Characterization of Roasted Coffee and Coffee Beverages by Solid Phase Microextraction−Gas Chromatography and Principal Component Analysis

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A method that differentiates between different roasted coffees and coffee beverages is described. Characterization is achieved by applying principal component analysis (PCA) to the chromatographic results obtained by solid phase microextraction−gas chromatography (SPME−GC), applied to the vapor phase in equilibrium with coffee (HS−SPME), or to coffee extracts (LS−SPME). The capabilities of this method are illustrated by some examples defining the differentiation of coffee samples of different origins or in mixtures of different compositions.

Keywords: *Roasted coffees; beverages; solid phase microextraction (SPME); GC; principal component analysis (PCA)*

Solid phase microextraction (SPME) is a recent sampling technique based on absorption, which was developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990). With SPME, the analytes are absorbed from the liquid or gaseous sample onto an absorbent-coated fused-silica fiber, which is part of the syringe needle, for a fixed time. The fiber is then inserted directly into a GC injection port for thermal desorption or into the HPLC injection valve for solvent desorption (Chen and Pawliszyn, 1995). SPME is a solvent-free technique, which is sensitive because of the concentration factor achieved by the fiber and selective because of different coating materials that can be used. One of the advantages of SPME is the possibility to directly sample the vapor phase in equilibrium with the matrix (headspace (HS)−SPME) or the matrix extract or solution (liquid sampling (LS)−SPME) directly, provided that suitable fibers are available.

Previously, SPME was mainly used for the analysis of pollutants, in particular for environmental water samples. Several applications involving flavor analysis in different matrices have also recently been reported (Yang and Peppard, 1994; Pelusio *et al.*, 1995; Picque *et al.*, 1995; Coleman, 1996; Field *et al.*, 1996; Harmon, 1997; Matich *et al.*, 1997). SPME has also been applied in the analysis of coffee: Hawthorne *et al.* (1992) directly determined caffeine concentration in coffee, tea, and carbonated beverages with quantitative reproducibilities of about 5%; Yang and Peppard (1994) reported the HS−SPME−GC of espresso-roast ground coffee; Wang *et al.* (1996) described the determination of Veltol (2-methyl-3-hydroxy-4-pyrone) and Veltol Plus (2-ethyl-3-hydroxy-4-pyrone) in several matrices including coffee beverages.

For some years, we have been engaged in a project aiming to use the chemical profile of one or more coffee fractions (e.g., HS, hot-water extract, phenolic fraction, sulfurated fraction, etc.) to obtain an unbiased evaluation of a coffee. Static headspace GC (S−HSGC) and/or total Mocha extract HPLC−UV patterns (Bicchi *et al.*, 1993) or chlorogenic acid HPLC−UV patterns (Bicchi *et al.*, 1995) in combination with principal components analysis (PCA) (Word, 1987; Statgraphic manual) have been used to discriminate green or roasted coffees from different origins or plantations, those that have been submitted to different technological treatments, or coffee blends of different composition. This paper reports on the method capability of discriminating between roasted coffees of different origins, those that have undergone different technological treatments, or blends of different composition by applying PCA to the SPME−GC patterns of either their headspaces or their hot-water extracts.

EXPERIMENTAL PROCEDURES

Coffee Samples. Roasted coffees of different origins, different blends of coffees, and coffees submitted to different technological treatments were supplied by Lavazza SpA, Torino, Italy. Commercially available blends were from the market. At least five samples (50 g) of each coffee variety or blend were hermetically sealed under vacuum in nonpermeable polypropylene/aluminum/polyethylene packages and stored at −20 °C after roasting until used for chemical analysis.

Unless otherwise specified, all coffee samples were roasted for 6 min at 270 °C in a Probat laboratory roasting device (Emmerich, Germany). Additional details about the samples analyzed are given under Results and Discussion.

SPME Device. The SPME device was purchased from Supelco Co. (Bellefonte, PA) as were the fused-silica fibers coated with a 100-μm polydimethylsiloxane film for headspace sampling or a 7-μm polydimethylsiloxane bonded film for liquid sampling.

SPME Sampling. Before sampling, the 100-μm polydimethylsiloxane fiber was reconditioned for 30 min in the GC injection port at 250 °C, while the 7-μm polydimethylsiloxane bonded fiber was reconditioned for 60 min at 300 °C; they were used immediately. The fiber was pushed out of its stainless steel housing and exposed to the ground roasted coffee headspace or plunged into the coffee infusion for 5 min. After

