Quality control of raw hazelnuts by rapid and non-invasive fingerprinting of volatile compound release

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ABSTRACT

Although hazelnuts are mostly consumed after toasting and mixed with other ingredients, for manufactures it is important to have efficient quality control tests on the raw product that they purchase from farmers and suppliers. This study explores the possibility to predict sensory quality of raw hazelnuts, classified according to industrial sensory evaluation, using volatilome analysis through Proton Transfer Reaction Mass Spectrometry (PTR-MS) rapid fingerprinting. Firstly, the link between volatile markers for different visual and sensory defects was investigated. Uncompliant hazelnuts showed higher concentrations for a larger number of volatile organic compounds (VOCs) than compliant samples, including some key hazelnuts odorants like 5-methyl-4-heptanone, 5-propyldihydro-2(3H)-furanone, octanal, 2,4-nonadienal and hexanal. Secondly, by mixing defective and good quality hazelnuts, the method sensitivity in recognizing defects percentage was determined. For about 13% of the detected mass peaks, the method was able to discriminate samples containing 20% of hazelnuts with unacceptable quality from good quality samples. Finally, unsupervised data clustering of VOCs fingerprints obtained with different precursor ions (H_3O^+, NO^+ and O_2^+) provided a correct classification rate higher than 90% for all ions. The applied methodology is suitable to support sensory quality control programs of raw hazelnuts in confectionary industries.

1. Introduction

Hazelnuts (*Corylus avellana* L.) have a relevant role in agroindustry due to their nutritional and their unique and distinctive flavour (Ciarmiello et al., 2014; Wang et al., 2018) which makes them appreciated as ingredient in a variety of food products. More than one million tons of hazelnuts were produced worldwide in 2017, being Turkey the main producer (67.1%) followed by Italy (13.1%). Only 5% of hazelnuts production is intended for direct consumption while about 95% is used and processed by confectionary, chocolate and bakery industries (Eskandari, Kermani, Kouravand, & Zarafshan, 2018).

Hazelnuts market standards imply severe quality control: cultivar, cultural techniques, geographical origin, harvesting time, post-harvest management and processing, morphological and physio-chemical characteristics and aroma are the main parameters monitored to assess the final hazelnut quality (Cubero-Leon, Peñalver, & Maquet, 2014; Klockmann, Reiner, Cain, & Fischer, 2017; Locatelli et al., 2011).

The "rotten hazelnut" is one of the major defects affecting commercial quality, yield losses and market values since it is associated with negative sensory attributes, such as mold, old, bitter and earthy tastes (Battiliani et al. 2018). In commercial evaluation, rotten includes defects like brown spotted or mouldy kernels and can originate along the supply chain, especially during harvest and post-harvest stages. Different fast and non-invasive sorting technologies for hazelnuts defects were tested in agroindustry (Moscetti et al., 2015), however, most of them consider only visible defects which represent less than 50% of uncompliant products (Battilani et al., 2018). Investigation of raw hazelnuts "volatilome" - the main responsible of hazelnuts flavour perception – could be a valid alternative for quality control. Surprisingly, it has been evaluated only marginally as most of the studies have been focusing on volatile organic compounds (VOCs) and aroma produced after the roasting process. Burdack-Freitag and Schieberle (2010) quantified 37

odour-active compounds in raw nuts, calculated the odour activity values of 19 odorants and tested them through aroma recombination experiments (Burdack-Freitag & Schieberle, 2012a, 2012b). Recently, Rosso et al. (2018) presented a comprehensive two-dimensional gas chromatograph approach coupled with mass spectrometry detection (GC × GC-MS) to evaluate high-quality hazelnuts volatilome during the production chain and described the effect of drying temperatures and storage on hazelnut aroma. Despite being the benchmark analytical method for VOCs identification and quantification, GC-MS techniques are not designed to perform on-line measurements due to their low time resolution that, even by using high-speed GC, is at best in the minutes range (Ellis & Mayhew, 2013). Moreover, a sampling and pre-treatment phase often introduces concentration time averages of the measured mixture (Blake, Monks, & Ellis, 2009; Dewulf & Van Langenhove, 2002). Direct injection mass spectrometry (DIMS) methods have been developed to overcame some of these GC-MS drawbacks, due to the possibility to perform rapid, non-invasive, direct analysis without any or little need in terms of sample preparation and preconcentration. These characteristics make the DIMS methods more suitable for quality control industrial applications. Proton Transfer Reaction Mass Spectrometry (PTR-MS) is one of the available DIMS which, thanks to its high-sensitivity and rapidity, has been already applied in different agroindustry applications (Biasioli, Yeretzian, Gasperi, & Mark, 2011; Pedrotti, Spaccasassi, Biasioli, & Fogliano, 2019). For example, headspace PTR-MS fingerprint approach was applied to monitor food VOCs evolution as a function of time (*e.g.* shelf life, ageing, post-harvest storage, ripening and fermentation), as a function of ingredients reformulations (*e.g.* change of ingredient and concentrations), for classification challenges (*e.g.* geographical origin, and cultivar) and for quality control (*e.g.* freshness, adulteration, quality classification) (for an overview see: Ellis & Mayhew, 2014). Some of the most recent PTR-MS applications examples are in saffron (Nenadis, Heenan, Tsimidou, & Van Ruth, 2016) and anhydrous milk fat (Pedrotti et al., 2018, 2020) quality control, for evaluating shelf life of poultry meat (Wojnowski et al., 2018) and lactose free milk (Bottiroli et al., 2020) and for botanical and geographical origins characterization of both cocoa (Acierno, Yener, Alewijn, Biasioli, & Van Ruth, 2016) and coffee beans (Yener et al., 2014, 2015).

In this paper, industrial sensory evaluation was coupled to volatile fingerprinting obtained by PTR-ToF-MS coupled to an autosampler (Capozzi et al., 2017), a selective reagent ionization system (SRI) (Lanza et al., 2015), tailored data analysis and data mining tools to build predictive models for raw hazelnuts quality. This research includes three experiments with different sample sets aiming at (i) identifying VOCs markers linked to visual defects (light, dark and mouldy rotten), (ii) determining method sensitivity by mixing different percentages of good quality and uncompliant products and (iii) setting efficient models to predict the sensory quality of raw hazelnuts based on non-invasive and rapid PTR-MS fingerprint.

2. Material and methods

2.1. Samples

All hazelnuts samples were obtained from a selection operated by the industrial partner through industrial quality protocols and parameters. The procedure is described in the following paragraphs. The samples obtained through this procedure are a representation of the quality variability levels that agroindustry operates with.

2.1.1. Visual defects experiment

Raw hazelnuts samples (*Corylus avellana* L.*)* were obtained from different lots of chopped Turkish hazelnuts (*Akçakoca* region, 2017) after visual inspection according to industry quality standards (Fig. 1). These visual inspections were conducted by trained inspectors in quality control from the industrial partner during one year. The inspection divided the samples in good quality (YES) and rotten samples. Rotten samples were divided by industrial evaluation in three different classes (LIGHT, DARK and MOLD rotten) according to the type and the degree of the defect (Battilani et al., 2018; Pscheidt, Heckert, Wiseman, & Jones, 2019): samples with internal discoloration which tend to opaque/white to translucent, buttery yellow colour where assigned to the "LIGHT" class, samples with darker colour/black spots to the "DARK" class and samples with white and green molds to the "MOLD" class.

