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DOI:10.1007/s00216-017-0832-6

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Journal:	*Analytical and Bioanalytical Chemistry*
Manuscript ID	Draft
Type of Paper:	Research Paper
Date Submitted by the Author:	n/a
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Keywords:	comprehensive two-dimensional gas chromatography, hazelnuts Corylus avellana L., volatile metabolome, post-harvest practices, storage conditions, potent odorants

Evolution of potent odorants within the volatile metabolome of high-quality hazelnuts (*Corylus avellana* L.): evaluation by comprehensive two-dimensional gas chromatography coupled with mass spectrometry

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Abstract

Within the pattern of volatiles released by food products (volatilome), potent odorants are bio-active compounds that trigger aroma perception by activating a complex array of odor receptors (ORs) in the *regio olfactoria*. Their informative role is fundamental to select optimal post-harvest and storage conditions and preserve food sensory quality.

This study addresses the volatile metabolome from high-quality hazelnuts (*Corylus avellana* L.) from Ordu region (Turkey) and Tonda Romana from Italy, and investigates its evolution throughout the production chain (post-harvest, industrial storage, roasting) to find functional correlations between technological strategies and product quality.

The volatile metabolome is analyzed by headspace solid-phase microextraction combined with comprehensive two-dimensional gas chromatography and mass spectrometry. Dedicated pattern recognition, based on 2D data (targeted fingerprinting), is used to mine analytical outputs, while principal component analysis (PCA), hierarchical clustering, and analysis of variance are used to find *decision makers* among the most informative chemicals.

Low-temperature drying (18-20°C) has a decisive effect on quality; it correlates negatively with bacteria and mould metabolic activity, nut viability, and lipid oxidation products (2-methyl-1-propanol, 3-methyl-1-butanol, 2-ethyl-1-hexanol, 2-octanol, 1-octen-3-ol, hexanal, octanal and (*E*)-2-heptanal). Protective atmosphere storage (99% N_2-1% O_2) effectively limits lipids oxidation for 9-12 months after nut harvest.

The combination of optimal drying and storage preserves the aroma potential; after roasting at different shelf-life, key-odorants responsible for *malty* and *buttery* (2- and 3-methylbutanal, 2,3-butanedione and 2,3-pentanedione), *earthy* (methylpyrazine, 2-ethyl-5-methyl pyrazine and 3-ethyl-2,5-dimethyl pyrazine) and *caramel-like* and *musty* notes (2,5-dimethyl-4-hydroxy-3(2H)-furanone - furaneol and acetyl pyrrole) show no significant variation.

Key-words

comprehensive two-dimensional gas chromatography; hazelnuts *Corylus avellana* L.; volatile metabolome; post-harvest practices; storage conditions; potent odorants

1. Introduction

The chemical fingerprint of a food sample can be used to correlate the distinctive distribution of components (primary and secondary metabolites, products generated by thermal treatments and/or enzymatic activity) present in the raw material and/or formed during post-harvest practices, storage, and transformation processes. When properly characterized by complementary analytical techniques, these chemicals can be treated comprehensively by pattern analysis, to establish functional correlations with biological properties. This approach, adopted by system biology [1], is appropriate for the modern *omics* strategies, combining multidimensional techniques, *sensu latu*, for the productive global investigation of food (i.e., foodomics) [2]. Moreover, when the analysis addresses the sensory-active compounds responsible for multimodal perceptions (aroma, taste, texture, etc.) sensomics becomes the reference discipline, and its protocols provide a rationale for productive and conclusive investigations [3].

Aroma perception is triggered by food volatiles, usually hydrophobic, some of which are present at trace levels (mg/Kg to µg/Kg). These chemicals interact with the complex array of Odorant Receptors (ORs) expressed by Olfactory Sensory Neurons (OSNs) in the olfactory epithelium [3–5]. Perception is thus the result of the simultaneous activation of ORs generating a complex pattern of signals (i.e., the Receptor Code) sent to the central nervous system. The chemical characterization of OR ligands is thus fundamental to understand the chemical code underlying olfactory perception, and to objectify food aroma evaluation.

This study focuses on the volatile metabolome of hazelnuts (*Corylus avellana* L.) [6–9] as it is generated and modified along the production chain before industrial processing. Fresh, shelled, unroasted hazelnuts have a distinctive signature of volatiles related to the cultivar(s) and the geographical origin [6, 10–13]; post-harvesting practices, such as drying and storage, have a further impact on the volatile metabolome, providing information about oxidative status (photo-chemically and/or enzymatically driven), the development of moulds and bacteria, and nut viability/germination [14]. Thus the volatile metabolome can be mined to better understand the chemical code behind hazelnuts' overall quality [6, 13, 15, 16].

Among volatiles, potent odorants are of great interest for the confectionery industry: positive and pleasant odors, as well as off-odors, contribute to defining a distinctive aroma profile.

Multidimensional analytical platforms support comprehensive investigations of the volatile metabolome by combining: (a) effective gas chromatographic (GC) separations based on a single, or a combination of different, discrimination probes (e.g. volatility, polarity, and partition coefficient), (b) Mass Spectrometry (MS) for identification and quantitation; and (c) olfactometric detection, whereby human assessors detect odor-active compounds as they elute from a GC column [17–19]. In particular, comprehensive two-dimensional GC (GC×GC) coupled with MS detection is the technique that currently offers the highest separation power and sensitivity, fundamental for detailed profiling and accurate fingerprinting of volatiles, while also including most of the odor-active (aroma) compounds that are closely related to the perceivable quality of food [20, 21].

This study aims to find reliable correlations between some key independent variables (botanical and geographical origin, post-harvest practices, and storage) and odor-active volatiles that may affect product quality. Thanks to the power of GC×GC-MS for detailed chemical profiling, within the complex hazelnut volatile metabolome, potent odorants are mapped and their peculiar distribution adopted as a fingerprinting tool. Volatile organic compounds (VOCs) fingerprints are mined to monitor the evolution of potent odorants as a function of hazelnut origin, post-harvest practices, storage conditions, and shelf-life. The aroma potential is evaluated by applying standardized lab-scale roasting to develop characteristic odorants, as a function of the distribution of non-volatile precursors in the raw material.

2. Materials and methods

2.1 Reference standards and solvents

Pure reference compounds for key-odorant identity confirmation were purchased from Sigma-Aldrich (Milan, Italy); they are listed in **Table 1** and connoted by an asterisk. The homologue series of *n*-alkanes (from *n*-C9 to *n*-C25) for Linear Retention Index (I^T_s) determination were also from Sigma-Aldrich (Milan, Italy). Solvents (toluene and n-hexane) were all HPLC-grade, from Sigma-Aldrich (Milan, Italy).

2.2 Hazelnut samples

Commercial samples of raw hazelnuts (*Corylus avellana* L.) from the 2014 harvest, with a selected caliber of 13-14 mm, were supplied by Soremartec Srl (Alba-CN, Italy). Samples included the mono-cultivar *Nocciola Romana* (*TR*), also known as Tonda Gentile Romana, a Protected Denomination Origin - PDO product (EU Quality registration code IT/PDO/0005/0573), and a Turkish blend harvested in the *Ordu* region made up different cultivars, predominantly *Tombul, Palaz,* and *Çakildak*.

Hazelnut samples, with an average kernel humidity of 25%, were collected in-field immediately after their optimal harvest, and submitted to two different drying processes (D1 and D2) in order to reach a final kernel humidity of 6%, a condition that keeps the product stable throughout its shelf-life. D1 consisted of traditional procedures. For nuts of Ordu origin, consisting of long husk varieties, nuts were husked and dried in shell at ambient temperatures between 30-35°C during summer. For TR - D1, a short husk variety that does not require husking, the nuts were dried in shell at 35-38°C in artificial driers, to mimic the traditional procedure. The TR-D2 procedure consisted of lower temperature drying, at 18-20°C in artificial driers.

Storage was under controlled temperature (5 and 18°C ± 0.1) and atmosphere (regular atmosphere - NA: 78% N_2-21% O_2 or modified atmosphere - MA 99% N_2-1% O_2) with 65% of ERH (equilibrium relative humidity). **Table 1** summarizes sample characteristic and sample acronyms.

Samples stored for 4, 9 and 12 months were roasted in lab-scale conditions with hot-air ventilation to evaluate the aroma potential of hazelnuts throughout their shelf-life. Time and temperature followed a previously optimized protocol, which ensured the development of a pleasant aroma, taste, brown color, and crunchy texture [22, 23]. In particular, 40.0 ± 0.05 grams of shelled nuts of uniform size were roasted at 160°C for 15 minutes. Roasting was conducted in two replicate batches (batch #1 and #2) and samples immediately frozen with liquid nitrogen to stop thermal reactions and avoid any possible loss of volatiles. Frozen hazelnuts were stored at -80°C if not analyzed immediately.

2.3 Headspace Solid Phase Microextraction (HS-SPME) devices and sampling conditions

Automated HS-SPME sampling was run on a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) installed on the GC×GC-MS systems. SPME fibers, Divinylbenzene/Carboxen/ Polydimethyl siloxane (DVB/CAR/PDMS) d_f 50/30 µm - 2 cm, were from Supelco (Bellefonte, PA, USA). Fibers were conditioned before use as recommended by the manufacturer.

The ISTD (α-thujone) used for peak response normalization was pre-loaded into? onto? the SPME fiber before sampling, by exposing the extraction device (i.e. the SPME fiber) to 5 µL of ISTD standard stock solution for 20 minutes at 50°C [24].

