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Solid-phase microextraction–gas chromatography mass spectrometry and multivariate analysis for the characterization of roasted coffees

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Abstract

HS-SPME–GC–MS in combination with PCA was employed to discriminate different arabica/robusta blends having different geographical origins. HS-SPME confirmed to be an effective and reproducible sampling technique for routine characterization of coffees. In addition, the chemometric approach permitted to find parameters suitable for the differentiation of the different blends and the determination of the real quality of the products. Finally, the proposed methods have been successfully applied to some commercial coffee blends.

Keywords: Solid-phase microextraction; Headspace-SPME; GC–MS; Principal component analysis; Coffee

1. Introduction

The commercially available coffee beans derive from two plants: *coffea arabica* and *coffea canephora* var. *robusta* that are cultivated in many tropical countries. A coffee beverage is made from roasted beans of *arabica* or *robusta* or blends *arabica*/*robusta*. The former variety is considered more valuable and is consequently the most expensive. It is common practice to sell coffees on the basis of their quality and/or geographical origin [1]; as a consequence, simple, rapid and inexpensive methods to characterize coffee varieties are highly desirable. These methods could assess the real quality of the product in case of fraudulent of accidental mislabeling. However, this is not an easy task, since the chemical composition of coffee varieties is very similar and it is then necessary to find parameters suitable for their differentiation. Some authors have proposed interesting solutions to characterize coffee varieties that consider the analysis of the volatile fraction by different approaches, the on-column injection [2], the headspace (HS) sampling using a static sampler [3] or the purge and trap technique [4,5].

An alternative approach could be represented by the use of solid-phase microextraction (SPME) [6]; since its introduction, it has been shown to be an excellent sampling method, allowing simultaneous extraction and pre-concentration of analytes from sample matrix. Hundreds of SPME applications relevant to the determination of residues of chemicals in various environmental [7–9], biological [10–13] and food [14] matrices can be found in the literature; in particular, it has been widely employed in the HS sampling mode and in conjunction with gas chromatography, for the determination of flavor compounds in various foods, i.e. wine [15], cheese [16], cooked meat [17], oil [18], coffee [19–21] and many others.

Multivariate analysis has traditionally been employed for food quality evaluation. Principal component analysis (PCA), an unsupervised multivariate technique used to reduce data dimensionality to a smaller set of orthogonal factors of easier interpretation [22], is one of the most suitable and simple statistical tools for classification, searching similarities and finding relationships. Moreover, this method does not require

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information for classification as it clusters individual samples on the basis of similarity among their data.

HS-SPME in combination with PCA was successfully employed [19,21] for the characterization of roasted coffee and coffee beverages.

In the present paper, HS-SPME–GC–MS in combination with PCA was successfully employed to discriminate different arabica/robusta blends and different geographical origins.

2. Experimental

2.1. Apparatus

GC–MS analysis was performed with an HP 5890 series II gas chromatograph equipped with a HP 5890 GC split/splitless injector and interfaced, by a GC transfer line, to a VG Trio-2000 quadrupole mass spectrometer (VG BIOTECH, Altrincham, UK).

The GC chromatographic column consisted of a Supelcowax fused silica capillary column (30 m length, 0.25 mm i.d. with 0.25 μm film thickness, Supelco). The carrier gas was helium.

2.2. Chromatographic and detection conditions

GC–MS: the optimized oven temperature program was 40 °C (1 min) to 150 °C (15 min) at 3 °C/min, then 150–250 °C at 5 °C/min (final temperature held for 5 min).

A column head pressure of 15 psi and an injector temperature of 250 °C were used. The GC transfer line was maintained at 250 °C.

The mass spectrometer was operated in the electron impact positive ion (EI^+) mode with a source temperature of 200 °C. The electron energy was 70 eV and the filament current 200 μA.

Mass spectra were acquired in the mass range from *m*/*z* 50 to 350, using a scan time of 0.45 s and an inter-scan time of 0.05 s.

2.3. Coffee samples

Coffee samples (100% robusta or 100% arabica) having a well known geographical origin were used for constructing the training set; beans were grinded and sealed under vacuum until the time of analysis. Commercial grinded coffees were sealed in vacuum packages and were opened immediately before analysis. All these samples, supplied by SAICAF SpA, Bari, Italy, had the same roasting profile.

2.4. Solid-phase microextraction

A silica fiber coated with 100 μm thick polydimethylsiloxane (PDMS) film and a manual SPME device (Supelco) were employed. One fiber was used throughout all the experimental work.