2.1.2. Sensory defects experiment

For this experiment, two classes of ground raw hazelnuts samples were obtained from the industrial partner: good quality samples (YES) and bad quality samples (NO). The sampling was based on industrial sensory evaluation (described in the sensory analysis section) and originated from 2016 harvest of Turkish hazelnuts (*Akçakoca* region) from different suppliers. All samples had a selected calibre of 13–14 mm.

A) OK **B) LIGHT ROTTEN**

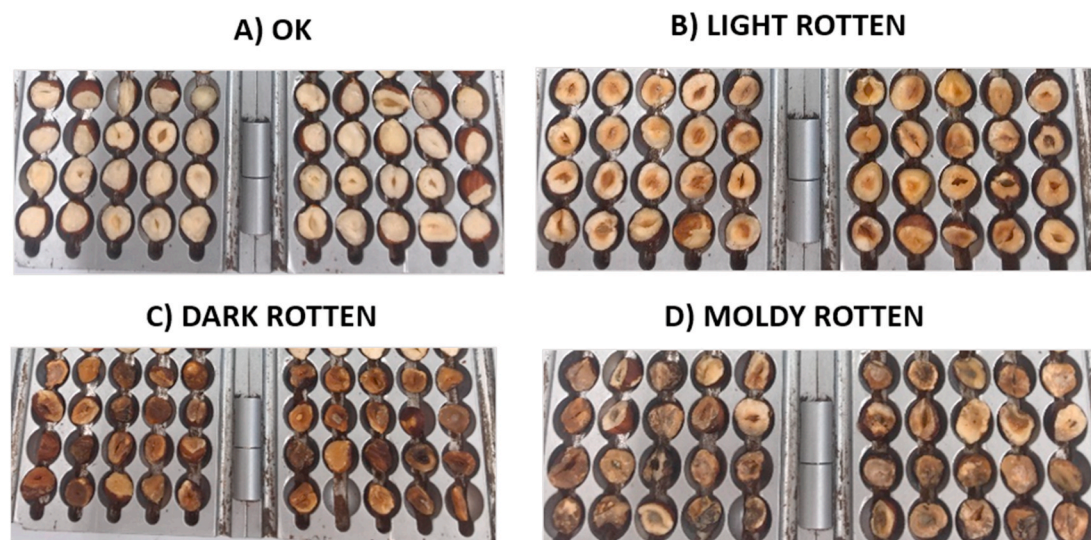**C) DARK ROTTEN** **D) MOLDY ROTTEN**

Fig. 1. Example of raw hazelnuts samples analyzed in the visual defects experiment. The rotten samples were divided in three classes of defects: light rotten ("LIGHT"), dark rotten ("DARK"), mouldy rotten ("MOLD").

2.1.3. Blind classification experiment

Raw hazelnuts samples (a total of 44, approximately 1 kg each) were provided by the industrial partner in blind. These samples were four biological replicates of 11 different lots divided in classes according to industrial evaluation: good quality (YES, 5 samples), bad quality (NO, 5 samples) and high quality reference (REF, 1 sample). All hazelnuts had a selected calibre of 13–14 mm and were stored at 5.0 ± 0.1 °C with a controlled atmosphere (78% N_2–21% O_2) and with 65% of ERH (equilibrium relative humidity) except for the ones of the "YES" sample ("I") that were stored at 5 °C but in a modified atmosphere (99% N_2– 1% O_2). "REF" samples resulted from 2017 harvest and were from mono-cultivar 'Tonda Gentile Trilobata'. "NO" hazelnut samples resulted from 2015 to 2016 harvests while "YES" samples from 2016 to 2017 harvests. Both "YES" and "NO" were a Turkish blend harvested in the *Ordu* and *Akçakoca* regions. Additional details can be found in Table 2.

All raw hazelnuts from the three experiments, once collected from the industrial partner were stored at - 20 °C $\pm$ 1 °C. An overview of the samples measured is presented in Table 1. For the sake of clarity, in the paper we will refer to the experiments as: visual defects, sensory defects and blind classification experiments.

2.2. Sensory analysis

For both the sensory defects and the blind classification experiment, sensory evaluation was carried out by the industrial partner for dividing the samples in the different quality classes. Sensory analysis was performed in multiple sessions by 30 internal judges (aged between 25 and 50 years, 13 women) according to the standard "A – not A" test (ISO 8588:2017). After a training where the panel inspected multiple examples of compliant ("A") and not compliant ("not A") hazelnuts, panellists received samples according to a replicated mixed design and were asked whether each one is either compliant ("A") or not ("not-A"). For the evaluation, panellists were instructed to firstly evaluate samples odour and then their flavour by tasting them. After each evaluation, judges were asked to rinse the mouth with water. For the blind classification experiment a flash profiling (Dairou & Sieffermann, 2002; Delarue & Sieffermann, 2004) with 15 panellists (aged between 30 and 50 years, 6 women) was also conducted to give an indication of the aroma defect for the "NO" samples.

2.3. PTR\SRI-ToF -MS analysis

2.3.1. Samples preparation

For the sensory defects experiment, grain hazelnuts were already

Table 1
Sample description for the three experiments. For each sample is indicated the grain percentage that has been used, the number of biological replicates and Proton Transfer Reaction Mass Spectrometry (PTR-MS) ionization mode for PTR-MS measurements. Each biological replicate was obtained by sampling 3 g of hazelnut grain obtained by grinding approximately 15 hazelnuts (40–50 g) randomly picked from each lot.

Code	Number of samples (percentages)	Replicates (biologic)	PTR-MS ionization mode
Experiment 1: visual defects			
YES	1 (100%)	4	H_3O^+
LIGHT	4 (10, 50, 80, 100%)	4	H_3O^+
DARK	4 (10, 50, 80, 100%)	4	H_3O^+
MOLD	4 (10, 50, 80, 100%)	4	H_3O^+
Experiment 2: sensory defects:			
YES	1 (100%)	5	H_3O^+
NO	6 (5, 10, 20, 50, 80, 100%)	5	H_3O^+
Experiment 3: blind classification (for details see Table 2)			
YES	5 × 4 (blind replicates)	3 × 2 technical	H_3O^+, NO^+, O_2^+
NO	5 × 4 (blind replicates)	3 × 2 technical	H_3O^+, NO^+, O_2^+
REF	1 (Tonda Gentile Trilobata) x 4	3 × 2 technical	H_3O^+, NO^+, O_2^+

available and five replicates of 3.00 ± 0.05 g grain were prepared. In this case, the grains were mixed to create different levels of defects to simulate real industrial applications (Table 2).

For the visual defects and blind classification experiments grain hazelnuts were produced from whole unshelled hazelnuts. In this case, for each sample 15 hazelnuts (approx. 40–50 g) were ground by a IKA®A11 basic analytical mill (IKA Werke, Staufen im Breisgau, Germany) under liquid nitrogen and 3.00 ± 0.05 g grain were then transferred into 20 mL vials. For each sample biological replicate, the procedure was repeated with 15 new hazelnuts to obtain a new grain.

