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Artificial Intelligence decision-making tools based on comprehensive two-dimensional gas chromatography data: the challenge of quantitative volatilomics in food quality assessment

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ABSTRACT

Effective investigation of food volatilome by comprehensive two-dimensional gas chromatography with parallel detection by mass spectrometry and flame ionization detector (GC×GC–MS/FID) gives access to valuable information related to industrial quality. However, without accurate quantitative data, results transferability over time and across laboratories is prevented. The study applies quantitative volatilomics by multiple headspace solid phase microextraction (MHS-SPME) to a large selection of hazelnut samples (*Corylus avellana* L. $n = 207$) representing the top-quality selection of interest for the confectionery industry. By untargeted and targeted fingerprinting, performant classification models validate the role of chemical patterns strongly correlated to quality parameters (*i.e.*, botanical/geographical origin, post-harvest practices, storage time and conditions). By quantification of marker analytes, Artificial Intelligence (AI) tools are derived: the augmented smelling based on sensomics with blueprint related to key-aroma compounds and spoilage odorant; decision-makers for rancidity level and storage quality; origin tracers. By reliable quantification AI can be applied with confidence and could be the driver for industrial strategies.

1. Introduction

Consumer acceptability of food is significantly impacted by its pleasant aroma shaped by the presence of key-aroma compounds and potent odorants within the complex fraction of volatile organic compounds [1]. The so-called aroma *blueprint* unequivocally evokes in humans the product's identity [2]. Although the majority of volatile compounds do not exert a direct effect on aroma perception, their potential in informing about product quality and authenticity/identity is relevant. Analytical strategies capable of effectively investigating the so called *food volatilome* (or volatome) which "*contains all of the volatile metabolites as well as other volatile organic and inorganic compounds that originate from an organism*" super-organism, or ecosystem [3], are of great interest for the food industry. By this fraction, primary materials, semi-finished and finished products can be monitored based on their botanical/geographical characteristics (*i.e.*, the phenotype), the impact of processing, or shelf life.

In hazelnuts (*Corylus avellana* L.), a primary material used by the confectionery industry, the volatilome encrypts information about many quality features. Native volatiles, such terpenes and terpenoids [4], are a direct expression of the plant phenotype, informing also for the presence of bacteria and moulds [5]. Monoterpenoids in raw hazelnuts are α-pinene, β-pinene, *(E)*-p-2-menthen-1-ol, camphene, δ-carene, α-thujene, γ-terpinene, sabinene, limonene, *cis*-sabinene hydrate, α-terpinolene, β-phellandrene, and *p*-cymene [6–8].

Another class of volatiles with great informative power on hazelnut quality and sensory profile is that of secondary products of lipid oxidation. They derive from lipids autoxidation by β-scission and hydroperoxide epi–dioxide decomposition of fatty acids hydroperoxides (*i.e.*, primary oxidation products) [9]. Within this class, linear saturated aldehydes, unsaturated aldehydes, and methyl-ketones might have sensory relevance conferring unpleasant aroma qualities (*e.g., rancid, fatty,* and *waxy* notes). Moreover, although connoted by high odor thresholds (*i.e.,* lower odor potency), alcohols, and short-chain fatty acids [9,10] are also formed

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during hydroperoxides degradation; they give a contribution to the informative potential of the volatilome as markers of lipid stability during storage of hazelnuts [6,11,12].

Post-harvest practices are known to have a role in kernel stability over time; high water activity (a_w), uncontrolled temperatures during drying, and substrates availability might promote enzymatic activation of the seed as well as the development/growth of bacteria and moulds further increasing the volatilome chemical complexity. Moreover, inappropriate post-harvesting is generally correlated to the off-flavors due to the presence of highly impacting odorants belonging to the classes of primary and secondary alcohols, carboxylic acids [6], lactones, furans, and some aromatics (benzaldehyde, phenyl ethyl alcohol, phenyl ethyl acetaldehyde, alkylated phenol derivatives) [5,11,13,14].

Nowadays, raw hazelnuts are checked for their quality and the absence of sensory defects by visual inspection by trained staff and evaluation by a sensory panel. Representative samples from all incoming batches are tested before their acceptance or rejection. This approach, although highly specific since performed by a direct measure of the target quality feature (*i.e.*, morphological defects or presence of off-flavors), is strongly influenced by the operator (*e.g.* level of experience, eyes sensibility, generically human variability), time-consuming [15], and hardly standardisable over time and across-panels.

This study aimed at developing and validating an accurate quantification method targeted to robust quality markers of raw hazelnuts to be used by the industry as an objective support tool for quality control (QC) purposes. Volatile makers within the detectable volatilome were selected based on their information potential: aroma blueprint odorants [11,16], spoilage indicators [5], botanical/geographical tracers, secondary products of lipid oxidation [10], and storage time/conditions indicators [6].

To achieve suitable separation efficiency, resolution, sensitivity, and quantification accuracy, an analytical platform based on comprehensive two-dimensional gas chromatography with parallel detection by mass spectrometry and flame ionization detector (GC×GC–MS/FID) was designed. Moreover, intending the methodology suitable for industrial laboratories, fully automated sample preparation was by exhaustive extraction by multiple headspace solid-phase microextraction (MHS-SPME) to achieve accurate quantitative results [17–19] due to the solid nature of hazelnut kernels.

The method has the potential of the sensomics-based expert system (SEBES) for efficient and quick screening of key-odorants in food [20]. As an Artificial Intelligence (AI) smelling machine, the aroma blueprint of food can be monitored objectively. Moreover, by extending method capabilities to a larger set of quality markers, it might support AI decision-making tools once reference values are defined for each marker/pattern of markers [21].

To prove the method's suitability, a large set of hazelnut samples from industrial batches ($n = 72$) were selected. Samples were from different botanical/geographical origins ($n = 4$), undergone different post-harvesting (optimal or high moisture), and were stored in different conditions (5 °C with air or under vacuum) for twelve months (time 0–4–6–9–12 months).

2. Materials and methods

2.1. Reference standards and solvents

Pure standards of *n*-alkanes (from *n*-C7 to *n*-C30) used for Linear Retention Indices (I^T) calibration; of α/β-thujone used as Internal Standards (ISs); of hexanal, heptanal, octanal, nonanal, decanal, 4-heptanone, 2-pentanol, 2-heptanol, γ-hexalactone used for external calibration; and solvents (cyclohexane and dibutyl phthalate – 99% of purity) were all from Merck (Milan, Italy).

2.2. Reference solution and calibration standards

Standard stock solutions of reference analytes were prepared at a concentration of 10 g/L in cyclohexane and stored at −18 °C for one week.

The *n*-alkanes solution for I^T calibration was prepared by mixing suitable amounts of standard stock solutions of pure standards to reach the concentration of 0.050 g/L using cyclohexane as solvent.

ISs working solution for standard-in fiber pre-loading was prepared at 0.100 g/L in dibutyl phthalate and stored at −18 °C in sealed vials. ISs were used for validation purposes (method precision and repeatability) and to normalize the analytes' absolute responses (*i.e.*,% normalized response).

The reference working solution for analytes external calibration was prepared by mixing suitable amounts of standard stock solutions using dibutyl phthalate as solvent. Calibration curves were set to cover analyte absolute amounts within the range 1–2.5–5–10–25–50–100–250–500 ng. External standard calibration by MHS-SPME was conducted on FID while the MS trace was used to confirm analytes' identity and exclude co-elutions. Sampling conditions were those applied to hazelnut samples (50 °C – 50 min sampling) with 4 consecutive extraction steps, achieving an almost exhaustive extraction for calibrated analytes [22].

2.3. Hazelnut samples

Raw hazelnuts from 2020 harvest year, were provided by Soremartec Italia Srl (Alba, Cuneo, Italy). Samples collected at each time point (see below) were stored at −18 °C away from UV exposure until analysis.

The functional variables characterizing the sample set included four different cultivars/geographical origins (*Tonda Gentile Trilobata* – TGT, *Tonda Gentile Romana* – TGR, Akçakoca mix – AKC, Giresun mix – GIR) informing about the phenotype expression in the volatilome. Post-harvest conditions were tested on *ad hoc* samples from AKC and GIR, where hazelnuts were-dried on multiple layers with humidity kept between 15 and 20% in order to artificially simulate a rainy season (*Bad Post-Harvest* drying – GIRbPH and AKCbPH) and compared to an optimal in-field sun-drying on a single layer kept at humidity levels under 6% (*Good Post-Harvest* drying – GIRgPH and AKCgPH) [6].

Hazelnuts were then stored until 12 months after their harvest, and samples were collected and analyzed at 0,4,6,9, and 12 months. Storage, impacting sensory quality and off-flavor development, was at 5 °C and 65% of equilibrium relative humidity with a standard atmosphere (SA) or under vacuum (UV).

A summary of samples characteristics and acronyms is provided in Supplementary Table 1 – ST1.

2.4. Headspace solid-phase microextraction: sample preparation, devices and conditions

Multiple HS-SPME was conducted by a multipurpose sampler, model MPS-2 (Gerstel, Mülheim a/d Ruhr, Germany), with a 2 cm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) d_f 50/30 μm fiber from Merck (Milan, Italy), conditioned beforehand as recommended by the manufacturer. The standard-in-fiber procedure on 5 μL of IS placed in a 20 mL glass sealed vial was applied to pre-load the IS on the SPME; it was conducted at 50 °C for 5 min.