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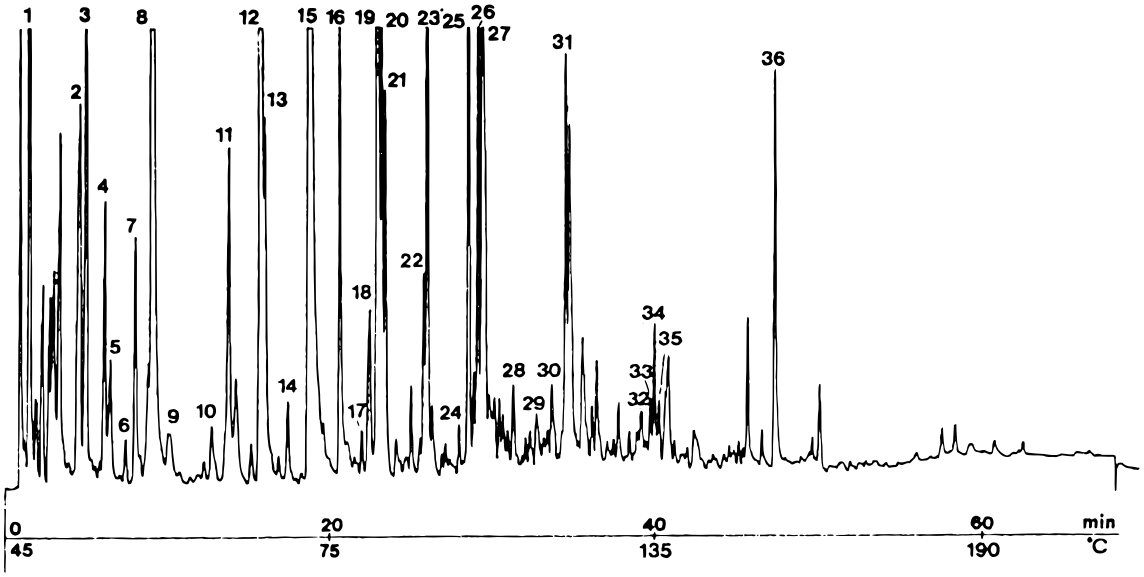

Figure 1. HS–SPME–GC pattern of a ground, roasted 50% Arabica–50% Robusta blend.

Table 1. Components Identified in 50% Arabica–50% Robusta Coffee Blend Analyzed by HS–SPME–GC (See Figure 1)

peak	name	peak	name
1	propan-2-one	19	2,6-dimethylpyrazine
2	2-oxopropanal	20	ethylpyrazine
3	2-methylbutanal	21	2,3-dimethylpyrazine
4	2,3-pentandione	22	hexane-3-one
5	3-hydroxy-2-butanone	23	5-methyl-2-furancarboxyaldehyde
6	2-ethylfuran	24	phenol
7	pyrazine	25	2-furanmethanol acetate
8	pyridine	26	2-ethyl-6-methylpyrazine
9	dimethyldisulfide	27	2-ethyl-3-methylpyrazine
10	cyclopentanone	28	1-hydroxy-3-methyl-2-cyclopentanone
11	2-methyl-3(2*H*)-furanone	29	dihydro-3-methylen-2(3*H*)-furanone
12	methylpyrazine	30	1-(1*H*-pyrrol-2-yl)ethanone
13	2-furancarboxyaldehyde	31	2-propanamine
14	methylphenol (isomer)	32	2-methoxyphenol
15	2-furanmethanol	33	hydroxypyridine (isomer)
16	3(2*H*)-furanone	34	3-hydroxy-2-methylpyran-4(4*H*)-one
17	2-methyl-2-cyclopenten-1-one	35	2-acetyl-3-methylpyrazine
18	1-(2-furyl)ethanone	36	2,4,6-trimethyl-3-benzenediamine

extraction, the SPME device was retracted into its housing, removed from the sample vial, immediately inserted into the GC injector, pushed outside its housing, and thermally desorbed for 5 min at 250 °C.

HS–SPME–GC Analysis. *Sample Preparation.* Each coffee package was equilibrated at room temperature for 30 min. At least six 1-g samples of each coffee were hermetically sealed in 10-mL vials and returned to storage at −20 °C. Before analysis, each vial was again left for 30 min to reach room temperature and then equilibrated for 2 h in the thermostatic bath of the HS injector at 60 °C before headspace injection. HS–SPME of coffee beverages was made by hermetically sealing 3 mL of a freshly prepared beverage, obtained as reported below, in a 10-mL vial. Equilibration conditions were the same as those adopted for ground coffee.

HS–SPME Sampling. HS–SPME sampling was carried out as described above.

CGC Analysis. An FSOT-high temperature silylated OV-1 column (df, 5 μm; i.d., 0.32 mm; *l*, 25 m) prepared in the authors' laboratory was used. Chromatographic conditions were as follows: injection system, splitless; time, 60 s; injector temperature, 250 °C; temperature program, from 45 °C (10 min) to 190 °C at 3 °C/min; detector, FID; temperature, 250 °C; carrier gas, hydrogen, flow rate, 2 mL/min.

LS–SPME–GC Analysis. *Sample Preparation.* Coffee beverages were prepared under strictly standardized conditions in the so-called Turkish mode. One hundred milliliters of boiling water was poured onto 20 g of roasted coffee in a 250-mL beaker. The resulting suspension was boiled for another 20 s, left for 9 min, and filtered under vacuum, measuring the volume of the resulting filtrate. Eight milliliters of the filtrate was transferred into a 10-mL vial. The vial was then hermetically sealed, conditioned for 30 min at 40 °C with stirring (Berg and Penton, 1996), and submitted to SPME sampling.