For the visual defects experiment, defective samples (LIGHT, DARK and MOLD classes) were measured as pure (100%) or mixed with different quantities of "YES" sample (90%, 50%, 20%) after being ground (Table 1). These measurements were performed in four replicates by preparing four different grains for each sample. For each sample of the blind classification experiment, three hazelnuts grains were measured in duplicate. All samples were kept at 6 °C until PTR-MS analysis.

2.3.2. Measurement

All measurements were performed by using a multipurpose GC sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) connected to PTR-ToF-MS through a heated PEEK capillary tube (D = 1 mm, T = 110 °C) as previously described (Yener et al., 2014). Before the experiment, few tests were run to optimize both the sample preparation method and the measurement procedure. PTR-MS instrumental parameters and incubation temperature were optimized for obtaining the best PTR-MS signal in terms of sensitivity and at the same time avoiding signal saturation. Incubation, sampling and waiting time were optimized to maximize headspace equilibration, to reduce total measurement time and to avoid memory effects.

As result of this optimization procedure, all samples were incubated at 50 °C for 25 min for headspace equilibration and then measured for 60 s with an acquisition rate of one spectrum per second and a flow rate of 35 cm³/min. The measurement order was randomized and a waiting time of 3 min was set to prevent memory effects.

A commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) in its standard configuration (V mode) was used. The instrument was equipped with a SRI system that allowed operation in H_3O^+, NO^+ or O_2^+ mode as described elsewhere (Cappellin et al., 2014; Sánchez del Pulgar et al., 2013). SRI was used only for the blind classification experiment. The instrumental conditions were as following: drift pressure 280 Pa, drift temperature 110 °C, ion source current and drift voltages were adjusted according to the ion mode to get the optimal instrument conditions. Ion source current was set at 3.5 mA for H_3O^+ mode, 5.0 mA for NO^+ and O_2^+ ones. A drift voltage of 537, 548 and 458 V was used for H_3O^+, NO^+ and O_2^+, resulting in an reduced electric field (E/N) value of 128, 132 and 105 Td (Townsend, 1 Td = 10^{21} V * m²) respectively. For the visual and the sensory defects experiments a radio frequency ion funnel to improve sensitivity (Brown et al., 2017) was used which resulted in a different drift voltage (628 V) and an ion funnel voltage of 18.2 V. In all cases, the mass resolution (m/Δm) was at least 3800.

2.3.3. Data processing

PTR\SRI-ToF-MS spectra were processed according to Cappellin et al. (2010, 2011). Dead time correction and peak extraction were performed to reach a mass accuracy of ~0.001, sufficient for determining sum formula of volatile compounds. Peak intensities from the mass spectra were converted in concentrations in ppbV (parts per billion by volume) according to Lindinger, Hansel, and Jordan (1998), assuming a constant reaction rate coefficient (k = 2 × 10^{-9} cm³ s⁻¹) which leads to a systematic error in the concentration estimation below 30% (Cappellin et al., 2012). For each sample, the average of the first 40 spectra of the measurement were obtained and the concentrations were converted in μg/L.

Table 2
Additional details on the samples of the blind classification experiment. Different hazelnuts from different regions, different harvest years and with a different storage (modified atmosphere) were selected. Each hazelnut lot was sent as four blind replicates (tag replicates) by the industrial partner resulting in a total of 44 hazelnuts samples, each consisting of approximately 1 kg of hazelnuts.

Code	Origin	Year	Storage atmosphere	Tag replicates				Sensory	Sensory description
A	Akçakoca	2016	78% N_2–21% O_2	1	2	3	4	NO	Rancid
B	Ordu	2016	78% N_2–21% O_2	5	6	7	8	NO	Weak old
C	Akçakoca	2016	78% N_2–21% O_2	9	10	11	12	NO	Old
D	Ordu	2015	78% N_2–21% O_2	13	14	15	16	NO	Mold, rancid
E	Akçakoca	2015	78% N_2–21% O_2	17	18	19	20	NO	Mold, rancid
F	Akçakoca	2016	78% N_2–21% O_2	21	22	23	24	YES	
G	Akçakoca	2016	78% N_2–21% O_2	25	26	27	28	YES	
H	Ordu	2016	78% N_2–21% O_2	29	30	31	32	YES	
I	Akçakoca	2016	99% N_2–1% O_2	33	34	35	36	YES	
L	Akçakoca	2017	78% N_2–21% O_2	37	38	39	40	YES	
REF	Piedmont	2017	78% N_2–21% O_2	R1	R2	R3	R4	REFERENCE	Tonda Gentile Trilobata

2.3.4. Data analysis

A mass peaks selection procedure (Pedrotti et al., 2020) was applied to extract relevant information and reduce noise signal associated to PTR-ToF-MS measurements. This procedure allowed to select 179 peaks (first experiment), 212 peaks (second experiment), 120, 104 and 105 peaks (third experiments H_3O^+, O_2^+ and NO^+) for further statistical analysis.

Principal components analysis (PCA) was performed after logarithmic transformation and mean centering of the mass peaks for data visual inspection. One-way ANOVA ($P < .005$) and post-hoc test (Tukey honest significant difference) were performed to evaluate VOCs emissions differences among samples classes and to select a manageable number of peaks to discuss further. In this case, the confidence level is merely an indication of the magnitude of the difference. Further noise reduction was obtained by considering only mass peaks above 0.5 µg/L for at least one of the hazelnuts classes. For the visual defects experiment, the classes with 100% of defects were used. The results for the visual and sensory defects experiments were summarized on tables. Tentative peak identification was performed by using the in-house library developed by the authors and through literature review (Burdack-Freitag & Schieberle, 2010, 2012a, 2012b; Cialiè Rosso et al., 2018; Kiefl & Schieberle, 2013).

The reduced data sets obtained with the different ionization modes from the blind classification experiment, were scaled (mean centering and unit variance) and represented as heat maps. Firstly, a K-mean clustering (with $k = 2$) was performed on the samples (columns) to divide between "YES" and "NO" classes followed by hierarchical clustering (Manhattan distances, Ward method), (Murtagh & Legendre, 2014). For mass peaks (rows) only hierarchical clustering was applied. The same was applied also to the data from other ionization modes (NO^+ and O_2^+).

All the statistical analysis were run in R software v3.6.3 (Gu, Eils, & Schlesner, 2016; R Core Team, 2016) and related packages (ChemometricsWithR, mixOmics, multcomp, vegan, matrixStats, ComplexHeatmap, ggplot2).