Raw and roasted hazelnuts were frozen before milling, using liquid nitrogen, to ensure uniform particle size distribution. Samples were weighed exactly (1.500 ± 0.001 g) in glass headspace vials (20 mL) and submitted to headspace extraction for 40 minutes at 50°C.

2.4 GC×GC-MS instrument set-up

The GC×GC system consisted of an Agilent 7890B GC coupled to an Agilent 5975C fast quadrupole MS detector (Agilent, Little Falls, DE, USA) operating in EI mode at 70 eV. The GC transfer line was set at 280°C. The MS was tuned using the Autotune (*Atune*) option. The scan range was set to m/z 40-240 with a scanning rate of 12,500 amu/s to obtain a spectra generation frequency of 28 Hz.

Injections for I^T_S determination were carried out with the MPS-2 auto sampler under the following conditions: injection mode split, split ratio 1:40, injection volume 1 µL, and injector temperature 270°C.

Fiber thermal desorption into the GC injector port was under the following conditions: split/splitless injector in pulsed split mode, and split ratio 1:5.

The system was equipped with a two-stage KT 2004 loop thermal modulator (Zoex Corporation. Houston. TX) cooled with liquid nitrogen and controlled by Optimode™ V.2.0 (SRA Instruments, Cernusco sul Naviglio, Milan, Italy). Hot jet pulse time was set at 250 ms, modulation period (P_M) was 4 s; the cold-jet total flow was progressively reduced with a linear function from 40% (12.5 L/min) of Mass Flow Controller (MFC) at initial conditions to 5% at the end of the run. A deactivated fused silica capillary loop (1 m × 0.1 mm d_c) was installed in the modulation slit.

The column set was configured as follows: ^{1}D SolGel-Wax column (100% polyethylene glycol) (30 m × 0.25 mm d_c, 0.25 µm d_f) coupled with a ^{2}D OV1701 column (86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl) (1 m × 0.1 mm d_c, 0.10 µm d_f). The ^{1}D column was from SGE (Melbourne, Australia) whereas the ^{2}D column was from Mega (Legnano, Milan, Italy).

The carrier gas was helium, at a constant flow rate of 1.5 mL/min (initial head pressure - relative was 251 KPa). The oven temperature program was: 40°C (1 min) to 190°C at 3.0°C/min and to 260°C at 50°C/min (10 min).

Data were acquired by an Agilent MSD ChemStation version D.02.00.275 and processed using GC Image GC×GC Software version 2.7 (GC Image, LLC Lincoln NE, USA).

2.5 Targeted profiling by pattern recognition approaches

Targeted profiling was carried out by the *template matching* approach, introduced by Reichenbach and co-workers in 2009 [25] and successfully adopted to investigate the chemical complexity of several food commodities [26–28]. The approach uses metadata collected from 2D peak patterns (retention times, MS fragmentation patterns, retention indexes, and detector responses) and establishes reliable correspondences between the same chemical entities across multiple chromatograms. The output is a data matrix of aligned 2D peaks and related metadata (^{1}D and ^{2}D retention times, compound names, fragmentation pattern, and single ion and/or total ion response) that are available for comparative purposes and further processing.

Targeted analysis focused on 133 compounds identified by matching their EI-MS fragmentation patterns (NIST MS Search algorithm, ver 2.2, National Institute of Standards and Technology, Gaithersburg, MD, USA, with Direct Matching threshold 900 and Reverse Matching threshold 950) with those collected in commercial (NIST2014 and Wiley 7n) and in-house databases. As a further check on identification, experimental Linear Retention Indices (I^{T}_{S}) were computed and compared to the tabulated indices.[29]

3. Results and discussion

The following sections deal with: (*a*) raw hazelnut volatile fraction composition and its evolution as a function of key variables related to harvesting practices and industrial storage; (*b*) roasted hazelnut volatile signature and its evolution as a function of storage conditions, with emphasis on potent odorant evolution; (*c*) the ways in which GC×GC-MS could offer prompt and effective pattern recognition tools, based on visual features, to monitor fingerprint changes.

3.1 Raw hazelnuts: influence of drying and storage conditions on odor active compound signature

A comprehensive and informative investigation (i.e., profiling) of volatiles should provide an effective and unbiased mapping of all detectable analytes, including potent odorants, secondary products of lipid oxidation, and compounds deriving from reactions (enzymatically catalyzed or not) occurring on non-volatile precursors in consequence of post-harvest and storage conditions.

In this study, volatile sampling conditions and tools were set to achieve a sensitivity appropriate for most of the potent odorants describing the main aroma notes, while maintaining the complexity, and thus the informative power of the sampled volatile. Raw hazelnuts from Ordu region (Turkey) and Tonda Romana (Italy) were described by the 133 known volatiles listed in **Table 2**. **Figure 1A** is an illustrative 2D pattern from TR D1 raw hazelnuts.

Insert Figure 1 here

A preliminary explorative Principal Component Analysis (PCA) was run on the entire dataset from raw hazelnuts (133 targets × 110 samples) to map the natural conformation of sample groups and sub-groups. Results are shown in **Figure 2A** as score plot on the first two Principal Components (F1 and F2) accounting for the 44.35% of the total variance. The two most relevant variables driving sample clustering are origin (botanical/geographical) and drying process. Tonda Romana samples (purple and green indicators - TR D1 and TR D2) are grouped independently of those from Turkey (O D1). Confidence ellipses inform about the influence of some latent variables, like storage conditions and time.

Within Italian samples, the effect of drying (18°C - D1 vs. 45°C - D2) is clear, as the two groups cluster independently and are well separated along both PCs.

Insert Figure 2 here

The wider dispersion of Ordu samples (blue indicators in **Fig. 2A**) could reasonably be explained by their lack of uniformity, since the blend is composed of different cultivars. The distribution of samples along F1 indicates a positive correlation of this PC with storage time.

Supervised Discriminant Analysis (DA), driven by post-harvest drying (D1 *vs.* D2), was the next data mining step, with the aim of selecting those volatiles with greater informing power concerning the drying process. DA was run on all samples, independently on their origin. Analytical replicates were kept, removed from the training set, and included in the validation set to verify model adequacy. The confusion matrix for the estimation samples gave 100% correctness, as did that of the validation samples. The results indicate the most informing variables, with $p < 0.0001$ and Fisher ratio between 202 and 22, as being a series of linear and branched alcohols (2-heptanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-ethyl-1-hexanol, benzyl alcohol), esters (ethyl acetate, butyl butanoate, 2-methyl-butyl propanoate) and acetic acid.

Most of these compounds have been correlated with nut ripening and/or fermentation processes occurring in vegetables [30]. For instance, 3-methyl-1-butanol (i.e., isoamyl alcohol) is a fermentation product in grapes and wines, where it is formed from L-leucine, and 2-methyl-1-propanol has L-valine as precursor [31]. 2-Heptanol is formed during tomato ripening, from β-ketoacids hydrolysis and subsequent decarboxylation [32], while 2-ethyl-1-hexanol has been found in fermented soybean foods [33].

Raw hazelnut aroma is described as the combination of different notes: *fruity*, *nutty*, *green*, *citrus-like*, *earthy*, *flowery*, *malty*, *popcorn-like*, *potato-like*, *sour,* and *phenolic* [11, 13]. Key odorants responsible for these notes were characterized by sensomics on the basis of their relevance through the odor activity value (OAV) [3, 10]. They are: hexanal (*green, grassy*), octanal (*soapy*), acetic acid (*sour*), linalool (*flowery*), 2 and 3-methylbutanal (*malty*), 5-methyl-(*E*)-2-hepten-4-one (i.e. filbertone) and 5-methyl-(*Z*)-2-hepten-4-one (*nutty, fruity*), 2-acetyl-1-pirroline (*popcorn-like*), 3,6-dimethyl-2-ethyl pyrazine and 3,5-dimethyl-2-ethyl pyrazine (*earthy*, *roasty*), 2,3-butanedione and 2,3-pentanedione (*buttery*), and phenylacetaldehyde (*honey*, *flowery*).

If the investigation is limited to potent odorants, the results are still consistent and confirm most of the above observations. Potent odorants were selected on the basis of their odor thresholds (OT) within the entire dataset of 133 targeted 2D peaks. Reference data on OT were collected from the existing literature and, when possible, were referred to orthonasal perception from fatty matrices (oil). **Table 2** reports published data and the relative reference papers. The dataset was reduced to 37 analytes (odorants) so that the resulting data matrix dimension was 37 × 110 (samples). The resulting PCA is illustrated in **Figure 2B;** the total explained variance rose to 61.91%, with sample sub-classification that confirmed the dominant role of drying conditions above origin. Ordu (O D1) and Tonda Romana (TR D1) samples submitted to conventional drying (blue and green indicators) now overlap, and storage time (samples spreading along F1) prevails over hazelnut origin. As already observed for the entire dataset, storage time is still positively correlated with F1.

The most potent odorants (OT values up to 2500 µg/L) correlated closely (> 0.800) with storage time were: 1-heptanol (*green*, *chemical*), 2-octanol (*metal*, *burnt*), 1-octen-3-ol (*mushroom*), (E)-2-heptenal (*fatty*, *almond*), hexanal (*leaf-like*, *green*), heptanal (*fatty*), octanal (*fatty*) and nonanal (*tallowy*, *fruity*). The histograms in **Figure 3** illustrate the evolution over time of these components, as a function of storage atmosphere (normal - NA or modified - MA) and temperature (5°C and 18°C). Analyte relative abundance was normalized over values obtained from raw hazelnuts, analyzed at time zero (T0). An arbitrarily fixed value of 100 counts was assigned for those analytes that reported an instrumental response below the Limit of Detection (LOD).

