JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Group-Type and Fingerprint Analysis of Roasted Food Matrices (Coffee and Hazelnut Samples) by Comprehensive Two-Dimensional Gas Chromatography

CHIARA CORDERO,* CARLO BICCHI, AND PATRIZIA RUBIOLO

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, I-10125 Torino, Italy

The present study is focused on the volatile fraction of roasted hazelnut and coffee samples, differing in botanical origins, morphological characteristics, and roasting treatments, selected as challenging matrices. Volatile components, sampled by headspace solid phase microextraction (HS-SPME), were analyzed by GC×GC-qMS, and separation results were adopted to classify, correlate, and/or compare samples and evaluate processing effects. The high-complexity sample profiles were interpreted through different methods: a group-type characterization, a direct fingerprint comparison, and a template matching to extract useful and consistent information, and advantages and limits of each specific approach were critically evaluated. The group-type analysis, focused on several known botanical and technological markers, enabled sample comparison and characterization based on their quali-quantitative distribution; it is highly reliable, because of the authentic standard confirmation, and extends the comparative procedure to trace and minor components. Fingerprint approaches (i.e., direct fingerprint comparison and template matching), on the other hand, extended sample comparisons and correlations to the whole volatiles offering an increased discrimination potential and improved sensitivity due to the wider analyte pattern considered. This study demonstrates the ability of comprehensive GC to further explore the complexity of roasted samples and emphasizes the advantages of, and the need for, a comprehensive and multidisciplinary approach to interpret the increased level of information provided by GC×GC separation in its full complexity.

KEYWORDS: GC×GC; orthogonality; group-type analysis; fingerprint analysis; roasting matrices; comparative analysis; roasted hazelnuts; roasted coffee

INTRODUCTION

Over recent decades, consumers' preferences have tended to favor healthier and more flavorsome food with higher nutritional value: the driving force has been food quality, where "quality" is a challenging and complex issue. The primary condition for food quality is its safety, which is closely related to compliance with established legal standards concerning human health risks, the environment, animal welfare, protection of natural resources, and ethical requirements, although a further and equally important aspect is the sensory impact due to flavor, smell, and appearance. In such a context, some products achieve further added value thanks to origin and manufacturing [quality labels, i.e., Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialty Guaranteed (TSG)] and processing practices. A clear example is roasting, which is one of the key processes in transforming a raw matrix into a food end-product and which should be carefully monitored and controlled to obtain products of the required quality standard.

It is well-known that roasting induces several chemical reactions, the control of which is fundamental to optimize flavor, color, and texture. These reactions involve specific precursors following known and unknown pathways, to originate a complex mixture of more than 20 different groups of substances, most of them contributing to the total flavor: furans, pyrazines, ketones, alcohols, aldehydes, esters, pyrroles, thiophenes, sulfur compounds, aromatic compounds, phenols, pyridines, thiazoles, oxazoles, lactones, alkanes, alkenes, and acids.

Comprehensive two-dimensional gas chromatography (GC×GC) offers the effective separation of such extremely complex volatile mixtures in a single run (*1*−*3*) because (a) it has high practical peak capacity; (b) it provides the possibility of applying two different separation principles, one for each chromatographic dimension; GC×GC can give rationalized spatial domains for chemically correlated groups of substances, and specific separation patterns useful for component identification; (c) it can

* Author to whom correspondence should be addressed (e-mail chiara.cordero@unito.it; telephone +39 011 670 7662; fax +39 011 2367662).

10.1021/jf801001z CCC: $40.75
Published on Web 08/06/2008

Table 1. Hazelnut and Coffee Markers Adopted for the Group-Type Characterization Approach[a]

			column set 1			column set 3				
ID	compound name	identification	RI	1D (min)	2D (s)	RI	1D (min)	2D (s)	coffee	hazelnut
	Acids									
1	formic acid	*tentative*	534	3.40	1.00				x	x
2	acetic acid	RF	548	4.00	0.95	1452	21.53	0.63	x	x
3	propionic acid	RF				1520	25.13	0.67	x	x
4	butanoic acid	RF	767	4.80	1.52	1565	28.67	0.71	x	x
5	pentanoic acid	RF	809	6.60	2.19	1638	32.80	0.83	x	x
6	3-methylbutanoic acid	RF	883	9.73	2.71	1586	30.33	0.71	x	x
7	2-methylbutanoic acid	RF	849	8.27	2.43				x	x
8	hexanoic acid	RF	856	8.60	2.43	1745	36.73	0.92	x	x
9	heptanoic acid	*tentative*	983	14.00	2.86				x	x
10	octanoic acid	RF	1074	18.20	2.86				x	x
	Alcohols									
11	2-methyl-1-propanol	RF	748	4.00	0.71				x	x
12	1-pentanol	RF	803	6.33	1.19				x	x
13	2,3-butanediol	RF	811	6.67	1.86				x	x
14	1-hexanol	RF	873	9.33	1.52				x	x
15	1-heptanol	RF	969	13.40	1.71				x	x
16	1-octanol	RF	1072	18.07	1.81				x	x
17	benzyl alcohol	RF	1036	16.40	2.43				x	x
18	4-ethyl-2-methoxyphenol	RF	1272	27.40	1.96				x	x
19	phenethyl alcohol	RF	1114	20.07	2.54				x	x
20	4-ethenyl-2-methoxyphenol	RF	1311	29.13	0.79				x	x
	Aldehydes									
21	2-butenal	*tentative*	761	4.53	0.81	1009	6.40	0.71	x	x
22	2-methylbutanal	RF	758	4.40	0.86	895	4.73	0.75	x	x
23	hexanal	RF	823	7.20	1.14	1076	7.93	0.96	x	x
24	benzaldehyde	RF	959	13.00	1.86	1508	24.13	0.92	x	x
25	(*E*)-2-heptenal	RF	956	12.87	1.71				x	
26	benzeneacetaldehyde	RF	1047	16.93	1.95				x	x
27	octanal	RF	1003	14.87	1.57	1289	14.93	1.29	x	x
28	(*E*)-2-nonenal	RF	1161	22.27	1.95				x	x
29	nonanal	RF	1106	19.67	1.71	1395	19.20	1.42	x	x
	Pyrazines									
30	pyrazine	RF	788	5.67	1.10	1209	11.93	0.79	x	x
31	2-methylpyrazine	RF	841	7.93	1.29	1262	13.93	0.92	x	x
32	2-ethenylpyrazine	*tentative*				1432	20.73	0.96	x	
33	2-ethylpyrazine	RF	916	11.13	1.48	1331	16.60	1.04	x	
34	2,5-dimethylpyrazine	RF	912	11.00	1.48	1320	16.13	1.08	x	x
35	2,6-dimethylpyrazine	RF				1326	16.40	1.08	x	x
36	2,3-dimethylpyrazine	RF	919	11.27	1.48	1344	17.13	1.04	x	x
37	2-ethenyl-5-methylpyrazine	*tentative*	1019	15.60	1.67	1485	22.93	1.08	x	x
38	2-ethenyl-6-methylpyrazine	*tentative*	1021	15.73	1.67	1490	23.13	1.08	x	x
39	2-isopropenylpyrazine	*tentative*	1107	19.73	1.92	1545	27.07	1.08	x	x
40	2-ethyl-6-methylpyrazine	RF	999	14.67	1.57	1384	18.73	1.21	x	x
41	2-ethyl-5-methylpyrazine	RF	1087	18.80	1.62	1390	19.00	1.21	x	x
42	2-ethyl-3-methylpyrazine	RF	1007	15.07	1.52	1402	19.47	1.21	x	x
43	2,3,5-trimethylpyrazine	RF	1003	14.87	1.57				x	
44	2-propylpyrazine	RF				1415	20.00	1.17	x	x
45	2-acetylpyrazine	RF	1023	15.80	1.91				x	x
46	(*E*)-2-methyl-5-(1-propenyl)-pyrazine	*tentative*	1197	24.00	1.91				x	x
47	2-methyl-3-propylpyrazine	RF				1471	22.33	1.38	x	x
48	3-ethyl-2,5-dimethylpyrazine	*tentative*	1081	18.53	1.62	1444	21.20	1.38	x	x
49	2-ethyl-3,5-dimethylpyrazine	*tentative*	1093	19.07	1.62				x	x
50	2-ethyl-3,6-dimethylpyrazine	*tentative*	1081	18.53	1.62	1444	21.20	1.38	x	x
51	2,6-diethylpyrazine	*tentative*				1432	20.73	1.38	x	x
52	2,3-diethylpyrazine	RF	1084	18.67	1.62					
53	2-methyl-5-propylpyrazine	RF				1473	22.40	1.33	x	x
54	2-acetyl-6-methylpyrazine	*tentative*	1113	20.00	2.00	1592	30.80	1.04	x	x
55	2,3-diethyl-5-methylpyrazine	RF	1156	22.07	1.62	1496	23.20	1.54		
56	3,5-diethyl-2-methylpyrazine	RF				1507	24.07	1.50	x	x
57	2,5-dimethyl-3-propylpyrazine	*tentative*				1508	24.13	1.5	x	x
	Furans									
58	2-methylfuran	RF	745	3.87	0.62					x
59	3-methylfuran	RF	747	3.94	0.62				x	
60	2(5*H*)-furanone	RF	916	11.13	3.00				x	x
61	2(3*H*)-furanone	*tentative*	918	11.22	3.00				x	
62	dihydro-2(3*H*)-furanone	RF				1559	28.20	0.92		x
63	2-furancarboxaldehyde	RF	847	8.20	1.67					x
64	2-furanmethanol	RF	863	8.87	2.00	1577	29.60	0.75		x
65	2-acetylfuran	RF	912	11.00	1.81					x
66	5-methyl-2-furancarboxaldehyde	RF	964	13.20	2.05	1536	26.33	0.92	x	x
67	2-acetyl-5-methylfuran	RF	970	13.47	2.96				x	
68	2-butylfuran	RF					12.73	1.17		x
69	furfuryl formate	*tentative*				1495	23.33	0.92		x