3. Results

3.1. VOCs linked to visual defects

The first two components of PCA analysis (Fig. 2) on the mass peaks explained 79% of the total variability and the defective hazelnuts classes were distinguishable from the "YES" class already when 20% and 50% of defective hazelnuts were present in the samples. The different hazelnuts were distributed in the space as clusters: PC1 separated "YES" samples from "DARK" and "LIGHT" ones, while PC2 separated the "YES" from
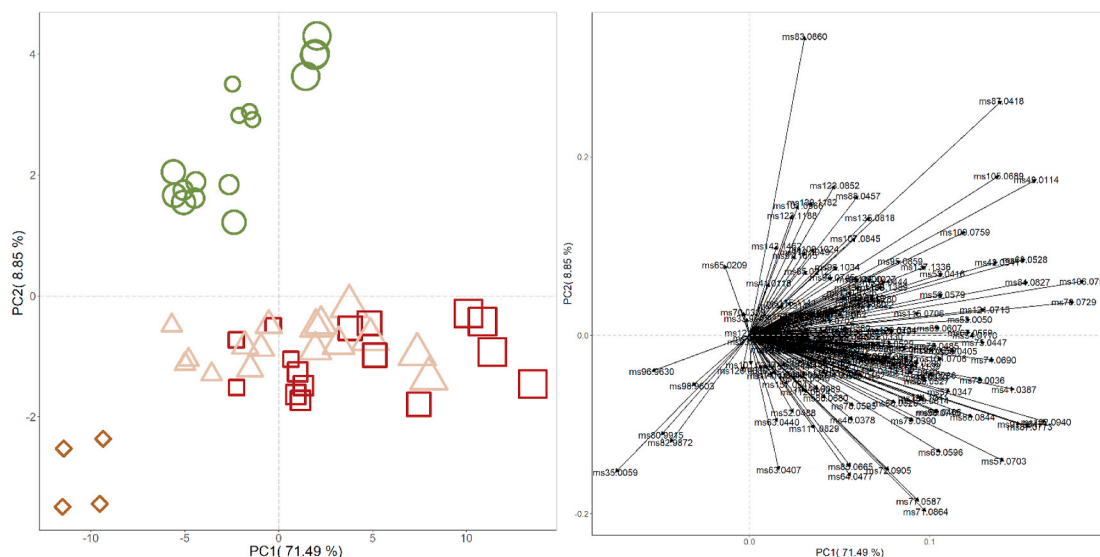

Fig. 2. Score and loading plots of explorative principal component analysis (PCA) for the 179 mass peaks obtained from the visual defects experiment. The first two PC are shown. Different colors and shape indicate different sample classes and in particular: = DARK, = MOLD, = LIGHT, = YES. Different sizes represent different percentage of mixture of the samples. Larger the point, larger is the percentage of defective hazelnuts present in the hazelnuts ranging from 0% (100%" YES") to 20%, 50%, 90% and 100%. For each sample the four biological replicates are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

"MOLD". According to the loadings plot (Fig. 2), most of the mass peaks contributed to PC1 and had a higher concentration for the "DARK" and "LIGHT" classes which were grouped together and were not separated by the analysis. Research on specific causes of mold in hazelnuts is still scarce but most probably these visual defects are caused by different microorganisms. While *Mycospharella punctiformis* has been associated to kernel tip necrosis (black spots) and *Nematospera coryli* to kernel dark spots, *Diaphorte* genus and *Septoria ostryae* are commonly associated to internal kernels' discoloration (Battilani et al., 2018). By looking at Fig. 2, giving the same percentage of defected hazelnuts in the sample, the "DARK" class had a higher concentration of some mass peaks characterizing the PC1 including *m/z* 41.039, 70.073, 87.077, 91.065, 115.071, and 121.072 than the "LIGHT" class. The "MOLD" class was characterized by few specific mass discussed more in details in the next section, while the "YES" class had a lower concentration of most VOCs.

The distinction of the defective classes observed with the PCA was confirmed by the 1-way ANOVA analysis and the post-hoc test. This univariate data analysis found 105 mass peaks significantly different for at least one of the class ($P < .005$) and were reported in Table S1 (supplementary materials). Table 3 is a reduced version of Table S1, where a selection of the key hazelnuts compounds (Burdack-Freitag & Schieberle, 2010, 2012a, 2012b; Cialiè Rosso et al., 2018; Kiefl & Schieberle, 2013; Nicolotti et al., 2013) characteristic for each class is reported.

No peak was significantly higher for "YES" samples. This result was in line with the previous observation from the PCA where "YES" samples were characterized by low intensities for most of the measured VOCs. The "DARK" class showed the highest portion of mass peaks significantly higher than the other classes (70%). The mass peaks 41.039, 42.034 and 87.077 tentatively identified respectively as an alkyl fragment, a fragment of nitrogen-containing VOCs and 2/3-methylbutanal/2-pentanone/pentanal were the most concentrated compounds. The "LIGHT" class had 18 mass peaks with the highest concentration. Among these *m/z* 81.07, 89.061, 99.083, 101.058 and 155.148 were associated to linalool/monoterpenes fragment, butanoic acid/ethyl acetate/acetoin, 2,3-pentanedione and 2-decenal/linalool. The presence of specific volatile markers for the "DARK" and the "LIGHT" classes corroborates the hypothesis that the two classes originates from different microorganisms which produce different aroma compounds. These microorganisms could be responsible for the production of the detected volatiles or could have affected hazelnut's metabolic pathways. For example, monoterpenes such as linalool are formed by fruits directly from geranyl diphosphate via the isoprenoid pathway (Lewinsohn et al., 2001). The fungi infection may have altered the pathway. Moreover, some molds which occur on hazelnuts (*e.g., Penicilium*) are capable of forming terpenes, such as limonene, myrcene, and valencene (Demyttenaere, Moriña, & Sandra, 2003). Moreover, recently one *Diaphorte* spp, *Diaporthe apiculatum,* was also found capable of monoterpenes production (Song et al., 2019) and therefore it could also be responsible for the higher levels measured in the "LIGHT" class.

"LIGHT" and "MOLD" samples had also high levels of *m/z* 115.114 tentatively identified as a mixture of different compounds, including prenyl ethyl ether. This compound, which has been associated to mold activity, is responsible for a metallic, solvent like off-flavour in hazelnuts when present together with significant concentrations of hazelnuts terpenes (Amrein, Schwager, Meier, Frey, & Gassenmeier, 2010, 2014). "MOLD" class had other 6 mass peaks that showed higher concentrations than the other classes: *m/z* 83.086, 87.042, 101.097, 109.102, 139.118 and 143.146. Most of these mass peaks were tentatively identified as aldehydes and ketones like *m/z* 101.097 which could be hexanal, 3-methyl-2-pentanone, its isomer 2-hexanone or a contribution of all the three different molecules. The measurements performed on the third experiment with NO$^+$ as primary ion - able to separate aldehydes and ketones (Yener et al., 2015) - can give further indications to disentangle the contributions of each compound. *M/z* 99.082 resulting from the hydride ion transfer reaction of the aldehyde with NO$^+$, presented a

comparable concentration to *m/z* 101.097 (H$_3$O$^+$ dataset). On the other hand, *m/z* 100.086 and 130.087 in the NO$^+$ dataset, that could correspond respectively to 3-methyl-2-pentanone deriving from the charge-transfer reaction and to 2-hexanone deriving from the ion-molecule association reaction with NO$^+$ (depending on their different ionization energy) (Smith & Španěl, 2005), had lower concentrations than the aldehyde. Hexanal and its fragment (*m/z* 83.086) originate from oxidation of unsaturated fatty acids, in hazelnuts mostly oleic and linoleic acids (Amaral et al., 2006; Xu, Yu, Li, Chen, & Wang, 2018). Fungal growth, together with other factors, is related to the occurrence of oxidative processes and changes in the activity of hydrolytic enzymes (Amaike & Keller, 2011; Moscetti et al., 2015). The higher concentration of hexanal in the "MOLD" class possibly derives from an augmented hydrolysis of fatty acids due to fungal activity. This leads to an increased production of free fatty acids that, through auto-oxidation reactions, could lead to develop of rancidity, off-flavors and bitterness (Köksal, Artik, Şimşek, & Güneş, 2006).

