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Reliable characterization of coffee bean aroma profiles by automated headspace solid phase microextraction-gas chromatography-mass spectrometry with the support of a dual-filter mass spectra library

This investigation is based on the automated solid phase microextraction GC-MS analysis of the volatile fraction of a variety of coffee bean matrices. Volatile analytes were extracted by headspace (HS)-SPME which was achieved with the support of automated instrumentation. The research was directed towards various important aspects relating to coffee aroma analysis: monitoring of the volatile fraction formation during roasting; chromatographic differentiation of the two main coffee species (Arabica and Robusta) and of a single species from different geographical origins; evaluation of the influence of specific industrial treatments prior to roasting. Reliable peak assignment was carried out through the use of a recently laboratory-constructed "flavour and fragrance" library and a dual-filter MS spectral search procedure. Further emphasis was placed on the automated SPME instrumentation and on its ability to supply highly repeatable chromatographic data.

Key Words: Coffee beans; Coffee volatiles; SPME; GC-MS; LRI

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1 Introduction

Coffee, both as a beverage and a plant, originates from north-eastern Africa. The plant is a woody perennial evergreen and is produced mainly in economically developing countries. Coffee beans are initially processed by removing the outer layer of fleshy pulp. This may be accomplished by a dry or a wet procedure. The wet (or washing) process is the more complex and time-consuming procedure but leads generally to a higher quality final product [1, 2]. Green coffee beans cannot be consumed as such but need to undergo the process of roasting which is essential for the formation of coffee aroma. The different degrees of roasting (light, medium-light, medium, medium-dark, dark, very dark) produce different aroma profiles and, thus, a variety of coffee beverages. Only two species of coffee are extensively cultivated: *Coffea arabica* and *canephora*, each comprising a large number of varieties, including Robusta, the most important variety of the *C. canephora* species. The Arabica class is the most valuable as it produces a better tasting beverage and, as such, it is subject to a greater risk of adulteration. While the raw beans of the two species present different characteristics, making visual differentiation quite easy, such distinction is much more difficult with the roasted product [1–3]. The detection of fraud in the latter is usually achieved by the determination of predominant components in one of the two species [4–6].

Research dedicated to the volatile fraction of raw and, particularly, roasted beans throughout the years has been extensive. The green bean aroma profile is certainly the less complex while the roasted bean is characterized by several hundreds of components in a vast concentration range. Recent comprehensive two-dimensional gas chromatography (GC × GC) applications have highlighted the high complexity of this matrix [7, 8]. The main classes of compounds that have been identified in roasted beans are: furans, pyrazines, ketones, alcohols, aldehydes, esters, pyrroles, thiophenes, sulfur compounds, benzenic compounds, phenolic compounds, phenols, pyridines, thiazoles, oxazoles, lactones, alkanes, alkenes, and acids. The coffee bean chemical composition depends upon a variety of factors, such as species and variety of bean, geographic origin, soil conditions, storage of the beans, time and temperature of the roasting procedure [1–3].

GC-MS is commonly employed for the analysis of volatiles in raw and roasted beans. It should be added that GC-olfactometry has also been applied to this type of

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matrix [9, 10]. The main differences, in terms of analytical approach, lie in the solute extraction procedures. Distillation techniques (steam, vacuum, etc.) have commonly been used in coffee applications [11, 12]. Distillation can be useful when solute concentration is necessary but the use of organic solvents for compound extraction and exposure to high temperatures can cause artefact formation. Purge and trap techniques have also been used as a coffee sample introduction system for GC analysis [13]. Static headspace sampling is, at present, the most widely used method for coffee volatile entrapment prior to GC analysis [14, 15]. This procedure allows the preparation of a sample that is a near to a true representation of the coffee odorants perceived by the consumer. An enrichment of headspace analytes, when necessary, can be achieved by adsorbents such as activated charcoal or porous polymers [16]. Excellent coffee analyte recoveries obtained through headspace sorptive extraction and stir bar sorptive extraction [17] and solid-phase aroma concentrate extraction [18] have recently been reported in the literature.