Table 1. Continued

			column set 1			column set 3				
ID	compound name	identification	RI	1D (min)	2D (s)	RI	1D (min)	2D (s)	coffee	hazelnut
70	2,5-dimethyl-3(2*H*)-furanone	RF	1057	17.40	2.86				x	
71	2-pentylfuran	*tentative*				1241	12.87	1.33		x
72	furan-2-ylmethyl acetate	RF				1520	25.07	1.00	x	
73	furan-2-ylmethyl propanoate	RF				1550	27.47	1.00		x
	Pyrroles									
74	1*H*-pyrrole	RF	797	6.07	1.43	1504	23.87	0.67	x	x
75	2-methyl-1*H*-pyrrole	*tentative*				1136	9.58	0.86	x	
76	1-methyl-1*H*-pyrrole	RF				1134	9.53	0.83	x	x
77	1-methyl-2-pyrrolidinone	RF	1053	17.20	2.62					x
78	2-acetylpyrrole	RF	1063	17.67	2.71				x	x
79	1-methyl-1*H*-pyrrole-2-carboxaldehyde	RF	1007	15.07	2.05	1557	28.00	0.96	x	x
80	1-furfurylpyrrole	RF				1719	35.80	1.08	x	x
	Pyridines									
81	pyridine	RF				1180	10.87	0.83	x	x
82	2-methylpyridine	RF				1214	12.13	0.92	x	x
83	3-methylpyridine	RF				1292	15.00	1.00	x	
84	2,4-dimethylpyridine	RF				1356	17.60	1.04	x	x
85	3-ethylpyridine	RF	1060	17.53	3.21	1379	18.53	1.13	x	x
86	3-methoxypyridine	RF				1537	26.47	1.00	x	
87	2-acetylpyridine	RF				1548	27.27	1.08	x	
88	2-butyl-6-methylpyridine	*tentative*				1562	28.40	1.13	x	x

[a] ID number, chemical name, retention index (RI), ^{1}D and ^{2}D retention times referred to column sets 1 and 3 and the matrix where they occurred. Markers were identified on the basis of their linear retention indexes and MS-EI spectra compared with those of authentic standards (indicated with "RF") or tentatively identified through their MS-EI fragmentation patterns and retention indices.

describe and define a specific two-dimensional fingerprint of each sample that provides more information than is offered by a 1D profile; and, last but not least, (d) its sensitivity enables sample comparisons and investigations including also trace and minor components. The chemical composition of the volatile fraction of a thermally treated food matrix (i.e., roasted hazelnuts and coffee samples) offers a challenging subject for two specific GC×GC approaches: group-type and fingerprint-type analyses; these approaches have successfully been adopted to classify samples in the petrochemical field (*4*) through specific criteria and rules to determine groups of analytes, displayed in clearly ordered structures over the 2D plane, on the basis of their retention times and fragmentation patterns.

The group-type approach is in general adopted when identification of all individual components of a complex fraction is neither necessary nor possible, and the interest is focused on specific classes of compounds [e.g., petrochemical samples (*5*) or fats and oils (*6*)]. The 2D fingerprint analysis, that is, the description of the specific 2D profile produced by all of the separated and detected components of a sample, is useful as a primary tool to compare samples versus a reference model and to correlate them without the need to identify all of their components.

This study aims to evaluate the specific abilities of GC×GC coupled with fast-scanning quadrupole MS detection (qMS) in chemical speciation, differentiation, and correlation of fractions in complex food matrices. In particular, the interest is focused on the possibility of applying the analytical results approaches specific to GC×GC based on the bidimensionality of the separation, where each run provides different information, that is, ^{1}D and ^{2}D retention times, detector response, and the MS spectrum, when an MS system is available. GC×GC can provide unique opportunities for data processing that are related to the analyte distribution of the sample components over the chromatographic plane (fingerprint), abstracting it (or not) from the sample chemical composition. These specific methods are known as group-type characterization, direct image comparison, and template matching. In this study, each of these methods is tested experimentally and applied to the study of the volatile fraction of roasted coffee and hazelnuts, to evaluate its ability to differentiate samples on the basis of characteristics such as botanical and geographical origin or entity of thermal treatments and roasting profile(s). The object of these investigations were (a) roasted hazelnuts (*Corylus avellana* L.), in particular, the variety known as 'Nocciola del Piemonte' or 'Tonda Gentile delle Langhe', which is a PDO product from Piedmont (Italy) that has different morphological characteristics and sensory impact (*7*) and a chemical composition of the volatile fraction that is supposedly different from samples of other origins and (b) roasted coffee samples, studied as a reference in view of the extensive and exhaustive studies dealing with the chemical speciation of coffee's volatile fraction and botanical correlations (*8*).

The volatile fraction of these two matrices was sampled by headspace solid phase microextraction (HS-SPME), which both the authors' previous studies (*9*) and the extensive literature available (*10* and references cited therein) have shown to be sensitive and effective for routinely characterizing the roasted coffee volatile fraction.

Potential and limits of the GC×GC specific analysis approaches adopted will be critically evaluated on the basis of the consistency and reliability of the provided results; the advantages of a comprehensive and multidisciplinary approach to interpretation of the result will also be discussed, providing a possible scenario for a rational and fruitful investigation of the complexity of volatiles from roasted matrices.

MATERIALS AND METHODS

Standard Samples and Solvents. Pure standard samples of *n*-alkanes (from *n*-C9 to *n*-C25), *n*-C12 as internal standard (ISTD), and pure reference compounds adopted for the group-type characterization of samples, listed in **Table 1**, were supplied by Sigma-Aldrich (Milan, Italy) except 2-methyl-3-propylpyrazine and 2-butylfuran, which were supplied by VWR International (Milan, Italy). Standard stock solutions were stored at −18 °C and used to prepare standard working solutions at suitable concentrations, likewise stored at −18 °C.

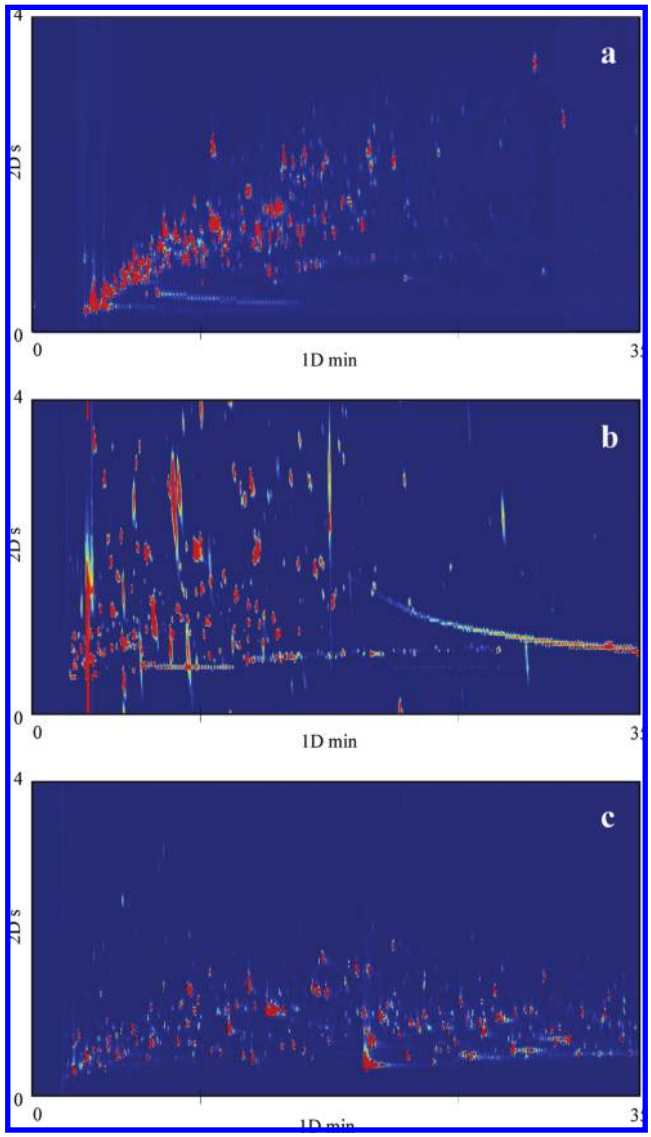

Figure 1. 2D patterns of the volatile fraction of a standard roasted *Corylus avellana* L. sample from Piedmont obtained with different column configurations: (**a**) column set 1; (**b**) column set 2; (**c**) column set 3. For experimental conditions, see text.