Insert Figure 3 here

As a general consideration, all analytes showed increasing trends over time, with maximum values at 12 months post-harvest. Secondary products of lipid oxidation (hexanal, octanal and (*E*)-2-heptanal) connoted by *fatty* and *green-leafy* odors are well known markers of hazelnut storage quality [34, 35] and their increase was thus expected. In Tonda Romana samples subjected to drying process D2 - TR D2 (45°C up to 6% of moisture), their evolution/formation over time was very limited: on average, at 12 months the relative abundance of hexanal and octanal was respectively 2.6 and 2.8 times lower compared to standard drying (D1) of the same product. (*E*)-2-heptenal and heptanal (data not shown) were present in TR D2 samples at levels below the method LOD in all cases.

Eight-carbon-atom alcohols, 2-octanol and 1-octen-3-ol, are known products of linoleic acid cleavage, which are generally promoted by fungal lipoxygenase/hydroperoxide liase enzymes [36]. With the exception of 1-octen-3-ol, which was not detected in Tonda Romana hazelnuts and was below the LOD in TR D2 samples, the increasing trend of these alcohols was quite informative, and might be correlated to the occurrence of off-odors related to *metallic* and *mushroom*-like notes.

The experimental results on raw hazelnuts clearly indicated the decisive effect of post-harvest drying conditions on volatile distribution and evolution over time. Interestingly, within the entire set of detectable analytes, those with high informative power were not potent odorants (OTs above 2500 µg/L) but known products of the metabolic/enzymatic activity of bacteria and moulds. If the fingerprinting potential is limited to odor-active analytes, post-harvest drying still dominates sample sub-classification, and blurs the signature of botanical/geographical origin. Among potent odorants, secondary products of hydroperoxide cleavage were very informative. This interesting outcome evokes the interesting hypothesis that important flavor-related volatiles in vegetable food are derived from essential nutrients and healthpromoting compounds, including amino acids, fatty acids, and carotenoids [37]. The development of unpleasant odors, such as those deriving from the oxidative cleavage of linoleic and oleic acids, could thus be related to a loss of nutritional value.

3.2 Aroma potential and volatile fingerprint evolution over time

The next step dealt with profiling volatiles and potent odorants in fresh and stored hazelnuts, after roasting in standardized conditions. This part of the study was motivated by the requirements of the confectionery industry, which needs to process high-quality hazelnuts all year regardless of the harvest season. Since in the case of raw hazelnuts drying and storage played decisive roles in defining distinctive signatures of volatiles, a similar effect was expected on the precursors that react and develop characteristic patterns of VOCs under thermal stress conditions (roasting) [13, 16, 22, 23, 28, 38, 39].

Roasting induces several chemical reactions that produce a complex array of compounds, and the volatile metabolome is enriched by moderate-to-high polarity chemicals, namely alcohols, aldehydes and ketones, acids, esters and lactones, sulphur derivatives, together with several heterocycles (furans, pyrazines, pyrroles, thiophenes, aromatic compounds, phenols, pyridines, thiazoles, oxazoles). These compounds combine to define the characteristic hazelnut flavour [7, 11, 12, 15, 16, 27, 40, 41].

Odor notes characterizing roasted hazelnuts are due to the presence of: 2-acetyl-1-pyrroline, 2-propionyl-1-pyrroline, 2-acetyl-1,4,5,6-tetrahydropyridine, and 2-acetyl-3,4,5,6-tetrahydropyridine (*roasty*, *popcorn-like*); 3,6-dimethyl-2-ethylpyrazine, 3,5-dimethyl-2-ethylpyrazine, and 2,3-diethyl-5-methylpyrazine (*earthy*); filbertone and 3-methyl-4-heptanone (*nutty*, *fruity*); 4-ethenyl-2-methoxyphenol, 2-methoxyphenol (*smoky*, *clove-like*); 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 4-hydroxy-3-methoxybenzaldehyde (*sweet*, *caramel-like*); (*E,E*)-2,4-decadienal (*fatty*); hexanal (*green, grassy*); 2-phenylacetaldehyde (*honey-like*); 3-methylthio-propionaldehyde (*potato-like*); 2- and 3-methyl butanoic acid (*sweaty*) odours.

Raw hazelnuts were thus roasted after storage (T0, T4, T9 and T12) under specific atmosphere/temperature conditions. Roasting conditions were defined on the basis of previous work [22] and carried out under mild conditions (160°C for 15 minutes) in a ventilated oven, to facilitate differentiation between samples.

2D patterns from roasted samples were connoted by more abundant volatiles and distinctive chemical classes formed by Maillard reactions, sugar degradation, and heat-triggered lipid oxidation [10, 27, 28, 38, 39]. **Figure 1B** shows the 2D plot of a Tonda Romana sample roasted immediately after drying at T0; structured patterns of homologue series and classes are highlighted.

The data set of aligned 2D peaks (133 target peaks × 110 samples) was submitted to an explorative PCA, shown in **Figure 2C**. The first two principal components explain 40.2% of the total variance; sample sub-classification confirms previous tendencies observed in raw hazelnuts: the drying process contributes to defining a clear and distinctive signature of volatiles that prevails over that of botanical/geographical origin. Tonda Romana D1 (TR D1 - green indicators) and Ordu D1 (OR D1 - blue indicators), although minimally overlapping, are closer (along F1 with 25.71% over 40% of the total variance) compared to the TR D2 cluster. Interestingly, roasting has a different impact on the two origins: raw samples from the Ordu region were widely spread across the Cartesian plane (**Fig. 2A**) indicating the presence of some other latent variables influencing the volatile distribution (i.e. storage conditions and timing) while, after roasting, the VOC signature appeared more uniform and samples were more closely grouped. Inversely, after roasting, TR D1 samples appeared widely spread across the Cartesian plane. Within latent variables, normal atmosphere storage and timing are those explaining the distribution of samples along F2 (red dotted lines indicate normal and modified-atmosphere samples).

As was done in the case of raw hazelnuts, the most potent odorants were selected within the set of 133 known volatiles, and a further PCA was conducted on the resulting data matrix (70 × 110 - odorants × samples). **Figure 2D** shows the loadings plot based on the first two components (F1 and F2) accounting for 45.6% of the total variance. The storage atmosphere was included as supplementary variable (Normal NA or Modifies MA) in addition to the origin and drying process (D1 and D2). Confidence ellipses (95% of confidence level) delineated with dotted lines include samples stored in MA, while continuous lines indicate those stored in NA.

The results clearly show the marked effect of storage atmosphere on the odorant fingerprint; this variable prevails over the others (origin and drying) and, above all, has a decisive role in minimizing volatile distribution differences throughout storage time. This preliminary data provide convincing indications concerning possible strategies for optimal storage, aimed at preserving hazelnut aroma quality before and after roasting.

The next point that was investigated concerned the effect of drying and of storage atmosphere on hazelnut aroma potential. Briefly, the issue concerns the evolution of selected odorants, responsible for aroma notes, when hazelnuts are subjected to a standard roasting procedure after different storage times. This interesting point arises from the observation that, with mild drying and/or a less preservative storage atmosphere, volatiles from raw hazelnuts provide information about extensive enzymatic activity (native or exogenous enzymes) and autoxidation reactions. In consequence, it was expected that there would be an effect on the relative distribution of known Maillard reaction and Streker degradation products, as the result of the depletion of their main precursors, namely fructose, glucose, sucrose, and several L-aminoacids in the raw nuts.

The results from Tonda Romana samples subjected to D1 drying are of particular interest to verify this hypothesis. The subset of samples includes, as independent variables, storage atmosphere (NA and

MA), temperature (5 and 18°C) and timing (0, 4, 9 and 12 months). Potent odorants are represented as a heat-map, and subjected to hierarchical clustering based on Euclidean distances, to locate chemical variables with similar/dissimilar behavior. The heat-map in **Figure 4** illustrates the relative abundance of the 70 selected odorants within the sample set; colors indicate abundance, from blue (low) to red (high). The Normalized 2D Volumes were set to percentage; data were averaged and centered in rows.

Insert Figure 4 here

A first group of variables, clustered together in two steps, is related to the autoxidation of the lipid fraction (clusters are marked with the $ symbol in **Fig. 4**). Linear saturated aldehydes (from C5 to C10), unsaturated aldehydes ((*E*)-2-heptenal, (*E*)-2 octenal ad (*E*)-2 decenal), short chain fatty acids (pentanoic, octanoic and nonanoic acid) and linear alcohols (from C5 to C8) are all secondary products of hydroperoxide cleavage [42]. Their presence is negligible in freshly roasted hazelnuts and in those stored in a modified atmosphere (MA), but in samples stored in a normal atmosphere (NA) they increase over storage time, also depending on temperature (5 or 18°C) and shelf-life, as additional stress factors. Conversely, odorants already present in freshly roasted hazelnuts (T0, first two columns) cluster together (symbol £) and show an increasing trend (predominance of white to red spots) throughout shelf-life. The only exception is for normal atmosphere (NA) and ambient temperature (i.e., 18°C) storage, when their relative abundance (compared to the entire fingerprint) decreases. Fingerprint changes can be tracked on 2D-plots of **Figures 1 A-C**. In particular, **Fig. 1C** clearly shows the increased complexity of the volatile metabolome, when storage and roasting exert their concurrent effects.