In general, SPME has proved to be a valuable tool for headspace and aqueous sample extraction. This sample preparation method exploits the high sorption power of a fused silica fiber coated with a specific absorbent in contact with the analytes [19]. SPME in combination with mass spectrometric detection has recently been reviewed [20]. The choice of a SPME fiber is dependent on the specific physico-chemical characteristics of the target solutes to be extracted. This valid sampling procedure has been employed for the extraction of coffee analytes prior to GC-MS analysis [10, 17, 18, 21]; in all cases, the SPME step was carried out manually. A large part of modern analytical method development is currently directed towards the reduction of human intervention through automation with the aim of gaining a number of undisputed advantages: lower time-consumption; lower probability of sample contamination; and higher analytical repeatability. The present research focuses on the application and evaluation of a fully automated HS-SPME-GC-MS method in the analysis of coffee beans. Positive peak assignment was carried out with the support of a recently developed MS library. With respect to analyte quantitation, pure standard components were not employed; instead, semi-quantitative data were derived from GC-FID (flame ionization detector) applications.

2 Experimental

2.1 Samples

The following samples were supplied by *Mauro Caffè S.p.A.* (Reggio Calabria, Italy): five Arabica coffee bean samples (labelled as A1, A2, A3, A4, A5) obtained at various roasting temperatures (A1 is raw while A2 to A5 are characterized by a progressive degree of roasting); Arabica (Ecuador) and Robusta (Vietnam) roasted coffee beans; Arabica and Robusta roasted coffee beans of a different geographical origin: Brazil (Santos), El Salvador, Costa Rica, India, Vietnam, and Togo; Robusta (India) coffee beans processed by wet and dry methods. Four sub-samples were derived from each sample (one sub-sample was analyzed by GC-MS, while the remaining three sub-samples were analyzed consecutively by GC-FID) for a total of 60 sub-samples. The roasting process, in all cases, was carried out by the company that supplied the samples. The samples were stored in a freezer at –18°C upon receipt. The coffee beans were brought to room temperature in sealed vials before carrying out HS-SPME-GC analysis.

2.2 SPME operating conditions

A Shimadzu AOC-5000 auto injector (Shimadzu, Milan, Italy) was used for the HS-SPME operations. Approximately 2 g of coffee beans, in a sealed 10 mL vial, was subjected to a pre-equilibration period of 10 min at 60°C. The vial was agitated in an alternate clockwise-anticlockwise rotation mode at 500 rpm. The SPME fiber used was a triple phase 50/30 μm DVB/Carboxen/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane) provided by Supelco (Milan, Italy). The fiber was exposed to the coffee headspace for 40 min at 60°C and agitated as described above. The fiber was desorbed in the GC injection port for 5 min at 260°C in the splitless mode (the fiber was held in the injection port for an additional 5 min in the split mode). Blank samples, which were run every twenty applications (under the coffee bean analytical conditions), provided negligible responses. The same fiber was used in all applications.

2.3 GC-FID conditions

The GC system consisted of a Shimadzu GC 2010 equipped with a split-splitless injector (260°C) and FID detector (280°C). Sampling time was 5 min in splitless, followed by 5 min in the split mode (20 : 1). The column, an Omegawax 250 (polyethylene glycol), 30 m × 0.25 mm ID × 0.25 μm (stationary phase thickness) (Supelco, Milan, Italy) was temperature programmed as follows: 40°C for 5 min, to 230°C at 4 K/min, to 280°C at 50 K/min (2 min). Helium was used as carrier gas at constant linear velocity (35 cm/s). The following gases were used for the FID system: makeup gas was N_2 at a flow rate of 50 mL/min; the H_2 flow rate was 50 mL/min; the air flow rate was 400 mL/min. Data were collected by GC Solution software (Shimadzu, Milan, Italy).

2.4 GC-MS conditions

GC-MS analyses were carried out on a Shimadzu QP2010 (Shimadzu, Milan, Italy) equipped with a split-

splitless injector and a laboratory-constructed "flavour and fragrance" MS library. The same column, injection conditions and temperature program as for GC-FID analyses were used. The carrier gas was He which was delivered at a pressure of 40.3 kPa and a linear velocity of 34 cm/s. The interface temperature was 230°C; the ionization energy was 1.5 kV; the acquisition mass range was 40–400; acquisition was carried out in the scan mode; the scan interval was 0.5 s. Data were collected by GCMS Solution software (Shimadzu, Milan, Italy).

3 Results and discussion

3.1 Coffee bean roasting process monitoring

As already mentioned, coffee aroma consists of a wide range of volatiles which are mainly formed through the roasting procedure. The mechanisms involved are quite complex and are not completely known. The accurate monitoring of this process can be considered of fundamental importance for the coffee industries. Applicable analytical techniques, in this field, must possess flexibility, rapidity, reliability, and repeatability.