Solvents (cyclohexane, *n*-hexane, acetone) were all of HPLC grade from Riedel-de Haen (Seelze, Germany).

Hazelnuts. Commercially representative samples of *C. avellana* L. from different geographical origins, 'Tonda Gentile Romana' from Lazio (Italy), 'Tonda Gentile delle Langhe' from Piedmont (Italy), and Turkish hazelnuts from the Akcakoca region, all harvested in 2006, were selected and submitted to two thermal treatment (roasting) profiles in a Probat laboratory roasting device (Emmerich, Germany). Roasted samples were then classified as standard or over-roasted depending on the degree of roasting. The roasted nuts were hermetically sealed under vacuum in nonpermeable polypropylene/aluminum/polyethylene packages and stored at −20 °C, until required for chemical analysis. Hazelnuts were kindly supplied by Prof. Vincenzo Gerbi, DiVaPRA (Dipartimento Valorizzazione e Protezione delle Risorse Agroforestali), University of Turin, Italy.

Coffee. Green beens of *Coffea arabica* and *Coffea canephora* var. *robusta* from two different geographical origins, Java (Indonesia) and Los Santos (Panama), supplied by Lavazza SpA (Turin, Italy), were submitted to two thermal-treatment (roasting) profiles in a Probat laboratory roasting device. Roasted samples were then classified as standard or over-roasted depending on the degree of roasting. The

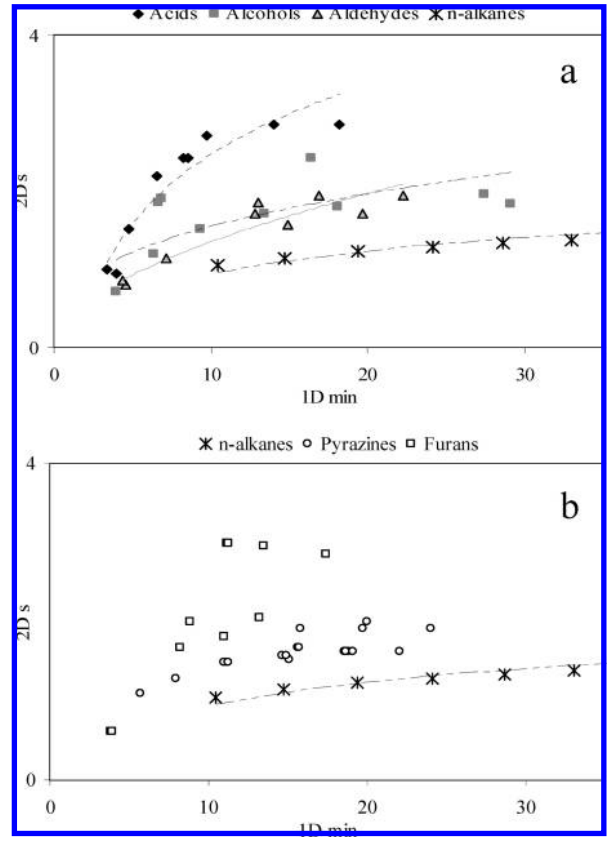

Figure 2. Group of components of the volatile fraction of the Piedmontese standard roasted sample located over the 2D plane with elution bands (dotted lines) analyzed with column set 1.

roasted beans were hermetically sealed under vacuum in nonpermeable polypropylene/aluminum/polyethylene packages and stored at −20 °C, until required for chemical analysis.

HS-SPME Sampling. The SPME device and fibers were from Supelco (Bellefonte, PA). A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) df 50/30 μm, 2 cm length fiber was chosen and conditioned before use as recommended by the manufacturer. Material was left to reach ambient temperature before sampling. Appropriate temperature and equilibration time to obtain significant headspace profiles were chosen through a series of experiments carried out by static headspace sampling (S-HS). Five hundred milligrams of each roasted coffee and 800 mg of roasted hazelnuts were ground immediately before sampling and hermetically sealed in a 12.5 mL vial together with 1 μL of the ISTD solution (1 mg/L) and equilibrated for 20 min at 60 °C. The SPME device was manually inserted into the sealed vial containing the sample prepared as described above, and the fiber was exposed to the roasted matrix headspace for 40 min during HS equilibration. The vial was vibrated for 10 s every 10 min with an electric engraver (Vibro-Graver V74, Burgess Vibrocrafters Inc., Brayslake, IL) to speed the analyte equilibration process between headspace and fiber coating. Only that part of the vial in which the solid sample was present was heated, in order to keep the SPME fiber as cool as possible, to improve the vapor phase/fiber coating distribution coefficient. After sampling, the SPME device was immediately introduced into the GC injector for thermal desorption for 10 min at 250 °C. Each experiment was carried out in triplicate.

GC×GC Instrumental Setup. GC×GC analyses were carried out on an Agilent 6890 GC unit coupled with an Agilent 5975 MS detector operating in EI mode at 70 eV (Agilent, Little Falls, DE). The transfer line was set at 270 °C. A standard tune option was used, and the scan range was set at *m*/*z* 35−240 with the fast scanning option applied (10000 amu/s) to obtain a number of data points for each chromatographic peak suitable to make their identification and quantitation reliable.

The system was provided with a two-stage thermal modulator (KT 2004 loop modulator from Zoex Corp., Houston, TX) cooled with liquid

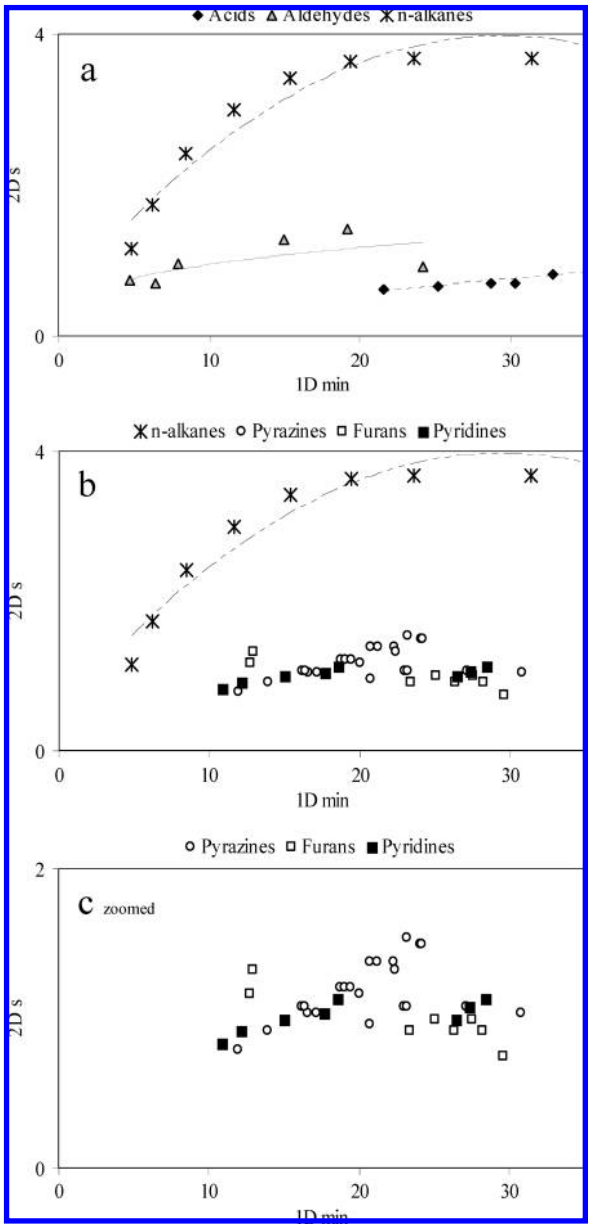

Figure 3. Group of components of the volatile fraction of the Piedmontese standard roasted sample located over the 2D plane with elution bands (dotted lines) analyzed with column set 3. Panel **c** represents an enlarged area of the 2D plot in which group location can be better appreciated.

nitrogen and with the hot jet pulse time set at 250 ms with a modulation time of 4 s adopted for all experiments. Fused silica capillary loop dimensions were 1.0 m length and 100 μm i.d.

Column sets adopted were configured as follows: column set 1 consisted of a ^{1}D SE52 column (95% polydimethylsiloxane, 5% phenyl) (30.0 m × 250 μm o.d., 0.25 μm df) coupled with a ^{2}D OV1701 column (86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl) (1.0 m × 100 μm ID, 0.10 μm df); column set 2 consisted of a ^{1}D SE52 column (30.0 m × 250 μm o.d., 0.25 μm df) coupled with a ^{2}D OV17 column (50% polydimethylsiloxane, 50% phenyl) (1.0 m × 100 μm i.d., 0.10 μm df); column set 3 consisted of a ^{1}D CW20 M column (100% polyethylene glycol) (30.0 m × 250 μm i.d., 0.25 μm df) coupled with a ^{2}D OV1701 (1.0 m × 100 μm i.d., 0.10 μm df). All columns were from MEGA (Legnano (Milan), Italy).