MHS-SPME was performed on 100 ± 0.2 mg of finely ground hazelnuts (*i.e., granella*) prepared with a lab-scale grinder at Soremartec. The average dimensions of the powder were 500 µm (±50).

Sampling was at 50 °C for 50 min. MHS-SPME was applied with 4 consecutive extraction steps on a 15% of samples randomly distributed over the analytical batch to define reliable decay constants (β) for accurate quantification [18,23–25].

2.5. GC×GC–MS/FID: instrument setup and conditions

Analyses were performed on an Agilent 7890B unit coupled with an Agilent 5977B fast quadrupole MS detector (Agilent Technologies, Little Falls, DE, USA) with a high-efficiency source (HES) working in electron ionization mode at 70 eV. The scan range was between 40 and 250 *m/z* achieving an acquisition frequency of 28 Hz. The MS source was set at 230 °C, the quadrupole at 150 °C, and the transfer line temperature was at 280 °C. Parallel detection was achieved with an FID set at 300 °C base temperature, the H_2 flow was 40 mL/min, the air flow was 450 mL/min, and the sampling frequency was 200 Hz.

The split-splitless injector was set at 250 °C, operating in pulsed-split mode at 250 kPa until 2.5 min with a 1:5 split ratio. The carrier gas was helium with a differential flow set at 0.4 mL/min for the first dimension (^{1}D) column and 10 mL/min in the second dimension (^{2}D).

Modulation was by differential-flow modulator via the reverse-inject dynamics, it was realized on the capillary flow technology™ CFT (G4573A, Agilent Technologies). The modulation period (P_M) was 2.5 s and pulse time was 0.25 s.

The column configuration was the following: ^{1}D DB-HeavyWax™ (100% polyethylene glycol - PEG; 20 m × 0.18 mm d_c × 0.18 μm d_f) coupled with a ^{2}D DB17 ((50%-phenyl)-methylpolysiloxane; 1.8 m × 0.18 mm d_c × 0.18 μm d_f) both from Agilent Technologies. After the ^{2}D column, the flow was split using a three-way unpurged capillary microfluidic splitter (G3181B, Agilent Technologies). The connections toward the MS and FID consisted of deactivated silica capillaries (Agilent Technologies) 0.5 m × 0.1 mm d_c and 1.1 m x 0.18 mm dc respectively, resulting in a split ratio of 70:30 FID/MS. The bleeding capillary (5.81 m × 0.1 mm d_c) consisted of deactivated silica, dimensions were calculated using a validated calculator [22,26].

The oven temperature program was the following: 40 °C (2 min) to 130 °C (0 min) @ 4 °C/min, and to 260 °C (10 min) @ 8 °C/min.

2.6. Data acquisition and processing

Data were acquired using MassHunter WorkStation (Agilent Technologies) and processed with GC Image GC×GC Software ver. 2021r2 (GC Image, LLC, Lincoln, NE, USA). Data mining was performed using Matlab R2021a (The MathWorks, Inc., Natick, Massachusetts, United States) with the following packages: PCA toolbox (v1.5) [27] and Classification toolbox (v6.0) [28], and XLSTAT 2014 by Addinsoft (New York, USA).

3. Results and discussion

The analytical workflow employed in this study, based on the combined untargeted and targeted (UT) fingerprinting of the detectable volatilome of hazelnuts, has a great informative potential which can be exploited for answering many questions related to the samples properties. For instance, by UT fingerprinting and machine learning, informative patterns of UT features and their correlations to quality can be revealed and then used for quality assessment and benchmarking. At the same time, thanks to the accurate quantification of an extended range of volatile components, robust data is collected for their adoption in long-term and/or inter-laboratory studies. The combination of both, the informative potential and the accurate quantification would be the basis for AI decision-making strategies [21].

Next sections will focus at first on the results related to the informative fingerprinting potential and its relation to hazelnut quality (Section 3.1), and then on the robust and transferable outcomes of quantitative fingerprinting with an insight on the augmented smelling capability (Sections 3.2 and 3.3).

3.1. Raw hazelnut volatile fingerprint

Hazelnut volatilome was explored through the combined UT fingerprinting procedure [29–31] with previously validated processing parameters for the UT template construction [32], *i.e.*, S/N threshold of 50 datapoints (dp), distance threshold of 10 dp in both FID and MS channels, and additional direct match factor (DMF) threshold of 700 according to the NIST similarity algorithm for the MS channel only [33]. The resulting feature template was characterized by 44 reliable peaks and 164 peak regions for the MS channel and by 271 reliable peaks and 439 peak regions for the FID channel, of which 117 were putatively identified with the aid of I^T (± 15 tolerance) and spectrum similarity (> 900 DMF) for the MS channel. The resulting discrepancy in the number of reliable peaks between the two channels is attributed to both, the higher specificity of MS *vs*. FID that provides positive matches just for candidate peaks with a spectral similarity above the 700 DMF threshold, and the split ratio of 70:30 FID/MS which impacts on the absolute channel sensitivity.

The contour plot of the detected volatiles from a cumulative image resulting from all fresh (T0) hazelnut samples is shown in Fig. 1, while the list of targeted peaks is provided in Supplementary Table 2 – ST2 together with their retention times and experimental and tabulated $^1D\ I^T$. The complexity of the matrix is shown in an exemplary heatmap in Supplementary Figure 1 – SF1 where all UT features are represented in a color scale (from blue to red) according to their relative abundance (*i.e.*, normalized responses *vs.* internal standard). Hierarchical clustering of variables was by Pearson similarity.

The detectable volatilome was explored using chemometrics to verify the known correlations between biological properties/functional variables and chemical patterns, and possibly highlight new potential markers to be used for screenings at industrial level. Both unsupervised (Principal Component Analysis PCA) and supervised (Partial Least Squares Discriminant Analysis PLS-DA) algorithms were adopted.

Fig. 2A shows the PCA scores on the first two principal components (PC1 and PC2 for a total explained variance of 32%) obtained computing FID normalized responses of 439 UT peak regions collected from all samples (including 3 replicates each, n = 203); samples are colorized according to storage time. Results confirm previous findings of large variability of the volatile fraction of raw hazelnuts as a function of botanical/geographical origin and post-harvest practices [6]. Fresh raw hazelnuts have a distinctive volatile signature which is mainly driven by the genetic and pedoclimatic characteristics of the harvest region (phenotype). However, along with storage, these distinctive traits are fuzzed by the impact of autoxidation which produces a large amount of volatile secondary products (saturated and mono-unsaturated linear aldehydes above all). Note that raw hazelnuts have a 50–60% of the gross composition represented by fat [34,35].

Besides natural clustering shown by the PCA, supervised algorithms were used to understand the differential impact of functional variables left hidden by this preliminary approach. The classification of samples according to the storage months is improved with the supervised algorithm, as shown in Fig. 2B. Scores plot for geographical origin macro-regions (*i.e.,* Italy *vs.* Turkey) and storage conditions' (SA *vs*. UV) models are displayed in Fig. 2C and 2D,