In the present research, the triple phase coating (DVB/Carboxen/PDMS) fiber proved to be the most suitable as it covered the wide range of analyte-properties present in this type of sample. This was determined in previous SPME research work [7, 8]. HS-SPME-GC-MS and -GC-FID applications were applied to the five Arabica coffee bean samples [A1, A2, A3, A4, A5 (see Section 2.1)]. Unfortunately, exact information regarding each roasting time and the related temperature were not provided by the local company which supplied the coffee samples (for industrial secrecy reasons). The total ion current GC-MS chromatograms relative to the green Arabica bean and to the final commercial roasted product are illustrated, respectively, in **Figure 1.a** and **Figure 1.b**. As expected, sample A5 presented a much more crowded chromatogram: 145 peaks with a signal-to-noise ratio of more than three were counted (against 58 components counted in sample A1).

With regards to peak assignment, 27 were identified in green coffee while 57 were identified in the roasted product (peak identification is reported in **Table 1**). Reliable MS identification was achieved through the employment of a laboratory-constructed "flavour and fragrance" MS library and a dual-filter library search process. The library was created recording pure mass spectra for standard and well-known simple matrix components. Linear retention index (LRI) values were calculated for each component on a polar and an apolar stationary phase. The chromatographic information, such as LRI, can be used interactively to filter MS results, enabling a simpler and more reliable peak assignment. An additional filter, concerning the degree of spectral similarity, can be applied for the exclusion of the low probability matches. The twin filter worked as follows: the LRI value relative to the unidentified peak is calculated before library matching. The library software automatically deleted matches with lower than 90% similarity (filter one) and with a reference LRI, in respect of the experimental value, outside a ±10 unit LRI range (filter two). Obviously, both the degree of similarity and the index range are chosen by the analyst. The relatively wide LRI window applied in this investigation was linked to the fact that polyethylene glycol phases are characterized by higher LRI variations with respect to apolar phases. It has to be highlighted that, in many cases, only one possible library match was provided by the software. In this specific investigation, for example, the use of a conventional unfiltered search for peaks 29 (2,5-dimethylpyrazine), 30 (2,6-dimethylpyrazine), and 33 (2,3-dimethylpyrazine) would have probably been unfruitful: all three analytes have the same molecular weight (108) and altogether similar fragmentation patterns (and MS spectra). Through the use of the twin-filter library, which exploited substantial differences in the LRI values (see Table 1), this source of uncertainty was eliminated. This type of procedure, using commercial MS libraries, has been recently applied by Mondello et al. in a GC × GC-qMS experiment [22]. It must be added that while approximately 47% of the peaks present in the sample A1 chromatogram were positively assigned, only about 39% were identified in sample A5. This was because sample A1 was much less complex than A5 and, thus, a higher percentage of single component effluent bands were delivered to the MS system. Component co-elution was certainly more extensive for sample A5, which can be considered as highly complex. It is obvious that pure mass spectra (and above 90% library spectra similarities) cannot be obtained from multi-compound peaks in single column GC-MS (unless peak deconvolution techniques are used).

Altogether, 73 different compounds were identified if all five samples are considered (Table 1); 39, 46, and 53 peaks were positively assigned, respectively, in samples A2, A3, and A4. As expected, the complexity of the chromatographic profiles increased with the degree of roasting. All of the chemical classes observed have been reported in previous studies [1, 3, 7, 10]. A series of observations can be made concerning the qualitative and quantitative (mean relative percentage peak areas) data reported in Table 1. Volatiles such as ketones, pyrrole and derivatives, furan- and furfuryl compounds are formed only at an advanced roasting stage; they almost all appear in samples A3 and A4 and increase or remain at a constant concentration in the final roasted product. Also to be noted is the degradation undergone by the terpene chemical class as roasting proceeds; in most cases (peaks 8, 9, 19, etc.) they are not present in sample A5. Limonene undergoes a drastic reduction from 63.1% to