Coffee powders (1 g) were placed into 7 ml clear vials (Supelco), the vials were sealed with hole caps and PTFE-faced silicone septa (Supelco), equilibrated for 2 h in a thermostatic bath at 60 °C and subjected to HS-SPME. HS extraction were carried out for 5 min. Thermal desorption (5 min desorption time) was performed directly into the GC injection port maintained at 250 °C.

2.5. Data elaboration

The statistical analyses were carried out on a dataset represented by GC peak areas relevant to 32 components that could be identified in all the investigated samples.

The peak areas were obtained by integration using a Labbase (VG Biotech) software routine.

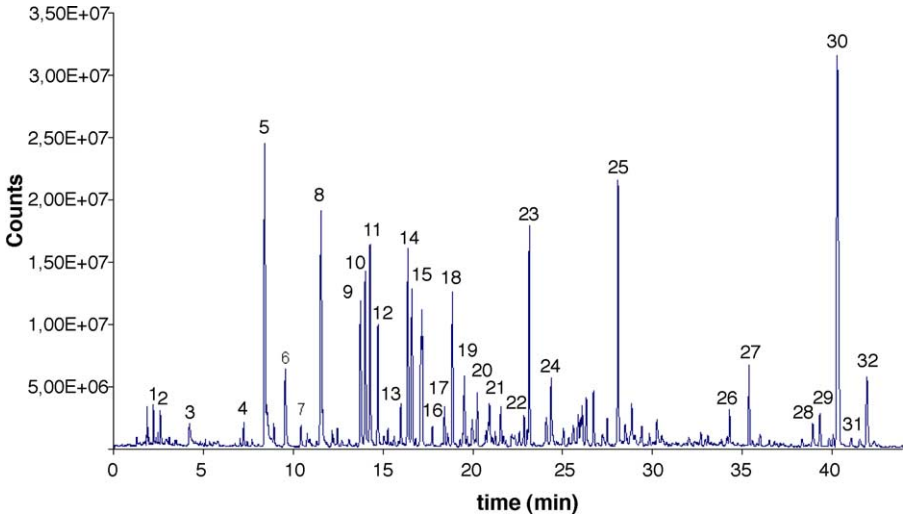

Fig. 1. Typical HS-SPME–GC–MS chromatogram of a commercial roasted coffee powder. For peaks identification see Table 1.

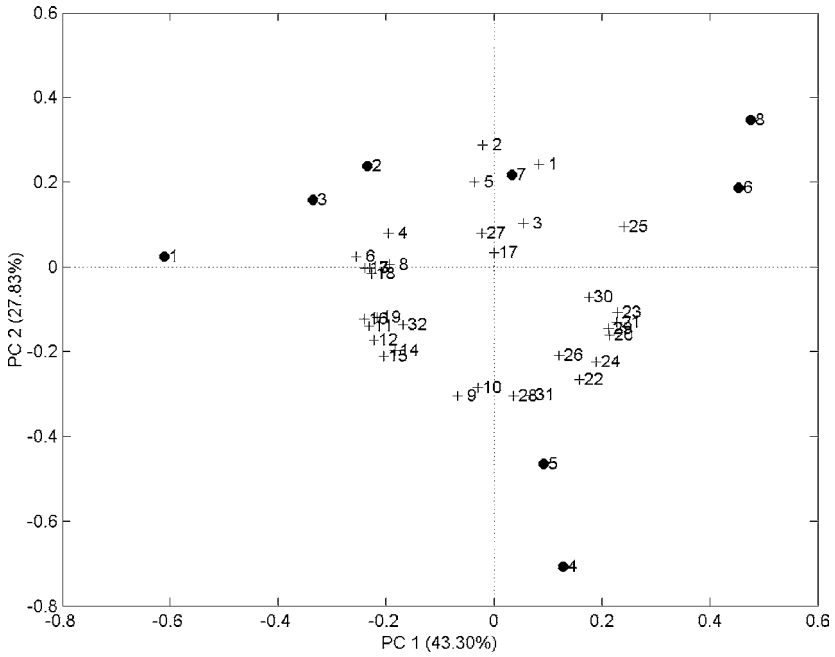

Fig. 2. Biplot for the dataset of Arabica and Robusta coffees. Principal Component 1 and 2 (PC1 and PC2) account for 43.3% and 27.8% of the variance in the data set, respectively. See Table 1 for loadings (+) and Table 2 for scores (●) identification, respectively.