These interesting outcomes were also confirmed for those key-odorants (marked with an asterisk in **Fig.4**) responsible for the *malty* and *buttery* (2- and 3-methylbutanal, 2,3-butanedione and 2,3-pentanedione), *earthy* (methylpyrazine, 2-ethyl-5-methyl pyrazine and 3-ethyl-2,5-dimethyl pyrazine) and *caramel-like* and *musty* notes (2,5-dimethyl-4-hydroxy-3(2H)-furanone - furaneol and acetyl pyrrole). They did not show any significant depletion during storage at lower temperatures (5°C) in either Tonda Romana or Ordu samples subjected to D1 drying. This might be due to the stable distribution of their precursors throughout shelf-life. In TR D2 samples, *nutty* odorants (5-methyl-(*Z*)-2-hepten-4-one and 5-methyl-(*E*)-2-hepten-4-one, filbertone), and in particular the *Z* isomer, showed an increasing trend over time, resulting in a more intense perception of this characteristic note.

Heat-maps corresponding to TR D2 and OR D1 samples are provided as supplementary material (Supplementary Figures SF1 and SF2). It is of note that the TR D2 samples show more uniform fingerprints, stress factors have less impact on VOC precursors, and several odorants related to off-flavor notes (e.g., γlactones and secondary products of lipid oxidation) were not detected (grey spots on the heat-map). These data are in good agreement with the PCA results given in **Fig. 2C** and **2D**.

3.3 Visual features fingerprinting

Visual features fingerprinting [43] was then applied as an additional tool to investigate pattern changes. This procedure tracks chemical changes on pre-processed 2D chromatograms, providing information on both pre-targeted and non-targeted 2D peaks across the pattern. This specific approach offers a direct comparison between 2D data points (e.g. single scans from fast quadrupole MS detection) while keeping all metadata information (i.e. compound names, retention times, MS fragmentation pattern and detector response). Metadata are fundamental to identify analytes subjected to quantitative variations. **Figures 5A** and **5B** show differential images obtained by computing TR D1 (Fig. 5A) and TR D2 (Fig. 5B) samples (*analyzed* images) stored for 9 months at 18°C in a normal atmosphere. 2D patterns corresponding to freshly roasted hazelnuts (T0) after D1 and D2 drying respectively were taken as *reference*.

Insert Figure 5 here

The pattern differences in Fig. 5 are computed as *colorized fuzzy ratio* rendering (GC Image v. 2.7), which uses the Hue-Intensity-Saturation (HIS) color space to color each pixel in the retention-time plane. The algorithm computing the difference at each data point, between the two aligned images, colors pixels indicating positive detector differences, and thus larger detector responses in the *analyzed* image, in green (TRD1_NA_18°C_T9 or TRD2_NA_18°C_T9). Red colored pixels indicate negative differences, and thus larger responses in the *reference* image (TRD1_T0 and/or TRD2_T0)). Brightness depends on the size of the difference, while white saturation indicates pixels where peaks have detector responses that are almost equal in the analyzed and reference images.

Fig. 5A shows very clear signatures of homologue series of secondary products of hydroperoxide cleavage (green pixels or peak-regions): linear saturated and unsaturated aldehydes, linear alcohols and short-chain fatty acids, from C6 to C9. Although less structured in the chromatographic space, low-molecular-weight ketones are also present in the T9 sample, with some potent odorants imparting negative odor notes. Conversely, alkyl-pyrazines (red pixels or peak-regions) are more abundant in freshly roasted samples, as are some other analytes easily retrieved from the heat-map in **Fig. 4**.

Not surprisingly, TR hazelnuts subjected to D2 drying have a very stable signature of volatiles; **Fig. 5B** shows few compositional differences for monoterpenoids, more abundant in the T9 sample, and for phenylacetaldehyde, which is less abundant when hazelnuts are roasted after 9 months of storage at 18°C in a normal atmosphere.

4. Conclusion

This study has systematically investigated the direct and indirect effects of some functional variables related with post-harvest management of hazelnuts that impacts the perceived aroma quality. From an industrial perspective, post-harvest drying and storage conditions (storage atmosphere, temperature, and timing) have been related to VOCs profile(s) treated as decision maker.

In particular, the effects of drying and storage atmosphere have been clarified, and related to nut viability and lipid fraction degradation, by interpreting the chemical information encrypted in raw hazelnut VOCs fingerprint and its evolution over time. The sample fingerprint of potent odorants informs about odor qualities and defects arising from inadequate storage practices.

As conclusive step, evaluation of the aroma potential, i.e. the actual development of potent odorants characterizing roasted hazelnut aroma, also provides indirect information about the impact of manufacturing practices on non-volatile precursors. Drying appears fundamental to inactivate enzymatic activity (exogenous and endogenous enzymes), leading to products that are more stable throughout their shelf-life, independently of storage atmosphere, temperature, and timing. With the same drying conditions (D1), samples of different origins (TR *vs.* OR) show similar VOCs patterns when stored in a more protective atmosphere (MA), while they differ significantly with storage at ambient temperature (NA - 18°C), providing a proof-of-concept for the rational management of industrial storage.

The possibility of mapping the evolution of the volatile fingerprint comprehensively, through sensitive and highly informative analyses, enables further dimensions of information to be exploited, and provides evidence of quality changes during products' shelf-life.

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Figure Caption

Figure 1. 2D patterns from Tonda Romana (TR) hazelnuts subjected to conventional drying (D1). **1A** raw hazelnuts immediately after harvest (T0); **1B** roasted hazelnuts obtained from fresh raw nuts (for roasting conditions see Experimental section); **1C** roasted hazelnuts obtained from nuts stored in normal atmosphere (NA - 78% N_2-21% O_2) and 18°C for 12 months.

Figure 2. Explorative Principal Component Analysis (PCA) results on hazelnut targeted analytes. Fig. 2A shows the score plot of the first two Principal Components (F1 and F2) from raw hazelnuts Normalized 2D Peak Volumes (133 targets × 110 samples); in Fig. 2B the processing is based on a selection of 37 potent odorants from among 133 targeted analytes. Fig. 2C PCA refers to the roasted hazelnut dataset, and includes all detected volatiles (133 targets × 110 samples), while 2D reports the results limiting the dataset to about 70 potent odorants.

Figure 3. Histograms illustrating the evolution over time of informative analytes closely correlated with product sensory quality, as a function of storage atmosphere (normal - NA or modified - MA) and temperature (5°C and 18°C). Analytes' relative abundance is normalized to values obtained at time zero (T0). An arbitrarily fixed value of 100 counts is assigned to analytes having an instrument response below the Limit of Detection (LOD).

Figure 4. Heat-map illustrating, from blue (low values) to red (high), the relative abundance distribution of 70 potent odorants from Tonda Romana (TR) hazelnuts subjected to conventional drying (D1), different storage conditions (NA and MA), and roasted at different stages of their shelf-life. The Normalized 2D Volumes are set to % and data averaged and centered in rows. Hirarchical clustering is based on Euclidean distances.

Figure 5. Visual feature fingerprinting represented by differential images, obtained by computing Tonda Romana TR D1 (Fig. **5A**) and TR D2 (Fig. **5B**) samples (*analyzed* images) stored for 9 months at 18°C in a normal atmosphere. As *reference* 2D patterns, those corresponding to freshly roasted hazelnuts (T0) after D1 and D2 drying, respectively, were taken.

Table Captions

Table 1: summary of sample characteristics, with acronyms used in the text.

Table 2: list of targeted analytes together with their retention times ($^{1}t_R$, $^{2}t_R$), 1D linear retention indexes (I^T_S), odor quality descriptors, and odor thresholds (OT µg/L) in oily matrices. References are given at the foot of the table.