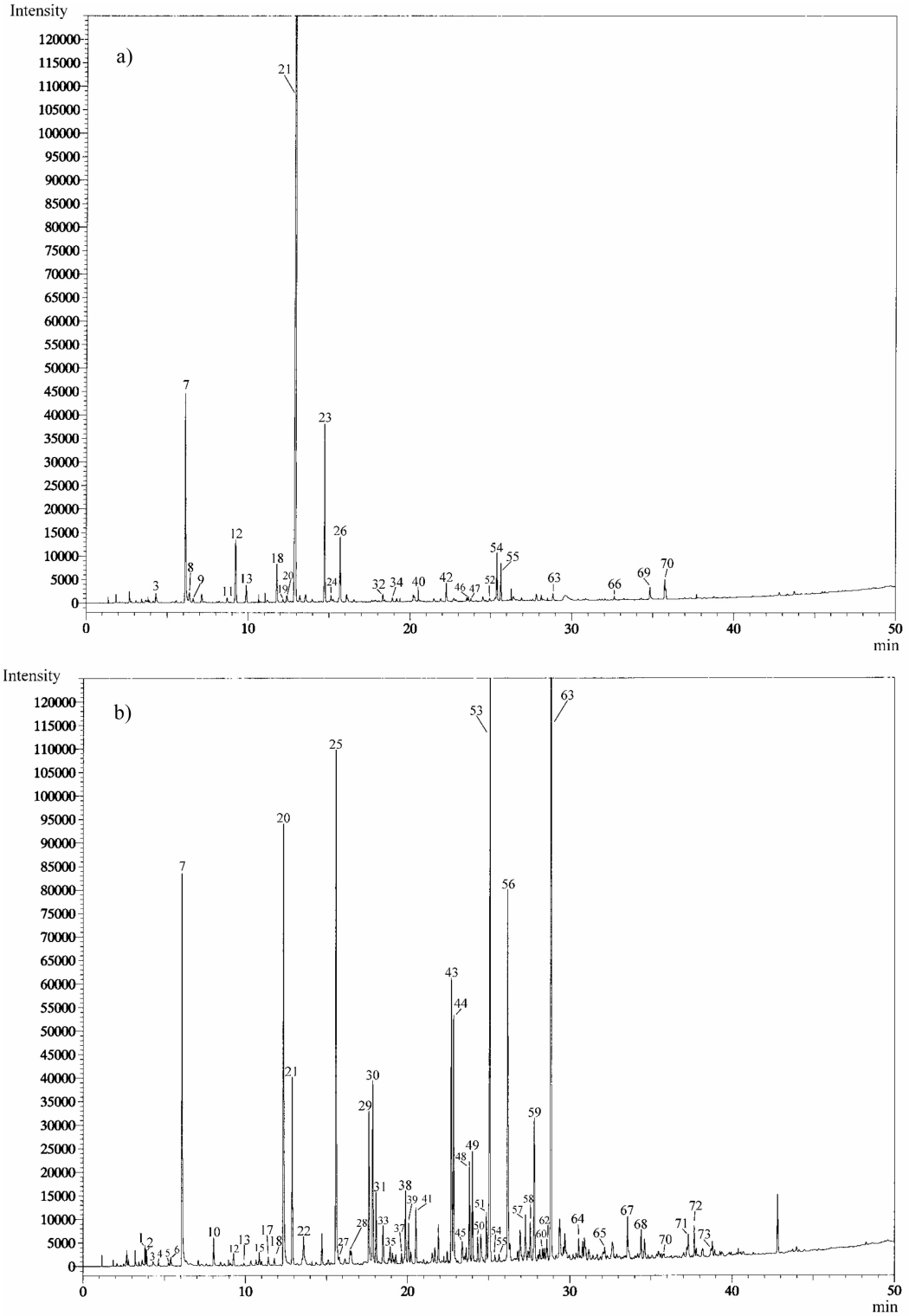

Figure 1. a) Upper chromatogram: HS-SPME-GC-qMS result for Arabica green coffee beans (sample A1), b) lower chromatogram: HS-SPME-GC-qMS result for Arabica roasted coffee beans (sample A5). For peak identification refer to Table 1.

2.6% in the final product. Methyl- and ethyl-disubstituted pyrazines appear after the first roasting step (A2), while other components, such as furfuryl alcohol and pyridine, are to be found in all samples, with amounts greatly rising in the last two roasting steps. The observed effects of the roasting process, in terms of chemical class formation-

Table 1. Peak identification, LRI values and mean relative percentage peak areas (rel.%) for all the five coffee samples (from A1 to A5). CV% values refer to sample A5.

