Journal of Chromatography A, xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Profiling food volatiles by comprehensive two-dimensional gaschromatography coupled with mass spectrometry: Advanced fingerprinting approaches for comparative analysis of the volatile fraction of roasted hazelnuts (*Corylus avellana* L.) from different origins

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ARTICLE INFO

Article history:
Received 6 May 2010
Received in revised form 22 June 2010
Accepted 1 July 2010
Available online xxx

Keywords:
GC × GC
Fingerprint analysis
Comprehensive template matching
fingerprinting
Roasted hazelnut
Corylus avellana L. volatile fraction
Key-aroma markers

ABSTRACT

This study examined how advanced fingerprinting methods (i.e., non-targeted methods) provide reliable and specific information about groups of samples based on their component distribution on the GC × GC chromatographic plane. The volatile fractions of roasted hazelnuts (*Corylus avellana* L.) from nine different geographical origins, comparably roasted for desirable flavor and texture, were sampled by headspace-solid phase micro extraction (HS-SPME) and then analyzed by GC × GC-qMS. The resulting patterns were processed by: (a) "chromatographic fingerprinting", i.e., a pattern recognition procedure based on retention-time criteria, where peaks correspondences were established through a comprehensive peak pattern covering the chromatographic plane; and (b) "comprehensive template matching" with reliable peak matching, where peak correspondences were constrained by retention time and MS fragmentation pattern similarity criteria. Fingerprinting results showed how the discrimination potential of GC × GC can be increased by including in sample comparisons and correlations all the detected components and, in addition, provide reliable results in a comparative analysis by locating compounds with a significant role. Results were completed by a chemical speciation of volatiles and sample profiling was extended to known markers whose distribution can be correlated to sensory properties, geographical origin, or the effect of thermal treatment on different classes of compounds. The comprehensive approach for data interpretation here proposed may be useful to assess product specificity and quality, through measurable parameters strictly and consistently correlated to sensory properties and origin.

1. Introduction

The term fingerprint, in its general meaning, refers to the "impression of a fingertip on any surface ... an ink impression of the lines upon the fingertip taken for the purpose of identification" and/or "something that identifies: as (a) a trait, trace, or characteristic revealing origin or responsibility; (b) analytical evidence (as a spectrogram) that characterizes an object or substance; in particular the chromatogram or electrophoretogram obtained by cleaving a protein by enzymatic action and subjecting the resulting collection of peptides to two-dimensional chromatography or electrophoresis." [1]. For chromatographers, this definition evokes the intrinsic potential of the bi-dimensional separation patterns, obtained by comprehensive methods, for sample characterization, differentiation, discrimination and, as a consequence, classification on the basis of the peculiar component distribution over the 2D plane. In particular, comprehensive two-dimensional gas chromatography (GC × GC) has proven to be a powerful tool for sample profiling, i.e., the exhaustive analysis of a complex mixture to characterize its chemical composition. GC × GC yields highly informative separation patterns because of its great practical peak capacity, sensitivity, and structure-retention patterns for chemically related groups of substances, produced by applying two different separation principles one for each chromatographic dimension. However, the improvement in information causes a large and complex dataset for each sample, consisting of bi-dimensional retention data, detector responses and MS spectra requiring suitable data mining (a) to interpret the higher level of

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doi:10.1016/j.chroma.2010.07.006

information and (b) to extract useful and consistent data on sample compositional characteristics.

Different approaches have been investigated to link raw data (i.e. separation data) with the chemical composition of samples, and their effectiveness has been demonstrated for different fields of application [2–6]. GC × GC approaches are commonly classified into two main groups: targeted and non-targeted methods [2]. Non-targeted methods often are based on chemometric techniques or on image processing procedures [2–6], but the multi-dimensionality of the GC × GC separation may only partially be exploited. The MS fragmentation pattern is a critical point for several approaches because it includes a number of variables (i.e. *m*/*z* fragments and intensities) whose control is difficult. On the other hand, interpretation of fragmentation patterns may be crucial for analyte identification and quantification. This is an area of active research. Cross-sample analyses with GC × GC include oil spill identification [7], metabolomic analysis of mouse tissue [8], chemical profiles of illicit drug samples [9,10], investigation of changes in cocoa bean volatiles caused by moisture damage [6], and profiles of impurities in a chemical weapon precursor [4]. Extracted features have been compared and analyzed using methods such as Fisher Ratio, PCA, and machine learning algorithms. An important problem in cross-sample analysis is feature matching, i.e., matching the same features across samples. For example, datapoint-to-datapoint analyses have been reported but that approach is subject to problems related to retention-time variability. Comprehensive matching of all peaks across complex chromatograms can account for retention-time variability but is intractable, even with mass spectrometry, so peaks are sometimes matched selectively rather than comprehensively. The challenge of automated comprehensive comparisons is addressed in this paper.

This study investigated (a) how advanced fingerprinting approaches can fully exploit the informative content of GC × GC-qMS patterns (^{1}D and ^{2}D retention times, detector responses, and MS spectra) and can profitably be applied to complex food samples investigations, and (b) which advantages they provide, by including in the discrimination process all the separation dimensions and maintaining intact the informative content. The food matrix here investigated is hazelnuts (*Corylus avellana* L.), which, besides their economic value [11] and potential health benefits [12,13], have a unique and distinctive flavor [14–20] and a crispy and crunchy texture [18] induced by a technological thermal treatment. Roasting is the key step in industrial hazelnut processing, inducing several chemical reactions on specific precursors, present at different concentrations in the raw material. It produces a mixture consisting of several groups of compounds (i.e., furans, pyrazines, ketones, alcohols, aldehydes, esters, pyrroles, thiophenes, sulfur compounds, aromatic compounds, phenols, pyridines, thiazoles, oxazoles, lactones, alkanes, alkenes, and acids among the others) whose complexity is challenging to explore, even with GC × GC-qMS. Roasting has to be monitored because sensory properties are influenced, on one hand, by the quali-quantitative distribution of aroma markers resulting from the thermal treatment due to lipid-oxidation, Maillard reactions, and Strecker degradation, and, on the other hand, by the geographical origin through primary and secondary metabolites, in particular terpenoids.

The number of volatiles effectively contributing to the aroma of a food is rather limited and complex analytical procedures are required to detect, identify, and possibly quantify odour active components occurring at trace level, sometimes below ppts (ng/kg), for a reliable characterization of the overall aroma. This is particularly true for analytes with very low odour-thresholds, called "key-aroma" markers, whose concentration-in-the-food-matrix/odor threshold ratio (also defined as odor activity value, OAV) is ≥1 [21]. GC × GC sensitivity was demonstrated to be crucial in characterizing the aroma profile of Arabica coffee samples, enabling study of the quali-quantitative distribution of key-aroma markers [22].

The potential of novel advanced fingerprinting methods are shown here to: (a) reveal samples compositional peculiarities, (b) delineate fingerprints with different discrimination potential, and (c) locate compounds (known and unknown) comparatively important for geographical origin and characteristics of technological treatment assessments. Fingerprinting results are additionally validated and confirmed through known markers, in particular aroma compounds, identified by GC-O and aroma extract dilution analysis (AEDA) [23], and other markers whose distribution greatly influence sample sensory properties or indicate the extent of thermal treatments, storage time, and conditions.

2. Materials and methods

2.1. Reference compounds and solvents

Standard samples of *n*-alkanes (from *n*-C9 to *n*-C25) and pure reference compounds were supplied by Sigma-Aldrich (Milan, Italy). Standard stock solution of *n*-dodecane, the internal standard (ISTD) was prepared in acetone at 1000 μg/mL, stored at −18 °C, and used to prepare standard working solutions in concentrations ranging from 70 to 7 μg/mL, likewise stored at −18 °C. Solvents (acetone, cyclohexane, *n*-hexane, dichloromethane) were all HPLC-grade from Riedel-de Haen (Seelze, Germany).