Table 1

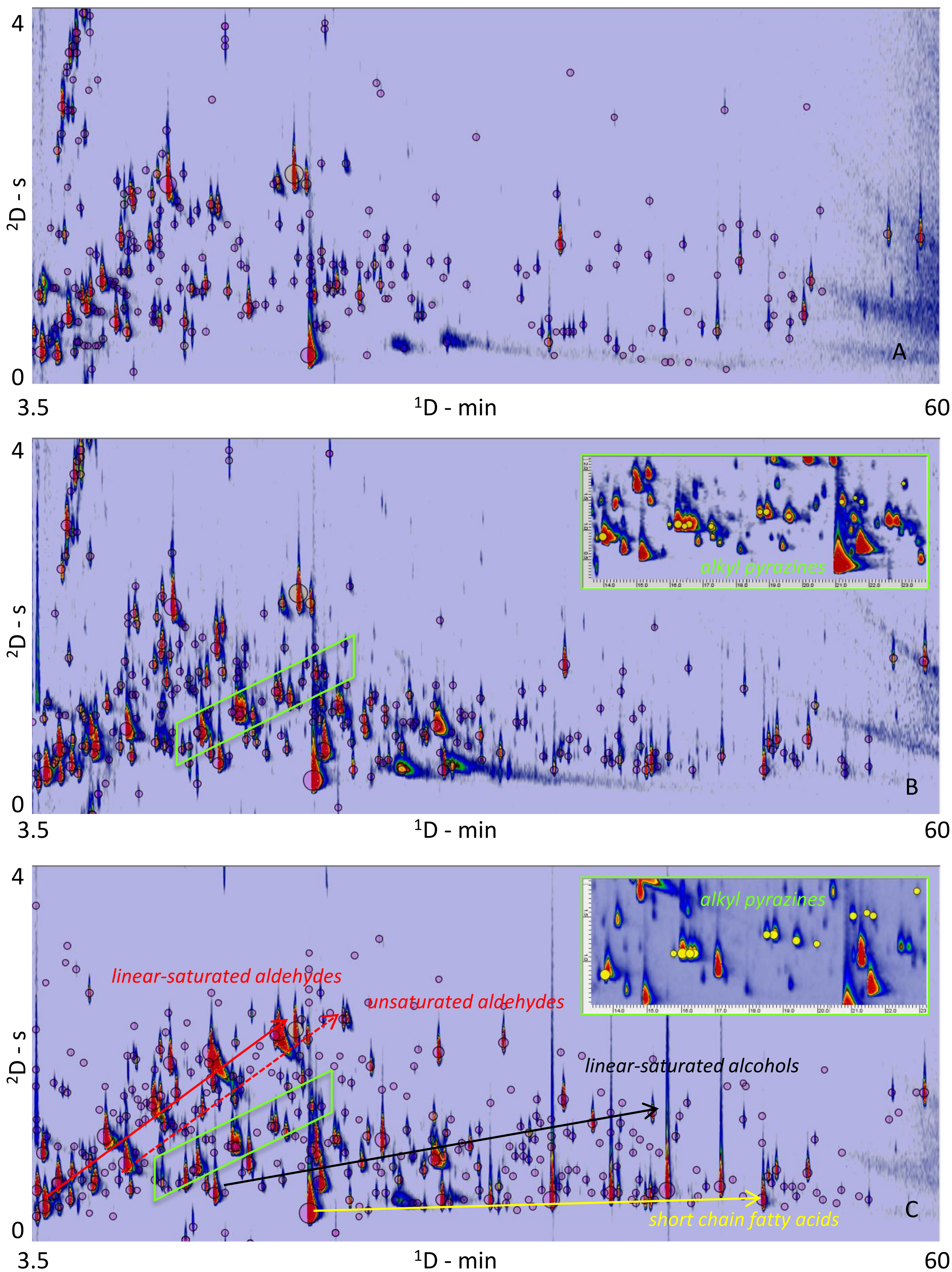
Hazelnut samples	Drying	Storage	Temperature	Acronyms	Timing
Ordu (*Tombul*, *Palaz* and *Çakildak*) harvest 2014 caliber 13-14 mm	35-38°C 6% moist	78% N_2-21% O_2 65% of ERH§	5 and 18 (±0.1) °C	OR_D1_NA_5 OR_D1_NA_18	(T) 0-4-9-12 months
		99% N_2-1% O_2 65% of ERH§	5 (±0.1) °C	OR_D1_MA_5	
Nocciola Romana harvest 2014 caliber 13-14 mm	30-35°C 6% moist	78% N_2-21% O_2 65% of ERH§	5 and 18 (±0.1) °C	TR_D1_NA_5 TR_D1_NA_18	
		99% N_2-1% O_2 65% of ERH§	5 (±0.1) °C	TR_D1_MA_5	
	18-20°C forced air 6% of moist	78% N_2-21% O_2 65% of ERH§	5 and 18 (±0.1) °C	TR_D2_NA_5 TR_D2_NA_18	
		99% N_2-1% O_2 65% of ERH§	5 (±0.1) °C	TR_D2_MA_5	

§: equilibrium relative humidity

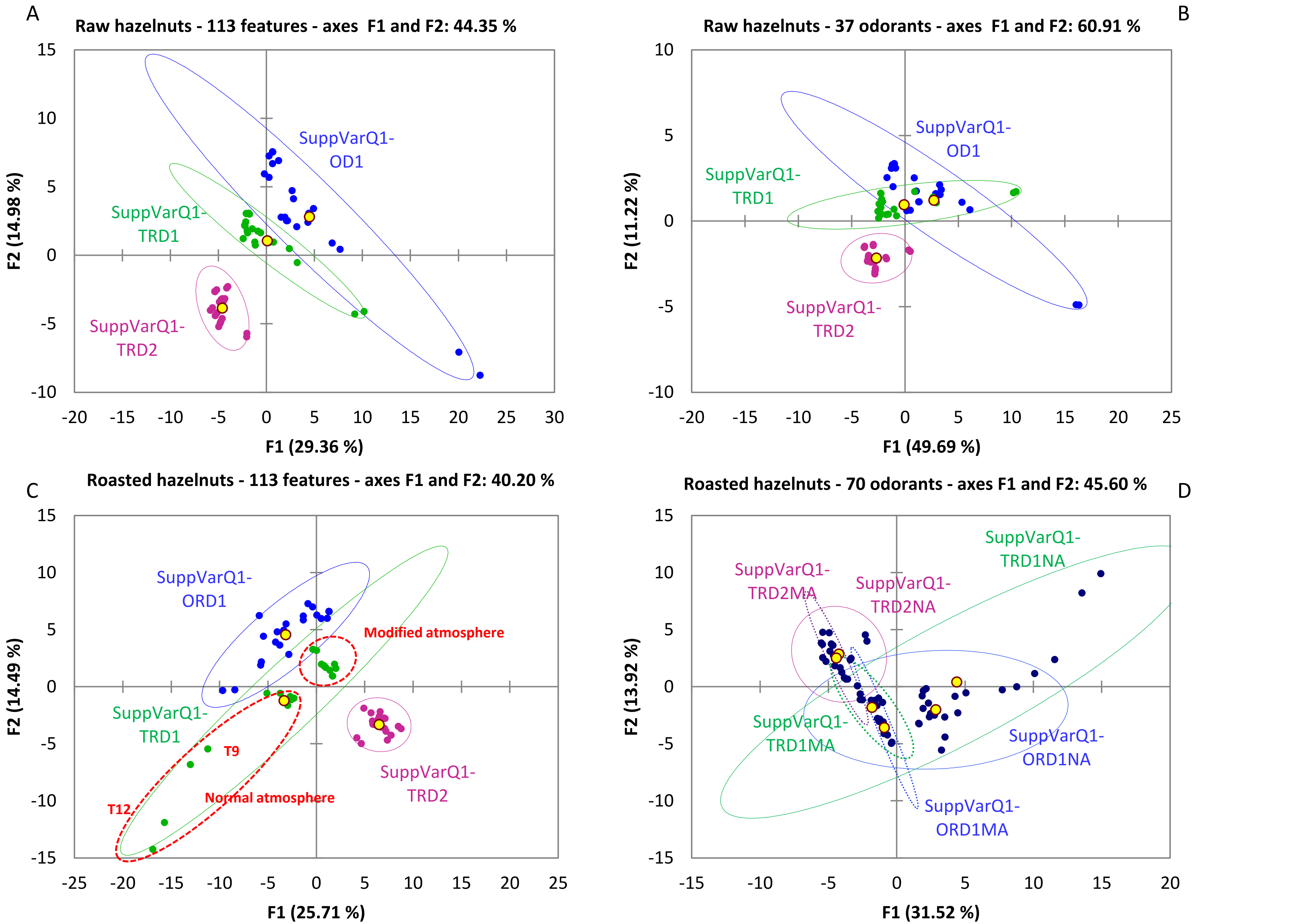
#	Compound Name	^1t_R (min)	^2t_R (sec)	I^T_S ^{1}D	Odor quality	Odor Threshold (µg/l)	Ref.
1	hexane	3.55	0.45	600			
2	butanal	3.65	0.40	605	green, pungent	9*	£
3	acetaldehyde	3.75	0.34	620	pungent, fruity	0.22	£
4	heptane	3.75	0.62	700			
5	octane	4.09	1.00	800			
6	2-methylpropanal	4.75	0.55	812	green, pungent	43	£
7	acetone	4.22	0.45	833			
8	ethyl acetate	4.75	0.55	949	solvent-like, fruity	500*	£
9	2-butanone	4.89	0.55	952	etheric	10000*	$
10	2-methylbutanal	5.02	0.69	956	green, almond-like	140	£
11	3-methylbutanal	5.09	0.69	958	malty	13	£
12	dichlorometane (solvent)	5.15	0.41	960			
13	2,5-dimethylfuran	5.49	0.69	970			
14	2-pentanone	5.55	0.93	970	fruity	70000*	$
15	*n*-butyl ether	5.69	1.62	976			
16	2,3-butanedione	5.82	0.52	979	buttery	10	£
17	pentanal	5.95	0.79	983	pungent, almond-like	240	£
18	acetonitrile	6.29	0.48	993			
19	*(Z)*-3-penten-2-one	6.55	0.72	1001			
20	3-methyl-2-pentanone	6.62	1.00	1003			
21	2-ethyl-5-methylfuran	6.75	1.00	1006			
22	methylbenzene	7.02	0.90	1014			
23	2-butenal	7.22	0.66	1020			
24	2,3,5-trimethylfuran	7.35	1.03	1023			
25	(*E*)-2-pentenal	7.38	0.68	1024	pungent, apple-like	240	£
26	2,3-pentanedione	7.42	0.72	1026	buttery	16	£
27	hexanal	8.02	1.17	1043	green, leaf-like	120	£
28	2-methyl-1-propanol	8.09	0.76	1050	solvent-like	1000*	£
29	2-pentanol	8.89	0.72	1068	light, seedy, sharp		
30	2,2-dimethyl-3-hexanone	8.95	1.34	1070			
31	sabinene	9.02	2.17	1072	terpeny	6300^	£
32	4-heptanone	9.22	1.59	1078			
33	*(E)*-3-penten-2-one	9.29	0.90	1080			
34	2-hexanone	9.69	1.45	1082			
35	2-methylbutanoate	9.45	1.63	1082	fruity, sweet	60*	£
36	1-butanol	9.62	0.62	1089	fruity	500*	£
37	2-methylbutyl propanoate	9.82	2.07	1095			
38	3-methyl-4-heptanone	9.95	1.97	1099			
39	2-heptanone	11.02	1.55	1130	soapy, fruity	140-3000	$
40	heptanal	11.09	1.59	1132	fatty	250	£
41	2-ethylhexanal	11.15	2.03	1134			
42	pyridine	11.15	0.76	1134			
43	*(Z)*-5-methyl-hept-2-en-4-one	11.49	1.69	1143	fruity, hazelnut-like		
44	3,5-dimethyl-4-heptanone	11.55	1.72	1145			
45	limonene	11.55	2.24	1145	citrus-like	200*-R(+) isomer	£
46	3-methyl-1-butanol	11.69	0.79	1149			
47	5-methyl-3,4-heptanedione	12.02	1.62	1159			
48	2-vinyl-5-methylfuran	10.02	1.03	1160			
49	butyl butanoate	12.22	2.14	1164	strongly fruity	100*	$
50	2-pentylfuran	12.62	1.76	1176	buttery, green bean-like	2000	£
51	methyl pyruvate	12.82	0.69	1182			
52	γ-terpinene	13.15	2.28	1191			
53	1-pentanol	13.22	0.72	1193	balsamic	4000*	$
54	methylpyrazine	14.02	0.83	1216			
55	4-isopropyl-1-methylbenzene	14.09	1.86	1218			
56	2,4-dimethyl-3-pentanol	14.55	1.10	1232			
57	3-hydroxy-2-butanone	14.62	0.66	1234	buttery	800*	£
58	2-octanone	14.75	1.90	1237			
59	octanal	14.89	1.93	1242	fatty, green	56	£
60	*(E)*-5-methyl-hept-2-en-4-one (filbertone)	14.95	1.72	1243	nutty	0.05*	£
61	1-hydroxy-2-propanone	15.15	0.55	1249			
62	2-hexenal,2-ethyl	15.35	1.86	1255			
63	3-hepten-2-one	15.35	1.45	1255			
64	5-methyl-2-heptanone	13.62	1.76	1256			
65	2-heptanol	15.95	1.07	1272	citrusy	263	€
66	2,5-dimethylpyrazine	16.22	1.07	1280	earthy		
67	*(E)*-2-heptenal	16.35	1.52	1284	fatty, almond-like	3750	£
68	2,6-dimethylpyrazine	16.49	1.03	1288	earthy		
69	ethylpyrazine	16.55	1.03	1290			
70	2-acetyl-1-pyrroline	16.77	1.18	1299	roasty, sweet	0.1	£
71	3-methyl-4-heptanol	16.78	1.32	1301			
72	1-hexanol	17.29	0.86	1311	green, flowery	2500*	$