Peak	Compound	LRI	A1 (rel.%)	A2 (rel.%)	A3 (rel.%)	A4 (rel.%)	A5 (rel.%)	CV%
1	2-methylbutanal	916	–	0.264	0.190	0.166	0.196	0.51
2	3-methylbutanal	920	–	0.196	0.063	0.088	0.150	2.33
3	ethyl alcohol	938	0.442	0.249	0.206	0.093	0.064	3.53
4	2,5-dimethylfuran	953	–	–	–	–	0.043	2.64
5	2,3-butanedione	981	–	–	–	–	0.041	5.98
6	3-methyl-2-butanone	987	–	–	–	0.080	0.119	0.33
7	acetonitrile	1011	8.644	7.763	6.988	4.363	6.131	0.46
8	α-pinene	1018	0.434	0.312	0.225	–	–	–
9	α-thujene	1025	0.160	0.104	0.075	–	–	–
10	2,3-pentanedione	1067	–	–	0.142	0.468	0.442	3.60
11	hexanal	1079	0.202	0.125	0.066	–	–	–
12	β-pinene	1102	3.187	2.159	1.535	0.391	0.212	2.12
13	sabinene	1118	0.759	0.558	0.413	0.096	0.038	0.58
14	2,3-hexanedione	1138	–	–	–	–	0.074	4.22
15	methyl-1*H*-pyrrole	1143	–	–	–	0.050	0.190	3.04
16	3,4-hexanedione	1147	–	–	–	0.050	0.057	1.57
17	2-vinyl-5-methylfuran	1158	–	–	–	0.092	0.143	1.42
18	β-myrcene	1167	1.634	1.423	1.172	0.272	0.111	2.95
19	α-terpinene	1177	0.123	0.105	0.090	–	–	–
20	pyridine	1182	0.321	0.346	0.738	1.539	7.781	1.33
21	limonene	1196	63.120	54.834	45.761	10.041	2.618	2.84
22	pyrazine	1215	–	–	–	0.208	0.400	2.69
23	γ-terpinene	1245	7.050	6.571	5.834	–	–	–
24	*trans*-β-ocimene	1256	0.222	0.286	0.283	–	–	–
25	methylpyrazine	1267	–	1.298	4.513	7.212	8.843	3.70
26	*p*-cymene	1271	2.414	2.551	2.220	–	–	–
27	2,5-dimethylpyrrole	1273	–	–	–	0.464	0.053	2.30
28	acetoin	1291	–	–	–	0.261	0.264	2.00
29	2,5-dimethylpyrazine	1324	–	2.306	3.685	2.948	2.283	1.66
30	2,6-dimethylpyrazine	1330	–	0.468	2.019	3.156	2.765	1.63
31	ethylpyrazine	1336	–	0.275	0.882	1.112	1.106	1.41
32	2-methyl-5-hepten-6-one	1344	0.287	0.221	0.165	–	–	–
33	2,3-dimethylpyrazine	1348	–	0.148	0.415	0.644	0.710	3.37
34	hexanol	1360	0.222	–	–	–	–	–
35	1-hydroxy-2-butanone	1361	–	–	–	0.094	0.236	3.21
36	2-methyl-2-cyclopentenone	1371	–	–	–	0.058	0.154	4.56
37	3-ethylpyridine	1381	–	–	–	–	0.156	1.15
38	2-ethyl-6-methylpyrazine	1388	–	0.362	1.211	1.257	1.031	1.70
39	2-ethyl-5-methylpyrazine	1394	–	0.609	1.006	0.785	0.658	1.46
40	nonanal	1397	0.154	0.105	0.212	–	–	–
41	2-ethyl-3-methylpyrazine	1407	–	0.632	1.357	1.161	0.906	1.23
42	1-octen-3-ol	1459	0.668	0.067	–	–	–	–

Table 1. Continued.

Peak	Compound	LRI	A1 (rel.%)	A2 (rel.%)	A3 (rel.%)	A4 (rel.%)	A5 (rel.%)	CV%
43	furfural	1473	–	2.740	2.932	13.765	4.512	2.36
44	acetol acetate	1477	–	–	0.134	2.851	3.768	1.62
45	2-furfuryl-5-methylsulfide	1493	–	–	0.130	0.198	0.400	0.80
46	2-decanone	1497	0.106	0.218	0.250	–	–	–
47	decanal	1498	0.178	0.183	0.241	–	–	–
48	furfuryl formate	1507	–	–	–	1.130	1.467	1.35
49	2-acetylfuran	1513	–	–	0.182	1.626	1.707	1.43
50	pyrrole	1523	–	–	0.186	0.186	0.390	0.86
51	3,3-dimethyl-2-butanone	1540	–	–	–	0.718	0.700	1.00
52	2-nonenal	1543	0.112	0.128	–	–	–	–
53	furfuryl acetate	1547	–	–	0.094	4.716	9.987	1.39
54	linalool	1557	1.707	1.843	1.557	0.512	0.092	2.53
55	linalyl acetate	1565	1.472	2.189	2.183	0.890	0.083	3.61
56	5-methylfurfural	1582	–	0.262	0.826	10.990	5.720	0.98
57	2-furfuryl furan	1618	–	–	–	0.377	0.886	1.76
58	*N*-methyl-2-formylpyrrole	1628	–	–	0.226	0.543	0.580	0.95
59	γ-butyrolactone	1637	–	0.461	0.463	1.409	2.657	0.84
60	4-(2-furyl)-2-butanone	1654	–	–	–	0.087	0.152	2.35
61	2,5-dihydro-3,5-dimethyl-2-furanone	1658	–	–	–	0.101	0.142	2.71
62	2-acetyl-1-methylpyrrole	1663	–	–	–	0.200	0.366	0.97
63	furfuryl alcohol	1671	0.317	1.017	2.207	11.708	14.913	1.50
64	N-acetyl-4(H)pyridine	1731	–	–	0.125	0.252	0.272	2.09
65	1-(5-methyl-2-furyl)-2-propanone	1787	–	–	–	0.237	0.122	3.54
66	butyl digol	1804	0.159	0.359	0.495	–	–	–
67	furfuryl pyrrole	1839	–	–	0.089	0.355	0.516	2.93
68	2-methoxyphenol	1871	–	–	–	0.131	0.366	1.44
69	benzyl alcohol	1886	0.704	0.104	–	–	–	–
70	phenylethyl alcohol	1921	0.912	0.086	0.031	0.023	0.026	0.99
71	2-acetylpyrrole	1983	–	–	0.137	0.253	0.271	1.98
72	furfuryl ether	1996	–	–	–	0.086	0.466	4.87
73	pyrrole-2-carboxaldehyde	2038	–	–	–	0.090	0.058	2.49