LS–SPME Sampling. LS–SPME sampling was carried out as described above.

CGC Analysis. An FSOT-high temperature silylated polyethylene glycol column (df, 0.5 μm; i.d. 0.25 mm; *l*, 30 m) (CP-Wax 52 CB, Chrompack, The Netherlands) was used. Chromatographic conditions were as follows: injection system, splitless; time, 60 s; injector temperature, 270 °C; temperature program, from 40 °C (1 min) to 150 °C (15 min) at 3 °C/min then to 250 °C at 5 °C/min; detector, FID; temperature, 250 °C; carrier gas, hydrogen; flow rate, 1.5 mL/min.

GC/MS Analysis. GC/EI–MS analyses were carried out on a Hewlett Packard 5988 A GC/MS system provided with a Hewlett Packard 5890 GC unit. Capillary GC separations were carried out with the same column and under conditions analogous to those reported in the previous paragraph. Carrier gas was helium.

Data Elaboration. The chromatographic data from HS–SPME–GC and LS–SPME–GC were processed on HP 3396A computing integrators and then transferred on-line to an HP Vectra 486DX personal computer (Hewlett Packard, Grenoble, France), where they were elaborated through a PCA program (Statgraphics, Statistical Graphics Corporation, Rockville, MD). A homemade program was used to convert the file format from the computing integrator into a form compatible with the statistics program. Routine statistical analyses

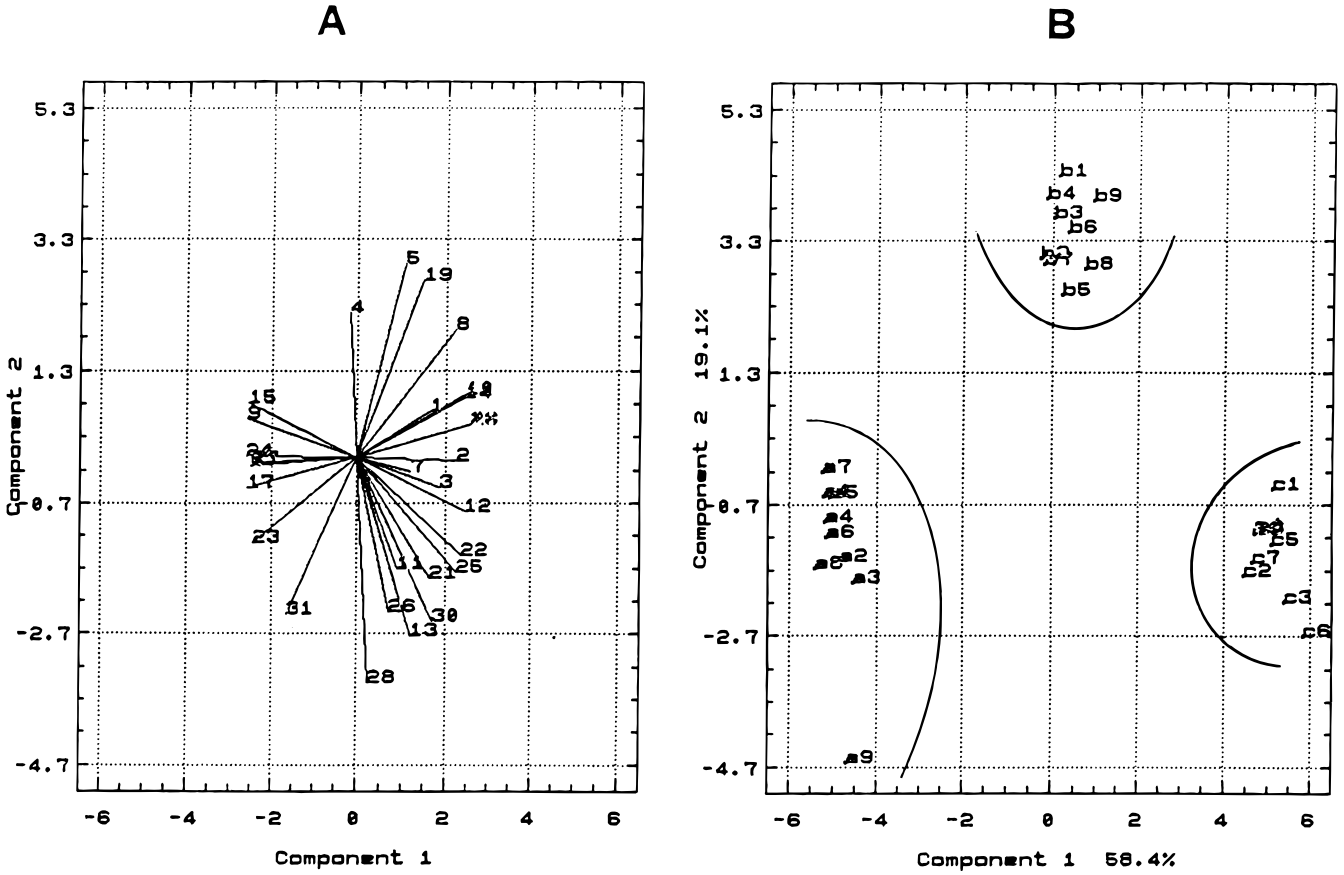

Figure 2. (A) Distribution of HS−SPME−GC loadings for PCA of 60% Arabica−40% Robusta (a), 50% Arabica−50% Robusta (b), 40% Arabica−60% Robusta (c) coffee blends. (B) HS−SPME−GC scatterplot of principal components of the same coffee samples; PC1 = 58.4%, PC2 = 19.1%.