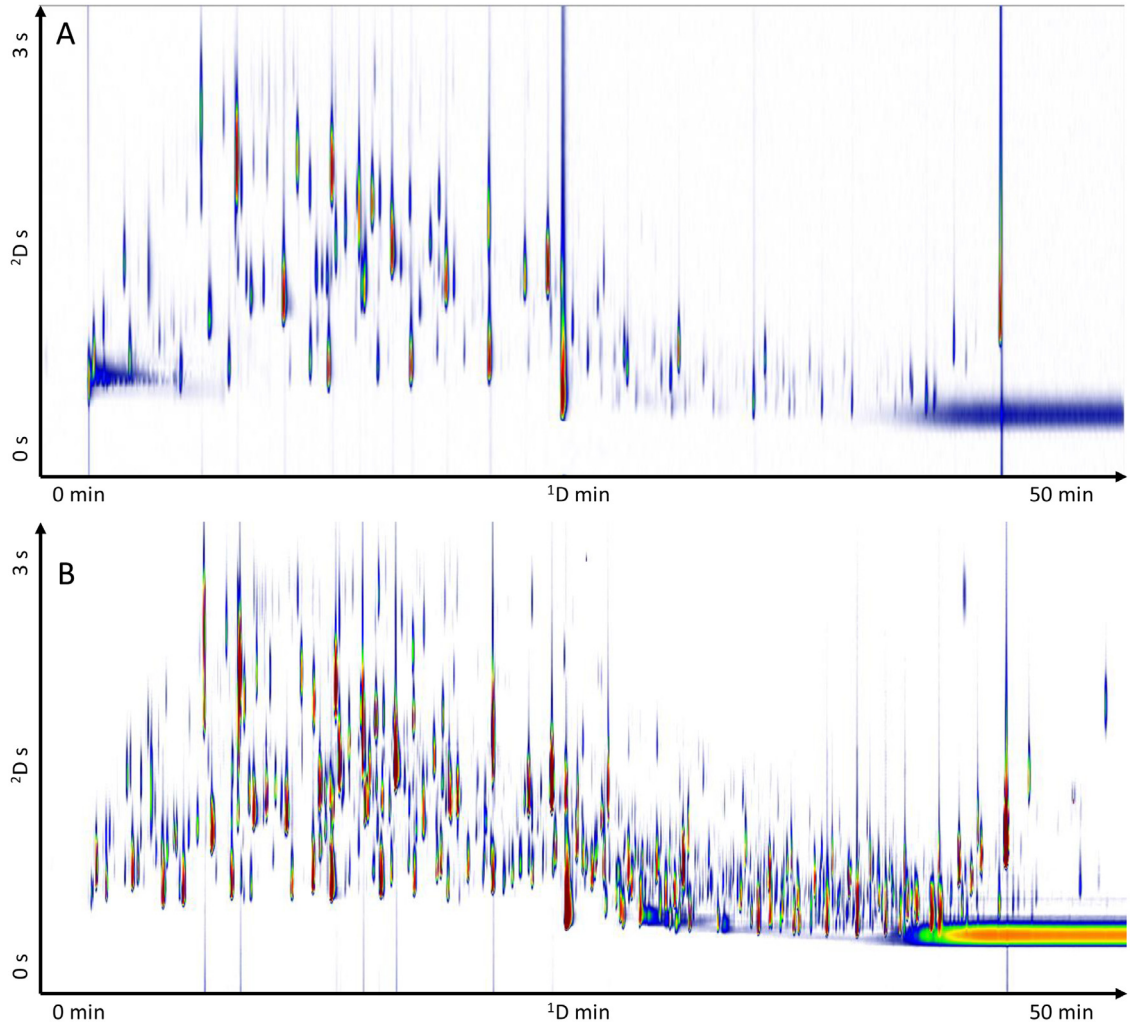

Fig. 1. Contour plots corresponding to the detectable volatilome of fresh hazelnuts (0 months of storage) analysed by a polar × semi-polar column combination (*i.e.*, HeavyWax × OV17) and resulting from the parallel detection by MS (1A) and FID (1B).

respectively. Classifications were validated according to the Montecarlo cross-validation algorithm which uses 20% of the total number of samples as the testing dataset and reiterates the validation procedure 1000 times with a random choice of test samples [36]. The confusion matrix and ROC curves are shown in Supplementary Figure 2 – SF2. Moreover, variable importance in the projection (VIP) scores were examined to confirm the role of markers associated with the investigated variables and eventually highlight new volatiles with a potential marker role. Forty-eight targeted analytes were characterized by VIPs greater than 1 on LV1 and LV3 in the storage months' model, while 40 of them were discriminant in the storage conditions model. The complete list is reported in Supplementary Table 3 – ST3 with an arrow indicating whether the analyte is positively or negatively contributing to the probability for an unknown sample to be classified as a member of a particular class. Note that the PLS-DA multi-class models were based on UT features% responses (*i.e.*, 2D peak response normalized over the IS and then over the total chromatographic response – MSTUS method). Therefore, an analyte could have positive/negative probability coefficients as a function of the specific class.

The combination of the information collected from classification models and variables interaction showed that short-chain saturated aldehydes registered higher normalized responses in samples undergone sub-optimal storage conditions (SA) with a positive correlation with storage months. Being secondary products of lipid autoxidation, their role as rancidity markers is validated by further supporting their use in the AI decision-making tool [37]. Moreover, at longer shelf-life times (9–12 months), the extent of oxidative processes converts carbonyl species into carboxylic acids, where an increase is registered for both octanoic and nonanoic homologs. Both chemical classes (carbonyls and carboxylic acids) are negatively impacting the storage classification model for the UV class. Other discriminant chemical species are primary alcohols (*e.g.,* 1-hexanol), which are also associated with SA storage, and are the final degradation products of linoleic acid [9,10,38–50]. These findings are aligned with previous studies on storage stability by Kinderlerer *et al.* [10], on Turkish cultivars/blend quality by Alasalvar *et al.* [51,52], by Cialiè Rosso *et al.* [6] and Cordero and coworkers [14,53] when focusing on high-quality hazelnuts for confectionery.

Monoterpenes did not show significant evolution along shelf-life, although they have a weak influence on the SA storage model, most probably due to a differential impact of storage ambient to the seed metabolism.

3.2. Multiple markers quantitative analysis: accuracy and precision

Quantification is one of the most difficult tasks when HS sampling is applied to a solid matrix/sample. The physical form of the matrix prevents the adoption, at least for accurate quantifica-

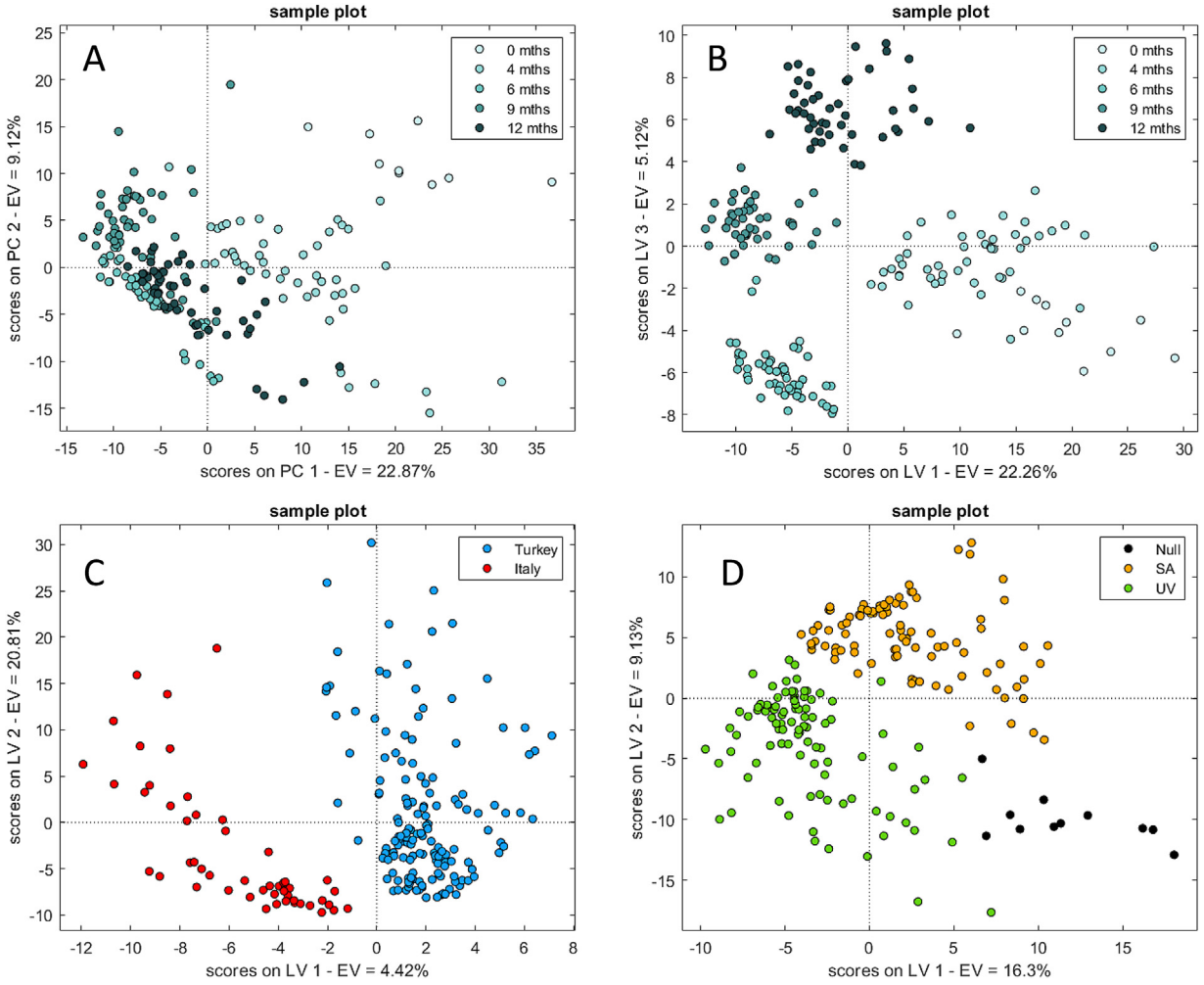

Fig. 2. (2A) PCA score plot of untargeted and targeted (UT) reliable peaks (203 samples x 439 variables) based on normalized responses after Z-score normalization, displaying the storage months (*i.e.*, 0 months, 4 months, 6 months, 9 months, 12 months with lighter to darker shades of green). PLS-DA score plot of untargeted and targeted (UT) reliable peaks (203 samples x 439 variables) based on normalized responses after Z-score normalization, displaying the storage months (2B), the geographical area (*i.e.*, Turkey – blue, Italy – red) (2C), and the storage conditions (*i.e.*, Null – black, Standard Atmosphere (SA) – orange, Under Vacuum (UV) – green) (2D).

tion purposes, of any equilibrium-based approach since the analyte(s)' release from the condensed phase to the HS is governed by a combination of sorption/desorption and adsorption mechanisms [25,54], the latter affected by displacement issues. Moreover, HS linearity, *i.e.*, the absence of the HS saturation, is compulsory especially if HS sampling is conducted in non-equilibrium conditions. To achieve a suitable accuracy level, external calibration should also take into account the matrix effect, as done by standard addition procedure with or without the use of stable isotope-labelled standards [18]. Note that standard addition provides accurate results in absence of HS saturation or for equilibrium sampling [17,18,55,56].