2.2. Hazelnut samples

Commercially representative samples of *C. avellana* L. (harvest years 2007 and 2008) from different cultivars/varieties and geographical origins were analyzed. Monovarieties from Italy were "Tonda Gentile Romana" (named *Romana*), "Nocciola di Giffoni" (*Giffoni*), "Nocciola del Piemonte" (*Piemonte*) and "*Mortarella*", while Turkish hazelnuts from "Akçakoca", "Giresun", "Ordu", and "Trabzon" regions were blends of different cultivars. Akçakoca hazelnuts are composed mainly by *Tombul*, *Mincane*, *Foşa* and *Cakildak* cultivars; Giresun by *Tombul* and *Kalinkara*; Ordu by *Tombul*, *Palaz* and *Kalinkara*; and Trabzon by *Mincane*, *Tombul*, and *Foşa*. The "Cile" sample is representative of an experimental plantation of Mediterranean varieties of *C. avellana* L. in Cile. Raw hazelnuts were selected on the basis of their dimensions (caliber within 12–13 cm) and submitted to roasting in an industrial plant at different time/temperature ratios consistent with their desirable final sensory characteristics. Roasted samples were then hermetically sealed under vacuum in non-permeable polypropylene/aluminum/polyethylene packages and stored at −20 °C until their chemical analysis. Hazelnuts were supplied by Nocciole Marchisio Cortemilia (CN), Italy.

2.3. Isolation of the volatiles by solvent assisted flavor evaporation (SAFE) extraction

Roasted hazelnuts (100 g) were frozen in liquid nitrogen and then grinded by a commercial blender (Moulinette, Quelle, Nürnberg, Germany). The hazelnut powder (50 g) was extracted for 3 h at 40 °C with diethyl ether (600 mL) under constant stirring, dried over anhydrous sodium sulfate, and concentrated to 200 mL using a Vigreux column (50 cm × 1 cm internal diameter). The concentrate then was submitted to solvent assisted flavor evaporation (SAFE) [24–26] to remove the nonvolatile fraction, the resulting distillate was reduced to 200 μL by means of a Vigreux column, and the odor-active compounds were evaluated by aroma extract dilution analysis, AEDA [27].

Please cite this article in press as: C. Cordero, et al., J. Chromatogr. A (2010), doi:10.1016/j.chroma.2010.07.006

2.4. GC-O/FID and aroma extract dilution analysis (AEDA)

GC analyses were performed on a Trace GC-Ultra gas chromatograph (Thermo Fischer Instruments, Mainz, Germany) with a SE-54 (5% phenyl–95% polydimethylsyloxane), and a FFAP (100% polyethylene glycol) column both 30 m × 0.32 mm ID, 0.25 μm df (J&W Scientific, Folson, CA (USA)). Samples were introduced by cold on-column injection at 40 °C. After 2 min, the temperature of the oven was raised at 6 °C/min to 240 °C and held for 5 min. Analyses were performed at constant pressure (90 kPa) with helium as carrier gas. The linear retention indices (I^{T}_{S}) were calculated using *n*-alkanes as reference.

The flavor dilution (FD) factors [24] of the odorants were determined by AEDA. An aliquot of each distillate (0.5 μL of 200 μL) was submitted to GC analysis on the FFAP column, the effluent was split to both the FID and the sniffing port (1:1 by vol.), and the odor-active regions and the odor qualities were assigned by three assessors (GC-O). The extract was stepwise diluted with diethyl ether (1:1 by vol) and aliquots of the diluted solutions (0.5 μL) were again evaluated by three assessors.

2.5. Headspace-solid phase microextraction (HS-SPME) devices and sampling conditions

The SPME device and fibers were from Supelco (Bellefonte, PA, USA). 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. Roasted hazelnuts (1.0 g) were ground, immediately sealed in a 20 mL vial and equilibrated for 20 min at 50 °C before sampling. The Internal Standard loading procedure onto the SPME fibre [28,29] was as follows: the SPME device was manually inserted into a 20 mL sealed vial containing 4 mL of ultra-pure water to which 2 μL of *n*-undecane (ISTD) standard working solution at 7.0 μg/mL was added. The fiber was then exposed to the headspace at 50 °C for 20 min. After ISTD loading, the fiber was exposed to the matrix headspace at 50 °C for another 20 min. The vial was vibrated for 10 s every 5 min with an electric engraver (Vibro-Graver V74, Burgess Vibrocrafters Inc., Brayslake, IL) to speed up 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 cold 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: the resulting relative standard deviation (RSD%) referred to the identified analytes on the normalized 2D-peak volumes was always below 15%.

2.6. GC × GC-qMS analyses

GC × GC analyses were performed on an Agilent 6890 GC unit coupled with an Agilent 5975 MS detector operating in EI mode at 70 eV (Agilent, Little Falls, DE, USA). The transfer line was set at 280 °C. A Standard Tune option was used and the scan range was set at *m*/*z* 35–250 with the *fast scanning* option applied (10,000 amu/s) to obtain a number of data points for each chromatographic peak suitable to make its identification and quantitation reliable. The system was provided with a two-stage thermal modulator (KT 2004 loop modulator from Zoex Corporation, Houston, TX, USA) cooled with liquid nitrogen and, with the hot jet pulse time set at 400 ms, a modulation time of 4 s was applied to all experiments. A 1.0 m × 100 μm ID fused silica capillary loop was used. The column set consisted of a ^{1}D CW20 M column (100% polyethylene glycol) (30 m × 0.25 mm ID, 0.25 μm df) coupled with a ^{2}D OV1701 column (86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl) (1 m × 0.1 mm ID, 0.10 μm df) from MEGA (Legnano (Milan)-Italy).

One micro liter of the *n*-alkanes 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: 1/100; and injector temperature: 280 °C. The HS-SPME sampled analytes were recovered through thermal desorption of the fiber for 10 min directly into the GC injector under the following conditions: injector: split/splitless; mode: split; split ratio: 1/50; injector temperature: 250 °C; carrier gas: helium at a constant flow of 1.0 mL/min (initial head pressure 280 kPa); temperature program: from 50 °C (1 min) to 260 °C (5 min) at 2.5 °C/min; modulation period: 4 s.

Data were acquired by Agilent MSD ChemStation ver D.02.00.275 (Agilent Technologies, Little Falls, DE, USA) and processed using GC Image GC × GC Software, version 2.0 (GC Image, LLC, Lincoln NE, USA).

3. Results and discussion

This study develops an integrated approach based on advanced fingerprinting methods and extended target analysis to provide information on the quali-quantitative distribution of volatiles in hazelnut samples (*C. avellana* L.) of different varieties and geographical origin, submitted to thermal treatment.

In the first part, samples were submitted to non-targeted data-processing methods, i.e., fingerprint analysis, that demonstrated high specificity and sensitivity in revealing compositional differences and similarities between samples by extending the discrimination potential to the entire chromatographic profile [30,31]. In the second part, fingerprinting results were analyzed in depth by identifying analytes and correlating their distribution with sample sensory properties, thermal stress, and geographical origin in view of sample quality assessment.

3.1. Hazelnut volatiles advanced fingerprinting

3.1.1. General concepts

A new, effective, specific, and reliable non-targeted analysis approach for complex samples was adopted [31] for a comparative analysis of two-dimensional chromatographic data. This approach does not rely on sample chemical speciation, but instead relies on the information provided by the GC × GC separation (i.e. analyte relative retention, detector response and MS fragmentation patterns) in toto. This approach, known as "template-based fingerprinting", is inspired by biometric fingerprinting [31]. Most existing automatic biometric fingerprint verification systems are based on the fact that human fingertips have unique characteristics, e.g., ridge bifurcations and endings that can be localized and extracted from inked impressions or detailed images of the fingertip. These characteristics are called "minutiae features" and are cross-matched with a set of stored templates [32,33].