Data matrices were assembled so that each row represented a coffee blend and each column a variable, i.e. the peak area of a specific component. The data were then elaborated and processed by a chemometric software (Statistica '99, Statsoft), running on a PC.

3. Results and discussion

The first step of the work involved sampling and instrumental parameters optimization. Conditions adopted were those giving the most abundant and reproducible chromato-

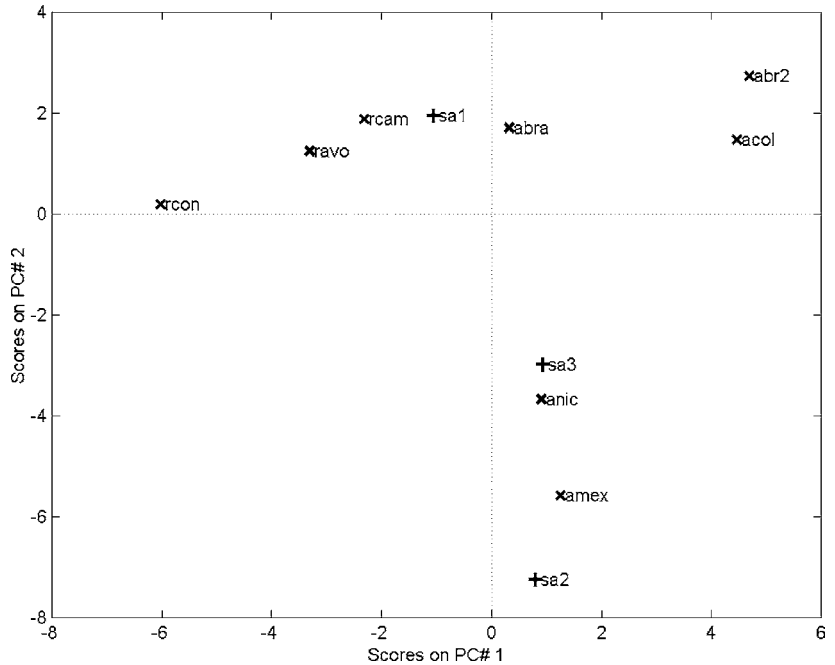

Fig. 3. Projection of score plot of three different commercially available blends (sa1, sa2, sa3) onto the model of Fig. 2. The scores of coffee samples used to build the model (see Table 2 for legend) have also been plotted.

Table 1
Compounds identified from the chromatographic peaks in Fig. 1 by comparison of their MS spectra with those of authentic standard and/or library match

Peak	Compound	Identification mode	Main *m/z* ions observed in MS spectra
1	2-Methylfurane	Library	82, 53
2	2-Methylbutanal	Library	57, 86, 71
3	3,3-Dimethylbutanone	Library	57, 85, 100
4	1-Methyl-1H-pyrrol	Library	81, 66, 53
5	Pyridine	Standard	79, 52
6	Pyrazine	Standard	80, 53
7	2-Metoxy-methylfurane	Library	112, 81, 53
8	4-Methylpyrimidine	Library	94, 67, 53
9	4,6-Di methylpyrimidine	Library	108, 81, 66
10	4,5-Di methylpyrimidine	Library	108, 81, 66
11	Ethylpyrazine	Library	107, 80, 53
12	2,3-Dimethylpyrazine	Library	67, 108, 52
13	Ethylpyridine	Library	92, 107, 65
14	2-Ethyl-6-methylpyrazine	Library	121, 94, 56
15	2-Ethyl-5-methylpyrazine	Library	121, 94, 56
16	Propylpyrazine	Library	94, 107, 122
17	2,6-Diethylpyrazine	Library	135, 108, 53
18	3-Ethyl-2,5-dimethylpirazine	Library	135, 108, 121
19	2-Ethyl-3,5-dimethylpirazine	Library	136, 108, 121
20	2,5-Di methylfurane	Library	96, 67, 53
21	Ethanediole diacetate	Library	86, 116, 73
22	Ethanediole propanoate	Library	57, 100, 87
23	3-Furan-methanol acetate	Library	140, 81, 98
24	5-Methyl-furancarbossialdeide	Library	110, 53, 81
25	Furanmethanol	Standard	98, 81, 69
26	1-(2-Furanil-methyl-)-1-H-pyrrol	Library	81, 147, 53
27	4-Metoxyphenol	Standard	109, 124, 81
28	1-(1H-pyrrol-2-il)-etanone	Library	94, 109, 66
29	2-2′Ossibis(methylen)bisfurane	Library	81, 53, 178
30	Phenol	Standard	94, 66, 55
31	1H-pyrrol-carboxyaldeide	Library	95, 66, 55
32	4-Ethyl-2-metoxyphenol	Library	137, 152, 121