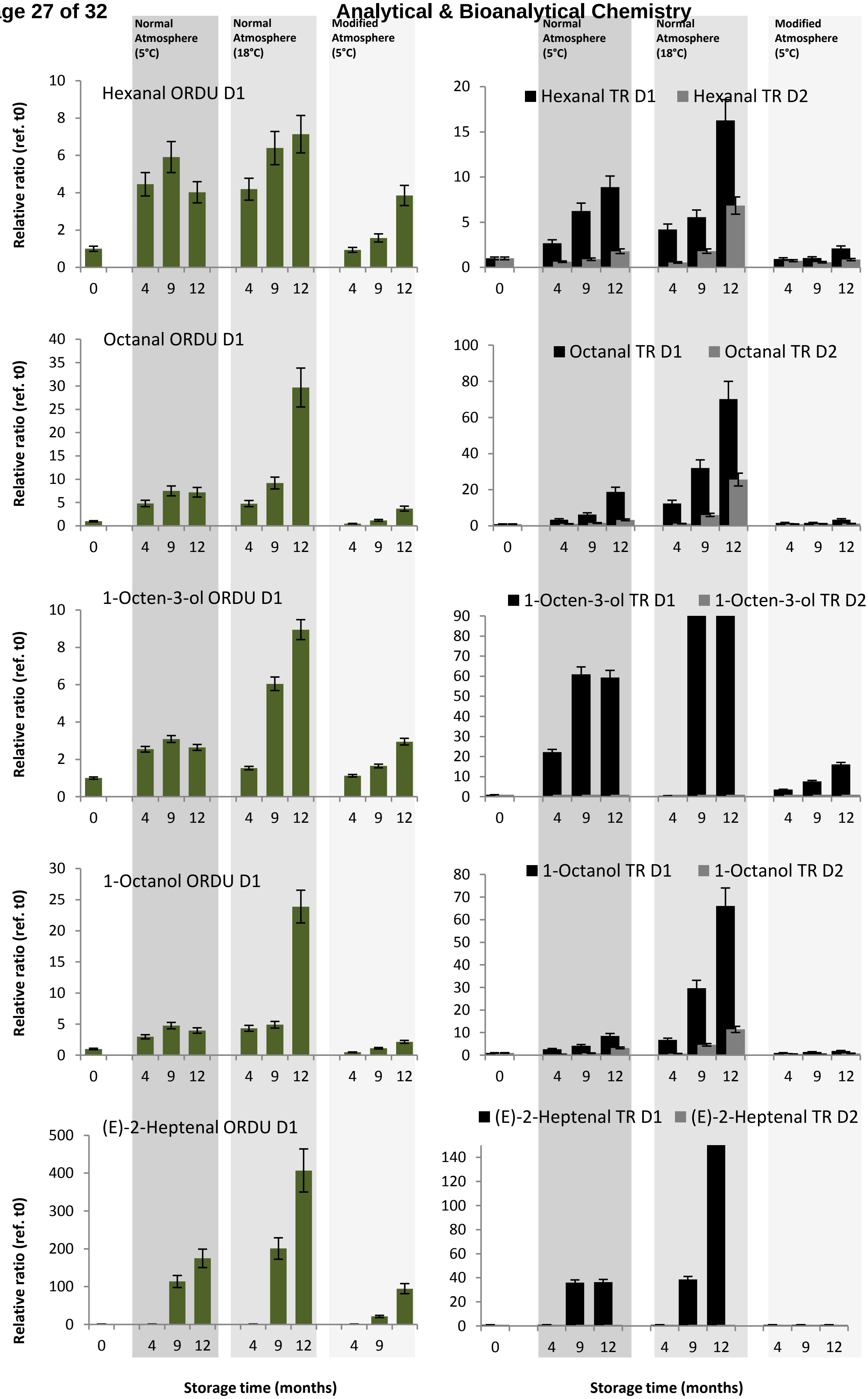
73	2-ethyl-6-methylpyrazine	18.69	1.28	1351			
74	2-nonanone	18.89	2.14	1357	fruity, soapy	200*	£
75	2-ethyl-5-methylpyrazine	18.95	1.24	1359			
76	nonanal	19.09	2.17	1363	tallowy, fruity	1000	£
77	1-hepten-3-ol	19.22	1.72	1367			
78	2-methyl-3(2H)furanone	19.35	0.72	1371			
79	2,3,5-trimethyl pyrazine	19.62	1.17	1378	potato-like, musty	91*	£
80	α-thujone (ISTD1)	20.15	2.21	1394			
81	2,4-dimethyl-3-pentanol	20.35	1.38	1400			
82	2-ethyl-3,5-dimethylpyrazine	20.55	1.31	1404	potato-like	2.2	£
83	*(E)*-2-octenal	20.62	1.69	1407	fatty, nutty	7000	£
84	β-thujone (ISTD2)	20.89	2.17	1415			
85	acetic acid	21.02	0.38	1419	vinegar-like, pungent	124	£
86	3-ethyl-2,5-dimethylpyrazine	21.15	1.45	1423	potato-like	24	£
87	1-octen-3-ol	21.22	1.00	1425	mushroom-like	34	£
88	1-heptanol	21.42	1.00	1430	cucumber, citrus-like	3*	$
89	furfural	21.69	0.69	1438	sweet	3000*	£
90	2-ethyl-1-hexanol	22.82	1.10	1471			
91	2-decanone	23.15	2.31	1481			
92	decanal	23.35	2.35	1486	orangeskin-like, flowery	6700	£
93	2-acetylfuran	23.42	0.79	1488			
94	1H-pyrrole	23.55	0.48	1492			
95	benzaldehyde	24.29	0.86	1513			
96	3-methyl-3-pentanol	24.62	1.14	1523			
97	*(E)*-2-nonenal	24.82	1.86	1533	fatty, cucumber-like	900	£
98	2-methyl-1h-pyrrole	25.15	0.59	1538			
99	sabinene hydrate	25.16	1.31	1540			
100	1-octanol	25.55	1.10	1550	chemical, metal, burnt	0,11-0,13	$
101	3-methyl-1H-pyrrole	25.75	0.55	1556			
102	5-methyl furfural	26.22	0.83	1569			
103	2-cyclopenten-1,4-dione	26.62	0.69	1581			
104	3-methyl-2-ciclohexen-1-one	27.09	1.24	1594			
105	dihydro-2(3H)-furanone	28.15	0.83	1625			
106	butanoic acid	28.22	0.41	1628	sweaty, rancid	135	£
107	phenylacetaldehyde	28.82	0.93	1644	honey-like, flowery	22	£
108	(*E*)-2-decenal	29.02	2.00	1650	fatty, tallowy, orange-like	33800	£
109	2-furanmethanol	29.22	0.48	1656			
110	1-nonanol	29.49	1.21	1666			
111	5-ethyldihydro-2(3H)-furanone	31.02	1.10	1708	coumarin, sweet	1600*	$
112	pyrazinamide	31.55	0.83	1723			
113	4-methyl-2-furfuryl alcohol	31.62	0.55	1725			
114	pentanoic acid	32.29	0.52	1745	sweaty	2100*	£
115	2(3H)furanone	32.82	0.66	1760			
116	5-propyldihydro-2(3H)-furanone	34.69	1.17	1814			
117	hexanoic acid	36.09	0.52	1855	goat-like, sweaty	5400	£
118	4-benzyloxypentanal	36.82	1.52	1876			
119	benzyl alcohol	37.09	0.62	1884	sweet, flower	10000*	$
120	2-phenylethanol	38.29	0.69	1918	honey-like, spicy	211	£
121	5-butyldihydro-2(3H)-furanone	38.62	1.28	1928			
122	2-(1-pyrrolyl)ethanol	39.35	0.59	1949			
123	heptanoic acid	39.82	0.55	1963			
124	acetylpyrrole	40.29	0.59	1976	nutty, anisic, sweet	170000*	$
125	2-formylpyrrole	41.95	0.48	2024			
126	4-hydroxy-2,5-dimethyl-3(2H)-furanone	42.22	0.59	2032	strawberry-, caramel-like	25	£
127	5-pentyldihydro-2(3H)-furanone	42.35	1.38	2036	coconut, peach	400	$
128	2-pyrrolidinone	42.55	0.62	2042			
129	octanoic acid	43.29	0.62	2063	sweaty	3000*	£
130	nonanoic acid	46.69	0.62	2161	green, fat	3000*	$
131	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	49.29	0.48	2236			
132	decanoic acid	49.89	0.69	2254	soap-like, fatty	10000	£
133	isobenzofuranone	52.09	0.79	2317			