3.2. Sensory defects linked to VOCs

In Table 3 and S1 are presented the results from the univariate data analysis, where 69 mass peaks were significantly different in at least one of the mixtures ($P < .005$). Also in this experiment samples with higher percentage of "NO" (defective) sample had higher VOCs emission. When comparing the 100% "YES" *vs* 0% "YES", all mass peaks but two - *m/z* 101.058 and 129.081 tentatively identified as 2,3-pentanedione and 5-propyldihydro-2(3H)-furanone - showed a significantly higher concentration in the 0% "YES" class. It may be that when these compounds are over a certain concentration, the samples are penalized by industrial sensory evaluation and indicated as non-compliant.

Some mass peaks were tentatively identified as key hazelnuts aroma compounds (Burdack-Freitag & Schieberle, 2012a, 2012b; Kiefl & Schieberle, 2013) as. *e.g. m/z* 129.128 associated to 3/5-methyl-4-heptanone, octanal or 2-octanone (Fig. 3A). 5-methyl-4-heptanone has a *fruity and hazelnuts-like* aroma, very low odour threshold (0.2 µg/kg in oil) and the highest odour active value after linalool in raw hazelnuts (Burdack-Freitag & Schieberle, 2012a, 2012b). The same authors hypothesized that this compound is biochemically formed in raw nuts because it decreases during roasting. This mass peak could be associated also to octanal (Burdack-Freitag & Schieberle, 2012a, 2012b; Cialiè Rosso et al., 2018). Again, to better determine the contribution of the aldehyde and the ketones it is useful to examine the NO$^+$ data in the blind classification experiment. M/z 158.118 corresponding to the compound obtained by the ion-ketone association reaction (M*NO+), has a concentration corresponding to 10% (on average 0.1 µg/L) of *m/z* 127.114 resulting from the charge-transfer reaction of the octanal with NO$^+$. For this reason, we hypothesize that *m/z* 129.128 showed in Fig. 3A is mainly representing octanal contribution. The contribution of the ketones mix (3/5-methyl-4-heptanone and 2-octanone) to the aroma may be still relevant due to their low odour threshold.

m/z 127.112 (Fig. 3B) was tentatively identified as 5-methyl-(E)-2-hepten-4-one, 2-octenal and/or 2-ethyl-2-hexenal. The 5-methyl-(E)-2-hepten-4-one, also known as "filbertone", is a key flavour compound in both raw and roasted hazelnuts and has been evaluated as quality marker for hazelnut pastes (Čížková, Rajchl, Šnebergrová, & Voldřich, 2013; Puchl'ová & Szolcsányi, 2018). Filbertone aroma has been described as *fruity, hazelnut* and *dried fruit* at low threshold (0.05 µg/L in water at 25 °C) (Belitz, Grosch, & Schieberle, 2009) while at higher concentrations (>25 µg/L in water) the compound tends to smell metallic (Guntert et al., 1991).

When looking at technique sensitivity in discriminating percentage of defective samples, the most common trend is the one showed by *m/z* 129.128 (Fig. 3A) where a significant difference was found between the 80% and 50% "YES" samples. About 58% of the mass peaks reported in Table S1 had the same trend, indicating that the technique can discriminate between samples contaminated with 20% and 50% ground

Table 3
Selection of tentatively identified mass peaks from the visual and the sensory defects experiments with a concentration higher than 0.5 μg/L. The concentrations are reported in μg/L.

