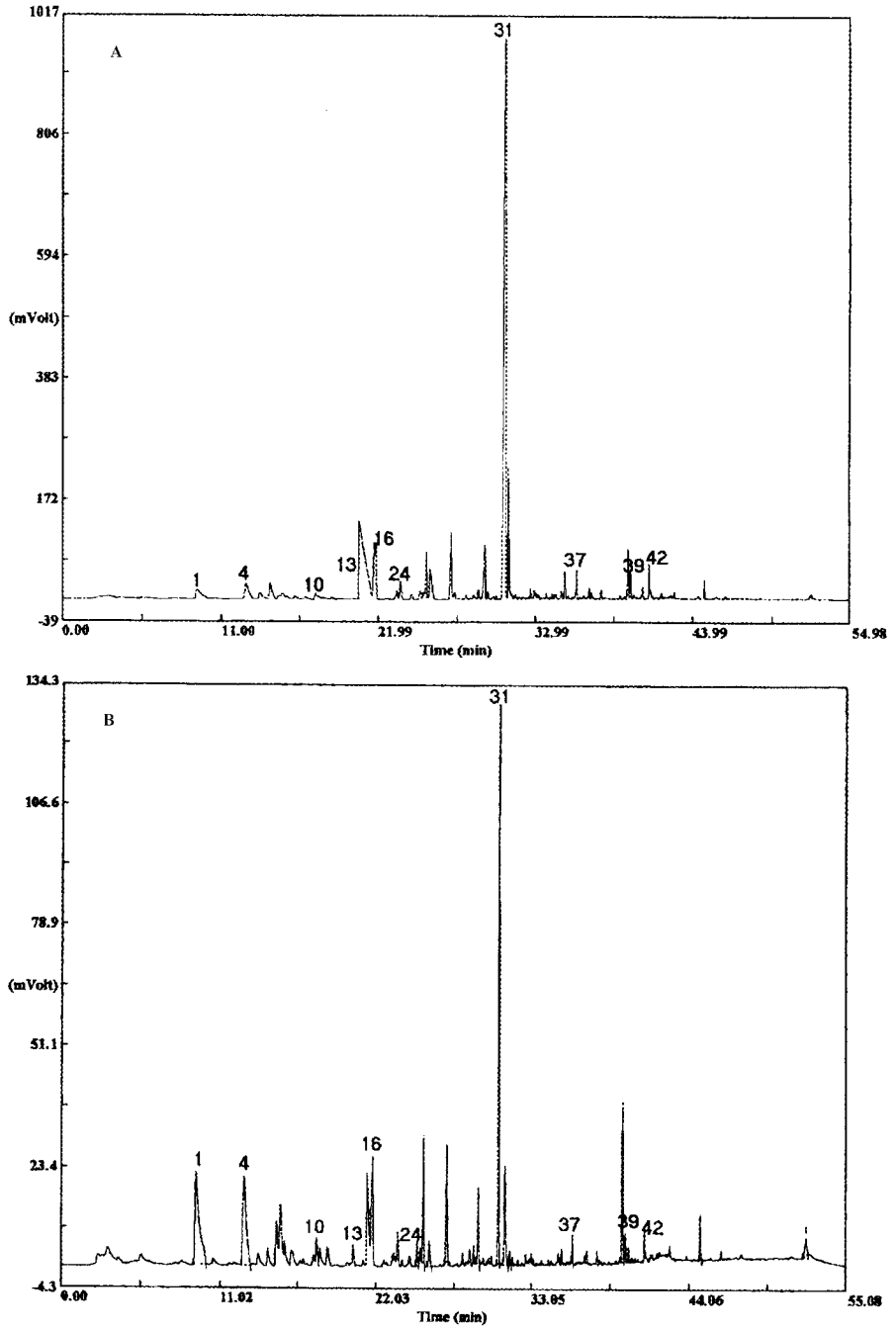

Figure 3. SPME-GC-MS chromatograms obtained from coffee samples extracted with, **A**, the CW-DVB fiber and, **B**, the PDMS fiber. Peak identification is as given in Table II.

these scores are plotted against the PC, similar samples tend to group together.