One microliter of the *n*-alkane sample solution was automatically injected into the GC instrument with an Agilent ALS 7683B injection system under the following conditions: injector, split/splitless; mode, split; split ratio, 1/50; injector temperature, 280 °C. The HS-SPME sampled analytes were recovered through thermal desorption of the fiber for 10 min into the GC injector under the following conditions: injector, split/splitless in split mode; split ratio, 1/20; injector temperature, 250 °C; carrier gas, helium at constant flow of 1.0 mL/min (initial head pressure of 280 kPa); temperature program, from 50 °C (1 min) to 260 °C (5 min) at 3 °C/min; modulation period, 4 s.

Data were acquired by an Agilent-MSD Chem Station ver. D.02.00.275 (Agilent Technologies) and processed using GC Image software, ver. 1.8b6 (GC Image, LLC, Lincoln, NE).

RESULTS AND DISCUSSION

The study examined the ability of GC×GC to provide further and specific information on a sample, or a group of samples, on the basis of component distribution on the chromatographic plane. The first part of the study involved optimizing the GC×GC column configuration to produce analytical results suitable for further processing; the second part examined a series of specific data-processing methods through dedicated approaches (group-type characterization, direct fingerprint comparison, and template matching) specific to GC×GC separations.

Optimization of GC×GC Column Set Configuration. The basic concept involved in group-type analysis and fingerprinting, as well as in target-type analysis, is orthogonality (*11*−*16* and references cited therein). Appropriate orthogonality is fundamental to achieve an optimal GC×GC separation, resulting in independent analyte retention between the two chromatographic dimensions, a maximization of usage of the separation plane, and, when possible, ordered structures (distributions) for chemically correlated compounds. Such ordered structures or distribution is the basis of group-type characterization for samples containing structurally related compounds, for example, homologues or isomers, as it is typical of roasted samples. It must, however, be stressed that an orthogonal column combination does not automatically provide structured chromatograms. Two conditions must contemporarily be satisfied for successful group-type analysis: (a) the sample must contain a suitable number of isomers, homologues, or congeners; and (b) the second-dimension stationary phase must be chosen with the physicochemical properties of the analytes to be grouped taken into account.

In this study, the possibility of adopting different orthogonal or nonorthogonal column combinations, to optimize separation conditions, was first investigated to select the column combination giving the most satisfactory group profiles, by evaluating their location on the chromatographic plane. Panels **a** and **b** of **Figure 1** report the 2D patterns of the volatile fraction of a standard roasted *C. avellana* L. sample obtained with two orthogonal (i.e., direct-type) column configurations; **Figure 1c** reports the 2D pattern obtained with a reverse-type column configuration, which is a nonorthogonal setup. **Figures 2a**,**b** and **3a**,**b** show a graph visualization of the correlated groups of chemicals located on the 2D plane. Group selection was done on the basis of their importance in defining the aroma characteristic (i.e., key aroma compounds) or because of their known correlation with roasting.

The first column set was a direct-type configuration, consisting of a ^{1}D SE 52 column and a ^{2}D OV1701 column. Chemically correlated groups (*n*-alkanes, alcohols, aldehydes, acids, pyrazines, furans, and pyridines) were structurally ordered along the 2D plane, as shown in **Figure 2**, where the position of each component is given by its relative retention in each dimension, that is, a volatility-based separation in the ^{1}D and a volatility/polarity-based separation in the ^{2}D. Linear alcohols, aldehydes, and acids followed a molecular weight (MW) based separation, due to the apolar ^{1}D SE 52 column, and a ^{2}D differential retention based on the functional group polarity.

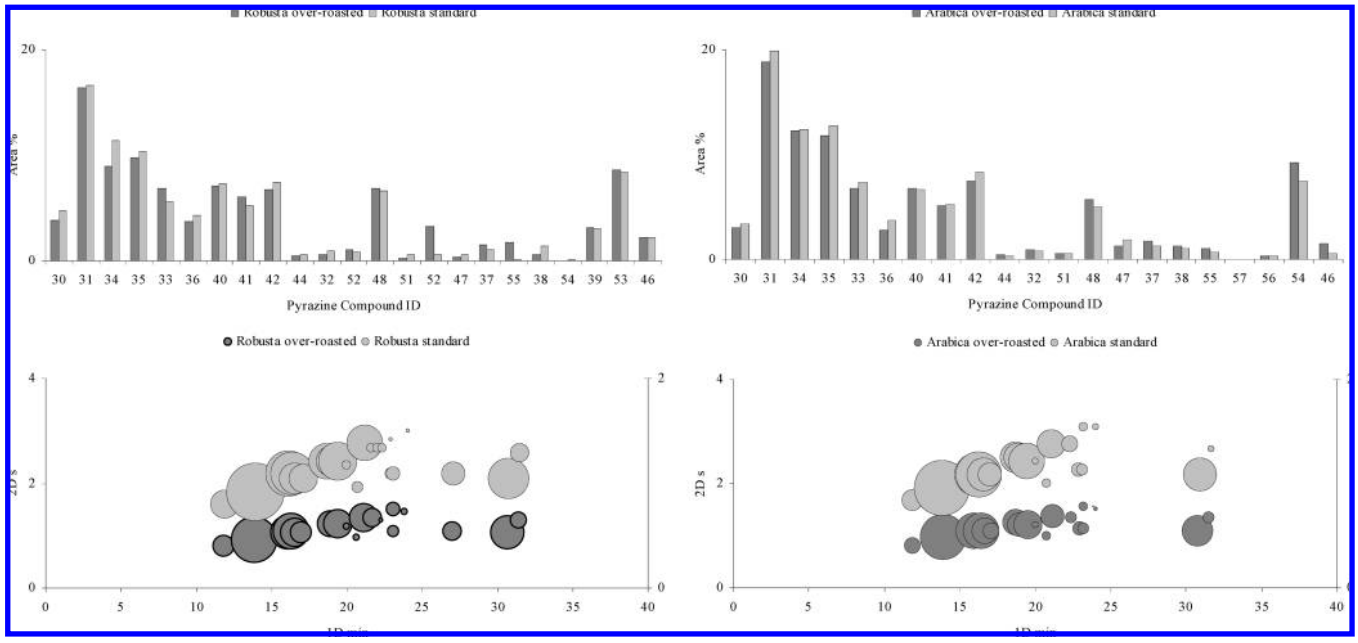

Figure 4. Pyrazine 2D patterns of roasted Robusta and Arabica coffee samples submitted to a standard and an over-roasted thermal treatment analyzed with column set 3. Histograms report the area percent of each congener (see ID in **Table 1**), whereas bubble plot graphs describe the components' location over the 2D plane.

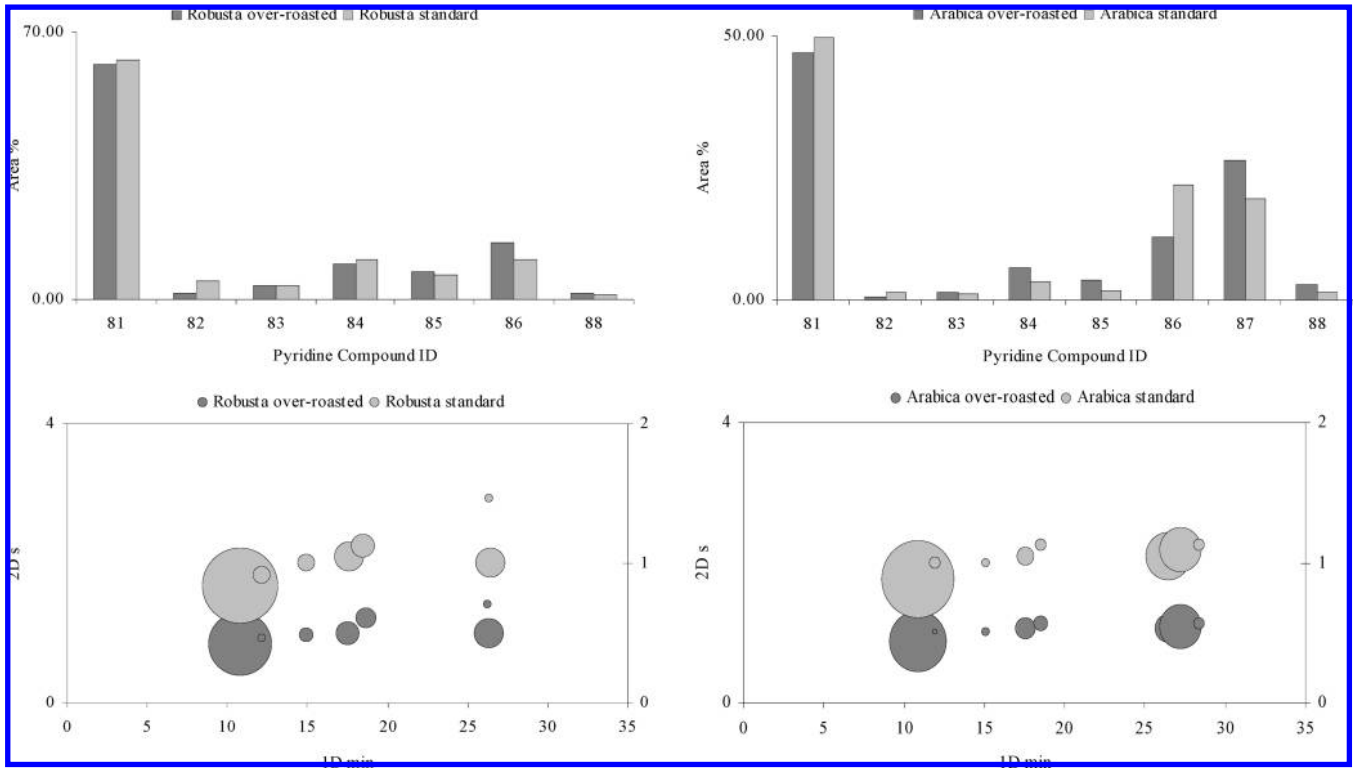

Figure 5. Pyridine 2D patterns of roasted Robusta and Arabica coffee samples submitted to a standard and an over-roasted thermal treatment analyzed with column set 3. Histograms report the area percent of each congener (see ID in **Table 1**), whereas bubble plot graphs describe the components' location over the 2D plane.