Mass peak	Chemical formula	Tentative ID	100% YES	95% YES	90% YES	80% YES	50% YES	20% YES	0% YES	YES	LIGHT	DARK	MOLD
41.039	C3H5+	Alkyl fragment	34±8a	37±5a	42±7a	48±6a	82 ± 16b	91 ± 14b	149 ± 29c	48±7a	517 ± 141b	880 ± 210c	198±4a
42.02			32±9 ab	27±9a	27±9 ab	36 ± 12	48 ± 15bc	54 ± 17c	61 ± 20c	15±2a	33±4c	38±2c	24±1b
49.011	CH4SH+	Methanethiol	0.1±0a	0.2±0 ab	0.3±0b	0.5±0c	1.1 ± 0.1d	1.6 ± 0.2e	1.7 ± 0.3e	1 ± 0.1a	15±2b	52 ± 11c	24±4b
68.053	C4H5NH+	Pyrrole								0.4±0a	4.7 ± 0.8b	18.9 ± 0.7c	4.6 ± 0.3b
74.069	C[13] C3H9O+	2-butanone isotope/2-methylpropanal isotope	1.9 ± 0.5a	1.8 ± 0.3a	1.9 ± 0.3a	2.0 ± 0.3a	2.3 ± 0.4 ab	2.2 ± 0.3a	2.7 ± 0.4b	3±2a	39 ± 10b	39 ± 12b	18±1a
81.07	C6H8H+	Linalool fragment/ monoterpenes fragment	5±1a	7±2a	9±2a	21±4b	59 ± 22c	87 ± 11d	95±6d	33±6a	131 ± 35b	123 ± 61b	77±9 ab
83.086	C6H11+	Hexanal fragment	6±2a	10±2 ab	14±5 ab	20±6b	91 ± 22c	104 ± 13c	134 ± 11d	7±2a	59 ± 18b	19±5a	88 ± 14c
85.067		3-penten-2-one/ (E)-2-pentenal								17±5a	18±5a	46±5b	11.4 ± 0.7a
85.099	C6H13+	1-Hexanol fragment (dehydratation)	0.5 ± 0.2a	0.6 ± 0.2a	0.8 ± 0.3a	1.5 ± 0.3a	4.5 ± 1.6b	4.6 ± 0.9b	10±2c	0.9 ± 0.2a	2.4 ± 0.1b	3.6 ± 0.8c	1.6 ± 0.3 ab
87.042	C4H6O2H+	2,3-butanedione	2.3 ± 0.7a	2.6 ± 0.5a	2.4 ± 0.3a	2.8 ± 0.4a	3±1 ab	4±1bc	4±2c	5±3a	67 ± 20b	115 ± 43c	121±9c
87.077	C5H10OH+	2/3-methylbutanal/2-pentanone/ pentanal	39 ± 10a	39±6a	42±8a	43±7a	54 ± 11b	49±9 ab	67 ± 18c	88 ± 21a	570 ± 150	1840 ± 160c	264±7a
89.061	C4H8O2H+	butanoic acid/ethyl acetate/acetoin	5±1 ab	4.6 ± 0.6a	4.5 ± 0.9a	5.5 ± 0.6 ab	6±1bc	7±1cd	8±2d	6±1a	54±9c	50±3c	32±1b
91.065	C4H10O2H+	2,3-butanediol	1 ± 0.4a	1.1 ± 0.2a	1.1 ± 0.2a	1.2 ± 0.2 ab	1.5 ± 0.3bc	1.6 ± 0.3c	2.2 ± 0.4d	1.7 ± 0.7a	14±5a	42 ± 15b	5.1 ± 0.2a
99.083	C6H10OH+	2-hexenal	0.5 ± 0.1a	0.6 ± 0.1a	0.6 ± 0.1a	0.7 ± 0.1a	0.9 ± 0.2b	1 ± 0.1b	1.4 ± 0.2c	0.9 ± 0.1a	1.8 ± 0.4c	1.5 ± 0.1bc	1.2 ± 0.2 ab
101.058	C5H8O2H+	2,3-pentanedione	1.2 ± 0.3b	1.1 ± 0.2b	1.1 ± 0.2b	1 ± 0.1b	1 ± 0.3b	0.9 ± 0.4b	0.5 ± 0.2a	1.7 ± 0.2a	5±2b	2.9 ± 0.3 ab	4.2 ± 0.1b
101.097	C6H12OH+	Hexanal, 3-methyl-2-pentanone, 2-hexanone	2.3 ± 0.8a	3.2 ± 0.5a	3.9 ± 0.9a	5±1a	16±5b	16±3b	34±8c	4.1 ± 0.7a	11±3bc	8±1b	15±2c
109.102	C8H13+	Monoterpene fragment	0.2 ± 0.1a	0.3±0a	0.2±0a	0.4 ± 0.1a	0.8 ± 0.3b	0.9 ± 0.1b	1.4 ± 0.2c	0.5 ± 0.1a	1.2 ± 0.2 ab	1.6 ± 0.1b	1.9 ± 0.7b
112.087	C6H9NOH+	2-acetyl-1-pyrolline/1H-pyrolle-2-ethanol								0.6 ± 0.1a	1 ± 0.1b	1.4±0c	0.5±0a
115.114	C7H14OH+	2/4-heptanone/ heptanal/prenyl ethyl ether	1.1 ± 0.3a	1.4 ± 0.2a	1.4 ± 0.2a	1.8 ± 0.3a	3.4 ± 0.8b	3.9 ± 0.6b	6 ±1c	2.1 ± 0.3a	4.1 ± 0.5b	3.2 ± 0.7b	4.3 ± 0.4b
127.112	C8H14OH+	5-methyl-(E)-2-hepten-4-one/2-octenal/2-ethyl-2-hexenal	0.3 ± 0.1a	0.3±0a	0.3±0a	0.4 ± 0.1a	0.7 ± 0.2b	0.8 ± 0.1b	1.2 ± 0.2c	0.5 ± 0.1a	1.2 ± 0.2b	2 ± 0.1c	1.1 ± 0.2b
129.081	C7H12O2H+	5-propyldihydro-2 (3H)-furanone	0.11 ± 0.03c	0.12 ± 0.02c	0.12 ± 0.01c	0.11 ± 0.02bc	0.09 ± 0.03b	0.08 ± 0.02b	0.04 ± 0.03a	0.2±0a	1.1 ± 0.1b	2.1 ± 0.3c	0.4±0a
129.128	C8H16OH+	3/5-methyl-4-heptanone/ octanal/2-octanone	0.6 ± 0.2a	0.8 ± 0.1a	0.9 ± 0.1a	1.2 ± 0.2a	3 ± 0.9b	2.9 ± 0.5b	5±1c	0.9 ± 0.6a	3.4 ± 0.5b	4±1b	3.1 ± 0.2b
131.104	C7H14O2H+	Ethyl 2-methylbutanoate/heptanoic acid	0.2±0a	0.2±0a	0.2±0a	0.2 ± 0.1a	0.4 ± 0.1b	0.5 ± 0.1b	0.6 ± 0.1c	0.4 ± 0.1a	2.6 ± 0.5b	2.9 ± 0.7b	0.8 ± 0.1a
135.119	C10H15+	p-cymene	0.1±0a	0.1±0a	0.1±0 ab	0.1±0b	0.2 ± 0.1c	0.3±0d	0.4 ± 0.1e	0.2±0a	0.9 ± 0.1c	1.3 ± 0.1d	0.6±0b
137.134	C10H17+	Monoterpenes, linalool fragment, 2-decenal fragment	1.7 ± 0.5a	2.3 ± 0.7a	3±1a	8±2a	26 ± 11b	44±8c	74 ± 18d	15±2a	85 ± 10b	169 ± 41c	93 ± 26b
139.118	C9H14OH+	2,4-nonadienal/2-pentylfuran	0.2±0a	0.2±0 ab	0.2±0 ab	0.3±0b	0.5 ± 0.1c	0.6 ± 0.1d	0.8 ± 0.1e	0.3±0a	0.8 ± 0.1b	1.2±0c	1.5 ± 0.2d
143.146	C9H18OH+	3,5-dimethyl-4-heptanone/2(3H)-furanone,5-butyldihydro/ nonanal/2-nonanone	0.2 ± 0.1a	0.2±0a	0.2±0a	0.3 ± 0.1a	0.7 ± 0.2b	0.6 ± 0.1b	1 ± 0.2c	0.3±0a	0.6 ± 0.1b	0.6 ± 0.1b	0.9 ± 0.1c

Table 3 (*continued*)

Mass peak	Chemical formula	Tentative ID	100% YES	95% YES	90% YES	80% YES	50% YES	20% YES	0% YES	YES	LIGHT	DARK	MOLD
153.127	C10H16OH+	2,4-decadienal								0.1±0a	0.5±0b	0.7 ± 0.1c	0.4±0b
155.148	C10H18OH+	2-decenal/linalool	0.02 ± 0.01a	0.02 ± 0.00a	0.02±0a	0.03±0a	0.1 ± 0.01b	0.1±0b	0.1±0c	0.02 ± 0.01a	0.17± 0.03c	0.16 ± 0.02c	0.11 ± 0.02b