Figure 4 shows plots of variable loadings and Figure 5 the plot of the scores obtained for the two first principal components of the *Arabica* and *Robusta* coffees. The data obtained by PCA analysis when the CW-DVB fiber was used indicated that the first two principal components explained more than 66% of sample variation. The loadings (Figure 5) show that variables 31, 13, 24, and 37 are sufficient to describe the samples (variables are identified in Table II). When the PDMS fiber was used the first two principal components explained 88% of sample variability and variables 1, 39, 16, 10, 4, and 42 were sufficient to describe this set of data. From Figure 5 it seems that the CW-DVB fiber can be used to differentiate the coffees according to their origin – the African coffees being located on the right of the plot and the American varieties at the left. Uganda coffee is the only African coffee more related to the American varieties. With these results we were unable to see any difference between *Arabica* and *Robusta* varieties. The Kenya B variety (*Arabica* from Africa) that failed the sensory tests (Table I) was mixed with the American varieties. Because the purpose is to identify compounds that account for the classification when the different fibers are used, compounds that affect classification were tentatively identified by GC-MS (Table II). For classification (Figure 4) we might say that variables 31 and 13 are those that account for geographic differentiation. Variable 31 characterizes mainly the American coffees and variable 13 the African coffees. Both compounds are considered as impact odorants of roasted coffee [25, 26]. Variables 24 and 37 are those responsible for the variance observed along the 2nd PC. These results are similar to those obtained by use of LS-SPME-GC [11], by use of which three coffees from Kenya, Colombia, and Guatemala, roasted under the same conditions, were separated by PCA according to geographic origin.

When the PDMS fiber was used for sampling very different results were obtained. The fermented sample from Kenya (Kenya B) was clearly separated from the others. Variable 4-(3-hydroxy-2-butanone) seemed to be responsible for this. In this analysis the coffees, instead of being separated according to geographic origin, were separated into the *Arabica* or *Robusta* varieties; this result is in agreement with the fact that different blends were separated when HS-SPME-GC and PCA were used for coffee characterization [11]. Kenya A, an African variety, is completely mixed with the American *Arabica* varieties. *Arabica* varieties seem to be differentiated by variables 39, 16, and 31 whereas the *Robusta* varieties are characterized by variables 1, 42, and 10 (the variables are identified in Table II). Once again variable 31, 2-furfurylthiol, and variable 10, an alkylpyrazine, play important roles in this differentiation [27]. This is an important result, because separation according to variety is very important for coffee blends [28, 29].

Conclusion

SPME is an effective and clean method for extraction and analysis of coffee aroma headspace. The fiber used is very important if the results obtained are to be used to separate coffee types according to variety, as is needed for coffee blends. Use

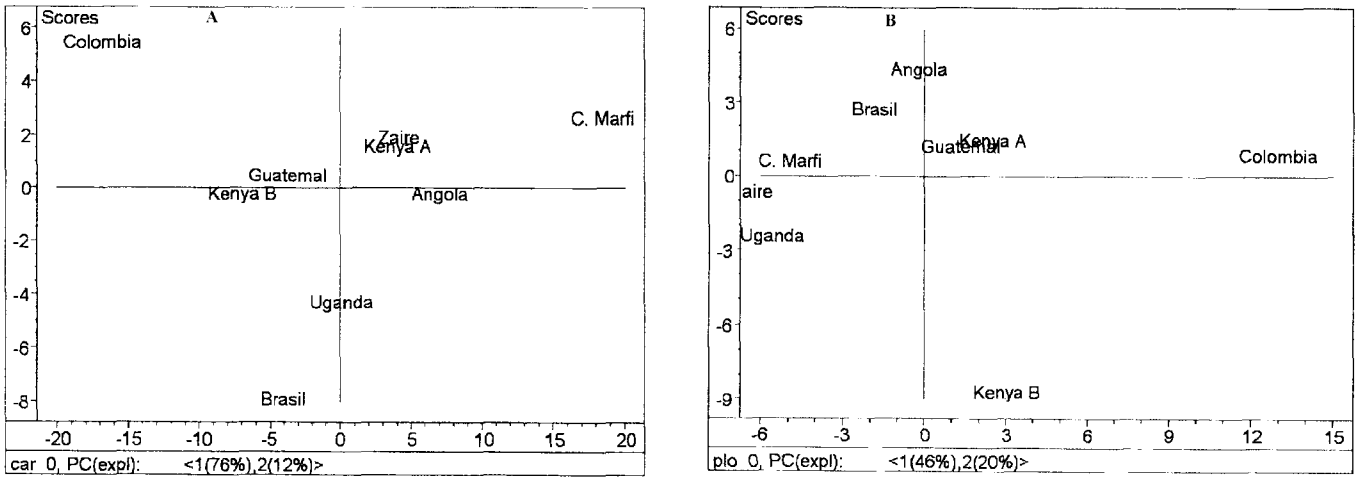

Figure 4. Plot of factor loadings for the test set of coffee samples extracted by SPME with, **A**, the CW-DVB fiber and, **B**, the PDMS fiber.