Linear acids were more strongly retained than alcohols or aldehydes with the same number of carbon atoms and showed higher ^{2}D capacity factors (^2k) than those observed for less polar functional groups. Components were located on the 2D plane following specific elution bands shown in dotted lines in **Figure 2**. Heterocyclic roasting markers, pyrazines and furans, for example, also followed structured patterns, in which the position of each component was closely correlated with functionality and ring substitution.

Occupation of the 2D plane was maximized by increasing the dissimilarity between the two chromatographic dimensions; this corresponds to an improved degree of orthogonality between the stationary phase coupling (as it is for ^{1}D SE52−^{2}D CW20M). However, the structured patterns of chemically correlated groups were partially and, in some cases, completely disordered, because the higher retention in the ^{2}D generated the phenomenon of wrap-around, in particular for the polar analytes such as alcohols and furans.

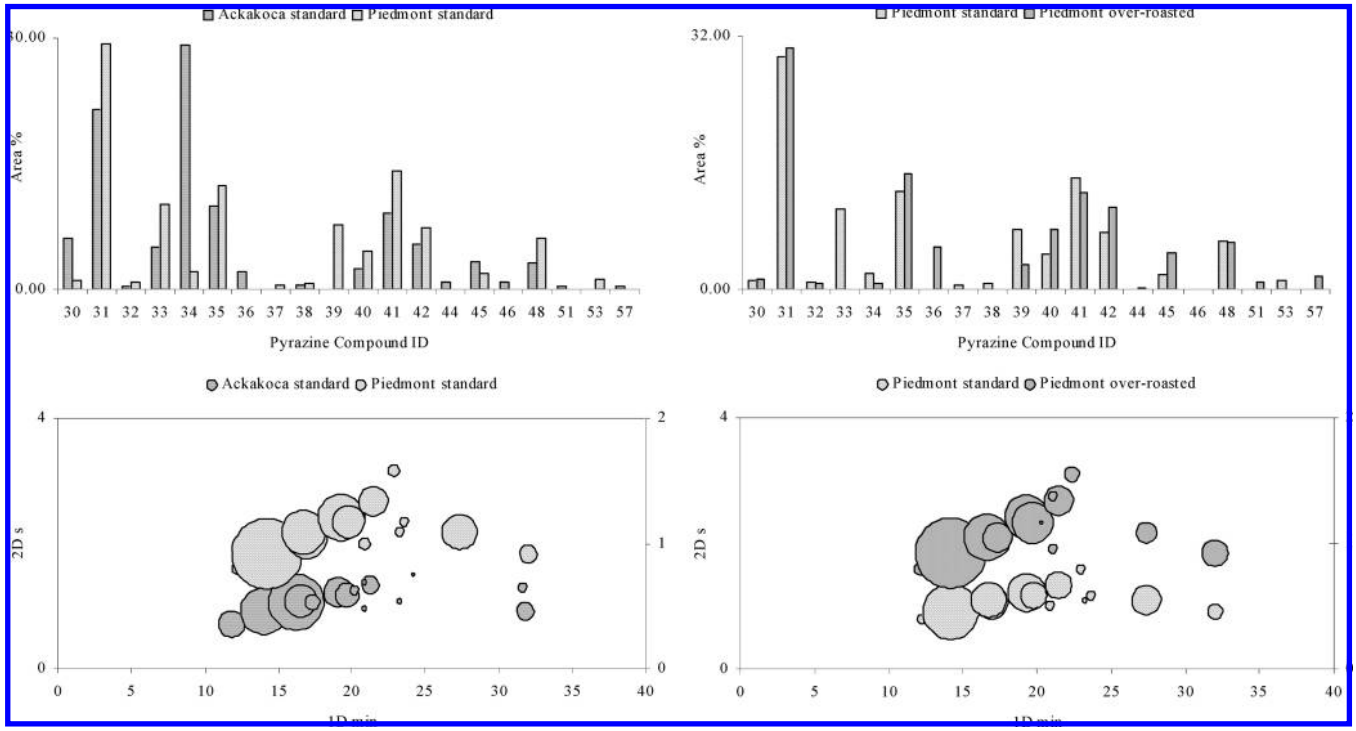

Figure 6. Pyrazine 2D patterns of roasted Ackakoca (i.e., Turkish) and Piedmontese hazelnut samples submitted to a standard and an over-roasted thermal treatment analyzed with column set 3. Histograms report the area percent of each congener (see ID in **Table 1**), whereas bubble plot graphs describe the components' location over the 2D plane.

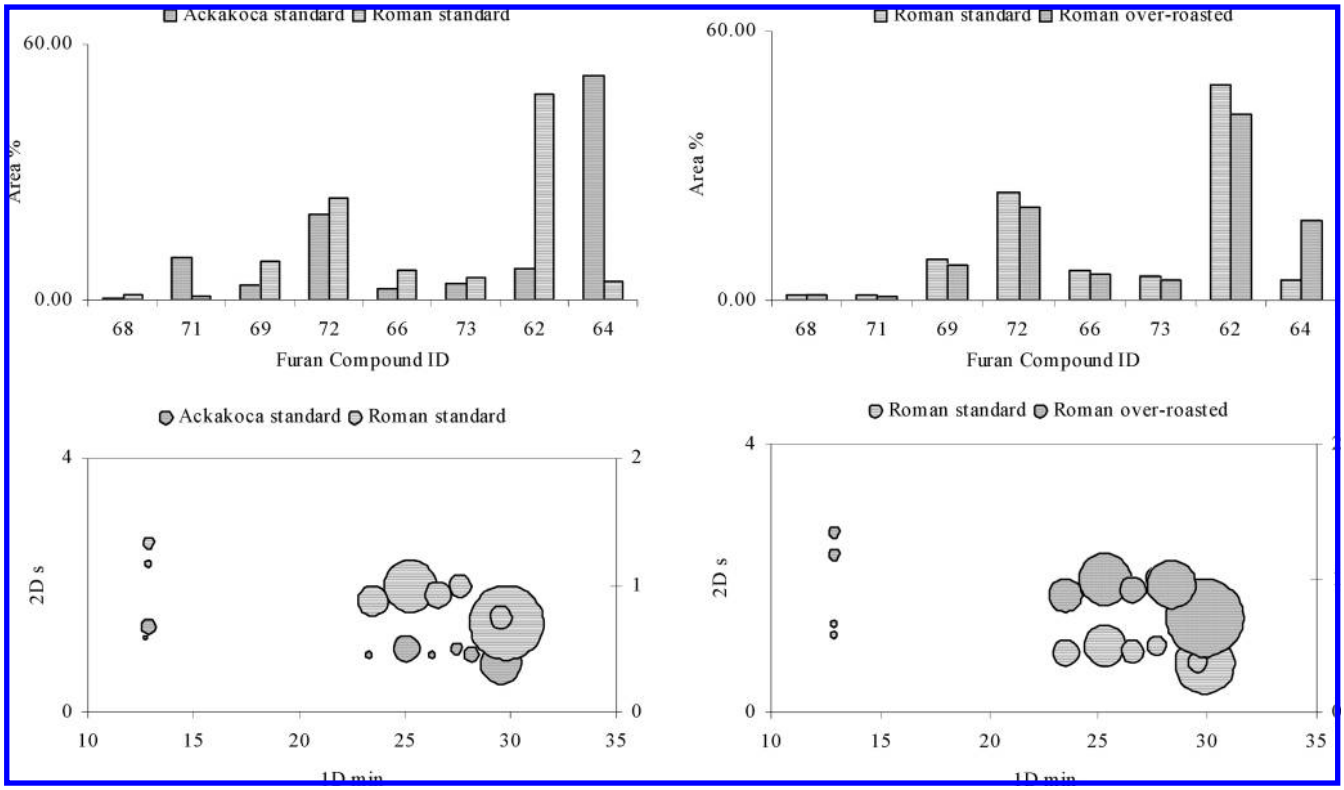

Figure 7. Furan 2D patterns of roasted Ackakoca (i.e., Turkish) and Roman hazelnut samples submitted to a 9 and an *9* thermal treatment analyzed with column set 3. Histograms report the area percent of each congener (see ID in **Table 1**), whereas bubble plot graphs describe the components' location over the 2D plane.