*Mean ± standard deviation of 4 and 5 replicates respectively; different letters in column mean significant difference among the classes of the same experiment (P. < 0.005, Bonferroni correction).
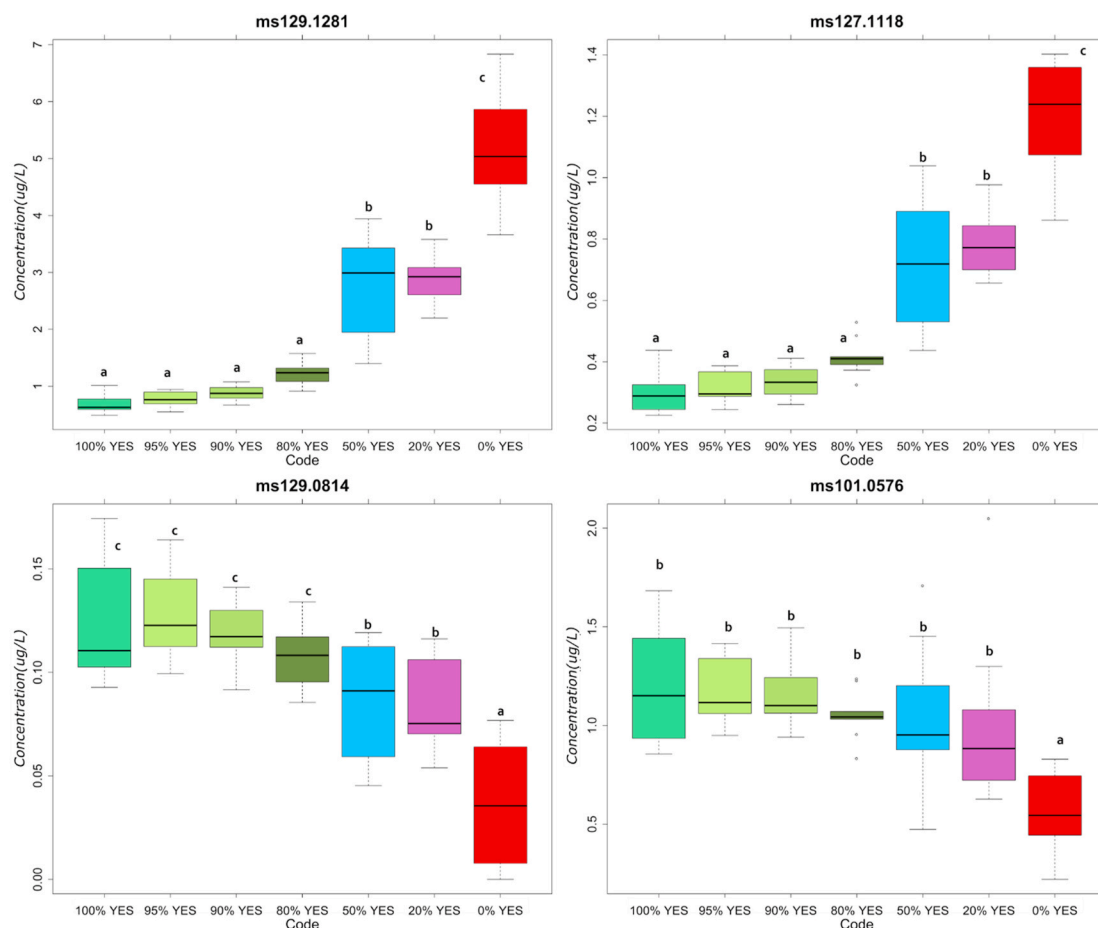
Fig. 3. Boxplots of four relevant mass peaks for the biological replicates of the samples from the sensory defects experiment. A) m/z 129.128 ($C_8H_{16}OH^+$) tentatively identified as 3/5-methyl-4-heptanone, octanal or 2-octanone. B) m/z 127.118 ($C_8H_{14}OH^+$) tentatively identified as 5-methyl-(E)-2-hepten-4-one, 2-octenal and/or 2-hexenal,2-ethyl. C) m/z 129.081 ($C_7H_{12}O_2H^+$) tentatively identified as 5-propyldihydro-2(3H)-furanone. D) m/z 101.058 ($C_5H_8O_2H^+$) tentatively identified as 2,3-pentanedione. For m/z 129.128 and 127.118 an increasing concentration was found when increasing the percentage of defective samples (from 100% "YES" to 0% "YES"). For m/z 129.081 and 101.058 the opposite was found.

hazelnuts of poor quality. However, about 13% of the mass peaks in Table S1 had a significant difference between samples made with 100% and 80% levels of "YES" samples like m/z 49.011, 81.070, 82.075, 83.086, 94.075, 95.086, 96.963, 135.119 and 139.118. Additionally, m/z 49.011 and 81.070 - tentatively identified respectively as methanethiol and as a fragment of linalool and/or as a fragment of monoterpenes – presented a significant difference in concentration also for the sample constituted of 10% and 20% defective hazelnuts. These markers have a more stringent cut-off value for discriminating samples quality and should be further investigated for potential applications in quality control.

Fig. 3C shows m/z 129.081 tentatively identified as 5-propyldihydro-2(3H)-furanone (or ɣ-heptalactone). This lactone, used as food additive to deepen fatty notes of nut flavours, has been identified in many different fruit species as well as in hazelnut (Cialiè Rosso et al., 2018). In

this case, even if at low concentrations, the trend was inverse: increasing the quantity of "NO" sample decreased the compound concentration. A similar trend was highlighted as well for m/z 101.058, tentatively identified as 2,3-pentanedione (Fig. 3D). This molecule, a sugar degradation product which gives a *sweet, buttery* and *caramel-like* odour (Alasalvar, Shahidi, & Cadwallader, 2003), was found to be significantly lower for the 0% "YES" sample (Table 3). This indicates that good quality hazelnuts need to have a minimal concentration of these compounds. Therefore, not only the presence or the absence of a key odorant is fundamental, but as well concentration levels play a role in determining the final raw hazelnuts quality. Future researches should further explore and clarify the concentrations thresholds of these key odorants beyond which they negatively affect hazelnut aroma, by also considering VOCs interactions and the matrix effect. This may be achieved through more targeted approach like the molecular sensory science

approach developed by Schieberle and co-workers (Burdack-Freitag & Schieberle, 2012a, 2012b; Kiefl, Pollner, & Schieberle, 2013).

3.3. Blind classification experiment

In Fig. 4 is presented the heat map for the selected mass peaks (H_3O^+ mode) of the blind classification experiment samples. The K-means

clustering (k = 2), separated the 44 samples into two main clusters corresponding to "NO" and "YES" (including the REF) classes. Only two samples (F23 and G26), corresponding to two different lots from the same harvest year (2016) and the same location (*Akçakoca*, Table 2), were misclassified leading to a classification accuracy of about 95%.

Two main clusters were visible in the mass peaks (C1 and C2 in Fig. 4). The C1 cluster contained most of the selected mass peaks that
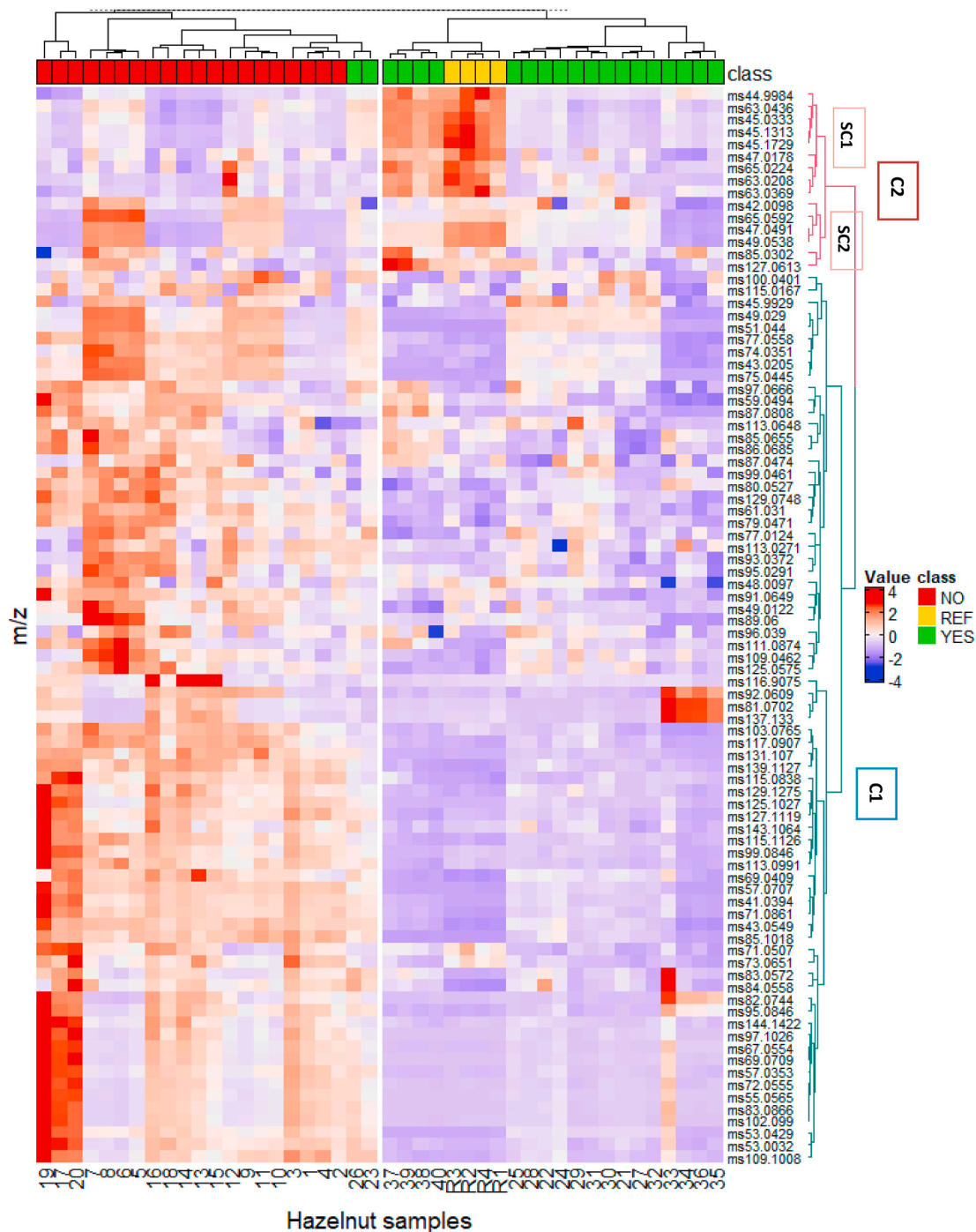