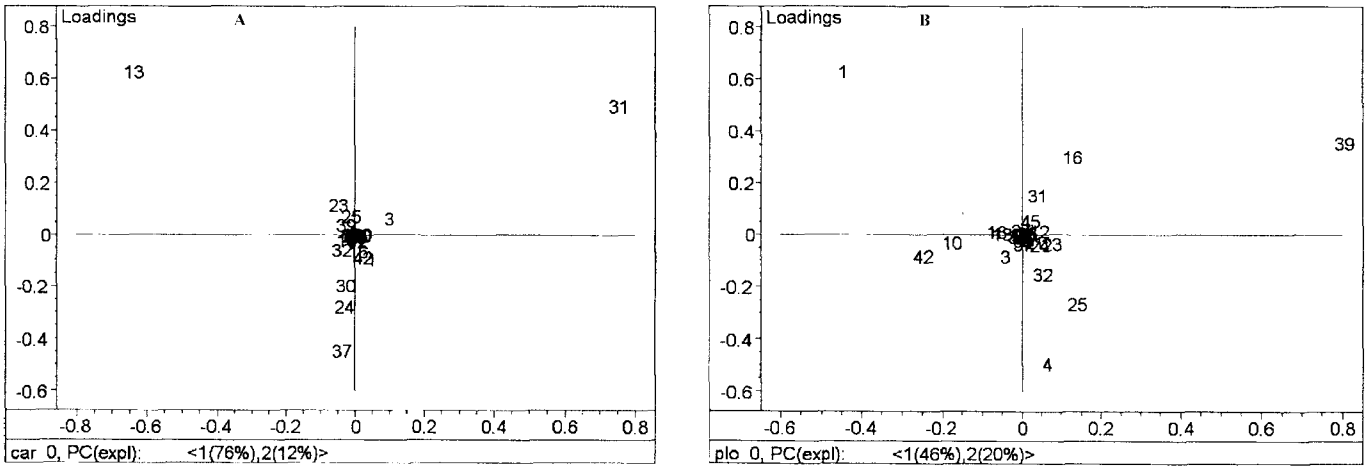

Figure 5. Extracted principal components as a function of thirty variables for the coffee samples analyzed, projected in the plane defined by the first two PCs. **A**. CW-DVB fiber; PC1 = 76%, PC2 = 12%; **B**, PDMS fiber; PC1 = 46%, PC2 = 20%.

Table I. Panel classification of the coffees studied (taste conditions: temperature 22 °C, RH 60%).[1]

Variety	Origin	Flavour	Classification[2]
Robusta	Angola	3	18.1
	Ivory Coast	3	17.9
	Uganda	3	17.9
	Zaire	3	17.9
Arabica	Brazil	4	18.1
	Colombia	4	18.1
	Guatemala	4	18.1
	Kenya A	5	18.7
	Kenya B	Failed; abnormal	Failed; abnormal

[1] Panel results were from the same organization that provided the coffee samples. The attributes considered were: A, organoleptic – color and visual impression; B, aroma; C, taste impressions – acidity, sweetness, metallic, salty, astringency, fermented; D, odor impressions; E, overall impressions – body and flavour; F, persistence – residual and persistence.

[2] The coffee panel considers all *Robustas* with a global classification ≥ 17.00 and all *Arabicas* with a flavor classification ≥ 4 to be 'very good'.

Table II. Tentative identification by GC-MS of the variables that account for the observed differentiation when PCA analysis is performed.

Variable no.	Compound name
1	Pyridine
4	3-Hydroxy-2-butanone
10	Ethylmethylpyrazine
13	Ethyldimethylpyrazine
16	1-acetyl(oxy)-2-propanone
24	Propanoic acid
31	2-Furfurylthiol
37	1*H*-Pyrrole-1(2-furanyl-methyl)
39	Acetylpyrrole
42	Methyl-2-pyrrole carboxaldehyde

of the PDMS fiber is better for this purpose, because the analytical results obtained enable differentiation between *Arabica* and *Robusta* varieties when HS-SPME-GC is used. When determination of geographic origin is the main purpose HS-SPME-GC with the CW-DVB fiber can be used. The differences observed clearly demonstrate the need of method evaluation whenever a new method/matrix is proposed.

Acknowledgments

The authors wish to thank Delta Cafés in Campo Maior, Portugal for the gift of the coffee samples and for the results from sensory testing, and Fundação para a

Ciência e Tecnologia P/AGR/1116/98 for funding.

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Received: May 21, 2001
Revised manuscript received: Jul 11, 2001
Accepted: Aug 21, 2001