A GC × GC separation pattern is composed of a number of 2D peaks spread over a two-dimensional plane. Each peak reasonably corresponds to a single compound, is potentially informative, and can be treated as a separate *minutiae* for a comparative pattern analysis, as is done for fingertip features. The goal of chromatographic fingerprinting is to catalog features of a chromatogram comprehensively, quantitatively, and in a way comparable across the samples.

This task can be performed in two ways: (a) by locating *minutiae features* extracting information from analytes distribution over the GC × GC chromatographic plane (i.e., *chromatographic fingerprinting*), or (b) by considering each individual 2D peak, together with its time coordinates, detector response, and MS fragmen-

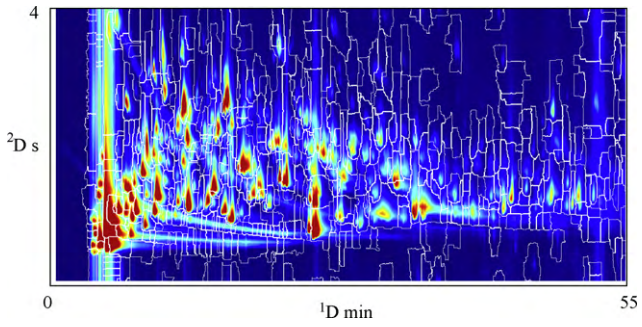

Fig. 1. Cumulative chromatogram for the nine samples of roasted hazelnuts and the regions of detected peaks used for chromatographic fingerprinting shown as white polygons. The number of chromatographic *features* is 411.

tation, as a potential *fingerprint minutiae* and including it in the sample template that can be used for a direct plot comparison (i.e., *comprehensive template matching fingerprinting*).

3.1.2. Chromatographic fingerprinting

The first approach aimed to locate and detect fingerprint *minutiae* in each GC × GC pattern of hazelnuts volatiles using features of a cumulative chromatogram from all analyzed samples to compare patterns reliably and/or to reveal differences between samples. First, a cumulative chromatogram was formed by summing all of the chromatograms of the set, with retention times alignment applied only where necessary [31]. Then, 2D chromatogram areas containing *features* were detected and treated as fingerprint *minutiae* to form the so called "consensus template", i.e., the collection of *minutiae* from the sample set. Fig. 1 shows the cumulative chromatogram for the nine samples of roasted hazelnuts and the regions of detected peaks used for fingerprinting. In this analysis, the number of chromatographic features was 411.

The *features* from the *consensus template* then were copied into each individual 2D chromatogram with the least-squares-optimal retention-times transformation (geometric scaling and translation) determined from peak matching. This elaboration keeps coherent the pattern of the *minutiae* in the retention-times plane and compensate for retention times shifts. The response in each *feature* (i.e., total ion current absolute abundance) was computed by summing the response at all datapoints in it. The result was a fingerprint obtained by grouping all the cumulative *minutiae* that reliably matched across the sample set and a semi-quantitative distribution based on an average percent response corresponding to each *feature* (i.e. the response within the *feature* divided by the response within the entire chromatogram). The fingerprinting results (Table 1), obtained by applying the cumulative fingerprint on each sample chromatogram, are useful for a preliminary analysis to focus the attention on those regions of the chromatogram, in which the detector response varied significantly, thereby indicating analytes with a highly informative role in this comparative process.

Fingerprint *minutiae* were sifted in various ways to generate tables of potentially significant *features*. In this application the first 20 *minutiae* with the largest average response, i.e., the response within the *feature* divided by the response within the entire chromatogram, were ranked. Table 1 lists the first 20 *minutiae* corresponding to the regions of the chromatogram with the largest average percent responses, presumably produced by compounds that are the major constituents of the sample. The cumulative results of the chromatographic fingerprinting are summarized at the bottom of the table as number of matched *features* with the *consensus template*, together with the percent of matching.

Cumulative results, in particular the percent of matching *features*, can be interpreted as an indication of similarity between samples, since they are obtained by matching the *consensus template,* formed by all the fingerprint *minutiae* collected from the cumulative chromatogram (i.e. the cumulative GC × GC plot obtained by summing chromatograms from the nine hazelnut varieties), with each single pattern of the sample set. As a general observation, Piemonte hazelnuts show the lowest matching percentage, 68.4%, with only 281 *features* over 411 corresponding to the template *minutiae*, while Cile (73.7%), Ordu (73.7%), Akçakoca

Table 1
First 20 *minutiae* with the largest average percent response (i.e., the response within the mesh panel divided by the response within the entire chromatogram) together with *feature* numbering (F_i), average retention times (^{1}D min – ^{2}D s) of the *feature* apex; *feature*'s average percent response relative standard deviation (RSD%) and average percent response from hazelnuts of nine origins. The largest value on each row is in bold while the smallest is in italics. Cumulative results, number of matched *features* with the *consensus template*, are expressed as percent of matching.

Feature no.	^{1}D (min)	^{2}D (s)	RSD%	Average percent response								
				Akçakoca	Cile	Giffoni	Giresun	Mortarella	Ordu	Piemonte	Romana	Trabzon
F1	4.42	0.53	51.65	8.23	22.73	16.86	20.87	29.97	*1.17*	27.57	**35.73**	26.28
F2	3.35	0.41	150.51	0.40	0.72	1.39	*0.01*	0.69	0.19	**9.59**	5.70	0.89
F3	7.55	1.03	174.64	0.04	0.04	0.04	**5.80**	*0.03*	0.04	1.20	3.45	0.03
F4	40.89	0.90	113.61	1.12	3.82	0.36	**4.94**	0.23	2.24	*0.19*	0.33	0.67
F5	4.75	0.66	37.55	4.70	2.57	**6.87**	*1.61*	4.73	3.38	4.25	3.67	3.79
F6	15.22	1.89	128.65	0.91	1.44	0.22	**4.14**	0.05	2.00	0.16	*0.15*	0.33
F7	3.95	0.41	39.04	**4.36**	1.99	3.60	*1.15*	3.94	1.96	4.19	2.51	2.59
F8	18.29	0.99	86.97	2.27	2.38	0.58	**2.66**	*0.21*	2.29	0.01	0.19	0.82
F9	20.02	2.18	174.40	0.58	0.86	0.08	**3.01**	*0.01*	0.25	0.02	0.05	0.12
F10	3.49	0.58	74.49	0.61	1.12	0.78	0.07	1.21	**1.95**	0.60	*0.06*	1.85
F11	3.82	0.86	91.16	0.62	1.54	0.50	0.28	0.30	0.32	**1.82**	*0.12*	0.43
F12	27.95	1.27	155.76	0.35	0.47	0.12	**1.72**	0.06	*0.01*	0.09	0.10	0.17
F13	5.62	0.74	55.33	1.22	**1.25**	0.47	1.32	0.61	*0.16*	0.29	0.97	0.72
F14	11.35	1.60	87.37	0.25	0.12	0.32	0.17	0.22	0.29	**1.11**	0.70	*0.17*
F15	36.29	0.82	107.10	0.16	0.58	0.10	**0.79**	*0.05*	0.34	0.05	0.07	0.11
F16	45.22	0.99	150.42	0.10	0.13	0.07	**0.85**	*0.04*	0.23	0.04	0.04	0.06
F17	3.15	0.90	87.27	0.25	0.12	**0.55**	*0.01*	0.19	0.40	0.04	0.04	0.41
F18	33.35	0.82	47.79	**0.58**	0.37	0.51	0.23	0.50	0.56	*0.07*	0.25	0.25
F19	6.89	0.70	52.61	0.26	0.24	0.35	*0.03*	0.19	0.18	0.38	**0.56**	0.33
F20	7.02	0.99	83.74	**0.33**	0.07	0.17	*0.04*	0.14	0.15	0.14	0.05	0.49

Chromatographic fingerprinting results	Akçakoca	Cile	Giffoni	Giresun	Mortarella	Ordu	Piemonte	Romana	Trabzon
Number of matched features (over 411)	300	303	327	325	317	303	281	293	325
Match %	72.99	73.72	79.56	79.08	77.13	73.72	68.37	71.29	79.08

(73.0%) and Romana (71.3%) samples showed similar matching rates.