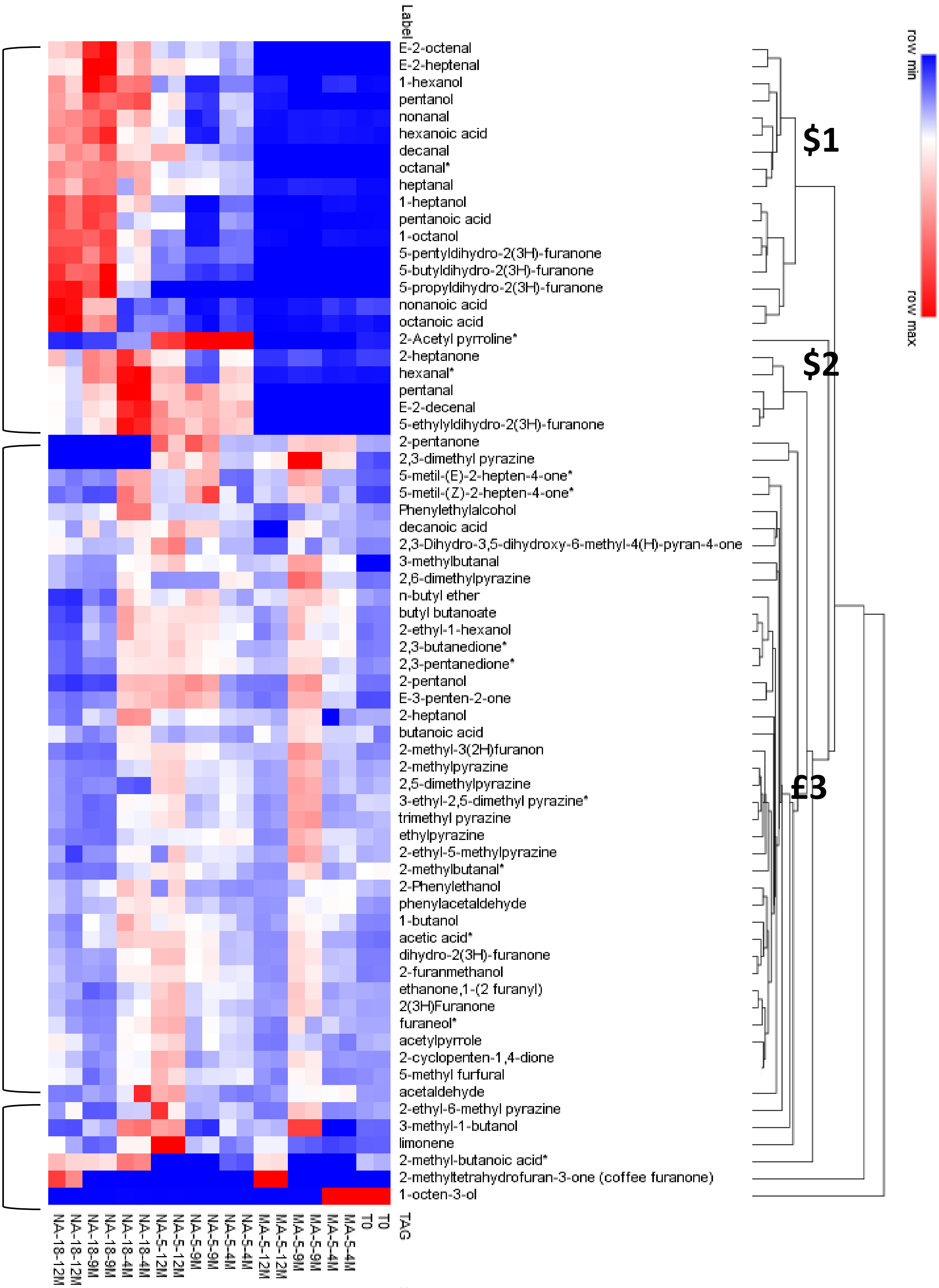
* = in water
^= in starch
£= Ref [7]
$= Ref [44]
€= Ref [45] J.Agric. Food Chem., Vol.56, No. 21, 2008



4
0
^{2}D - s
3.5
^{1}D - min
60
A
B
alkyl pyrazines
C
linear-saturated aldehydes
unsaturated aldehydes
linear-saturated alcohols
short chain fatty acids


A
Raw hazelnuts - 113 features - axes F1 and F2: 44.35 %
F2 (14.98 %)
F1 (29.36 %)
SuppVarQ1-OD1
SuppVarQ1-TRD1
SuppVarQ1-TRD2
B
Raw hazelnuts - 37 odorants - axes F1 and F2: 60.91 %
F2 (11.22 %)
F1 (49.69 %)
SuppVarQ1-OD1
SuppVarQ1-TRD1
SuppVarQ1-TRD2
C
Roasted hazelnuts - 113 features - axes F1 and F2: 40.20 %
F2 (14.49 %)
F1 (25.71 %)
SuppVarQ1-ORD1
Modified atmosphere
SuppVarQ1-TRD1
T9
T12
Normal atmosphere
SuppVarQ1-TRD2
D
Roasted hazelnuts - 70 odorants - axes F1 and F2: 45.60 %
F2 (13.92 %)
F1 (31.52 %)
SuppVarQ1-TRD1NA
SuppVarQ1-TRD2MA
SuppVarQ1-TRD2NA
SuppVarQ1-TRD1MA
SuppVarQ1-ORD1NA
SuppVarQ1-ORD1MA


Normal Atmosphere (5°C)
Normal Atmosphere (18°C)
Modified Atmosphere (5°C)
Hexanal ORDU D1
Hexanal TR D1
Hexanal TR D2
Octanal ORDU D1
Octanal TR D1
Octanal TR D2
1-Octen-3-ol ORDU D1
1-Octen-3-ol TR D1
1-Octen-3-ol TR D2
1-Octanol ORDU D1
1-Octanol TR D1
1-Octanol TR D2
(E)-2-Heptenal ORDU D1
(E)-2-Heptenal TR D1
(E)-2-Heptenal TR D2
Relative ratio (ref. t0)
Storage time (months)