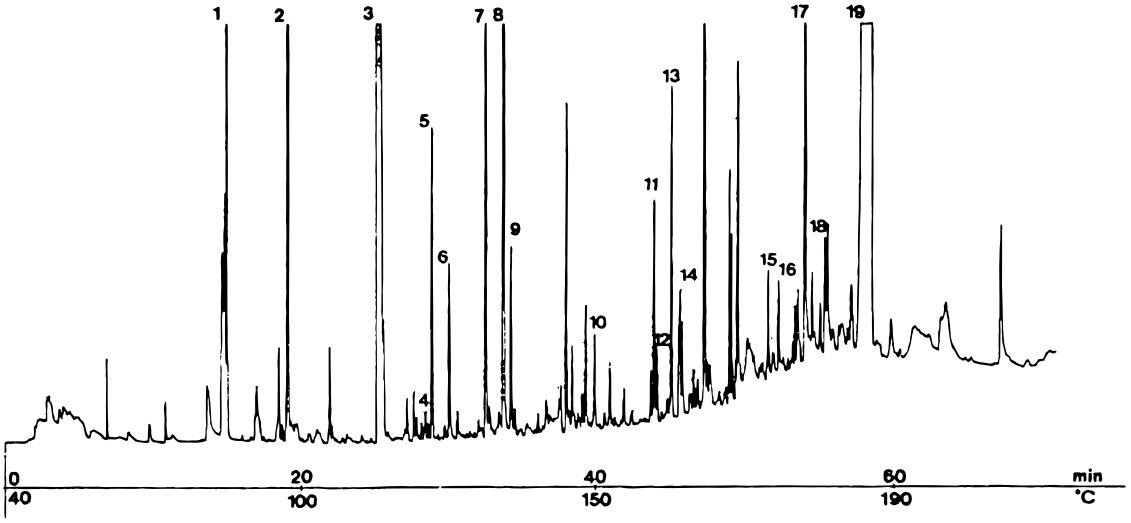

Figure 3. LS−SPME−GC pattern of a coffee extract obtained in the Turkish mode.

were carried out on peaks with detectable and reproducibly measurable areas common to the set of samples under investigation.

Sensory Evaluation. The beverages prepared with each sample of the six commercially available coffee blends, analyzed through LS−SPME−GC, were evaluated under the same conditions by a panel of 15 tasters trained in sensory evaluation of coffee (Gillette, 1990). The terms used by the panel to evaluate the samples reflected the characteristics of the coffees under investigation. The beverages for sensory evaluation were prepared in the Turkish mode under the strictly standardized conditions described above and tested at a temperature of 55 °C.

RESULTS AND DISCUSSION

The first part of this study concerned optimizing HS−SPME and LS−SPME sampling conditions and evaluating their reproducibility in view of the subsequent statistical elaborations.

HS−SPME Samplings. HS−SPME of both ground coffee and beverages was analyzed. The two matrices analyzed under the same conditions gave qualitatively similar profiles, but HS−SPME profiles of beverages were quantitatively not sufficiently significant for further statistical elaboration. Therefore, only HS−SPME of ground coffee was considered. The conditions adopted

Table 2. Components Identified in a Coffee Extract Analyzed by LS−SPME−GC (See Figure 3)

peak	name	peak	name
1	pyridine	11	3-hydroxy-2-methylpyran-4(4*H*)-one
2	1-hydroxy-2-propanone	12	3-ethyl-4-methylpyrrole
3	acetic acid	13	phenol
4	2-furanmethanol acetate	14	1*H*-pyrrole-2-carboxyaldehyde
5	propanoic acid	15	2-formyl-1-methylpyrrole
6	5-methyl-2-furancarboxyaldehyde	16	hydroxypyridine (isomer)
7	2(3*H*)-dihydrofuranone	17	hexadecanoic acid
8	2-furanmethanol	18	1,4-benzenediol
9	pentanoic acid (isomer)	19	caffeine
10	2-hydroxy-3-methyl-2-cyclopenten-1-one		