Results based on comprehensive chromatographic *features* have some limits as for example, they may define features incompletely (e.g., placing two important chromatographic peaks in the same fingerprint *feature*) or incorrectly (e.g., splitting a chromatographic peak into two fingerprint *features*) or worse establish inconsistent correspondences between peaks with different identities. On the other hand, this approach diminishes errors for mis-matched features related to unavoidable errors in detecting peaks, unmixing coeluting peaks, and distinguishing coincident peaks with the same retention indices across multiple images. And, the lower specificity of this approach enables an effective and less time consuming classification of samples especially when one has to process unknown patterns and consequently the need is to "scan" comprehensively all the chromatographic plane to find informative relevant variable regions.

3.1.3. Comprehensive template matching fingerprinting

The specificity of the fingerprinting process is clearly improved when positive matches are limited to those peaks resulting from the same analyte within a set of samples. Complex chromatograms, such as those from roasted hazelnuts volatiles (Fig. 1), may include hundreds of peaks and the identification of which peaks in a pair (or in a set) of chromatograms correspond for both relative retention (i.e., time position) and identity (MS fragmentation) is fundamental.

"Template-matching fingerprinting" was used successfully in previous investigations to identify target analytes in two-dimensional chromatograms [34]. This approach, implemented with the possibility to extend correspondences to the MS fragmentation pattern similarity, was, thus, adopted for a non-targeted analysis to try to reliably match as many peaks as possible in a set of chromatograms. The procedure first detects peaks in a source chromatogram to create a template that records the retention times, detector responses, and MS fragmentation patterns. Next, on one of the chromatograms to be compared, the matching algorithm determines the geometric transformation in the retention-times plane that best fits the expected peak pattern in the template and, in addition, evaluates the mass spectral match factor for the corresponding peaks. The correspondence is established, if a peak is detected within the retention-times window around the corresponding transformed template peak, also showing an MS fragmentation pattern with a proper match factor [35,36]. The effectiveness of the algorithm adopted for the template transformation has been extensively discussed in previous work [35,37].

This operation, applied to the entire set of sample chromatograms, generates a *consensus template* of non-targeted peaks that can be matched across all pairs of chromatograms within the set.

The following procedure was applied to establish reliable peak correspondences across the set of chromatograms:

1. Each chromatogram was baseline corrected in agreement with a specific algorithm whose peculiarities are discussed in detail in a previous paper [38].
2. 2D-peaks were detected. For explanatory purposes, the set of chromatograms denoted A, B, . . . I, are considered in which the detected peaks in chromatogram A are denoted A(i) where i is a unique peak ID.
3. A template was created for the first chromatogram. For each peak in the chromatogram, a peak was added to the chromatogram template together with its expected retention times. For example, the template for chromatogram A will have an expected peak denoted a(i) at the retention times of the detected peak A(i).

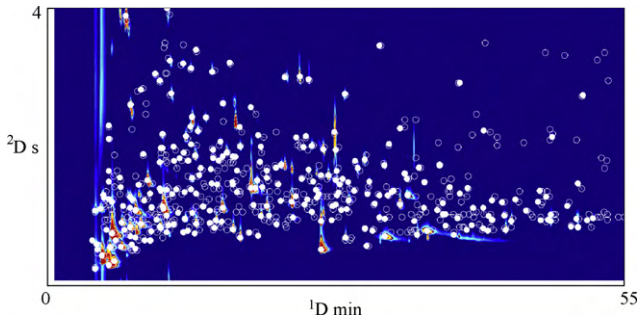

Fig. 2. GC × GC-qMS plot of Italian hazelnuts from Piedmont (i.e., Piemonte). Circles indicate the 422 peaks in the *consensus template*. The subset of 196 template peaks with matches in all nine chromatograms are shown with white filled circles.

4. For each peak in the template a rule was added to constrain MS matching using a CLIC^{TM} expression [30] such as:

   Match("<ms>") > match_factor

   where "<ms>" is the average mass spectrum of the template peak. The match function computes the match factor between the template spectrum and the detected peak spectrum, and the corresponding match-factor value should be the highest match-factor determined by considering all other peaks in the source chromatogram for the template by using the NIST MS Search algorithm [39]. In other words, the match-factor with the peak that has the most similar mass spectrum is determined and "accept" only those with a value higher than that in the rule [31].

5. Next, the template was matched to the detected peaks in the next chromatogram of the set. For example, when the template from chromatogram A is matched to the detected peaks in chromatogram B, template peak a(i) either matches some peaks B(j) or not. Then, for each unmatched peak in the chromatogram B, a template peak was added to the template, e.g., template peak b(j) for peak B(j).
6. Step 5 is repeated for every chromatogram, producing a comprehensive template with a peak for every detected peak in the set of chromatograms.

The comprehensive template was matched to each chromatogram and the set of peaks that matched at least for two chromatograms in the set, were included in a *consensus template*.

The automatic processing of samples, possible with the implemented tools present in the last software release, takes on average 2 min for each chromatogram (9–12 MB each data file) and outputs are given in different file formats.

Each peak in the *consensus template* was listed together with its expected retention times (i.e., averages of the retention times of the corresponding peaks in the set of individual templates), the mass spectrum (i.e. the average of the mass spectra) and the match factor value for the rule (i.e. the average of the match factor values). In the example, if A(i), B(j), and C(k) are matched peaks, then the consensus template peak denoted is t(i·j·k) = Average(A(i), B(j), C(k)).

Fig. 2 illustrates a GC × GC plot of Italian hazelnuts from Piedmont (i.e., Piemonte), with the locations of all 422 peaks in the *consensus template*. The subset of 196 template peaks with matches in all nine chromatograms are shown with white filled circles. Table 2 lists the first 20 2D-peaks that reliably matched across the set and were present in all nine varieties. Template peaks are listed in decreasing order of average normalized volume together with their retention times (^{1}D min – ^{2}D s) and relative standard deviation (RSD%). The first column indicates the peak numbering (M$_i$) and, where possible, the identity of the specific analyte. The largest

Table 2
First 20 *peaks* that reliably match across the sample set (retention times and MS fragmentation pattern) and present in all samples with the largest variability refereed to average normalized volume. Peaks, in decreasing order of average normalized volumes, are listed together with *peak* numbering (*Pi*), compound name, retention times (^{1}D min – ^{2}D s); *peak* normalized volumes relative standard deviation (RSD%) and normalized volumes from hazelnuts of nine origins. The largest value on each row is in bold while the smallest is in italics. Cumulative results, number of matched *peaks* with the *consensus template* are expressed as % of matching. Asterisk (*) indicates key-aroma markers (see text for details).