In the case of non-equilibrium samplings, MHE approach is the elective route [57,58]. It intrinsically overcomes the matrix effect by approaching/modeling exhaustive extraction of analytes from the sample HS. MHE was modelled by Kolb and Ettre [57] and successfully applied to HS-SPME sampling [18,24,25,54,59,60] as MHS-SPME. Principles of operation and fundamental equations are out of the scope of this manuscript, information is provided in reference studies [18,24,25,54,59,60].

In the present study, MHS-SPME was applied by external calibration on a selection of marker volatiles (hexanal, heptanal, octanal, nonanal, decanal, 4-heptanone, 2-pentanol, 2-heptanol, γ-hexalactone) selected for their informative role (lipid autoxidation secondary products, key-aromas, spoilage indicators) and also covering a suitable volatility/polarity range. External calibration was used in combination with FID-predicted relative response factors (RRFs) based on combustion enthalpies and molecular formula [61] to quantify additional volatiles not affected by HS saturation in the sampling conditions.

FID-predicted RFFs correlate the analyte response with its amount in the sample, relative to an internal standard (IS), taking into account the molecular weight and the structure (*e.g.*, number C–H-O and other heteroatoms, the number of aromatic rings) together with coefficients related to combustion enthalpy and IS identity. Equation 1 allows the estimation of the RRF for analytes whose formula is known. By predicted RRF accurate quantification without calibration is possible as long as IS quantification is consistent and accurate.

$$\mathbf{RRF} = 10^3 * \left(\frac{\boldsymbol{MW_i}}{\boldsymbol{MW_{IS}}}\right) * (-61.3 + 88.8\eta_C + 18.7\eta_H - 41.3\eta_O + 6.4\eta_N + 64.0\eta_S - 20.2\eta_F - 23.5\eta_{Cl} - 10.2\eta_{Br} - 1.75\eta_I + 127\eta_{benz})^{-1} \quad (1)$$

In this study, the IS for RRFs application was hexanal, which amount was estimated by external calibration in all samples.

Table 1
Quantified analytes together with their retention times (^1t_R, ^2t_R), experimental and tabulated ^{1}D I^T, odor qualities and odor threshold as listed in reference literature [46], calculated RRFs, β values (*i.e.*, constant representing the MHE exponential decay function [41]), and precision data on normalized responses over 3 analytical replicates from 12 different samples. For analytes included in the external calibration, linear function parameters (slope, intercept, and determination coefficient R^2) are also reported.

										External calibration			
Analyte	^1t_R min	^2t_R *sec*	Exp. I^T	tab i^t	odor quality	OT (ng/g)	RRFs	$\beta \pm$ SD		Uncertainty (% RSD)	Range (ng/g)	Slope	Intercept	R^2
Hexanal	11.5	0.93	1083	1080	green, grassy	276	0.967	0.662	0.026	10.8	1_50	0.030	0.038	0.998
											50_500	0.021	−0.079	0.986
Heptanal	15.3	0.98	1183	1186	oily, fatty	50	1.412	0.748	0.061	16.8	1_50	0.028	0.000	0.996
											50_500	0.024	0.129	0.983
Octanal	19.1	1.01	1288	1289	soapy	51	1.365	0.792	0.037	7.0	1_50	0.025	0.094	1.000
											50_500	0.021	0.037	0.983
Nonanal	22.8	1.03	1393	1390	fatty, waxy	610	1.329	0.892	0.022	10.9	1_50	0.031	0.227	0.932
											50_500	0.017	0.382	0.986
Butanoic acid	29.3	0.38	1621	1637	cheesy	135	2.357	0.928	0.007	10.5				
Decanal	26.1	0.91	1497	1496	orange peel like	75	1.301	0.837	0.064	14.3	1_50	0.035	0.073	1.000
											50_500	0.024	0.274	0.979
4-Heptanone	13.0	1.05	1124	1131	fruity, sweet	8.2	1.412	0.699	0.037	9.0	1_50	0.030	0.092	1.000
											50_500	0.035	−0.623	0.987
2-Pentanol	12.7	0.59	1115	1115	fruity	470	1.504	0.765	0.028	10.5	1_50	0.020	0.017	0.999
											50_500	0.033	−1.362	0.976
Nonanoic acid	38.0	0.38	2154	2144	fatty, waxy	2400	1.540	0.803	0.015	16.8				
α-pinene	9.3	1.67	1022	1022	terpenic	274	1.168	0.842	0.028	9.9				
3-Methyl-4-heptanone	14.0	1.17	1149	1178	nutty	0.86	1.365	0.875	0.045	8.8				
Octanoic acid	36.6	0.36	2048	2046	fatty waxy rancid	3000	1.608	0.781	0.021	16.1				
Pentanal	8.0	0.81	980	978	pungent, like bitter almond	150	1.574	0.684	0.057	12.6				
Hexanoic acid	33.4	0.38	1836	1839	pungent, musty	460	1.828	0.898	0.026	19.6				
1-Hexanol	21.1	0.60	1345	1340	fruity	400	1.425	0.768	0.046	9.8				
2,3-Butanediol	27.0	0.43	1530	1545	buttery, creamy	20,000	2.184	0.918	0.035	10.4				
Decanoic acid	39.3	0.35	2258	2265	fatty	230,000	1.487	0.861	0.035	8.8				
2-Heptanone	15.1	0.97	1180	1175	herbaceous	300	1.412	0.617	0.024	18.6				
4-Heptanol	18.7	0.68	1277	1281	alcoholic	410	1.371	0.685	0.053	10.9				
1-Pentanol	17.4	0.58	1242	1244	fruity	470	1.504	0.675	0.055	10.2				
γ-Butyrolactone	29.5	0.51	1630	1626	creamy, fatty, caramel	20,000	2.569	0.922	0.013	8.9				
Heptanoic acid	35.1	0.38	1943	1946	rancid, fatty	100	1.700	0.797	0.066	19.2				
3-Methylbutanoic acid	30.2	0.38	1663	1666	sweaty	22	2.024	0.865	0.086	10.9				
Propanoic acid	27.1	0.41	1535	1534	Sour	720	3.047	0.865	0.055	11.4				
Sabinene	12.8	1.52	1117	1120	woody, spicy, citrus	750	1.168	0.785	0.046	9.0				
Limonene	15.6	1.43	1193	1199	terpenic	14,700	1.168	0.838	0.046	9.7				
2-Heptanol	19.9	0.65	1312	1318	fresh, lemon	263	1.371	0.725	0.039	9.9	1_50	0.031	0.031	0.995
											50_500	0.042	−1.218	0.990
Pentanoic acid	31.5	0.39	1729	1733	sweaty, fruity	400	2.024	0.906	0.035	12.8				
2-Hexanol	16.4	0.63	1214	1211	herbaceous	15	1.425	0.745	0.038	10.0				
p-Cymene	18.3	1.23	1268	1268	woody, terpenic	18,000	1.190	0.828	0.025	9.9				
Benzaldehyde	26.9	0.64	1527	1530	sweet, oily	60	1.351	0.923	0.023	10.5				
δ−3-Carene	13.8	1.61	1144	1147	citrus, pine, herbal	770	1.050	0.829	0.069	9.6				
γ-Hexalactone	31.1	0.55	1704	1703	sweet, creamy	50	1.913	0.926	0.020	13.5	1_50	0.026	0.021	0.985
											50_500	0.033	−0.449	0.999
(E)−2-Heptenal	20.3	0.85	1322	1332	green, fatty, oily	14	1.457	0.800	0.032	12.6				
(E)−2-Hexenal					green, fatty, cheesy	0.42	1.535	0.812	0.023	8.8				
1-Octanol	27.5	0.55	1549	1546	waxy, green, citrus	900	1.331	0.888	0.009	12.7				
2-Decanone	25.9	0.76	1491	1495	orange, floral	8.3	1.301	0.778	0.127	18.4				
2-Nonanone	22.5	1.01	1387	1390	sweet green	100	1.329	0.825	0.035	8.3				
2-Octanone	18.8	1.12	1279	1281	herbal	510	1.365	0.742	0.011	12.5				
γ-Octalactone	34.8	0.51	1923	1916	sweet	120	1.657	0.928	0.004	20.7				
2-Decenal, (E)-	29.8	0.66	1642	1630	fatty	101	1.327	0.887	0.109	12.7				
(E)−2-Nonenal	27.2	0.77	1536	1543	fatty	4.1	1.359	0.933	0.010	11.5				
3-Methylbutanal	6.4	0.80	918	922	malty	5	1.574	0.848	0.012	11.5				

Table 1 lists all quantified analytes together with their retention times (^1t_R, ^2t_R), experimental and tabulated ^{1}D I^T, odor qualities and odor threshold as listed in reference literature [62], calculated RRFs, β values (*i.e.*, constant representing the MHE exponential decay function [57]), and precision data on normalized responses over 3 analytical replicates from 12 different samples. For analytes included in the external calibration, linear function parameters (slope, intercept, and determination coefficient R^2) are also reported.