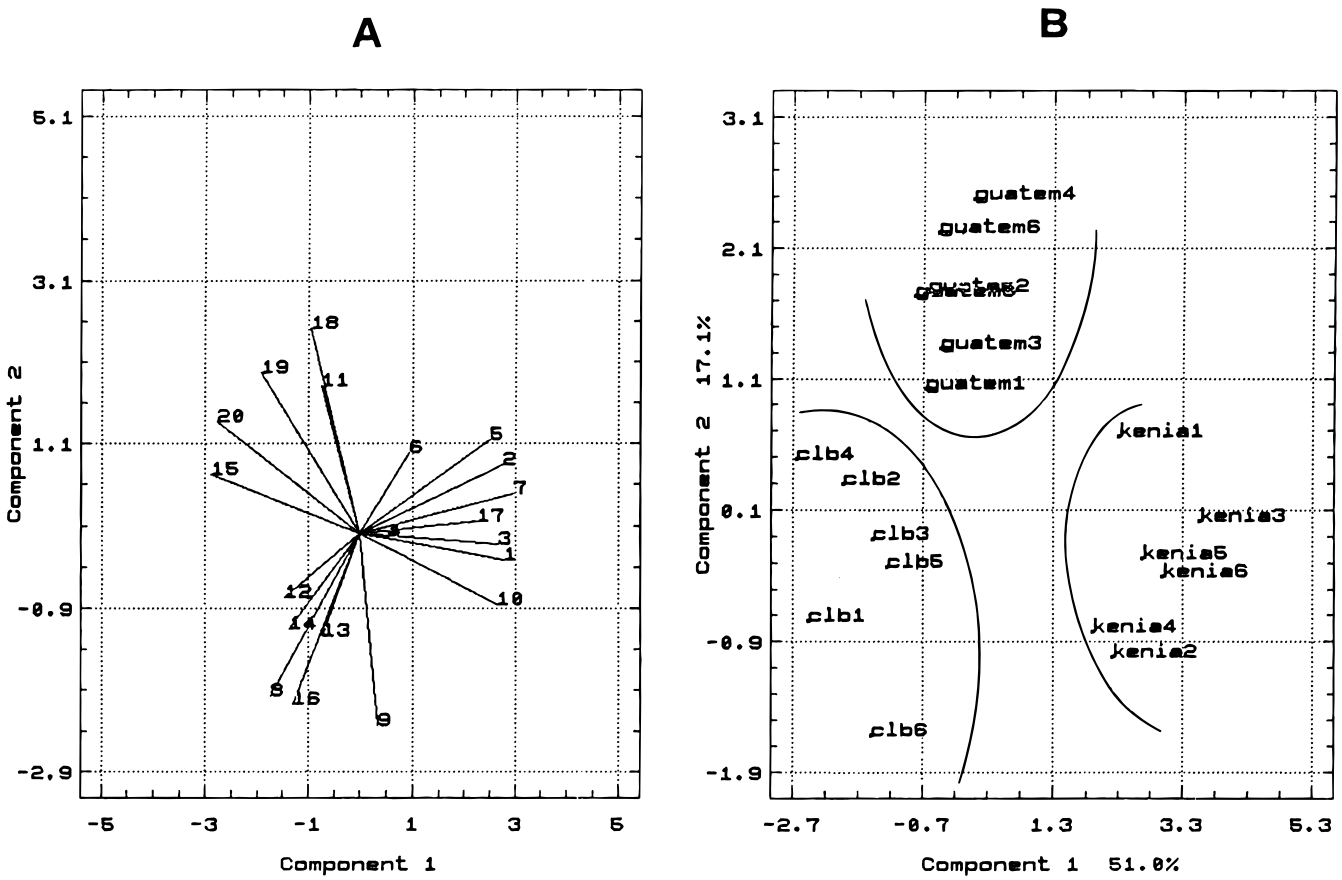

Figure 4. (A) Distribution of LS−SPME−GC loadings for PCA of three Arabica coffees of different origins [Kenya (Kenia), Colombia (clb), and Guatemala (guatem)] submitted to the same roasting profile; (B) LS−SPME−GC scatterplot of principal components of the 18 analyzed coffee samples; PC1 = 51.0%, PC2 = 17.1%.

(i.e., 2 h equilibration time at 60 °C, 5 min SPME sampling time) were those giving the richest and most reproducible GC profiles over time. Relative standard deviation varied from 7% for the most volatile components to 2.5% for the less volatile: the average value over all the components considered was about 4%. These variations did not influence statistical elaborations (see below).

LS−SPME Sampling. Mocha, Melitta, and Turkish modes of preparing coffee were evaluated. The coffee sample was extracted under strictly standardized conditions and then analyzed by LS−SPME−GC. The Turkish mode gave the most reproducible results. Moreover, pH and salt effects were studied in order to evaluate their influence on SPME sampling: sodium chloride and sodium sulfate salting out and pH values of 3 and 8 were tested in a series of experiments. The different conditions did not markedly influence the GC profile; therefore, the extracts were SPME sampled as such. The conditions adopted (i.e., 30 min thermostatization at 40 °C, 5 min SPME sampling time under constant stirring) gave significant and reproducible GC profiles. Relative standard deviation varied from 4% to 13%; the average value over all the components considered was about 7.5%. These variations were within the limits already reported by other authors (Wang *et al.*, 1996; Yang and Peppard, 1994). These variations only slightly interferred with statistical elaborations (see below).