Peak no.	Compound name	^{1}D (min)	^{2}D (s)	RSD%	Normalized volumes								
					Akçakoca	Cile	Giffoni	Giresun	Mortarella	Ordu	Piemonte	Romana	Trabzon
P1	Acetic acid*	23.16	0.66	38.40	8.29	8.99	15.36	5.77	14.72	*5.33*	11.94	**16.48**	9.65
P2	3-Methyl butanal*	4.76	0.66	40.52	4.07	2.38	**7.61**	*2.25*	5.98	3.45	5.63	5.62	3.40
P3	2-Propanone	3.96	0.41	43.27	3.77	1.84	3.99	*1.61*	4.99	2.00	**5.55**	3.83	2.32
P4	2-Furancarboxaldehyde	23.22	0.90	32.51	2.02	2.44	3.83	1.66	1.99	*1.26*	2.76	**3.14**	2.55
P5	Pentanol	13.62	0.86	91.00	1.51	3.92	0.63	**4.70**	0.43	2.58	*0.46*	1.00	0.64
P6	Hexanol	18.22	1.03	84.28	1.96	2.21	0.65	**3.73**	*0.27*	2.34	0.37	0.51	0.74
P7	5-Methyl-(E)-2-hepten-4-one (Filbertone)*	15.29	1.73	54.05	1.82	0.87	**2.84**	0.70	1.55	1.38	2.76	1.98	*0.40*
P8	Octanal*	15.22	1.89	150.25	0.79	1.33	0.25	**5.79**	0.06	2.04	*0.21*	0.23	0.30
P9	2-Methylpyrazine	14.22	0.95	33.20	*0.87*	1.25	**2.37**	0.87	1.62	1.27	1.85	1.40	1.34
P10	Heptanol	23.02	1.15	134.46	0.71	1.24	0.34	**4.70**	*0.11*	1.66	0.31	0.29	0.36
P11		15.82	0.74	38.12	0.65	1.36	1.54	0.78	1.25	*0.38*	1.07	**1.56**	1.01
P12	2-Furanmethanol	32.56	0.86	45.64	0.62	0.84	**1.60**	0.41	1.03	*0.31*	0.94	1.17	0.87
P13		3.69	0.78	175.24	*0.14*	0.24	0.22	**3.51**	0.24	0.17	0.32	0.53	0.22
P14	2,4-Dimethyl-1-heptene	4.42	1.15	70.97	0.39	0.73	0.53	0.78	0.72	*0.08*	**1.64**	0.19	0.68
P15	Octanol	27.89	1.32	146.99	0.30	0.44	0.13	**2.40**	*0.07*	0.78	0.13	0.16	0.15
P16		3.82	0.86	70.97	0.54	1.43	0.55	0.39	0.38	0.33	0.41	*0.18*	0.38
P17	Dihydro-2(3H)-Furanone	30.82	1.11	25.02	*0.32*	0.40	**0.70**	0.39	0.66	0.53	0.50	0.60	0.49
P18	2-Methyl-1-butanol	11.89	0.82	47.23	0.59	0.29	0.59	*0.05*	0.70	0.42	0.63	**0.78**	0.34
P19		5.56	0.53	33.40	0.37	*0.28*	0.62	0.51	0.61	0.33	0.43	**0.67**	*0.28*
P20	3-Methyl-2-pentanone	6.29	1.69	62.72	0.36	0.29	0.18	*0.20*	*0.20*	0.79	**0.87**	0.71	0.32

Comprehensive template matching results	Akçakoca	Cile	Giffoni	Giresun	Mortarella	Ordu	Piemonte	Romana	Trabzon
Number of matched peaks (over 422)	320	271	309	286	251	330	196	218	322
Match %	75.83	64.22	73.22	67.77	59.48	78.20	46.45	51.66	76.30

value on each row is in bold while the smallest is in italics. Cumulative results are summarized at the bottom of the table as number of matched peaks with the *consensus template* together with percent matching. Again, the number of matched peaks over the reference template, composed by 422 peaks that reliably matched across the set, indicate the degree of similarity of each sample pattern with the *consensus template*. In this case, matching results indicate unequivocally those peaks (i.e., analytes) that are present in, at least, two samples within the set and whose variation can be considered as a diagnostic tool for a better pattern discrimination or to correlate sample composition with known chemical descriptors. It is interesting to note that Piemonte hazelnuts still showed the smallest matching percentage, 46.4%, indicating here again a lower degree of similarity with the *consensus template*. On the other hand, results visualized in Fig. 3, are in agreement with those reported by the chromatographic fingerprinting, except for the Akçakoca and Ordu varieties. Differences between samples are larger than those reported from simple pattern recognition (i.e., chromatographic fingerprinting) and demonstrate that constraining positive correspondences to MS fragmentation similarity greatly improved the sensitivity and specificity of the method.

Because one of the goals of this study was also to evaluate abilities, and limits, of fingerprinting techniques in sample profiling with a focus on technological and aroma markers, the last step in data elaboration was the identification of discriminating analytes. *Minutiae features* significantly varying across samples were first examined then, on the basis of template-based fingerprinting results, reliably matched peaks were located on each sample profile and analytes identified. Results are summarized in Table 3 .

The list reports 79 analytes with a certain discrimination potential, confirmed by fingerprinting elaboration, and with a known role in defining sensory properties, as indicators of the intensity of ther-

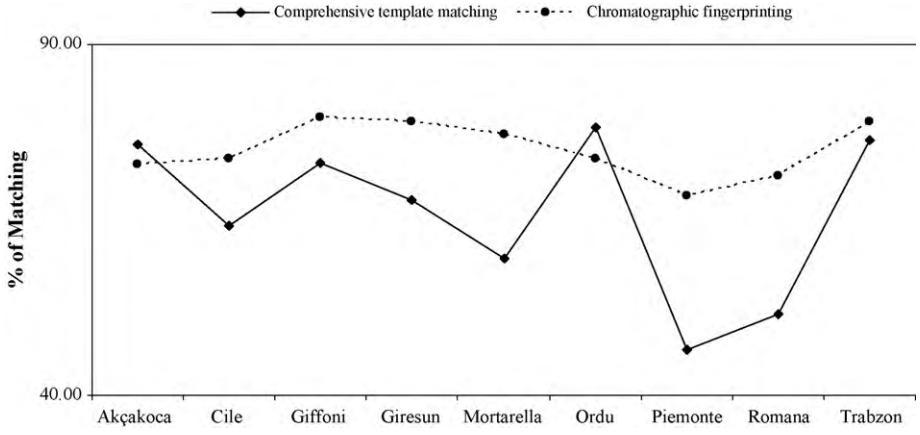

Fig. 3. Fingerprinting results expressed as % of matching with the *consensus template* (i.e., number of matched peaks divided by the total number of template peaks). Results are referred to chromatographic (- - -) and comprehensive template matching fingerprinting with MS approach (—).

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Table 3
List of analytes adopted to characterize the samples: chromatographic fingerprinting *features* numbering (F_i), identification number (#ID), compound name, odor quality for key-aroma (*) markers of roasted hazelnuts, ^{1}D and ^{2}D retention times, average normalized volumes for the nine geographical origins (average value of three replicates). Markers were identified on the basis of their linear retention indices and MS-EI spectra compared with those of authentic standards.