Due to the lack of certified reference materials, the quantification accuracy by MHS-SPME-GC×GC–MS/FID was verified using a previously validated and industrially certified method that quantifies linear saturated aldehydes (hexanal, heptanal, octanal, nonanal, decanal) by MHS-SPME-GC–MS [37]. The reference method is currently applied in QC industrial laboratories for rancidity assessment. To cover the full range of oxidation stages, therefore validating the current methodology within the entire dynamic range of application, results from nine samples were cross-compared and

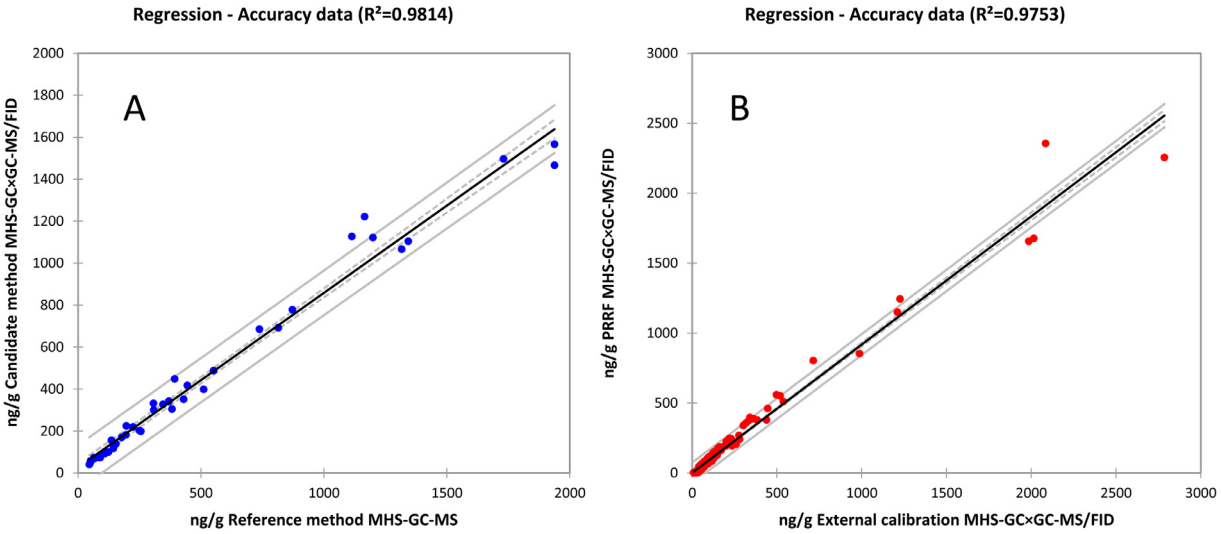

Fig. 3. Regression analysis comparing quantification results between methods. (3A) Correlation between the candidate method by MHS-SPME-GC×GC–MS/FID and the reference method by MHS-GC–MS. Analytes compared are the linear saturated aldehydes from C6 to C10. (3B) Correlation between the estimated amounts by predicted RRFs and the external calibration. Analytes compared are hexanal, 4-heptanone, 2-pentanol, 2-heptanol, and γ-hexalactone.

percent relative error (% RE) was calculated. Results indicate a quite good accuracy, RE was on average |11.6|% and ever below |20|% for all analytes which is considered acceptable by EU Commission indications for food safety analytical methods [63]. Results on accuracy are visualized as linear regression in Fig. 3A where the amount (ng/g) of target aldehydes estimated by the certified method is considered as the independent variable (x) and results obtained by external calibration with the candidate method (*i.e.*, MHS-SPME-GCxGC-MS/FID) are treated as the dependent variable (y). The coefficient of determination indicates a quite good linear correlation between methods with just a few outliers in the higher range, reasonably due to the achievement of the limit of HS linearity.

Given the high complexity of the hazelnut volatilome and the existence of multiple markers informing about quality, the ability to expand the analytical method's quantitation capability to a wide range of analytes while maintaining acceptable accuracy was appealing. Therefore, FID-structure's specific response factors based on combustion enthalpies were estimated using hexanal (IS) for all analytes falling within HS linearity condition. Table 1 lists RRFs calculated by Equation 1 for analytes which showed an exponential decay function by MHE at the sampling conditions. β values above 0.95 were excluded, according to Kolb and Ettre [57] above that limit, HS saturation occurs.

The RRF quantification accuracy was tested *vs.* the results obtained from external calibrations. Five test analytes were used to cover the full spectrum of chemical classes in the hazelnut volatilome (*i.e.,* octanal – aldehydes, 4-heptanone – ketones, 2-pentanol and 2-heptanol – alcohols, γ-hexalactone – esters). The%RE was calculated on 48 samples randomly distributed within all analytical batches. Results referred for acceptable accuracy, with median% RE values of 18.7 for octanal (% RSD 15), 15.8 for 4-heptanone (% RSD 12), 20 for γ-hexalactone (% RSD 17), and 10.5 for 2-heptanol (% RSD 8). Results were tested for correlation and linear regression and shown in Fig. 3B.

To further confirm the inaccuracy of internal standardization if adopted for estimating analytes amount in HS-SPME non-equilibrium sampling [18,19,22,55], results obtained by applying Equation 2 were compared to those from external calibration.

$$C_x = \frac{A_x * C_{IS}}{A_{IS}} \quad (2)$$

Where C_x is the amount or concentration of the analyte x in the analyzed sample, C_{IS} is the amount or concentration of the internal standard, A is the chromatographic area or response for the analyte (a_x) and the IS (A_{IS}). In practice, by internal standardization, the response factor of any target analyte is assumed to be equal to unity, and the matrix effect is neglected.

In the case of internal standardization, the median% RE values achieved 406 for octanal (% RSD 95), 646 for 4-heptanone (% RSD 108), 69 for γ-hexalactone (% RSD 36), and 633.5 for 2-heptanol (% RSD 108).

Once validated for its reliability, the quantitative results were used to develop an AI decision-making tool capable of augmented smelling with further potential to detect spoilage odors, rancidity while keeping its ability to correct classify samples according to origin and shelf-life.

3.3. AI decision-making tools: augmented smelling and robust classification

Aligned with the SEBES concept, which acts as an AI smelling machine [20], the extended quantification results were used to evaluate the putative contribution of potent odorants to the overall aroma profile of samples. Analytes that reported a median for the Odor Activity Value (OAV) > 1 were computed and OAV values visualized in the $\log_{10}$ scale as a spider diagrams. Fig. 4A shows the blueprint of fresh hazelnuts belonging to the three cultivar/geographical regions investigated.

Fresh samples (0 months) have similar profiles, with lower amounts of oxidative markers responsible for the *fatty* and *green* notes, and with the aroma blueprint dominated by 3-methyl-4-heptanone, which is responsible for the *nutty* quality. Along shelf life, samples have a different evolution, Fig. 4B illustrates the blueprint at 12 months of storage in SA. TGT samples, considered as a gold standard for their superior sensory quality, tend to maintain the original characteristics for longer periods without major changes in the aroma-active compound patterns while also eliciting stronger resistance to rancidity (*i.e.*, lower amounts of saturated aldehydes). On the other hand, samples from the other cultivar/geographical origins have a different evolution with the Turkey selection showing a general increase in the amounts of short-chain fatty acids likely impacting *rancid* and *musty* odors. Moreover, TGT

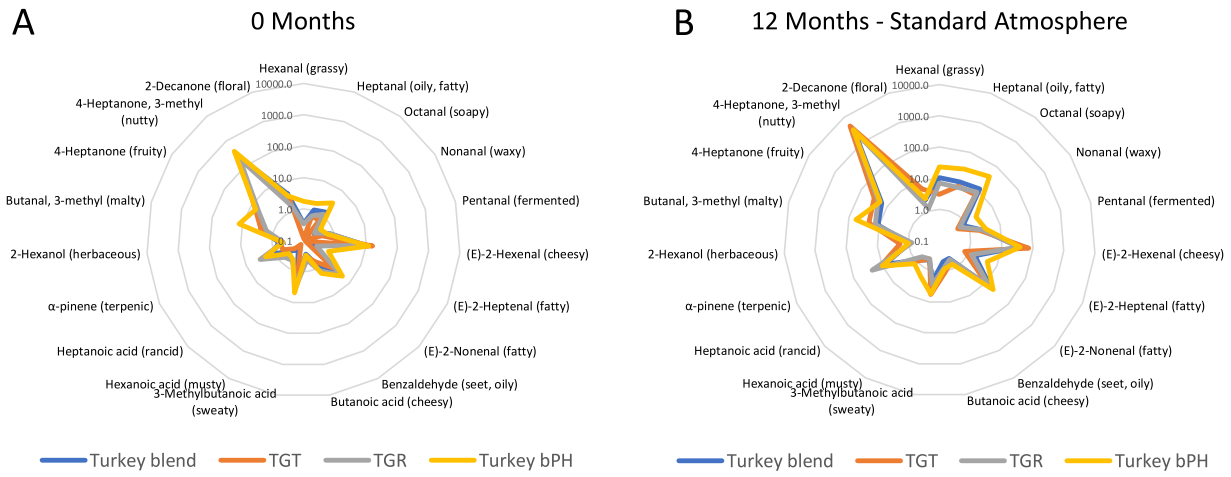

Fig. 4. Spider-diagrams showing OAVs for odorants related to the hazelnut aroma blueprint, spoilage, and rancidity. OAVs are reported in $\log_{10}$ scale. The shelf life changes are shown at 0 months, and 12 months with storage condition with standard atmosphere (SA); cultivar/geographical blend are the Tonda Gentile Trilobata (TGT), Tonda Gentile Romana (TGR), Turkey blend, and Turkey undergone bad post-harvest conditions (bPH).