graphic peaks over time. Fig. 1 reports, for instance, a typical HS-SPME–GC–MS chromatogram of a commercial roasted coffee powder. As apparent, the procedure was capable to extract and detect a great number of volatile compounds. Thirty-two components could be confidently identified by comparison of their MS spectra with those of authentic standard and/or through library match (see Table 1). It is worth noting that profiles similar to the one shown in Fig. 1 were obtained for all the analyzed samples. No particular "marker" capable of unambiguous discrimination between roasted coffees of different origins and/or composition and/or brands was found.

Then, it was investigated whether PCA could provide such discrimination. To this purpose the training set reported in Table 2 was used.

In order to assess whether GC–MS chromatograms could be acquired with reasonable precision, three different samples were analyzed five times by HS-SPME–GCMS, and peaks numbered 1–32 were quantitated through peak area measurement. In order to reduce the within-sample variance, data were pre-processed expressing the areas of each of the

Table 2
Description of the samples used for the training set

Sample number (label data)	Quality (100%)	Origin
1 (rcon)	Robusta	Congo
2 (rcam)	Robusta	Camerun
3 (ravo)	Robusta	Ivory coast
4 (amex)	Arabica	Mexico
5 (anic)	Arabica	Nicaragua
6 (acol)	Arabica	Colombia
7 (abra)	Arabica	Brazil (Santos)
8 (abr2)	Arabica	Brazil (Maragogipe)

32 individual peaks in Table 1 as a percentage of their total area; this kind of "internal normalization" should correct for the variance associated with the sampling step. Indeed the values of the relative standard deviations (R.S.D.) calculated for the selected variables after row profiling were definitely lower than those calculated on the original not pre-processed data. Moreover the data were auto-scaled before statistical analysis.

Principal component analysis was carried out first on a 8×32 data matrix of three 100% Robusta and five 100% Arabica coffees (see Table 2). The first two principal components (PCs) explained the 43.3 and 27.8% of the chromatographic variance, respectively. The biplot of the samples under investigation on the first two PCs is presented in Fig. 2 where the effectiveness of this chemometric approach to the HS-SPME–GC–MS data is clearly illustrated. As can be seen, the Arabica samples constitute a broad group spread along the positive values of PC1 (x axis), while the Robusta coffees are all found on the negative side of this axis.

As can be seen from the PC1 biplot in Fig. 2, the aroma components that determine the scores on PC1 are mainly furan and pyrazine derivatives (positive and negative side of the PC1, respectively).

While the PC1 helped in distinguishing Robusta from Arabica variety, the information contained in both the first and the second PCs could be applied to discriminate coffees of different geographic origins. Indeed the Robusta samples (all of African origin as they come from Congo, Cameroon and Ivory Coast) are characterized by negative PC1 and positive PC2. In spite of their similar geographic origin, the five Arabic samples are more spreaded than the Robusta ones. The Arabica samples coming from the South America have PC1 and PC2 both positive whereas those from central America (Mexico and Nicaragua) have negative PC2 and, obviously, positive PC1. These results are in agreement with those reported by Bicchi et al. [19], showing that three different samples from Kenya, Colombia and Guatemala could be distinguished.

The model obtained so far was quite interesting respect to the information it could permit to infer. In fact several different commercially available blends of roasted coffee were analyzed and results interpreted according to the described model. For instance, the scores relevant to three samples have been plotted in Fig. 3 together with those of the Arabica and Robusta coffees. As can be seen in Fig. 3, while sample 1

projection falls between Robusta and Arabica areas (it is a coffee blend used in a coffee distributing machine), samples 2 and 3 appear to be mixtures of Arabica coffees; this result is in agreement with the product labels where the exclusive presence of Arabica variety is claimed. As to the geographical origin, the negative PC2 scores of samples 2 and 3, possibly indicate that different Central America Arabica coffees have been blended.

4. Conclusions

The results reported above shows that HS-SPME is an effective and reproducible sampling technique for routine characterization of coffees. Coupled to GC–MS and PCA it could efficiently be applied to sample characterization. Further studies are under way using MS spectral data obtained on the HS without any chromatographic step.

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