Feature ID	#ID	Compound name	Odor quality	^{1}D (min)	^{2}D (s)	Normalized volumes								
						Akçakoca	Cile	Giffoni	Giresun	Mortarella	Ordu	Piemonte	Romana	Trabzon
F2, F7	1	2-Propanone		3.95	0.41	3.77	1.84	3.99	1.61	4.99	2.00	5.55	3.83	2.32
	2	4-Methyl octane		4.22	1.23	0.23	1.16	1.32	0.78	0.00	2.03	1.25	0.29	0.34
F1	3	2,4-Dimethyl-1-heptene		4.42	1.15	0.73	0.53	0.78	0.72	0.08	1.64	0.19	0.68	0.73
F5	4	3-Methylbutanal*	Malty	4.75	0.66	4.07	2.38	7.61	2.25	5.98	3.45	5.63	5.62	3.40
	5	Ethanol		4.95	0.45	0.00	0.05	0.00	0.00	0.04	0.37	0.56	0.08	0.02
	6	2,2-Dimethyl decane		5.28	2.42	0.41	0.00	0.54	0.34	0.00	0.11	0.45	0.00	0.11
F13	7	2-Methylbutanal*	Malty	5.62	0.78	1.23	2.12	0.55	0.00	0.79	0.48	0.30	4.86	3.32
	8	3-Methyl-2-pentanone		6.29	0.94	0.36	0.29	0.18	0.20	0.20	0.79	0.87	0.71	0.32
	9	α-Pinene*	Terpene-like	6.35	1.70	0.37	0.29	0.17	0.20	0.23	0.76	0.66	0.70	0.32
F19	10	(E)-2-Butenal		6.82	0.66	0.23	0.22	0.39	0.08	0.24	0.19	0.55	0.86	0.29
F20	11	2,3,5-Trimethylfuran		7.02	0.99	0.28	0.07	0.19	0.05	0.18	0.16	0.19	0.11	0.44
	12	2,3-Pentanedione*	Buttery	7.15	0.70	0.40	0.19	0.83	0.00	0.65	0.00	0.38	0.93	0.44
F3	13	Hexanal*	Green	7.75	1.11	8.63	1.30	2.16	18.66	0.14	1.40	1.21	0.35	0.52
	14	2-Methyl-1-propanol		7.95	0.62	0.09	0.00	0.00	0.00	0.08	0.06	0.00	0.07	0.00
	15	**n-Undecane**	**ISTD**	8.15	3.74	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
	16	β-Pinene		8.29	1.97	0.03	0.04	0.00	0.04	0.00	0.04	0.04	0.11	0.00
	17	Sabinene		8.75	1.93	0.11	0.14	0.08	0.11	0.25	0.11	0.28	0.30	0.22
	18	2-Pentanol		8.82	0.70	0.62	0.26	0.59	0.00	0.70	0.43	0.23	0.99	0.57
	19	3,3-Dimethyl-1-butene		9.02	0.86	2.73	2.49	3.30	1.57	3.60	3.27	1.33	3.88	3.83
	20	4-Heptanone		9.02	1.40	0.24	0.21	0.36	0.06	0.26	0.14	0.06	0.20	0.33
	21	δ-3-Carene		9.55	2.10	0.11	0.17	0.00	0.10	0.75	0.27	0.02	0.69	0.28
	22	3-Methyl-4-heptanone		9.75	1.81	0.32	0.19	0.41	0.06	0.33	0.20	0.25	0.25	0.21
	23	α-Terpinene		10.62	2.18	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.04
	24	Pyridine		10.69	0.82	0.00	0.00	0.37	0.00	0.00	0.00	0.12	0.00	0.00
	25	Heptanal		10.95	1.56	0.82	0.66	0.41	4.22	0.10	0.28	0.22	0.12	0.14
F14	26	Limonene		11.29	2.10	0.10	3.39	0.09	0.13	0.45	2.17	0.08	0.63	0.66
F14	27	5-Methyl-(Z)-2-hepten-4-one*	Fruity, hazelnut-like	11.49	1.68	1.31	0.38	1.27	0.24	1.04	0.42	1.12	1.04	0.69
	28	2-Methyl-1-butanol		11.89	0.82	0.59	0.29	0.59	0.05	0.70	0.42	0.63	0.78	0.34
	29	2-Pentylfuran		12.62	1.73	0.27	0.68	0.10	0.64	0.09	0.30	0.09	0.10	0.14
	30	γ-Terpinene		13.15	2.18	0.00	0.03	0.00	0.03	0.17	0.02	0.00	0.08	0.30
	31	Pentanol		13.62	0.86	1.51	3.92	0.63	4.70	0.43	2.58	0.46	1.00	0.64
	32	2-Methylpyrazine		14.22	0.94	0.87	1.25	2.37	0.87	1.62	1.27	1.85	1.40	1.34
	33	3-Hydroxy-2-butanone		15.15	0.78	0.29	0.32	0.60	0.23	0.71	0.19	0.36	0.56	0.31
F6	34	Octanal*	Fatty, green	15.22	1.89	0.79	1.33	0.25	5.79	0.06	2.04	0.21	0.23	0.30
	35	5-Methyl-(E)-2-hepten-4-one (Filbertone)*	Fruity, hazelnut-like	15.29	1.73	1.82	0.87	2.84	0.70	1.55	1.38	2.76	1.98	0.40
	36	1-Hydroxy-2-propanone		15.55	0.70	0.01	0.02	0.02	0.02	0.00	0.02	0.10	0.00	0.02
	37	2,5-Dimethylpyrazine		16.69	1.19	0.87	0.08	1.91	0.62	0.18	0.13	0.45	0.14	0.14
	38	(E)-2-Heptenal		16.75	1.56	0.82	0.66	0.41	4.22	0.1	0.28	0.22	0.12	0.14
	39	2,6-Dimethyl pyrazine		16.95	1.19	0.25	0.20	0.69	0.30	0.52	0.31	0.68	0.56	0.37
	40	2-Ethylpyrazine		17.15	1.19	0.37	0.29	0.74	0.85	0.53	0.38	0.52	0.57	0.43
	41	2,3-Dimethyl pyrazine		17.75	1.19	0.10	0.13	0.29	0.07	0.17	0.11	0.15	0.26	0.14
F8	42	Hexanol		18.22	1.03	1.96	2.21	0.65	3.73	0.27	2.34	0.37	0.51	0.74
	43	2-Ethyl-6-methyl pyrazine		19.55	1.40	0.14	0.10	0.37	0.11	0.29	0.15	0.31	0.16	0.18
	44	2-Ethyl-5-methyl pyrazine		19.82	1.44	0.42	0.28	0.73	0.29	0.72	0.46	0.24	0.13	0.45
F9	45	Nonanal*	Fatty, green	19.95	2.22	0.56	0.85	0.36	4.11	0.46	0.74	0.15	0.43	0.39
	46	2-Ethyl-3-methyl pyrazine		20.52	1.40	0.18	0.15	0.53	0.13	0.47	0.24	0.47	0.28	0.23
	47	(E)-2-Octenal*	Fatty, green	21.62	1.85	0.16	0.51	0.03	0.70	0.02	0.24	0.04	0.07	0.10
	48	3-Ethyl-2,5-dimethyl pyrazine		22.35	1.64	0.14	0.10	0.35	0.11	0.31	0.19	0.40	0.17	0.17
	49	2,5-Diethyl pyrazine		22.95	1.68	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00
	50	Heptanol		23.02	1.15	0.71	1.24	0.34	4.70	0.11	1.66	0.31	0.29	0.36
	51	2-Ethyl-3,5-dimethyl pyrazine*	Earthy	23.06	1.64	0.13	0.23	0.22	0.15	0.18	0.11	1.01	0.16	0.09

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Table 3 (*Continued*).