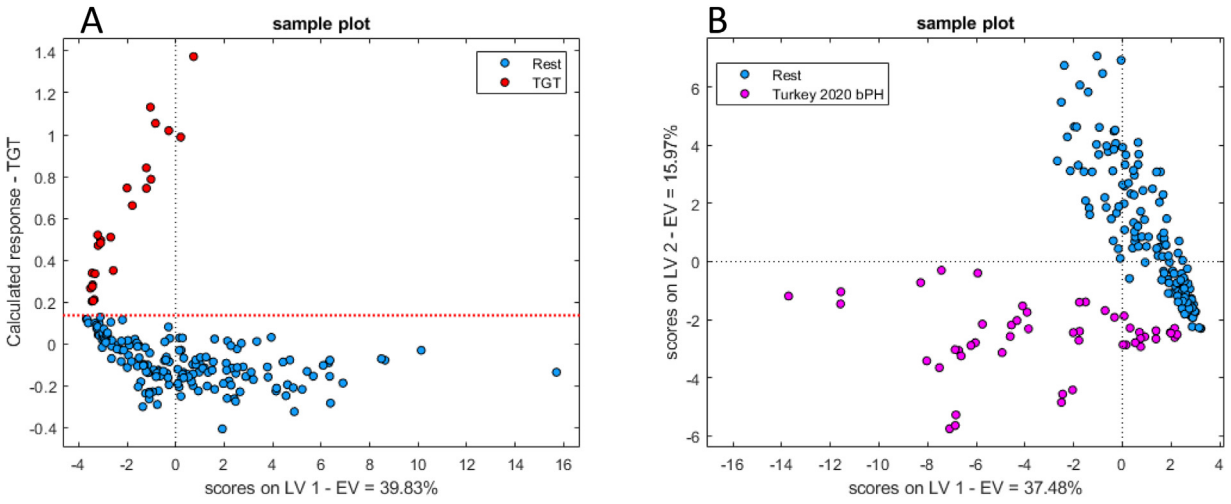

Fig. 5. PLS-DA score plot on quantified analytes (203 samples x 43 variables), displaying the discrimination model results for TGT (Red) *vs.* all (Blue) (5A), and Turkey bPH (Purple) *vs.* all (Blue) (5B).

samples are the ones with generally higher values of key-aroma compounds. To note, the spider diagrams are represented in $\log_{10}$ scale given the different order of magnitude of the OAV values. This means that minimal differences in the pictograms correspond to exponential variations; the complete list of average OAV values for all the samples at the different time-points is reported in Supplementary Table 4 –ST4.

Moreover, quantified data can be also processed with unsupervised and supervised chemometric tools. The latter are of particular interest given the possibility to generate models for quality benchmarking where diagnostic quantitative patterns of markers can be consistently used across many laboratories as AI decision-makers. PLS-DA models were created for two quality variables of interest for the industry: (a) discriminate the gold standard from the other cultivars/origins (*i.e.*, TGT *vs.* rest); and (b) discriminate the critical in-filed post-harvesting *versus* the optimal drying (*i.e.*, bPH *vs.* rest).

The first model, which model score plot is illustrated in Fig. 5A, was created to delineate the peculiar pattern of variables discriminating the gold standard TGT samples independently by the storage time. Odorous ketones, such as 4-heptanone, 2-decanone, and 3-methyl-4-heptanone are diagnostic markers for the TGT samples, while oxidation products as the carboxylic acids (C5 to C10), primary alcohols (C6 and C7), and both saturated and unsaturated aldehydes (C5 to C9) are the analytes discriminating all the other samples. These results are in keeping with previous observations on TGT cultivar and its distinctive volatile signature [14,16,53,64,65].

The second classification model was conducted to assess critical threshold values for pattern variables correlated to bad post-harvest practices (Fig. 5B). This is of particular importance for the industrial quality of kernels, as it was demonstrated to have a tremendous impact on their stability after long shelf-life periods [6]. Interestingly, eight variables were identified as the driving analytes for the classification algorithms: these are pentanoic and hexanoic acid (previously shown to be correlated with mouldy defect [5]), C6 to C8 saturated aldehydes, (*E*)–2-heptenal, 3-methylbutanal (correlated with higher oxidative levels probably due to the increased enzymatic activity correlated with increased moisture content), and two terpenoids, *i.e.*, p-cymene and sabinene (having a role in the plant defense against biotic stresses [66]).

4. Conclusions

The characterization of high-quality hazelnuts was done, for the first time, using a combination of chromatographic fingerprinting and multi-target quantification to achieve quantitative volatilomic as the basis of robust AI decision-making tools. Thanks to the improved separation power and chromatographic resolution compared to 1D-GC, fingerprinting is more specific and selective providing accurate quantification of several quality markers for product benchmarking and discrimination. Moreover, accurate quantitative data can be transferred over time and across laboratories enabling the validation of AI decision-making tools over many harvest years.

The use of comprehensive two-dimensional gas chromatography and parallel detection by MS/FID implemented in a commercial platform with automated sample preparation and data interpretation, a step forward in the objective evaluation of industrial quality is made. Although non-mandatory by law, as for safety requirements, the industry has a great interest in defining chemical quality traits for all incoming materials, since these traits are those that guarantee consumers' preferences/choices while supporting the industry's competitive advantage in the market. By a single analysis, it is possible to objectively capture relevant quality traits named: aroma blueprint, storage quality and time, geographical area of harvest, and spoilage. All these traits can be turned into decision-making tools driving and orienting industry strategic investments and value chains. Since edible crops are living organisms, their complexity is preventing any strategy for quality assessment based on simple, single-marker measurement.

The analytical strategy proposed is also of great interest in a scenario where climate change, market fluctuations, and geopolitical stability could impact the availability of crops with suitable quality. Robust tools can guide strategic warehousing and industrial storage while guiding breeding strategies in new promising geographical areas.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Nicola Spigolon, Dr. Giuseppe Genova, Dr. Giuseppe Castello, Dr. Irene Cincera are all employees of Soremartec Italia srl

CRediT authorship contribution statement

Simone Squara: Investigation, Formal analysis, Data curation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Andrea Caratti:** Investigation, Data curation, Writing – review & editing. **Angelica Fina:** Investigation, Data curation, Writing – review & editing. **Erica Liberto:** Data curation, Visualization, Writing – review & editing. **Nicola Spigolon:** Conceptualization, Writing – review & editing. **Giuseppe Genova:** Funding acquisition, Resources, Conceptualization, Writing – review & editing. **Giuseppe Castello:** Resources, Data curation, Writing – review & editing. **Irene Cincera:** Data curation, Writing – review & editing. **Carlo Bicchi:** Conceptualization, Supervision, Writing – review & editing. **Chiara Cordero:** Project administration, Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2023.464041.

References

[1] A. Dunkel, M. Steinhaus, M. Kotthoff, B. Nowak, D. Krautwurst, P. Schieberle, T. Hofmann, Nature's chemical signatures in human olfaction: a foodborne perspective for future biotechnology, Angew. Chem. Int. Ed.. 53 (2014) 7124–7143, doi:10.1002/anie.201309508.

[2] M. Granvogl, P. Schieberle, The sensomics approach: a useful tool to unravel the genuine aroma blueprint of foods and aroma changes during food processing, in: Compr. Anal. Chem., 1st ed., Elsevier B.V., 2022, pp. 41–68, doi:10.1016/bs.coac.2021.10.002.

[3] A. Amann, B. de L. Costello, W. Miekisch, J. Schubert, B. Buszewski, J. Pleil, N. Ratcliffe, T. Risby, The human volatilome: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva, J. Breath Res. 8 (2014) 34001, doi:10.1088/1752-7155/8/3/034001.

[4] P.M. Dewick, The biochemistry of plant phenolics, Phytochemistry 25 (1986) 2011–2012, doi:10.1016/s0031-9422(00)81203-0.

[5] F. Stilo, E. Liberto, N. Spigolon, G. Genova, G. Rosso, M. Fontana, S.E. Reichenbach, C. Bicchi, C. Cordero, An effective chromatographic fingerprinting workflow based on comprehensive two-dimensional gas chromatography – mass spectrometry to establish volatiles patterns discriminative of spoiled hazelnuts (Corylus avellana L, Food Chem. 340 (2021) 128135, doi:10.1016/j.foodchem.2020.128135.

[6] M. Cialiè Rosso, E. Liberto, N. Spigolon, M. Fontana, M. Somenzi, C. Bicchi, C. Cordero, 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, Anal. Bioanal. Chem. 410 (2018) 3491–3506, doi:10.1007/s00216-017-0832-6.

[7] L. Nicolotti, C. Cordero, C. Cagliero, E. Liberto, B. Sgorbini, P. Rubiolo, C. Bicchi, Quantitative fingerprinting by headspace-Two-dimensional comprehensive gas chromatography-mass spectrometry of solid matrices: some challenging aspects of the exhaustive assessment of food volatiles, Anal. Chim. Acta 798 (2013) 115–125, doi:10.1016/j.aca.2013.08.052.

[8] J. Kiefl, Differentiation of Hazelnut Cultivars (Corylus Avellana L.) by Metabolomics and Sensomics Approaches Using Comprehensive Two-dimensional Gas Chromatography Time-of-flight Mass Spectrometry (GCxGC/TOF-MS). PhD Thesis Editor:Verlag, Deutsche Forschungsanstalt für Lebensmittelchemie (DFA) (2013).

[9] H.D. Belitz, W. Grosch, P. Schieberle, Food Chemistry, 4th edition, Springer, Berlin, Heidelberg, 2009, doi:10.1007/978-3-540-69934-7.