Fig. 4. Heat map-illustrating, from blue (low values) to red (high values), the relative concentration (µg/L) of 88 mass peaks from the 44 raw hazelnuts samples of the blind classification experiment. The color annotation at the top of the heatmap indicates samples quality classification according to industry sensory evaluation (A-not A test). The data for each hazelnut sample were averaged on its biological replicates and the mass peaks were scaled (mean centering and unit variance). For the samples (columns) firstly, K-mean clustering (k = 2) was applied to split the samples in two groups followed by a hierarchical clustering on the Manhattan distances with a Ward method. The dashed line at the top of the samples' dendrogram indicates the K-mean clustering. For the mass peaks (rows) only hierarchical clustering was applied. Mass peaks were divided in two main clusters highlighted by different colors (C1 and C2) with the latter divided in two additional sub-clusters (SC1 and SC2). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

were also found in the previous two experiments. Most of them had higher concentrations for "NO" samples, confirming that good quality samples are characterized by low volatiles emissions. An exception was noticed for replicates 33–36 from sample "I", the one stored at modified atmosphere (99% N_2–1% O_2), which had the highest concentrations for mass peaks associated to fragment of 2-methyl-1H-pyrrole, linalool, 2-decenal and as monoterpenes. Previous studies not only showed the presence of α-pinene and limonene in raw hazelnuts (Burdack-Freitag & Schieberle, 2010; Cialiè Rosso et al., 2018), but they proved that reducing oxygen content in the storage atmosphere limits oxidation phenomena and maintains fruits quality (Ghirardello et al., 2013). Moreover, Rosso et al. (2018) reported lower levels of VOCs known to be secondary products of lipid oxidation like hexanal, octanal and 2-heptenal, in hazelnuts stored in modified atmosphere. In our case, we observed an increase of potent odourants such as linalool, responsible for *flowery* notes (Kiefl et al., 2013), meaning that modified atmosphere prevented its autoxidation.

For most of the mass peaks contained in cluster C1, samples 13–20 coming from replicates of sample "D" and "E", had the highest emissions. Both samples "D" and "E" belonged to 2015 harvest and were evaluated as "NO" samples (Table 2). This information highlighted aging effect on VOCs emissions. The cluster C2 corresponded to mass peaks mostly related to acetaldehyde, ethanol and methanol clusters, ethanethiol/dimethyl sulfide, methanethiol and m/z 42.001, 44.998, 45.033, 45.131, 45.173, 47.018, 47.049. In the cluster are also present m/z 74.035, 77.012, 85.03, 89.06, and 127.061. These mass peaks were divided into two different sub-clusters (SC1 and SC2). Samples R1-R4 (REF sample) and L37-40 were characterized by higher concentrations, especially for SC2 mass peaks. Although from different varieties and geographical origin, these samples were from the most recent harvest (2017). The reference samples were Piedmont hazelnuts know as 'Tonda Gentile Trilobata', a PDO product chosen by the industry as its reference for excellent quality. The mass peaks highlighted in SC2 are then good candidates for discriminating sample freshness and infer sample age.

Similar results were found for heatmaps built with PTR\SRI-ToF-MS data (see Fig. S1 and S2 in supplementary materials). Sample A2, A4, C9 and sample F23 were misclassified when NO^+ was used, leading to a similar classification accuracy (~91%). Also in this case, "NO" samples showed higher values for most of mass peaks with the exception of samples I33-36 that had higher levels for m/z 137.132 and its fragments and samples L37–40 and R1-R4 with mass peaks related to the SC2 described before. When using O_2^+ the same trend was highlighted: "NO" samples presented higher concentrations in more VOCs then "YES" samples and a similar classification accuracy (~93%) was found. Samples F23 and G26 were put it in the "NO" cluster while sample 2 was put in the "YES" cluster.

4. Conclusions

This study has successfully applied PTR/SRI-ToF-MS for rapid screening of raw hazelnuts (*Corylus avellana* L.) samples according to their quality evaluated through industrial quality control protocols. The novel approach described represents a great advantage for industrial manufactures who need to control quality of large numbers of raw materials, especially due to the technique rapidity and the low quantity of sample needed. In particular, the analysis identified specific mass peaks for different types of visual and sensory defects related to sensory/aroma. In all the experiments, defective hazelnuts were shown to have higher levels of most of detected VOCs including some key hazelnuts aroma compounds like 3/5-methyl-4-heptanone, prenyl ethyl ether, hexanal and linalool. Our analytical strategy was able to discriminate mostly between samples with 20% and 50% ground defective hazelnuts. For some mass peaks, a significant difference was observed also between samples made with 0–20% and 10–20% of non-compliant hazelnuts. These biomarkers should be further explored through a targeted

approach for confirming their identity, unravelling their origin and understand their critical concentration threshold. Finally, the possibility to predict sensory classification based on unsupervised clustering upon PTR/SRI-ToF-MS fingerprints was demonstrated by, at the same time, extrapolating information about harvest year and storage from VOCs fingerprints. Future studies should repeat the experiment on a larger hazelnut datasets and validate classification by applying more advanced supervised classification methods. Overall, the study proofed the proposed methodology to be a valid tool to support sensory quality control programs of raw hazelnuts in confectionary industries.

CRediT authorship contribution statement

M. Pedrotti: Methodology, Software, Formal analysis, Investigation, Data curation, writing and reviewing, Visualization. **I. Khomenko:** Methodology, Investigation, Software. **G. Genova:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Supervision, writing & reviewing. **G. Castello:** Conceptualization, Methodology, Formal analysis, Investigation, Resources. **N. Spigolon:** Resources, Project administration, Supervision. **V. Fogliano:** writing and reviewing, Supervision. **F. Biasioli:** Conceptualization, writing and reviewing, Supervision, Project administration, Funding acquisition, Resources.

Declaration of competing interest

o All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

o This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111089.

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