Feature ID	#ID	Compound name	Odor quality	^{1}D (min)	^{2}D (s)	Normalized volumes								
						Akçakoca	Cile	Giffoni	Giresun	Mortarella	Ordu	Piemonte	Romana	Trabzon
	52	Acetic acid*	Sour	23.10	1.66	15.36	5.77	14.72	5.33	11.94	16.48	9.65	14.96	9.61
	53	2-Furancarboxaldehyde		23.22	0.90	2.02	2.44	3.83	1.66	1.99	1.26	2.76	3.14	2.55
	54	1-(Acetyloxy)-2-propanone		23.49	1.03	0.14	0.00	0.36	0.00	0.21	0.16	0.12	0.00	0.00
	55	*trans*-Sabinene hydrate		23.49	1.60	0.13	0.05	0.07	0.13	0.19	0.22	0.00	0.18	0.09
	56	Decanal		24.89	2.47	0.16	0.00	0.00	0.17	0.00	0.05	0.00	0.00	0.04
	57	Pyrrole		25.55	0.78	0.14	0.14	0.52	0.07	0.25	0.10	0.30	0.24	0.21
	58	Benzaldehyde		25.82	1.15	0.46	0.05	0.65	0.26	0.04	0.03	0.37	0.15	0.02
	59	(E)-2-Nonenal*	Fatty, green	26.42	2.01	0.27	0.08	0.22	0.21	0.19	0.19	0.21	0.28	0.18
	60	2,4-Dimethyl-3-pentanol		26.69	1.40	0.24	0.07	0.21	0.19	0.14	0.18	0.28	0.20	0.18
	61	Propanoic acid		27.29	0.74	0.14	0.07	0.18	0.23	0.00	0.09	0.09	0.00	0.00
F12	62	Octanol		27.89	1.31	0.30	0.44	0.13	2.40	0.07	0.78	0.13	0.16	0.15
	63	5-Methyl-2-furancarboxaldehyde		28.22	1.15	0.05	0.09	0.04	0.06	0.05	0.05	0.00	0.00	0.08
	64	3-Methyl propanoic acid		28.69	0.99	0.17	0.08	0.24	0.16	0.23	0.13	0.18	0.23	0.12
F2	65	3-Methyl-2-cyclohexen-1-one		29.22	1.48	0.14	0.08	0.25	0.11	0.21	0.13	0.24	0.20	0.11
F2	66	2,3-Butanediol		29.55	0.82	0.31	0.49	0.42	0.19	0.63	0.23	0.10	0.65	0.13
	67	Dihydro-2(3H)-furanone		30.82	1.11	0.32	0.40	0.70	0.39	0.66	0.53	0.50	0.60	0.49
	68	2-Phenylacetaldehyde*	Honey-like	31.35	1.23	0.11	0.03	0.02	0.07	0.05	0.05	0.01	0.02	0.02
	69	Butanoic acid		31.42	0.82	0.12	0.02	0.11	0.26	0.11	0.18	0.02	0.11	0.08
	70	(E)-2-Decenal*	Fatty	31.49	2.18	0.03	0.01	0.00	0.00	0.00	0.14	0.00	0.00	0.00
	71	2-Furanmethanol		32.55	0.86	0.62	0.84	1.60	0.41	1.03	0.31	0.94	1.17	0.87
	72	2- and 3-Methyl butanoic acid*	Sweaty	33.09	1.07	0.03	0.02	0.04	0.01	0.04	0.07	0.04	0.03	0.02
F15	73	Pentanoic acid		36.29	0.86	0.26	1.07	0.11	1.12	1.20	0.73	0.05	0.18	1.07
	74	(E,E)-2,4-decadienal*	Deep-fried	36.95	1.89	0.00	0.51	0.00	0.10	0.10	0.45	0.00	0.31	0.10
F4	75	Hexanoic acid		40.89	0.94	1.08	15.98	0.43	6.55	2.74	11.77	0.19	4.89	0.35
	76	2-Phenylethanol*	Honey-like	43.02	1.19	0.18	0.40	0.12	0.20	0.46	0.48	0.07	0.48	0.23
F16	77	3-Acetylpyrrole		45.49	1.03	0.24	0.13	0.35	0.00	0.25	0.18	0.22	0.41	0.17
	78	1H-pyrrole-2-carboxaldehyde		47.49	1.03	0.20	0.11	0.39	0.19	0.25	0.12	0.18	0.33	1.06
	79	4-Hydroxy-2,5-dimethyl-3(2H)-furanone*	Sweet	48.15	1.02	0.63	0.27	0.51	0.76	1.20	0.37	0.31	0.69	0.25

Please cite this article in press as: C. Cordero, et al., J. Chromatogr. A (2010), doi:10.1016/j.chroma.2010.07.006

mal treatments or as components of vegetable origin (terpenoids) characteristic of the un-roasted hazelnut volatile fraction. Data interpretation can now be based on a limited number of known targets, thus affording a more effective and realistic discrimination process. It is interesting to observe that, with the exception of *features* 10, 17 and 18 (see Table 1 for *feature* numbering), the two fingerprinting approaches gave univocal results in indicating regions whose response variation over the sample set was high in both, chromatographic fingerprinting, and/or template matching of 2D peaks with MS. On the other hand, reliable peak matching provided more definitive results, because it also revealed peaks that were present in few samples (data not shown) representing a valuable qualitative diagnostic tool, in this case identifying marker analytes whose presence could be ascribed to specific geographical origins.

Terpenoids such as α-pinene, sabinene and limonene were detected in all hazelnuts patterns, but β-pinene, δ-3-carene, α- and γ-terpinene, and *trans*-sabinene hydrate were present in few samples and, in particular, δ-3-carene and *trans*-sabinene hydrate showed a high variability. Moreover, it has to be stressed that the reliability of a comparative analysis on samples, whose volatiles distribution is conditioned by several variables: botanical origin, pedo-climatic harvest conditions, post-harvest storage and roasting time/temperature ratios, has to be proved and updated constantly. In this perspective, the fingerprinting procedure appears to be a valuable methodology because of its potential to directly compare samples patterns and easily extract information on analytes distribution, including minor components. Results on technological markers and aroma compounds will be discussed in the next section.

3.2. Sample profiling: aroma and technological markers

Comprehensive template fingerprinting results were also used to define a more specific profile for each sample based on aroma and/or technological marker distribution, to be used as an additional informative tool for sample discrimination. The aim of this extended target analysis of the sample pattern was to see whether the comparatively significant analytes detected by the fingerprinting methods can be correlated to known markers and, in consequence, to sample properties, thus concurring to define their overall quality. Markers were identified on the basis of their linear retention indexes (I^T_S) and MS-EI fragmentation pattern similarity (fixed acceptable value above 850 referred to Identity Spectrum Match factor resulting from the NIST Identity Spectrum Search algorithm – NIST MS Search 2.0) with compounds collected in commercial and in-house databases or, where possible, with authentic standard confirmation.

The extended list of markers in Table 3 consists of: (a) analytes with the highest ranking in the template-based fingerprinting procedure (classification based on decreasing order of SD on average normalized volumes) and (b) analytes whose sensory, technological, and botanical significance is already known [13,15,25].

The results derived from the distribution of aroma markers are interesting. Several potent odorants were detected in the GC × GC patterns of the roasted hazelnuts under study. These compounds, isolated by solvent assisted flavor evaporation (SAFE) extraction from raw and roasted hazelnuts and identified by GC-O, and in particular with the AEDA screening technique [23], showed high flavor dilution (FD) factors indicating their prominent role in defining the characteristic aroma of the final product. This group of odorants, 56 in the raw and 57 in roasted hazelnuts, showed FD factors above 19 and can be defined as "*key-aroma*" compounds [24,25]. Table 3 reports the list of identified analytes together with *feature* numbering (F_i), derived by chromatographic fingerprinting, identification number (#ID), compound name, Odor Quality [40] for the sub-set

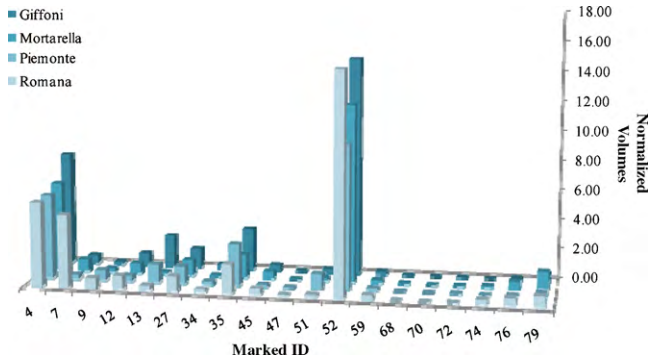

Fig. 4. Key-aroma pattern of the four Italian varieties (i.e., *Romana*, *Giffoni*, *Mortarella*, *Piemonte*) submitted to a standard roasting procedure. Results are reported as normalized 2D-Peak Volume over the ISTD. For analyte ID (*x*-axis) and full data of all investigated samples see Table 3.

of 16 key-aroma markers of roasted hazelnuts (indicated with an asterisk), ^{1}D and ^{2}D retention times and average normalized volumes for the nine geographical origins. Markers were identified on the basis of their linear retention indices (I^T_S) and EI-MS spectra compared to those of authentic standards.