degradation, are comparable with those previously reported [1–3 and references therein]. The analytical repeatability was fully satisfactory as can be observed from the CV% values (relative to sample A5) also listed in Table 1: only peak 5 (2,3-butanedione), amongst the 57 calculated, presented a CV% value of slightly over 5% (5.98%). This degree of analytical repeatability was also observed for the other four samples.

A single analysis, considering both sample preparation and GC separation, was achieved in approximately 100 min. The fiber desorption period (see Section 2.3) was probably a little more than necessary. This SPME operating condition, while slightly reducing the fiber lifespan, was necessary to avoid any possible chance of analyte carry-over effects. Automation of the entire analytical procedure enabled batch analysis and, thus, the possibility of overnight GC runs. Approximately 25 hours of continuous analyses were required for 15 samples (GC-FID).

3.2 Roasted coffee bean species, geographic origin, and processing differentiation

The differentiation of Robusta and Arabica coffee has been achieved through the determination of groups of volatile components and the use of statistical methods

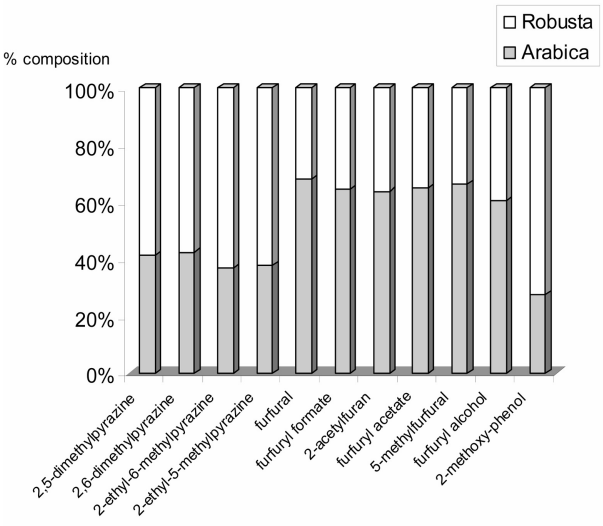

Figure 2. Mean relative percentage peak area ratios for eleven aroma-contributing compounds present in Arabica and Robusta samples.

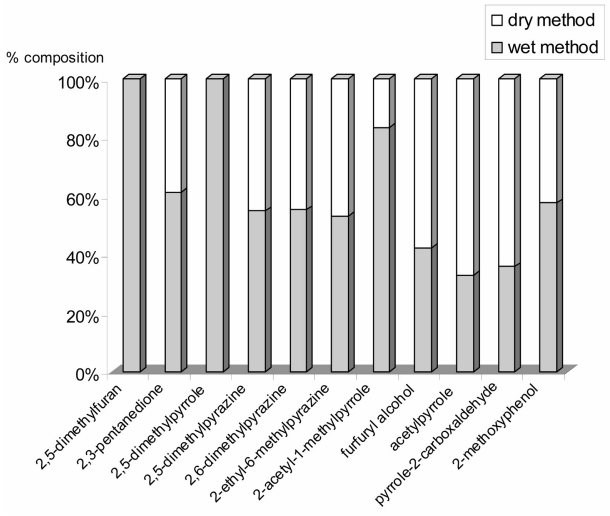

Figure 3. Mean relative percentage peak area ratios for eleven aroma-contributing compounds present in two India Robusta samples processed with the wet and dry method.