On the other hand, with the reverse-type configuration, consisting of a ^{1}D CW20 M column coupled with a ^{2}D OV1701 column, several classes of compounds were displayed in very clearly ordered structures and at the same time showed a good ability to handle sample complexity. **Figure 3** reports patterns of some groups of roasting markers, such as acids, aldehydes, pyridines, furans, and pyrazines, together with that of the *n*-alkanes to emphasize the reversion of the elution patterns over

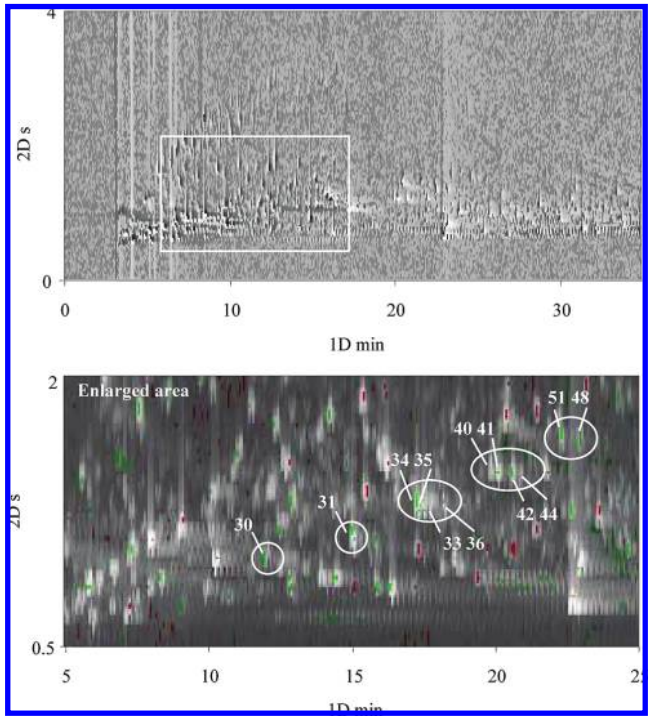

Figure 8. Resulting 2D fingerprint, that is, the difference or differential image, produced by comparing two Piedmontese hazelnut samples submitted to two different thermal processes, that is, *standard*−*analyzed* (i.e., *standard* Piedmont hazelnut) and *over-roasted*−reference (i.e., *over-roasted* Piedmont hazelnut). In the enlarged area of the 2D plot in the *fuzzy difference* visualization, brighter/green spots correspond to those analytes that were present in larger amount in the *over-roasted* Piedmont hazelnut sample. Dot-plot circles indicate pyrazine ID.

the 2D plane. With this setup, polar analytes, such as linear acids and aldehydes, were more strongly retained on the ^{1}D, because of the ^{1}D higher polarity, and the corresponding higher elution temperatures reduced the differential retention in the ^{2}D. On the other hand, heterocyclic compounds, such as pyrazines, furans, and pyridines, followed structured patterns in which their position in the chromatographic plane was again correlated with their functionality and ring substitution and, although occupation of the 2D plane was not maximized as for column set 1, this column configuration was adopted for the following experiments because of its suitable analyte resolution capability in both chromatographic dimensions.

In conclusion, orthogonal and nonorthogonal approaches resulted in (a) ordered structures for different chemical classes and (b) complementary patterns, between direct- and reverse-type column setups, which are a concrete aid for both more rational component identification and group-based sample characterization.

These aspects underlie the already existing rationalization of the data provided by GC×GC separations that include ^{1}D and ^{2}D retention times, peak area/height, and MS spectra; these data can be studied with approaches that can give specific information that is more illustrative than that obtainable from a normal 1D profile.

In the next sections, target analytes separated on the nonorthogonal column setup and belonging to chemically correlated groups were identified and quantified, and group differences were adopted to compare, correlate, or discriminate samples on the basis of the distribution of their components on the chromatographic plane.

Group-Type Characterization of Real-World Samples. Sample characterization was first carried out by selecting a suitable number of markers (targets) classified as a function of their significance for the purpose of describing botanical, technological, and sensory characteristics of the samples under study. **Table 1** reports the list of markers chosen for the group-type analysis, their ID numbers, group classification, linear retention indexes, ^{1}D and ^{2}D retention times corresponding to the separations obtained with column sets 1 and 3, and the matrix where they occurred. Each component was located in the 2D plot by its ^{1}D−^{2}D retention times and matching with the EI-MS fragmentation pattern of authentic standards (indicated in **Table 1** with the acronym RF); all of the other markers, where a reference was not available, were identified on the basis of their retention indices (apolar and polar columns, column sets 1 and 3, respectively) and with a suitable spectral similarity [fixed acceptable value above 900 referred to Identity Spectrum Match factor resulting from the NIST Identity Spectrum Search algorithm (NIST MS Search 2.0 ver. d) adopting commercial and in-house dedicated databases (indicated as *tentative* identification in **Table 1**)]. The relative percent area of each component, that is, area percent over the total area of group members, and its normalized area, that is, absolute area normalized versus the ISTD area, were used to compare samples differing in roasting treatment and/or botanical origin.

Figures 4−**7** report some results of group-type coffee and hazelnut sample characterizations. Bubble plot diagrams give marker location over the 2D plane; the area percent of each marker, corresponding to the bubble dimension and reported in the histogram, was calculated on the basis of the sum of the normalized absolute areas of the group members. For instance, **Figures 4** and **5** report pyrazine and pyridine 2D patterns of roasted Robusta and Arabica coffee samples submitted to standard and over-roasted thermal treatments. As expected, samples of the same species, harvested in the same geographical area and thought to have similar pyrazine and pyridine precursor chemical compositions, showed similar profiles for these two groups. The separation power of GC×GC is here evident in particular for pyrazines, where comparison was based on a large number of congeners that are difficult to separate with a one-dimensional GC system. A further positive aspect is the narrow peak widths, which increase the chromatographic sensitivity and enable us to reveal trace amounts of some analytes, such as alkylpyrazines (2-propylpyrazine, 2,3-diethylpyrazine, 2-acetyl-6-methylpyrazine, 2,5-dimethyl-3-propylpyrazine) and pyridines (2-methylpyridine, 2-butyl-6-methylpyridine). The area percent profile revealed small differences in the distribution of selected pyrazines when samples of the same origin but differing in roasting treatment (standard and over-roasted) were compared. Because the abundance of some markers is related to the extent of thermal treatment (*8*) and because samples submitted to a standard roasting treatment showed lower abundances (in terms of normalized areas) than those of over-roasted samples, an approach based on evaluating normalized areas of the roasting markers in different structural groups is more illustrative. These topics are discussed in greater detail in the next section, which deals with the direct fingerprint comparison, in which variations of both qualitative and quantitative distribution of separated components are emphasized.

The same approach was applied to the hazelnut volatile fraction; the results are reported in **Figures 6** and **7**. **Figure 6** shows the pyrazine patterns of two standard roasted

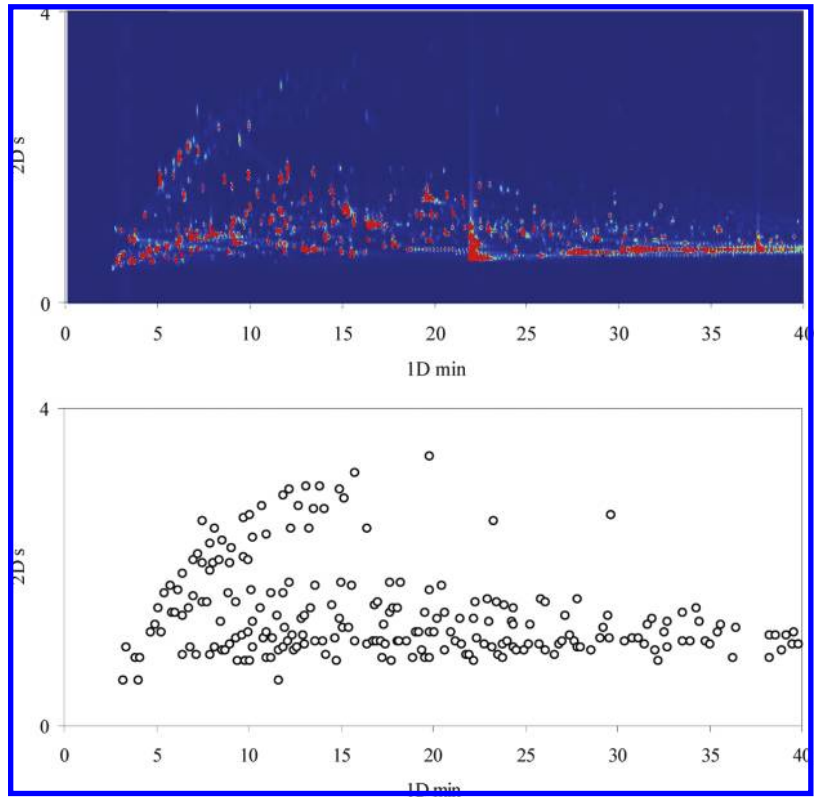

Figure 9. 2D plot and graphical representation of the 231 template peaks chosen from a standard roasted Roman hazelnut (i.e., arbitrarily considered as reference).