The distribution of potent odorants in the four Italian (i.e., *Romana*, *Giffoni*, *Mortarella*, *Piemonte*), standard roasted hazelnut samples is visualized in the histogram of Fig. 4. This profiling confirms the perceivable differences of the overall sensory impact provided by roasted samples of different origin [18,20,23,41]. In particular: 2- and 3-methylbutanal (*4* and *7*) and 2,3-pentanedione (*12*) concur to define the characteristic malty and buttery notes; 5-methyl-4-heptanone, 5-methyl-(*Z*)-2-hepten-4-one (*27*) and 5-methyl-(*E*)-2-hepten-4-one (filbertone) (*35*) are responsible for the fruity and nutty sensation; hexanal (*13*) and octanal (*34*) are perceived as green and fatty, respectively, while secondary lipid-peroxidation products such as (*E*)-2-heptenal (*38*), (*E*)-2-octenal (*47*), (*E*)-2-nonenal (*59*), (*E*)-2-decenal (*70*), (*E,E*)-2,4-decadienal (*74*) provide fatty sensations. The sweet and caramel like note can be ascribed to the presence of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (*79*), while phenylacetaldehyde (*68*) and 2-phenylethanol (*76*) elicit flowery and honey-like sensations. The highly variable abundance of some markers (e.g., 2- and 3-methylbutanal, hexanal, octanal, nonanal (*45*) and acetic acid (*52*)) is extremely informative of this aroma profiling assessment and provides a further valuable interpretation key for sample discrimination.

Aroma compounds are characterized by a very high concentration variability in roasted samples, ranging from traces (ng/g) to several percent (g/100 g), therefore sample pre-concentration is mandatory for a complete aroma profiling extended to the entire pattern of key-odorants. The literature refers to an average amount in roasted Romana hazelnuts ranging from 7 mg/kg of 3-methylbutanal, the most abundant, to about 2 μg/kg of (*E,E*)-2,4-decadienal [41]. However, thanks to its high sensitivity, GC × GC enabled us to identify and monitor the variation of 16 key-aroma compounds and semi-quantify them by their relative abundance in the sample set. Even though it's well known that HS-SPME is not representative of the "absolute" composition of the volatile fraction of a sample, after a careful standardization of the sampling procedure, it delivers reliable data, also avoiding long and artefact producing chemical treatments [42].

Further interesting groups of markers, useful to evaluate the thermal treatment and/or the post-harvest storage conditions, are compounds formed by the Maillard reaction, the Strecker degradation, and lipid-peroxidation, whose presence can be correlated to known precursors in the raw material. In addition, their abundance reflects the extent of thermal stress or exposure to oxidative conditions. Pyrazines for example, present a homogeneous distribution.

The highest variability was registered for 2,5-dimethylpyrazine (*41*) and 2-ethyl-3,5-dimethyl pyrazine (*51*), while 2,5-diethyl pyrazine (*49*) was detected in only one sample, the Piemonte origin. Despite their high odor thresholds and, as a consequence, low impact on sensory properties, alkyl pyrazines formation can successfully be correlated with the extent of thermal treatments representing a very sensitive tool for technological profiling.

Secondary products of lipid-peroxidation, such as saturated and unsaturated aldehydes can simultaneously provide information on aroma and technological profile. Lipid oxidation strongly affects shelf life and sensory characteristics of hazelnuts and depends on several factors such as the concentrations of unsaturated fatty acids, enzymatic activity, mineral composition, and amount of antioxidants [43,44]. Prolonged storage of hazelnuts induces the formation of volatile off-flavors, short chain fatty acids, and saturated and un-saturated aldehydes, such as hexanal and octanal, the most abundant lipid oxidation products that can increase up to tenfold their original concentrations [45]. The roasting procedure is also a factor promoting lipid oxidation. The homologous series of saturated aldehydes: hexanal, heptanal, octanal, nonanal, and decanal (the latter detected only in few samples) can, therefore, be diagnostic in this perspective, especially, because of their very high variability within the samples investigated. On the other hand, unsaturated aldehydes such as (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal and (*E*,*E*)-2,4-decadienal, present in very low concentrations, were only detected thanks to GC × GC sensitivity, emphasizing its ability to detect trace and minor components and include them in sample profiling. However, it has to be stressed that GC-O screening indicated the homologous series of (*Z*)-alkenals (i.e., (*Z*)-2-octenal, (*Z*)-2-nonenal, (*Z*)-2-decenal) as the highest impacting odorants responsible for the fatty and deep-fried notes in pan-roasted hazelnuts. This unusual behavior was ascribed to the procedure exposing grinded hazelnut to air before roasting, therefore increasing the possibility for unsaturated fatty acids to react with oxygen [23]. Industrial roasting, performed on fruits protected by kernel, reduces the exposure of fatty fraction to oxidative, degradation and, consequently, reduces the formation of (*Z*)-alkenals.

Aroma and technological marker profiles, extended to a wide range of analytes, are undoubtedly two very powerful diagnostic tools enabling correlation between quality descriptors (aroma and sensory properties) and process variables (post-harvest storage conditions, roasting treatment). Roasted hazelnut volatiles are a challenging fraction to evaluate how fingerprinting methods can guide towards a more profitable speciation of samples, improving the effectiveness of GC × GC targeted analysis.

4. Conclusions

Fingerprint analysis, whose results are based on the degree of similarity with a reference template, showed to be effective for sample comparison and classification of roasted hazelnuts. *Chromatographic fingerprinting*, in particular, was (a) effective as a "screening" method to locate informative relevant regions on the separation space, (b) versatile for processing of single channel detectors patterns (GC × GC-FID, GC × GC, GC × GC-ECD, etc.) and (c) less time consuming since the automatic processing of raw data took less than 1 min for each chromatogram. It may incompletely delineate features, but may have fewer mismatched features. Feature matching was constrained by retention times and MS fragmentation patterns to obtain consistent correspondences only for those analytes whose spectra referred a fixed degree of similarity with the corresponding template spectrum. The reliable peak matching procedure, implemented in the *comprehensive template matching fingerprinting* approach, enabled a successful screening of 2D peak distribution over the sample set, and the extraction of consistent information on analytes that were present in all or a few samples, suggesting the possible discrimination roles they can play in the comparative process. The cumulative matching results (percent matching) obtained with this approach showed, in fact, better specificity and sensitivity in discriminating samples differing for geographical origin than those obtained with chromatographic fingerprinting. The main limit concerns mismatching for those template peaks whose reference MS spectrum is qualitatively unacceptable (intensity below a given S/N) and, as a consequence matching values below the expected threshold.

Fingerprint analysis is an important tool to extend the informative potential of GC × GC; in particular in the flavor field, the fingerprint-assisted investigation of the distributions of known and unknown markers of a vegetable matrix can be very useful for the definition of the so-called *product signature* in terms of sensory properties, botanical/geographical origin and/or to study the modifications induced by thermal treatments on primary and secondary metabolites.

Acknowledgments

This research was carried out within the project entitled: "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).

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