HS−SPME−GC/PCA. Figure 1 reports a typical pattern of an HS−SPME−GC analysis of a ground, roasted 50% Arabica−50% Robusta blend. Table 1 lists the components identified in the sample.

The following example illustrates the effectiveness of the statistical methods applied to the HS−SPME−GC results. It concerns the discrimination of three commercially available blends mainly consisting of Arabica and Robusta coffees differing in their ratios and processed on different days. The following ratios were considered: 60% Arabica−40% Robusta (a), 50% Arabica−50% Robusta (b), 40% Arabica−60% Robusta (c). Sample a9 was prepared with a Robusta of different origins. Nine samples of each blend produced on different days were investigated. Each sample was analyzed six times by HS−SPME−GC. The HS profiles

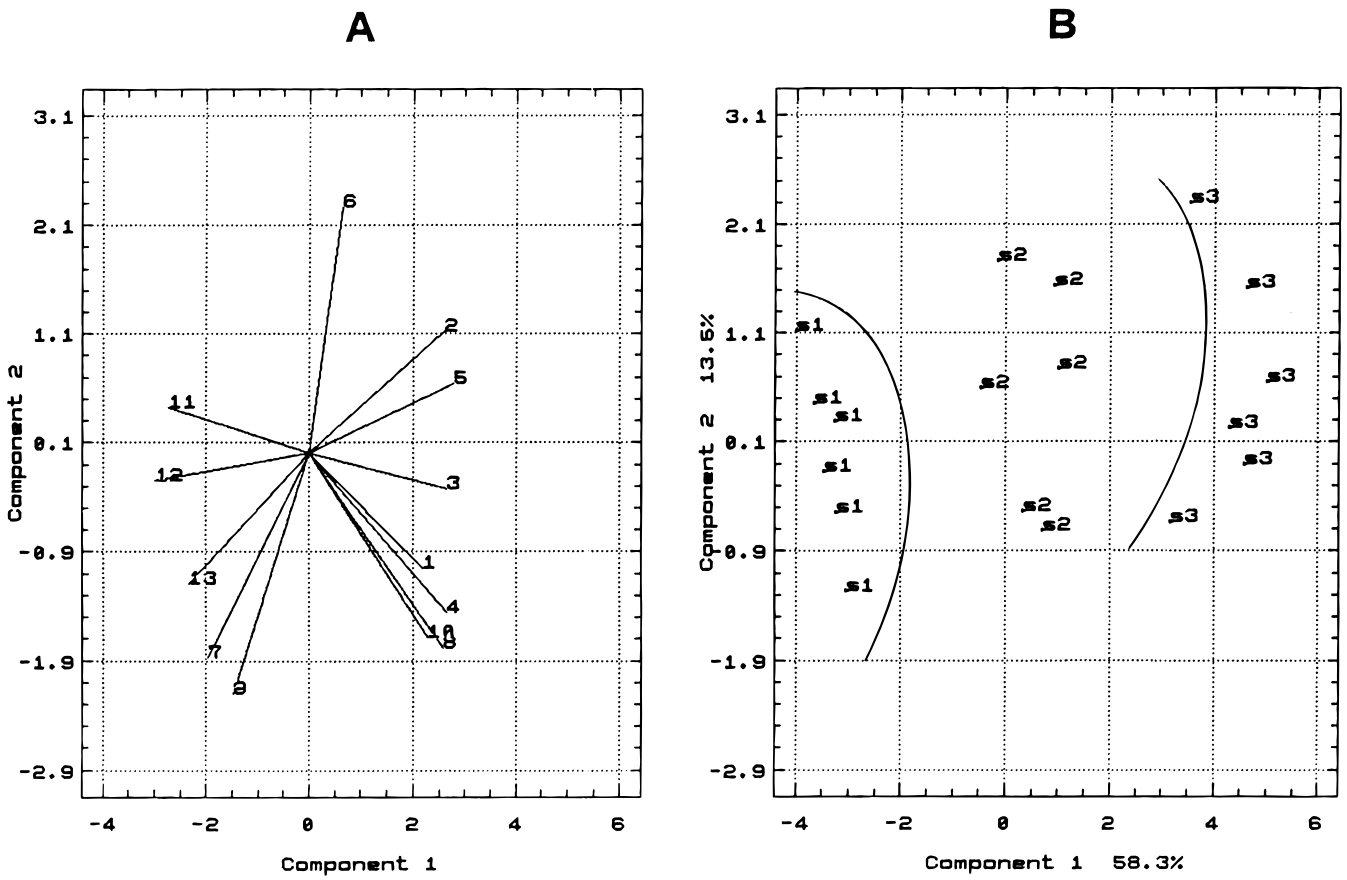

Figure 5. (A) Distribution of LS−SPME−GC loadings for PCA of three Santos samples from three different plantations in the same region; (B) LS−SPME−GC scatterplot of principal components of the 18 analyzed coffee samples; PC1 = 58.3%, PC2 = 13.5%.

were submitted to a detailed investigation using PCA. Routine statistical analyses were carried out on the 31 peaks, which showed detectable and reproducibly measurable areas in all the samples under investigation. PCA was applied to the mean areas, calculated over six analyses, of each peak from each sample so as to obtain a clearer scatterplot. Figure 2A reports the distribution of the loadings considered for PCA, and Figure 2B is the scatterplot of principal components of the coffees in question, which clearly distinguishes the different blends, and also the sample prepared with a Robusta of different origin (9a). For clarity, the different blends have been outlined by hand.