(principal component analysis) [6]. It must be emphasized, though, that concentration differences between volatiles in the two species are generally quite small and therefore, a high degree of analytical repeatability is fundamental for the attainment of reliable statistical data. In the present research, Arabica (Ecuador) and Robusta (Vietnam) end-product roasted samples (the roasting process was the same), both industrially processed by the dry method, were analyzed under the same operating conditions. The same 57 components as in sample A5 (see Table 1) were identified and mean peak areas were compared. CV% values again demonstrated excellent analytical precision, with no value over 5%. Peak area ratios, expressed in percentage values, for eleven important aroma components [1, 3 and ref. therein] in both species are reported in the graph shown in **Figure 2**. As it can be seen, the pyrazine content is slightly higher in Robusta than in Arabica (approx. 60% vs. 40%), while furan derivatives are more abundant in Arabica. The guaiacol (2-methoxyphenol) content was also evaluated, since it has been demonstrated by Semmelroch and Grosch [23] that this compound is a character impact odorant that gives a phenolic note to Robusta coffee aroma, where it is more concentrated. This last aspect was confirmed in the present research, where a 20:80 ratio was observed (Figure 2).

As mentioned above, coffee beans from different geographical areas are commonly characterized by different aroma profiles. Producers select and blend coffees on the basis of their specific volatile composition. Differentiation has been achieved through the determination of specific aroma components [13]. In the present research, three roasted samples from different countries for Arabica (El Salvador, Costa Rica, Santos) and Robusta (Togo, India, Vietnam) species were analyzed. All samples were industrially processed (wet method) and roasted in the same way. The mean relative percentage peak areas for 42 representative components determined in the Arabica and Robusta groups are reported in **Table 2**. Some brief observations can be made on the analyzed samples: as concerns the Arabica class, the El Salvador sample was characterized by higher amounts of ketones (peaks 5, 10, 35), especially diketones and aldehydes (peaks 1, 2, 43), in particular butanal derivatives. Costa Rica coffee presented the most abundant substituted pyrazine fraction (peaks 29, 30, 31, 38, 39, 41). Furthermore, it was characterized by the highest amount of guaiacol (peak 68: 0.526%) and the lowest amount of pyridine (peak 20: 5.181%). Santos coffee followed more or less the same behaviour with the exception of some of the furfuryl compounds (furfuryl alcohol, furfuryl ether) which were more concentrated in this matrix. Among the three Robusta samples, Togo coffee presented the lowest substituted pyrazine content (peaks 25, 29, 30, 31, 33, 38, 39, 41) and the highest percentage of some of the furfuryl compounds (peaks 57 and 63). Furthermore, it was characterized by the highest level of guaiacol (peak 68: 1.996%). The pyrazine and terpene fraction were respectively the most abundant and least present in India coffee. The Vietnam sample was poor in some of the characteristic aroma-contributing components such as furfuryl alcohol (8.930%) and guaiacol (0.983%).

As reported previously, coffee beans are processed by a dry or wet procedure. In the present research, two Indian

Table 2. List of 42 compounds identified and quantified [as mean relative percentage peak areas (rel.%)] in Arabica coffee beans (El Salvador, Costarica, Santos) and in Robusta coffee beans (Togo, India, Vietnam) of a different geographical origin.