hazelnuts differing in geographical origin (i.e., Ackakoca and Piedmont) and of two samples of the same origin submitted to different roasting treatments (i.e., Piedmont standard and over-roasted). The bubble plot diagram and related area percent histograms clearly show that the distribution and percent abundance of pyrazines were again different for samples from Turkey and Piedmont. Some components [2-propylpyrazine, (*E*)-2-methyl-5-(1-propenyl)pyrazine, 2,6-diethylpyrazine, 2,5-dimethyl-3-propylpyrazine], for example, were found in the Turkish sample and not in the Piedmontese one, whereas the differences in area percent with Piedmont hazelnuts submitted to different roasting time/temperature treatments were less evident. Furan derivatives were first considered not only because of their significant sensory impact (*17, 18*) but also for their importance in food-safety assessment (*19*); a standard roasting procedure for Turkish and Roman samples produced a very different distribution of these compounds (**Figure 7**, left). On the other hand, furans were similarly/qualitatively distributed in samples of Roman origin submitted to different roasting treatments, confirming the results reported above for coffee (**Figure 7**, right).

In conclusion, this approach showed the following advantages: (a) it is simple and easy to apply even without specific software support; (b) it is sensitive, its sensitivity depending on the selected markers, which in their turn are related to the specific analytical and/or practical interest; and (c) it can be applied to both qualitative and quantitative evaluations. On the other hand, the disadvantages concern (a) the need to predefine target compounds (markers) and, as a consequence, to know sample composition or speciation; (b) the need for a rigorous standardization of analytical conditions to make sampling and injection repeatable and to provide stable retention times (thus, suitable ISTDs must be adopted); and, last but not least, (c) the highly variable abundance of components throughout the sample set under study that may limit its effectiveness and, as a consequence, be carefully evaluated when area percent is compared.

Direct Fingerprint Comparison. The second approach, which operates by fingerprint evaluation, is based on comparative visualization achieved by specific software (GC Image vers. 1.8b6), the specifications and peculiarities of which were discussed by Hollingsworth et al. (*20*) and are outside the scope of this paper. Comparative visualization is considered to be an extension of conventional image comparison techniques, such as side-by-side comparison and flicker between images (*21*) or digital processing procedures (*22*−*24*). It consists of subtracting a *sample* or *analyzed* metadata from a *reference* metadata set. GC×GC raw data can be represented as an *a* [*m*, *n*] matrix, where *a* is the *analyzed* chromatogram with indexed pixels by ^{1}D retention time, *m*, and ^{2}D retention time, *n*; thus, to each pixel is assigned a value corresponding to the detector response that generates a three-dimensional image. The GC-Image software adopted for fingerprint comparison is provided with registration and scaling tools, to remove incidental differences in retention time, sample amount, and peak shape between chromatograms (*20*).

Figure 8 reports the resulting 2D fingerprint, that is, the difference, or differential image, produced by comparing two hazelnut samples of the same origin (Piedmont) submitted to two different thermal processes, that is, *standard*−*analyzed* (i.e., *standard* Piedmont hazelnut) and *over-roasted*−*reference* (i.e., *over-roasted* Piedmont hazelnut). The *gray scale difference* tool gives an image in which mid gray indicates no difference, darker grays indicate negative differences, and lighter grays represent positive differences between *analyzed* and *reference* metadata. In the enlarged area of **Figure 8**, that is, the bottom part of the 2D plot in the *fuzzy difference* visualization, those analytes that were present in larger amounts in the *over-roasted* Piedmont

Table 2. Results of Pattern Matching[a]

template		
Roman standard hazelnut	total template peaks	231
sample 1		
Piedmontese standard hazelnut	no. of peaks matched	165
	no. of peaks matched (normalized)	*0.71*
	matching max distance	*12.60*
	matching min distance	*0.62*
	matching av distance	*2.37*
sample 2		
Piedmontese over-roasted hazelnut	no. of peaks matched	176
	no. of peaks matched (normalized)	*0.76*
	matching max distance	*11.11*
	matching min distance	*0.40*
	matching av distance	*1.64*
sample 3		
Turkish standard hazelnut	no. of peaks matched	157
	no. of peaks matched (normalized)	*0.68*
	matching max distance	*11.23*
	matching min distance	*0.53*
	matching av distance	*4.89*
sample 4		
Turkish over-roasted hazelnut	no. of peaks matched	187
	no. of peaks matched (normalized)	*0.81*
	matching max distance	*12.58*
	matching min distance	*0.23*
	matching av distance	*3.30*

[a] Reference template (i.e., Roman standard hazelnut) and number of template peaks used for matching. Analyzed samples (i.e., standard and over-roasted Piedmontese and Turkish hazelnuts) together with template matching results: number of peaks matched, normalized number of peaks matched, and the matching maximum and minimum absolute distances.

hazelnut sample are depicted as brighter/green spots. Dot-plot circles indicate that pyrazines are more abundant in the *reference* sample (i.e., Piedmont *over-roasted*) than in the *analyzed* one (i.e., Piedmont *standard*), as was shown in the above group-type approach when normalized areas of congeners were compared. These results are compatible with the extent of the corresponding technological treatment and provide interesting information on analyte abundance in the sample; at the same time they show a representative pattern in which sample components can easily be located over the 2D plot, and differences are given through a simple graphical interpretation.

In conclusion, the comparative approach described here has some advantages: (a) it is intuitive and does not require preliminary sample speciation or analyte identification; (b) it requires minimal preprocessing of GC×GC data run by the dedicated software with a series of available tools; and (c) it offers increased discrimination potential compared to the group-based classification above-described. The present approach runs qualitative analysis (based on retention time location over the Euclidean 2D plane) and simultaneously a semiquantitative determination based on the detector intensity/response at the same time, supplying information on the sample composition and leaving out chemical identifications.

Direct fingerprint comparison may be considered to be complementary to group-based characterization, which in its turn is mainly an extended target-analysis approach; the resulting data visualization significantly improves interactive analysis and comparative evaluations and can be applied as a preliminary step toward complete sample speciation and analyte quantitation, when a comprehensive study on sample composition and discriminant analytes is necessary.

The main limits (*20*) of this approach are due to the influence of incidental differences in retention time and peak abundance or chromatographic distortion effects on the visual comparison. Specific software tools only minimally compensate for these effects, and the need for a rigorous standardization of analytical conditions makes the adoption of ISTD mandatory.

Template Matching. This approach consists of a pattern matching in which markers, or more generally separated analytes, of an unknown sample are identified in their GC×GC pattern by matching them to the corresponding reference peaks in the pattern of a reference sample (reference pattern) (*25*). The two patterns are indicated respectively as "template peak pattern" and "target pattern" (or target peak pattern). The template corresponds to a set of peaks chosen from a GC×GC data set consisting of all (or a selection) of the separated compounds of the reference sample, located and identified, in a first instance, through their two-dimensional retention times (^{1}D and ^{2}D) and peak values or intensities. The peak pattern matching procedure establishes correspondences between peaks in the template and peaks in the target peak pattern, and all matched compounds in the target peak pattern are then listed in a report and made available for classification and correlation purposes.

The main difficulty with this process is possible pattern distortions causing apparently different locations of the same compound in different images (retention time shifts). These distortions can be removed by specific tools available in dedicated software, which can compensate for oven temperature ramp rate and inlet gas pressure variations (*25*).

In this study, the set of separated and detected peaks in the 2D fingerprint of a specimen of Roman standard roasted hazelnuts was arbitrarily defined as the reference template. This peak pattern was then adopted as reference for other target patterns obtained by analyzing the volatile fractions of samples differing in origin and thermal treatment: Roman over-roasted, Piedmontese standard and over-roasted, and Turkish standard and over-roasted hazelnuts. The reference sample template becomes a discriminator allowing us to measure the degree of similarity between samples, for example, through the number of components of an unknown sample matching those of the reference template. **Figure 9** reports the 2D plot and a graphical representation of standard roasted Roman hazelnuts (i.e., arbitrarily taken as reference), the peak template (lower part) of which was adopted to evaluate similarities and differences versus Piedmontese and Turkish peak patterns. Black circles correspond to the 231 peaks included in the reference template. **Table 2** gives the pattern-matching results, indicating the number of separated components producing a significant match with those of the reference sample (i.e., number of peaks matched) when a template matching evaluation is performed, and a suitable correction of incidental differences that cause pattern distortions is adopted. The table also lists several matching descriptors such as the normalized number of peaks matched, obtained by dividing the number of matched peaks with the reference template peaks, and the matching maximum and minimum absolute distances, corresponding to the minimal and maximal distances between the reference and the analyzed peak, measured by the software transform applied for matching (*26*). Experimental results showed that the Turkish over-roasted hazelnut fingerprint had the highest degree of similarity versus the Roman standard hazelnut fingerprint, with a total of 187 peaks matching the reference

fingerprint (i.e., 81% of the total); on the contrary, the lowest degree of similarity was obtained for the same sample origin but submitted to a mild roasting treatment, with 68% of peaks matched.