[10] J.L. Kinderlerer, S. Johnson, Rancidity in hazelnuts due to volatile aliphatic aldehydes, J. Sci. Food Agric. 58 (1992) 89–93, doi:10.1002/jsfa.2740580115.

[11] S. Squara, F. Stilo, M. Cialiè Rosso, E. Liberto, N. Spigolon, G. Genova, G. Castello, C. Bicchi, C. Cordero, Corylus avellana l. aroma blueprint: potent odorants signatures in the volatilome of high quality hazelnuts, Front. Plant Sci. 13 (2022) 1–25, doi:10.3389/fpls.2022.840028.

[12] F. Ortega-Gavilán, S. Squara, C. Cordero, L. Cuadros-Rodríguez, M.G. Bagur-González, Application of chemometric tools combined with instrument-agnostic GC-fingerprinting for hazelnut quality assessment, J. Food Compos. Anal. 115 (2023) 104904, doi:10.1016/j.jfca.2022.104904.

[13] V. Cristofori, S. Ferramondo, G. Bertazza, C. Bignami, Nut quality and sensory evaluation of hazelnut cultivars, Acta Hortic. 845 (2009) 657–664, doi:10.17660/ActaHortic.2009.845.103.

[14] C. Cordero, E. Liberto, C. Bicchi, P. Rubiolo, P. Schieberle, S.E. Reichenbach, Q. Tao, Profiling food volatiles by comprehensive two-dimensional ga schromatography coupled with mass spectrometry: advanced fingerprinting approaches for comparative analysis of the volatile fraction of roasted hazelnuts (Corylus avellana L.) from different ori, J. Chromatogr. A 1217 (2010) 5848–5858, doi:10.1016/j.chroma.2010.07.006.

[15] A. Giraudo, R. Calvini, G. Orlandi, A. Ulrici, F. Geobaldo, F. Savorani, Development of an automated method for the identification of defective hazelnuts based on RGB image analysis and colourgrams, Food Control. 94 (2018) 233–240, doi:10.1016/j.foodcont.2018.07.018.

[16] J. Kiefl, G. Pollner, P. Schieberle, Sensomics analysis of key hazelnut odorants (Corylus avellana L. 'Tonda Gentile') using comprehensive two-dimensional gas chromatography in combination with time-of-flight mass spectrometry (GC×GC-TOF-MS), J. Agric. Food Chem. 61 (2013) 5226–5235, doi:10.1021/jf400807w.

[17] C. Cordero, A. Guglielmetti, B. Sgorbini, C. Bicchi, E. Allegrucci, G. Gobino, L. Baroux, P. Merle, Odorants quantitation in high-quality cocoa by multiple headspace solid phase micro-extraction: adoption of FID-predicted response factors to extend method capabilities and information potential, Anal. Chim. Acta 1052 (2019) 190–201, doi:10.1016/j.aca.2018.11.043.

[18] B. Sgorbini, C. Cagliero, E. Liberto, P. Rubiolo, C. Bicchi, C. Cordero, Strategies for accurate quantitation of volatiles from foods and plant-origin materials: a challenging task, J. Agric. Food Chem. 67 (2019) 1619–1630, doi:10.1021/acs.jafc.8b06601.

[19] H.H. Jeleń, M.N. Wieczorek, Commentary: "quantitative" *vs* quantitative headspace solid-phase microextraction (HS-SPME) in food volatile and flavor compounds analysis, J. Food Compos. Anal. 115 (2023) 104955, doi:10.1016/j.jfca.2022.104955.