Peak	Compound	El Salvador	Costarica	Santos	Togo	India	Vietnam
1	2-methylbutanal	0.373	0.207	0.235	0.263	0.264	0.105
2	3-methylbutanal	0.298	0.175	0.167	0.151	0.157	0.072
5	2,3-butanedione	0.183	0.172	0.125	0.119	0.08	–
10	2,3-pentanedione	0.512	0.287	0.241	0.056	0.153	0.118
12	β-pinene	0.124	–	0.149	0.606	0.230	0.826
15	methyl-1*H*-pyrrole	0.194	0.100	0.148	0.190	0.138	0.074
17	2-vinyl-5-methylfuran	0.169	0.108	0.086	0.088	0.100	–
20	pyridine	7.696	5.181	7.920	5.543	5.049	4.661
21	limonene	2.089	3.055	5.322	14.018	10.118	15.125
22	pyrazine	0.350	0.215	0.258	0.476	0.279	0.316
23	γ-terpinene	–	–	–	–	–	1.917
25	methylpyrazine	6.783	6.072	5.891	6.105	8.167	8.620
29	2,5-dimethylpyrazine	1.826	2.191	2.007	1.934	3.693	3.362
30	2,6-dimethylpyrazine	2.160	2.483	2.191	2.276	4.011	3.902
31	ethylpyrazine	0.856	0.925	0.816	1.322	1.740	1.758
33	2,3-dimethylpyrazine	0.649	0.639	0.607	0.712	0.913	0.807
35	1-hydroxy-2-butanone	0.224	0.189	0.174	0.198	0.216	0.177
37	3-ethylpyridine	0.177	0.182	0.240	0.191	0.257	0.180
38	2-ethyl-6-methylpyrazine	0.778	1.131	0.959	1.340	2.059	1.914
39	2-ethyl-5-methylpyrazine	0.548	0.666	0.589	0.771	1.357	1.158
41	2-ethyl-3-methylpyrazine	0.738	0.976	0.946	1.181	2.237	1.715
43	furfural	3.940	3.516	1.977	1.436	1.455	1.965
44	acetol acetate	3.837	3.582	2.762	1.731	2.216	1.813
45	2-furfuryl-5-methylsulfide	0.350	0.377	0.285	0.377	0.487	0.486
48	furfuryl formate	1.205	1.049	0.813	0.675	0.688	0.787
49	2-acetylfuran	1.872	1.774	1.302	0.725	0.827	0.936
50	pyrrole	0.374	0.347	0.423	0.421	0.321	0.296
53	furfuryl acetate	8.813	8.403	7.695	4.510	6.751	5.552
56	5-methylfurfural	6.120	6.005	3.991	2.024	2.608	2.880
57	2-furfurylfuran	0.914	0.931	0.703	2.442	0.989	0.884
58	*N*-methyl-2-formylpyrrole	0.586	0.602	0.519	0.526	0.495	0.506
59	γ-butyrolactone	2.772	2.799	3.349	2.029	2.906	1.503
60	4-(2-furyl)-2-butanone	0.156	0.170	0.137	0.128	0.162	0.144
61	2,5-dihydro-3,5-dimethyl-2-furanone	0.175	0.174	0.179	0.108	0.133	0.093
62	2-acetyl-1-methylpyrrole	0.406	0.444	0.442	0.471	0.555	0.464
63	furfuryl alcohol	17.741	19.418	20.600	12.011	10.414	8.930
64	*N*-acetyl-4(*H*)pyridine	0.322	0.327	0.344	0.333	0.326	0.241
67	furfuryl pyrrole	0.574	0.801	0.575	0.721	0.76	0.792
68	2-methoxyphenol	0.337	0.526	0.489	1.996	1.391	0.983
71	2-acetylpyrrole	0.724	0.877	0.896	0.867	0.834	0.529
72	furfuryl ether	0.480	0.585	0.669	0.454	0.645	0.408
73	pyrrole-2-carboxaldehyde	0.098	0.145	0.102	0.086	0.032	0.033

Robusta samples processed by different techniques but with the same degree of roasting were analyzed. Mean relative peak areas, for a series of characterizing aroma compounds (pyrazines, furans, pyrroles, 2,3-pentanedione, and 2-methoxyphenol) [1, 3 and references therein], were again compared (**Figure 3**). 2,3-Pentanedione is present in slightly larger amounts in the wet method processed product. 2-Methoxyphenol is hardly affected by the processing procedure employed. Further important volatiles seem to be in great part (2-acetyl-1-methylpyrrole) or completely eliminated (2,5-dimethylfuran and 2,5-dimethylpyrrole) by the dry method processing. The analytical repeatability for this series of applications, as for all others, was very good.

4 Concluding remarks

The aim of the present research was to develop an effective HS-SPME-GC-qMS method for the determination of the volatile fraction of one of the most complex and economically important food matrices. With respect to more conventional sample preparation techniques (i.e. static HS, purge and trap, etc.), SPME was confirmed as a valid alternative for coffee volatile isolation. The automation of the entire SPME sampling procedure greatly increased both the analytical precision and the daily sample throughput. Furthermore, the employment of a laboratory-constructed MS library in combination with a dual-filtered library search procedure enabled a more reliable identification of experimental MS spectra. The potential of the approach, with respect to various and important aspects of coffee analysis, has been demonstrated. Future research in this field will be devoted to further applications on other economically important matrices (both in-sample and headspace), to the continuing development of the flavor and fragrance library, and to the use of last generation automated instrumentation in other types of sample preparation procedures (i.e. derivatization of polar analytes prior to GC analysis).

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