This approach is useful (a) for a classification of sample fingerprints that is roughly based on the number of matched peaks (i.e., the degree of similarity based on the 2D plane location of separated components) and (b) for the location of unmatched peaks that may indicate potential differences and/or discriminant components through which to investigate the sample difference in greater depth. On the other hand, it requires proper tuning of the template matching algorithm settings and careful and critical evaluation of the absolute and normalized distances associated with each individual match between template and unknown sample peaks. Further tools, such as CLIC expression (Computer Language for Identifying Chemicals) and clustering (*26*), are available to improve the specificity of template matching; these approaches are under study and will be the object of a forthcoming publication.

Comprehensive Approach to Interpreting GC×GC Results. The methods and results reported here have shown that GC×GC not only possesses high separation power but also can further explore the complexity of the investigated sample thanks both to the higher level of information achievable from two-dimensional GC×GC separation data and to the correlation of sample components' distribution over the 2D plane. This is also made possible by its high sensitivity, which extends its ability to compare chromatographic fingerprints, taking also into account trace and minor components. This study has emphasized the advantages of, and the need for, a comprehensive and multidisciplinary approach to interpret the increased level of information that GC×GC separation, in its full complexity, can provide with some applications.

A suitable choice of experimental conditions can provide a rational GC×GC pattern, whereby a logical combined use of the above methods can be hypothesized, consisting of a preliminary fingerprinting comparison through template matching, which leaves out the sample chemical speciation, followed by the two complementary group-type and direct fingerprint comparison approaches, which give information on component distribution and chemical differences. The group-type characterization is easy to apply, even without specific software, it is highly sensitive provided that target markers are properly selected, it is compatible with qualitative and quantitative determinations, and it enables relative and/or absolute group quantitation. On the other hand, direct fingerprint comparison requires dedicated software and can be applied to only two samples contemporarily, but it does not require sample speciation.

LITERATURE CITED

(1) Tranchida, P. Q.; Dugo, P.; Dugo, G.; Mondello, L. Comprehensive two-dimensional chromatography in food analysis. *J. Chromatogr., A* **2004**, *1054*, 3–16.

(2) Ryan, D.; Shellie, R.; Tranchida, P. Q.; Casilli, A.; Mondello, L.; Marriott, P. Analysis of roasted coffee bean volatiles by using comprehensive two-dimensional gas chromatography−time-of-flight mass spectrometry. *J. Chromatogr., A* **2004**, *1054*, 57–65.

(3) Mondello, L.; Casilli, A.; Tranchida, P. Q.; Dugo, P.; Costa, R.; Festa, S.; Dugo, G. Comprehensive multidimensional GC for the characterization of roasted coffee beans. *J. Sep. Sci.* **2004**, *27*, 442–450.

(4) Welthagen, W.; Schnelle-Kreis, J.; Zimmermann, R. Search criteria and rules for comprehensive two-dimensional gas chromatography−time-of-flight mass spectrometry analysis of airborne particulate matter. *J. Chromatogr., A* **2003**, *1019*, 233–249.

(5) Adahchour, M.; Beens, J.; Vreuls, R. J. J.; Brinkman, U. A. Th. Recent developments in comprehensive two-dimensional gas chromatography (GCxGC) III. Applications for petrochemicals and organohalogens. *Trends Anal. Chem.* **2006**, *25* (7), 726–741.

(6) Tranchida, P. Q.; Donato, P.; Dugo, P.; Dugo, G.; Mondello, L. Comprehensive chromatographic methods for the analysis of lipids. *Trends Anal. Chem.* **2007**, *26* (3), 191–205.

(7) Botta, R.; Gianotti, C.; Richardson, D.; Suwanagul, A.; Sanz, C. L. Hazelnut variety organic acids, sugars, and total lipid fatty acids. *Acta Hortic.* **1994**, *351*, 693–701.

(8) Flament, I. *Coffee Flavor Chemistry*; Wiley: Chichester, U.K., 2001.

(9) Bicchi, C.; Panero, O.; Pellegrino, G.; Vanni, A. characterization of roasted coffee and coffee beverages by solid phase microextraction−gas chromatography and principal component analysis. *J. Agric. Food Chem.* **1997**, *45*, 4680–4686.

(10) Bicchi, C.; Cordero, C.; Liberto, E.; Sgorbini, B.; Rubiolo, P. Headspace sampling of the volatile fraction of vegetable matrices. *J. Chromatogr., A* **2008**, *1184*, 220–233.

(11) Adahchour, M.; Beens, J.; Vreuls, R. J. J.; Brinkman, U. A. Th. Recent developments in comprehensive two-dimensional gas chromatography (GCxGC) I. Introduction and instrumental set-up. *Trends Anal. Chem.* **2006**, *25* (5), 438–454.

(12) Liu, Z.; Patterson, D. G., Jr.; Lee, M. L. Geometric approach to factor analysis for the estimation of orthogonality and practical peak capacity in comprehensive two-dimensional separations. *Anal. Chem.* **1995**, *67*, 3840–3845.

(13) Venkatramani, C. J.; Xu, J. B.; Phillips, J. B. Separation orthogonality in temperature-programmed comprehensive two-dimensional gas chromatography. *Anal. Chem.* **1996**, *68*, 1486–1492.

(14) Phillips, J. B.; Beens, J. Comprehensive two-dimensional gas chromatography: a hyphenated method with strong coupling between the two dimensions. *J. Chromatogr., A* **1999**, *856*, 331–347.

(15) Schoenmakers, P.; Marriott, P.; Beens, J. Nomenclature and conventions in comprehensive multidimensional chromatography. *LC−GC Eur.* **2003**, (June), 335–339.

(16) Cordero, C.; Rubiolo, P.; Sgorbini, B.; Galli, M.; Bicchi, C. Comprehensive two-dimensional gas chromatography in the analysis of volatile samples of natural origin: a multidisciplinary approach to evaluate the influence of second dimension column coated with mixed stationary phases on system orthogonality. *J. Chromatogr., A* **2006**, *1132*, 268–279.

(17) Alasalvar, C.; Shahidi, F.; Cadwallader, K. R. Comparison of natural and roasted Turkish tombul hazelnut (*Corylus avellana* L.) volatiles and flavor by DHA/GC/MS and descriptive sensory analysis. *J. Agric. Food Chem.* **2003**, *51*, 5067–5072.

(18) Alasalvar, C.; Odabasi, N.; Demir, N.; Balaban, M. O.; Shahidi, F.; Cadwallader, K. R. Comparison volatiles and flavor of five Turkish hazelnut varieties as evaluated by descriptive sensory analysis, electronic nose, and dynamic headspace analysis/gas chromatography−mass specrometry. *J. Food Sci* **2004**, *69* (3), 99–106.

(19) Studer, A.; Blank, I.; Stadler, R. H. Thermal processing contaminants in foodstuffs and potential strategies of control. *Czech J. Food Sci.* **2004**, *22*, 1–10.

(20) Hollingsworth, B. V.; Reichenbach, S. E.; Tao, Q.; Visvanathan, A. Comparative visualization for comprehensive two-dimensional gas chromatography. *J. Chromatogr., A* **2006**, *1105*, 51–58.

(21) Lemkin, P. F.; Merril, C.; Lipkin, L.; Van Keuren, M.; Oertel, W.; Shapiro, B.; Wade, M.; Schultz, M.; Smith, E. Software aids for the analysis of 2D gel electrophoresis images. *Comput. Biomed. Res.* **1979**, *12*, 517–544.

(22) Gonzalez, R. E. C.; Woods, R. E. *Digital Image Processing*; Addison-Wesley: Reading, MA, 1992.

(23) Kunzel, A. In *Proceedings of the 13th International Congress and Exhibition on Computer Assisted Radiology and Surgery*, Paris, June 23−26; Lemke, H. U., Vannier, K. M. W., Inamura, A. G. K., Farman A. G., Eds.; Elsevier: Amsterdam, The Netherlands, 1999; pp 922.

(24) Shi, X. Q.; Eklund, I.; Tronje, G.; Welander, U.; Stamatakis, H. C.; Engstrom, P. E.; Engstrom, G. N. Comparison of observer reliability in assessing alveolar bone changes from color-coded with subtraction radiographs. *Dentomaxillofacial Radiol.* **1999**, *28*, 31–36.

(25) Ni, M.; Reichenbach, S. E.; Visvanathan, A.; TerMaat, J., Jr. Peak pattern variations related to comprehensive two-dimensional gas chromatography acquisition. *J. Chromatogr., A* **2005**, *1086*, 165–170.

(26) GC-IMAGE Users' Guide; http://www.gcimage.com/usersguide/.

Received for review March 31, 2008. Revised manuscript received July 7, 2008. Accepted July 10, 2008. This research was carried out within the project "Sviluppo di metodologie innovative per l'analisi di prodotti agroalimentari" (FIRB Cod.: RBIP06SXMR_002) of the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) (Italy).

JF801001Z