LS−SPME−GC/PCA. Figure 3 reports a typical pattern of an LS−SPME−GC analysis of an extract obtained in the Turkish mode. Table 2 lists the components identified in the sample.

The LS−SPME−GC operative conditions mentioned above were those giving the most reproducible quantitative results for coffee extracts, but in spite of the rigorously standardized sampling conditions, LS−SPME−GC gave a wider spread of the coffee samples on the PCA diagrams than HS−SPME−GC analysis.

The following examples illustrate the effectiveness of the statistical methods applied to the LS−SPME−GC results. The first concerns the distinction of three Arabica coffees of different origins (Kenya, Colombia, and Guatemala) but submitted to the same roasting profile. The beverages from six 20-g samples for each batch for a total of 18 samples were analyzed by LS−SPME−GC. Routine statistical analyses were carried out on the 20 peaks, which showed detectable and measurable areas in all the samples. Figure 4A reports the distribution of the loadings considered for PCA, and Figure 4B is the scatterplot of principal components of the 18 coffee samples. The three origins are clearly distinguished: as expected, the batches from Columbia and Guatemala were more similar to each other than to the Kenya samples. For clarity the different samples have been outlined by hand.

The second example concerns the discrimination between three Santos samples cultivated in the same region (Soul de Minas, Brazil), under the same soil and climate conditions but in three different plantations and classified as being of the same quality. The beverages from six 20-g samples for each plantation for a total of 18 samples were analyzed by LS−SPME−GC. Routine statistical analyses were carried out on the 13 peaks, which showed detectable and measurable areas in all the samples under investigation. Figure 5A reports the distribution of the loadings corresponding to the components used for PCA elaboration. Figure 5B reports the scatterplot of the principal components of the three coffees under investigation, which clearly distinguishes the samples in question. For clarity, the different samples have been outlined by hand. The scatterplot clearly shows how PCA discriminates between the different Santos samples.

The last example concerns the discrimination of six commercially available coffee blends from six different producers. The six samples were described by the test panel as reported in Figure 6. The descriptive profiles show that samples A−C were classified as being very similar (in particular samples A and B); samples D and E were slightly different, although similar to the previous ones; while sample F was different from the others,

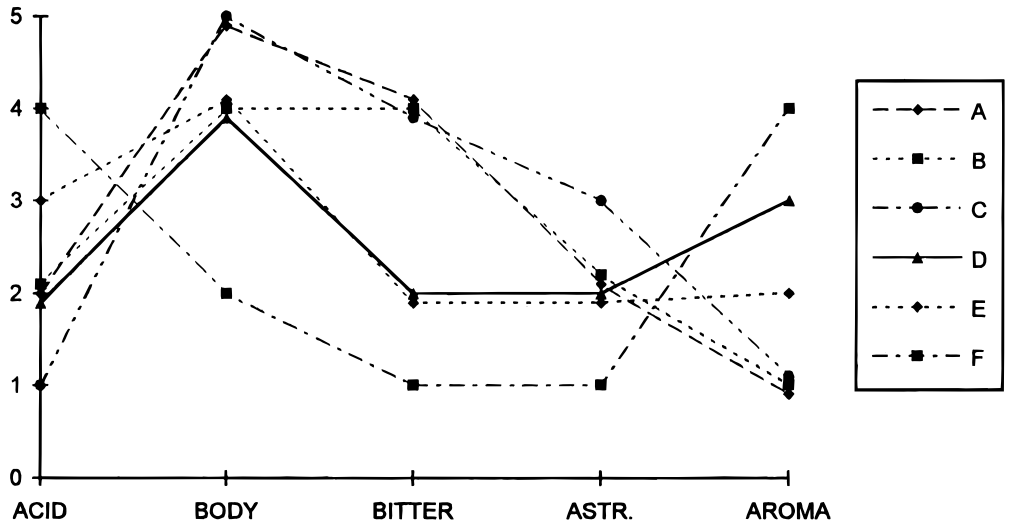

Figure 6. Descriptive analysis of six commercially available coffee blends from six different producers: 1 = low; 2 = medium low; 3 = medium; 4 = medium high; 5 = high.