Label
E-2-octenal
E-2-heptenal
1-hexanol
pentanol
nonanal
hexanoic acid
decanal
octanal*
heptanal
1-heptanol
pentanoic acid
1-octanol
5-pentyldihydro-2(3H)-furanone
5-butyldihydro-2(3H)-furanone
5-propyldihydro-2(3H)-furanone
nonanoic acid
octanoic acid
2-Acetyl pyrroline*
2-heptanone
hexanal*
pentanal
E-2-decenal
5-ethylyldihydro-2(3H)-furanone
2-pentanone
2,3-dimethyl pyrazine
5-metil-(E)-2-hepten-4-one*
5-metil-(Z)-2-hepten-4-one*
Phenylethylalcohol
decanoic acid
2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one
3-methylbutanal
2,6-dimethylpyrazine
n-butyl ether
butyl butanoate
2-ethyl-1-hexanol
2,3-butanedione*
2,3-pentanedione*
2-pentanol
E-3-penten-2-one
2-heptanol
butanoic acid
2-methyl-3(2H)furanon
2-methylpyrazine
2,5-dimethylpyrazine
3-ethyl-2,5-dimethyl pyrazine*
trimethyl pyrazine
ethylpyrazine
2-ethyl-5-methylpyrazine
2-methylbutanal*
2-Phenylethanol
phenylacetaldehyde
1-butanol
acetic acid*
dihydro-2(3H)-furanone
2-furanmethanol
ethanone,1-(2 furanyl)
2(3H)Furanone
furaneol*
acetylpyrrole
2-cyclopenten-1,4-dione
5-methyl furfural
acetaldehyde
2-ethyl-6-methyl pyrazine
3-methyl-1-butanol
limonene
2-methyl-butanoic acid*
2-methyltetrahydrofuran-3-one (coffee furanone)
1-octen-3-ol
NA-18-12M
NA-18-12M
NA-18-9M
NA-18-9M
NA-18-4M
NA-18-4M
NA-5-12M
NA-5-12M
NA-5-9M
NA-5-9M
NA-5-4M
NA-5-4M
MA-5-12M
MA-5-12M
MA-5-9M
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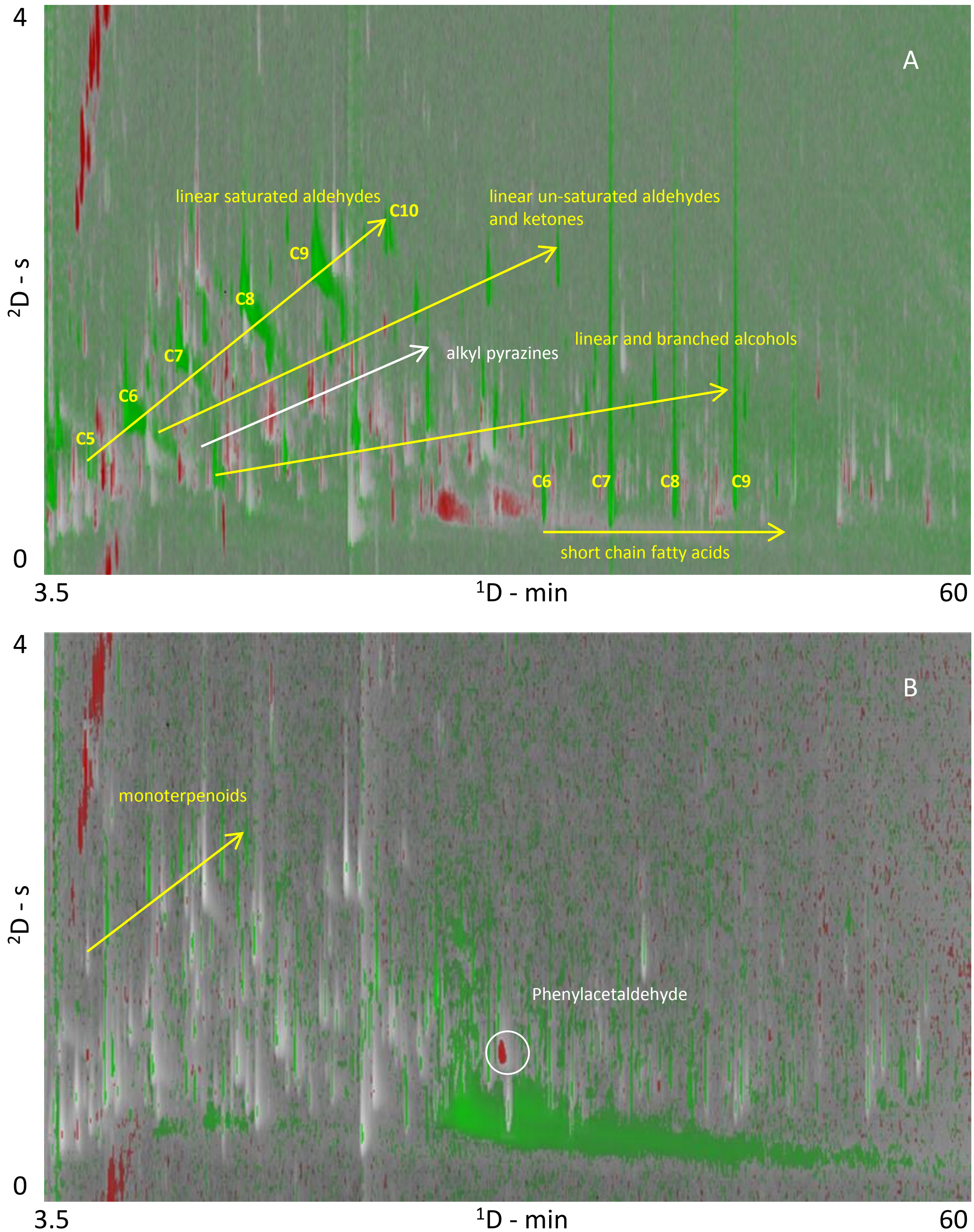
$1
$2
£3
row min
row max


4
A
linear saturated aldehydes
C10
C9
C8
C7
C6
C5
linear un-saturated aldehydes and ketones
alkyl pyrazines
linear and branched alcohols
C6
C7
C8
C9
short chain fatty acids
0
^{2}D - s
3.5
^{1}D - min
60
4
B
monoterpenoids
Phenylacetaldehyde
0
^{2}D - s
3.5
^{1}D - min
60

Supplementary data

Evolution of potent odorants within the volatile metabolome of high-quality hazelnuts (*Corylus avellana* L.): evaluation by comprehensive two-dimensional gas chromatography coupled with mass spectrometry

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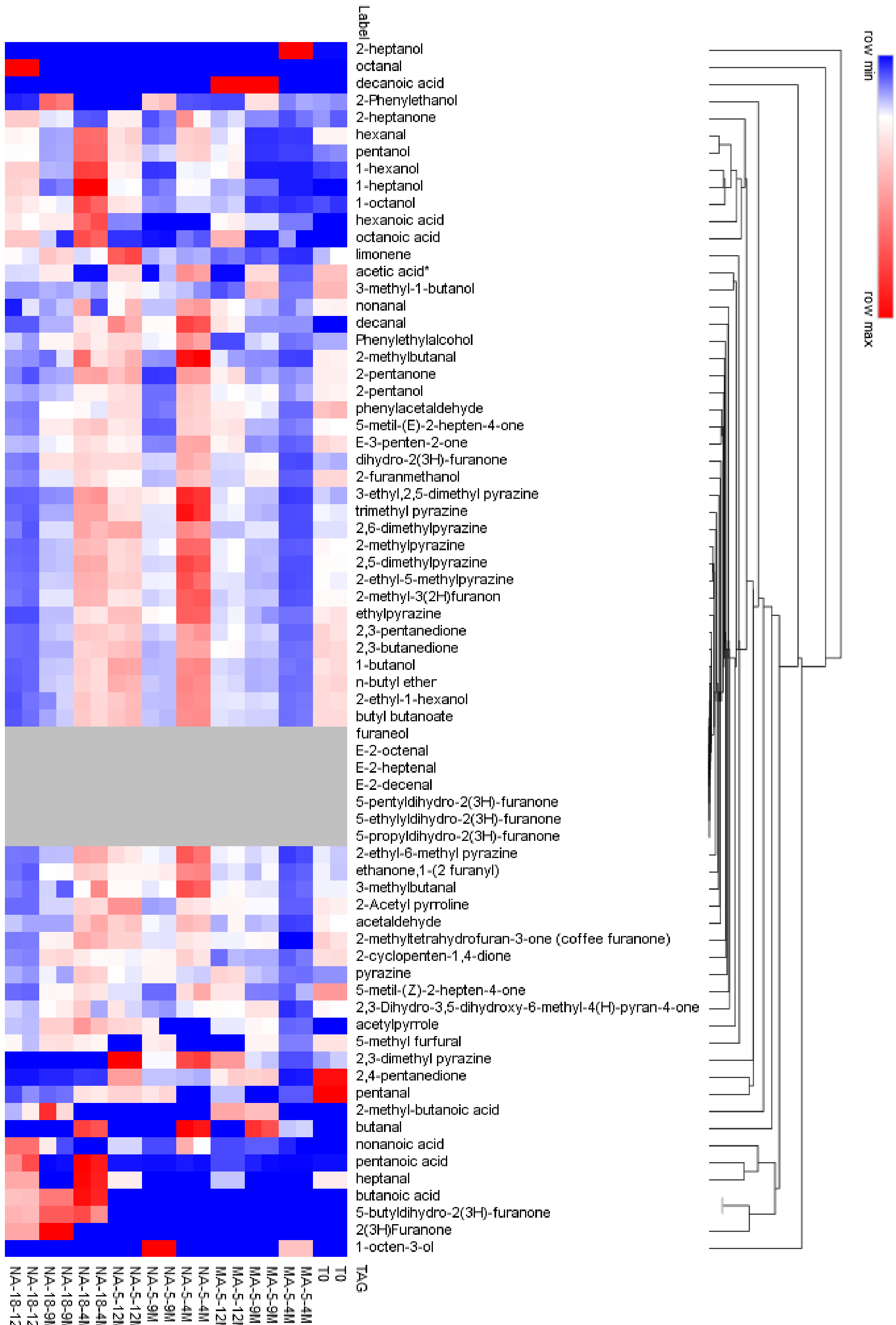
SF1: Heat-map illustrating, by color rendering from blue (low values) to red (higher values), the relative abundance distribution of 70 potent odorants from Tonda Romana (TR) hazelnuts subjected to low temperature drying (D2), different storage conditions (NA and MA) and roasted at different stages of their shelf-life. The Normalized 2D Volumes are set to % and data averaged and centered in rows. Hirarchical clustering is based on Euclidean distances.



SF2: Heat-map illustrating, by color rendering from blue (low values) to red (higher values), the relative abundance distribution of 70 potent odorants from Ordu (OR) hazelnuts subjected to conventional drying (D1), different storage conditions (NA and MA) and roasted at different stages of their shelf-life. The Normalized 2D Volumes are set to % and data averaged and centered in rows. Hirarchical clustering is based on Euclidean distances.