[20] L. Nicolotti, V. Mall, P. Schieberle, Characterization of key aroma compounds in a commercial rum and an australian red wine by means of a new sensomics-based expert system (SEBES) - an approach to use artificial intelligence in determining food odor codes, J. Agric. Food Chem. 67 (2019) 4011–4022, doi:10.1021/acs.jafc.9b00708.
[21] A. Aleta, F. Brighenti, O. Jolliet, E. Meijaard, R. Shamir, Y. Moreno, M. Rasetti, A need for a paradigm shift in healthy nutrition research, Front. Nutr. 9 (2022) 1–9, doi:10.3389/fnut.2022.881465.
[22] F. Stilo, M. Del P. Segura Borrego, C. Bicchi, S. Battaglino, R.M. Callejón Fernadez, M.L. Morales, S.E. Reichenbach, J. Mccurry, D. Peroni, C. Cordero, Delineating the extra-virgin olive oil aroma blueprint by multiple headspace solid phase microextraction and differential-flow modulated comprehensive two-dimensional gas chromatography, J. Chromatogr. A 1650 (2021) 462232, doi:10.1016/j.chroma.2021.462232.
[23] B. Kolb, L.S. Ettre, Static Headspace-Gas chromatography : Theory and Practice, Wiley-VCH, New York, 2006 https://books.google.com/books?hl=en&lr=&id=nGPmpb4VvEgC&oi=fnd&pg=PR5&ots=6SZHNIygx6&sig=lcvdWEsXW-3H8BzCWuKuK2PnjiI#v=onepage&q&f=false.
[24] B. Sgorbini, C. Bicchi, C. Cagliero, C. Cordero, E. Liberto, P. Rubiolo, Herbs and spices: characterization and quantitation of biologically-active markers for routine quality control by multiple headspace solid-phase microextraction combined with separative or non-separative analysis, J. Chromatogr. A 1376 (2015) 9–17, doi:10.1016/j.chroma.2014.12.007.
[25] R. Costa, L. Tedone, S. De Grazia, P. Dugo, L. Mondello, Multiple headspace-solid-phase microextraction: an application to quantification of mushroom volatiles, Anal. Chim. Acta 770 (2013) 1–6, doi:10.1016/j.aca.2013.01.041.
[26] M. Giardina, J.D. McCurry, P. Cardinael, G. Semard-Jousset, C. Cordero, C. Bicchi, Development and validation of a pneumatic model for the reversed-flow differential flow modulator for comprehensive two-dimensional gas chromatography, J. Chromatogr. A 1577 (2018) 72–81, doi:10.1016/j.chroma.2018.09.022.
[27] D. Ballabio, A MATLAB toolbox for Principal Component Analysis and unsupervised exploration of data structure, Chemom. Intell. Lab. Syst. 149 (2015) 1–9, doi:10.1016/j.chemolab.2015.10.003.
[28] D. Ballabio, V. Consonni, Classification tools in chemistry. Part 1: linear models, PLS-DA Anal. Methods. 5 (2013) 3790–3798, doi:10.1039/c3ay40582f.
[29] C. Cordero, A. Guglielmetti, C. Bicchi, E. Liberto, L. Baroux, P. Merle, Q. Tao, S.E. Reichenbach, Comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry featuring tandem ionization: challenges and opportunities for accurate fingerprinting studies, J. Chromatogr. A 1597 (2019) 132–141, doi:10.1016/j.chroma.2019.03.025.
[30] M. Cialiè Rosso, M. Mazzucotelli, C. Bicchi, M. Charron, F. Manini, R. Menta, M. Fontana, S.E. Reichenbach, C. Cordero, Adding extra-dimensions to hazelnuts primary metabolome fingerprinting by comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry featuring tandem ionization: insights on the aroma potential, J. Chromatogr. A 1614 (2020) 1–11, doi:10.1016/j.chroma.2019.460739.
[31] F. Magagna, L. Valverde-Som, C. Ruíz-Samblás, L. Cuadros-Rodríguez, S.E. Reichenbach, C. Bicchi, C. Cordero, Combined untargeted and targeted fingerprinting with comprehensive two-dimensional chromatography for volatiles and ripening indicators in olive oil, Anal. Chim. Acta. 936 (2016) 245–258, doi:10.1016/j.aca.2016.07.005.
[32] S. Squara, F. Manig, T. Henle, M. Hellwig, A. Caratti, C. Bicchi, S.E. Reichenbach, Q. Tao, M. Collino, C. Cordero, Extending the breadth of saliva metabolome fingerprinting by smart template strategies and effective pattern realignment on comprehensive two-dimensional gas chromatographic data, Anal. Bioanal. Chem. (2023), doi:10.1007/s00216-023-04516-x.
[33] N. Scientific, T. Databases, NIST/EPA/NIH Mass Spectral Library (NIST 14) and-NIST Mass Spectral Search Program (Version 2.2). Database available at. https://www.nist.gov/system/files/documents/srd/NIST1aVer22Man.pdf.
[34] C. Alasalvar, F. Shahidi (Eds.), Tree Nuts: Composition, Phytochemicals, and Health Effects (1st ed.), CRC Press, 2008, doi:10.1201/9781420019391.
[35] M. Cialiè Rosso, F. Stilo, S. Mascrez, C. Bicchi, G. Purcaro, C. Cordero, Shelf-life evolution of the fatty acid fingerprint in high-quality hazelnuts (corylus avellana l.) harvested in different geographical regions, Foods 10 (2021) 685, doi:10.3390/foods10030685.
[36] Q.S. Xu, Y.Z. Liang, Monte carlo cross validation, Chemom. Intell. Lab. Syst. (2001), doi:10.1016/S0169-7439(00)00122-2.
[37] S. Squara, A. Caratti, F.O. Gavilan, P. Bolzoni, N. Spigolon, G. Genova, G. Castello, M.G.B. González, L. Cuadros-Rodriguez, C. Bicchi, C. Cordero, Validation of a high-throughput method for the accurate quantification of secondary products of lipid oxidation in high-quality hazelnuts (Corylus avellana L.): a robust tool for quality assessment, J. Food Compos. Anal. 114 (2022) 104766, doi:10.1016/j.jfca.2022.104766.
[38] L.Q. Zepka, R. Wagner, E. Jacob-Lopes, M.M. Daltoé, A.B. Santos, A.F. Torri, J.Z. Donadel, M.I. Queiroz, Study of the Volatile Compounds Useful for the Characterization of Frozen Anchoita (Engraulis anchoita) By SPME-GC-MS, Elsevier Inc., 2014, doi:10.1016/b978-0-12-398549-1.00032-5.
[39] S. Kunyaboon, K. Thumanu, J.W. Park, C. Khongla, J. Yongsawatdigul, Evaluation of lipid oxidation, volatile compounds and vibrational spectroscopy of silver carp (Hypophthalmichthys molitrix) during ice storage as related to the quality of its washed mince, Foods. 10 (2021), doi:10.3390/foods10030495.
[40] R. Hung, S. Lee, J.W. Bennett, The effects of low concentrations of the enantiomers of mushroom alcohol (1-octen-3-ol) on Arabidopsis thaliana, Mycology 5 (2014) 73–80, doi:10.1080/21501203.2014.902401.
[41] D. Ghirardello, M. Bertolino, S. Belviso, B. Dal Bello, M. Giordano, L. Rolle, V. Gerbi, M. Antonucci, N. Spigolon, G. Zeppa, Phenolic composition, antioxidant capacity and hexanal content of hazelnuts (Corylus avellana L.) as affected by different storage conditions, Postharvest Biol. Technol. 112 (2016) 95–104, doi:10.1016/j.postharvbio.2015.09.039.
[42] M. Pedrotti, I. Khomenko, G. Genova, G. Castello, N. Spigolon, V. Fogliano, F. Biasioli, Quality control of raw hazelnuts by rapid and non-invasive fingerprinting of volatile compound release, LWT 143 (2021) 111089, doi:10.1016/j.lwt.2021.111089.
[43] S. Belviso, B. Dal Bello, S. Giacosa, M. Bertolino, D. Ghirardello, M. Giordano, L. Rolle, V. Gerbi, G. Zeppa, Chemical, mechanical and sensory monitoring of hot air- and infrared-roasted hazelnuts (Corylus avellana L.) during nine months of storage, Food Chem. 217 (2017) 398–408, doi:10.1016/j.foodchem.2016.08.103.
[44] M. Locatelli, J.D. Coïsson, F. Travaglia, M. Bordiga, M. Arlorio, Impact of roasting on identification of hazelnut (Corylus avellana L.) origin: a chemometric approach, J. Agric. Food Chem. 63 (2015) 7294–7303, doi:10.1021/acs.jafc.5b03201.
[45] M. Özdemir, F. Açkurt, M. Yildiz, G. Biringen, T. Gürcan, M. Löker, Effect of roasting on some nutrients of hazelnuts (Corylus Avellena L.), Food Chem. 73 (2001) 185–190, doi:10.1016/S0308-8146(00)00260-0.
[46] D. Ghirardello, C. Contessa, N. Valentini, G. Zeppa, L. Rolle, V. Gerbi, R. Botta, Effect of storage conditions on chemical and physical characteristics of hazelnut (Corylus avellana L.), Postharvest Biol. Technol. 81 (2013) 37–43, doi:10.1016/j.postharvbio.2013.02.014.
[47] M.A. Koyuncu, A. Islam, M. Küçük, Fat and fatty acid composition of hazelnut kernels in vacuum packages during storage, Grasas Aceites 56 (2005) 263–266, doi:10.3989/gya.2005.v56.i4.91.
[48] A. Turan, Effect of drying methods on fatty acid profile and oil oxidation of hazelnut oil during storage, Eur. Food Res. Technol. 244 (2018) 2181–2190, doi:10.1007/s00217-018-3128-y.
[49] S.M. Momchilova, S.P. Taneva, D. Zlatanov, G.A. Antova, M.J. Angelova-Romova, E. Blagoeva, Fatty acids, tocopherols and oxidative stability of hazelnuts during storage, Bulg. Chem. Commun. 49 (2017) 65–70.
[50] A. Memoli, D. Albanese, M. Esti, C. Lombardelli, A. Crescitelli, M. Di Matteo, I. Benucci, Effect of bug damage and mold contamination on fatty acids and sterols of hazelnut oil, Eur. Food Res. Technol. 243 (2017) 651–658, doi:10.1007/s00217-016-2778-x.
[51] C. Alasalvar, E. Pelvan, B. Bahar, F. Korel, H. Ölmez, Flavour of natural and roasted Turkish hazelnut varieties (Corylus avellana L.) by descriptive sensory analysis, electronic nose and chemometrics, Int. J. Food Sci. Technol. 47 (2012) 122–131, doi:10.1111/j.1365-2621.2011.02817.x.
[52] C. Alasalvar, A.Z. Odabasi, N. Demir, M.Ö. Balaban, F. Shahidi, K.R. Cadwallader, Volatiles and flavor of five Turkish Hazelnut varieties as evaluated by descriptive sensory analysis, electronic nose, and dynamic headspace analysis/gas chromatography-mass spectrometry, J. Food Sci. (2004), doi:10.1111/j.1365-2621.2004.tb13382.x.
[53] L. Nicolotti, C. Cordero, C. Bicchi, P. Rubiolo, B. Sgorbini, E. Liberto, Volatile profiling of high quality hazelnuts (Corylus avellana L.): chemical indices of roasting, Food Chem 138 (2013) 1723–1733, doi:10.1016/j.foodchem.2012.11.086.
[54] C. Bicchi, M.R. Ruosi, C. Cagliero, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, Quantitative analysis of volatiles from solid matrices of vegetable origin by high concentration capacity headspace techniques: determination of furan in roasted coffee, J. Chromatogr. A 1218 (2011) 753–762, doi:10.1016/j.chroma.2010.12.002.
[55] F. Stilo, C. Cordero, B. Sgorbini, C. Bicchi, E. Liberto, Highly informative fingerprinting of extra-virgin olive oil volatiles: the role of high concentration-capacity sampling in combination with comprehensive two-dimensional gas chromatography, Separations 6 (2019) 34, doi:10.3390/separations6030034.
[56] F. Stilo, E. Liberto, S.E. Reichenbach, Q. Tao, C. Bicchi, C. Cordero, Exploring the extra-virgin olive oil volatilome by adding extra dimensions to comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry featuring tandem ionization: validation of ripening markers in headspace linearity conditio, J. AOAC Int. 104 (2021) 274–287, doi:10.1093/jaoacint/qsaa095.
[57] B. Kolb, L.S. Ettre, Theory and practice of multiple headspace extraction, Chromatographia 32 (1991) 505–513, doi:10.1007/BF02327895.
[58] J. Pawliszyn, C.F. Ross, Headspace analysis, in: compr. sampl. sample prep, Elsevier (2012) 27–50, doi:10.1016/B978-0-12-381373-2.10036-5.
[59] Ó. Ezquerro, G. Ortiz, B. Pons, M.T. Tena, Determination of benzene, toluene, ethylbenzene and xylenes in soils by multiple headspace solid-phase microextraction, J. Chromatogr. A 1035 (2004) 17–22, doi:10.1016/j.chroma.2004.02.030.
[60] W.Q. Xie, Y.X. Gong, K.X. Yu, Enhancing the sensitivity of full evaporation technique using multiple headspace extraction analysis, Chromatographia 80 (2017) 1263–1268, doi:10.1007/s10337-017-3343-x.
[61] J.Y. De Saint Laumer, E. Cicchetti, P. Merle, J. Egger, A. Chaintreau, Quantification in gas chromatography: prediction of flame ionization detector response factors from combustion enthalpies and molecular structures, Anal. Chem. 82 (2010) 6457–6462, doi:10.1021/ac1006574.
[62] F. Stilo, M. Cialiè Rosso, S. Squara, C. Bicchi, C. Cordero, C. Cagliero, Corylus avellana l. natural signature: chiral recognition of selected informative components in the volatilome of high-quality hazelnuts, Front. Plant Sci. 13 (2022) 1–15, doi:10.3389/fpls.2022.844711.
[63] E. Commission, Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to, Off. J. Eur. Union. 180 (2021) 84–109 http://extwprlegs1.fao.org/docs/pdf/eur203999.pdf.

[64] J. Kiefl, P. Schieberle, Evaluation of process parameters governing the aroma generation in three hazelnut cultivars (Corylus avellana L.) by correlating quantitative key odorant profiling with sensory evaluation, J. Agric. Food Chem. 61 (2013) 5236–5244, doi:10.1021/jf4008086.
[65] M. Cialiè Rosso, F. Stilo, C. Bicchi, M. Charron, G. Rosso, R. Menta, S.E. Reichenbach, C.H. Weinert, C.I. Mack, S.E. Kulling, C. Cordero, Combined untargeted and targeted fingerprinting by comprehensive two-dimensional gas chromatography to track compositional changes on hazelnut primary metabolome during roasting, Appl. Sci. 11 (2021) 525, doi:10.3390/app11020525.
[66] B. Singh, R.A. Sharma, Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications, 3 Biotech 5 (2015) 129–151, doi:10.1007/s13205-014-0220-2.