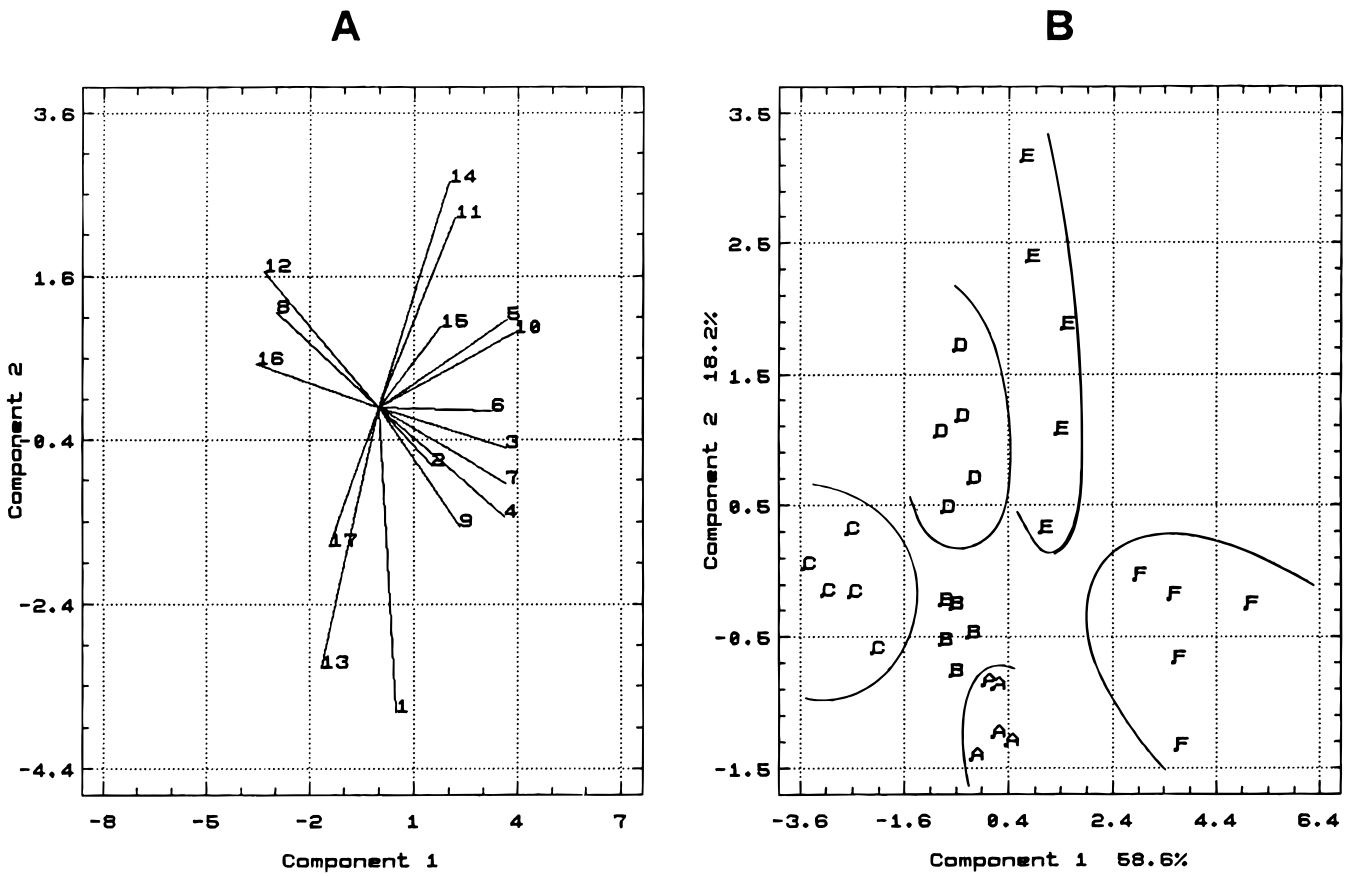

Figure 7. (A) Distribution of LS–SPME–GC loadings for PCA analysis of six commercially available coffee blends from six different producers; (B) LS–SPME–GC scatterplot of principal components of the 30 analyzed coffee samples; PC1 = 58.6%, PC2 = 18.2%.

probably because of the higher percentage of Arabica as compared to the others. Incidentally, samples A–E are commercially considered to be similar and competitive. The beverages from five 20-g samples for each commercial blend for a total of 30 samples were analyzed by LS–SPME–GC. From the LS–SPME–GC patterns, 17 peaks were considered for routine statistical analysis. Figure 7A reports the distribution of the loadings corresponding to the components used for PCA elaboration. Figure 7B reports the scatterplot of the principal components of the six coffees under investigation, which clearly distinguishes the samples in question. For clarity, the different samples have been outlined by hand. In this example, the distribution of the data of the different coffee blends by LS–SPME–GC/PCA is not only in agreement with the sensory evaluation but clearly discriminates sample F from the others (as expected) and samples D and E from samples A–C, when the two principal components are considered.

The results reported here show that SPME, with sampling both in vapor and liquid phase, is an effective and reproducible technique for routine characterization of coffees. Both HS– and LS–SPME–GC are suf-

ficiently to be applied to routine automated coffee control analysis in combination with PCA, although an increase in peak area reproducibility with LS−SPME would be desirable to improve PCA effectiveness. Further studies are under way in LS−SPME standardization.

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