Journal of Mass Spectrometry

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Journal:	*Journal of Mass Spectrometry*
Manuscript ID:	JMS-14-0207
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	09-Jul-2014
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Keywords:	Porcini (Boletus edulis), shelf-life, VOCs evolution, oxidation, Maillard reaction

Volatile compounds changes during shelf life of dried *Boletus Edulis*: SPME-GC-MS and PTR-ToF-MS as complementary techniques

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RUNNING TITLE: VOCs changes during dried *Boletus edulis* storage

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Abstract

Drying process is commonly used to allow long time storage of valuable porcini mushrooms (*Boletus edulis*). Although considered a stable product dried porcini flavour changes during storage. Monitoring of volatile compounds during shelf life may help to understand the nature of the observed changes. In the present work two mass spectrometric techniques were used to monitor the evolution of volatile compounds during commercial shelf life of dried porcini. SPME coupled to GC-MS allowed the identification of 66 volatile compounds, 36 of which reported for the first time, monitored during the commercial shelf life of dried porcini. PTR-ToF-MS, a direct injection mass spectrometric technique, was shown to be a fast and sensitive instrument for the general monitoring of volatile compound evolution during storage of dried porcini. Furthermore, PTR-ToF-MS grants access to compounds whose determination would otherwise require lengthy pre-concentration and/or derivatisation steps such as ammonia and small volatile amines.

The two techniques, both used for the first time to study dried porcini, provided detailed description of time evolution of volatile compounds during shelf life. Alcohols, aldehydes, ketones and monoterpenes diminish during the storage while carboxylic acids, pyrazines, lactones and amines increase. The storage temperature modifies the rate of the observed changes influencing the final quality of the dried porcini.

We show the complementarity of the two techniques, suggesting a strategy to be adopted to follow time evolution of volatile compounds in food products during shelf life, based on the identification of compounds by GC-MS and the rapid time monitoring by PTR-ToF-MS measurements in order to maximize the advantages of both techniques.

Keywords: Porcini (*Boletus edulis*), shelf life, VOCs evolution, oxidation, Maillard reaction

1. Introduction

Mushrooms are edible fungi of commercial importance for their nutritional value[1,2,3] and appreciated flavor and aroma.[4] *Boletus edulis*, commonly known as "porcino" or "cepe", is a prized ingredient in various recipes imparting a nutty and slightly meaty, and a distinctive aroma reminiscent of sourdough to any number of dishes. Fresh mushrooms are very perishable products with a limited shelf-life of 1 to 3 days at room temperature thus post-harvest treatments are mandatory to extend their shelf-life.[5] Dehydration is one of the important preservation methods employed for storage of mushroom, in particular for the Boletus family. Dehydrated porcini slices are suitable for long-term storage and have a recognized market value also because, unlike most mushrooms, maintain their flavour after drying.

Hundreds of volatile compounds have been identified in edible mushrooms that can contribute to aroma, such as derivatives of octane and octenes, lower terpenes, derivatives of benzaldehyde, heterocyclic compounds and sulphur compounds.[6] In dry porcini, Thomas identified about 70 volatile substances including 12 pyrazines and seven 2-formylpyrroles.[7] In a more recent work 53 volatile compounds were identified including numerous heterocyclic compounds formed in the course of the Maillard reaction during the thermal processing of mushrooms drying.[8]

Dried mushrooms are considered a stable product; however their characteristics change during the commercial life of the product that for Italian law cannot be longer than 12 months after packaging (DPR 14/7/1995 n. 376). While modification of drying process on volatile fraction of mushroom was previously investigated[9] data about the evolution of these volatile compounds during the shelf-life of the dried mushrooms are not available.

The aim of the present study is to investigate on the evolution of volatile compounds of dehydrated porcini mushroom during the commercial shelf-life (up to 12 months) at normal temperature of storage 20 °C and under stressed conditions at 37 °C.

Two complementary MS based techniques were used in the present study: A hyphenated technique namely GC-MS and a pure MS technique namely PTR-ToF-MS. Results obtained by the two techniques

were compared with the aim to elucidate their potentiality in the monitoring of volatile compounds during storage/shelf life and the complementarity of the information provided.

2. Materials and Methods

2.1 Samples

Mushrooms (*Boletus edulis*) were cut in thin slices and dried in tunnel dryer by a local producer (Dial srl, Pergine, Italy). At the end of drying process (residual humidity 12.20 ±0.28 %), mushrooms were packed in commercial polypropylene bags in portions of 100 g each and delivered to our laboratories in few hours. Once arrived, the samples were divided in three parts and stored at 3 different temperatures in dark conditions: 20 and 37 °C and -25 °C (as control group). A further sample was directly stored at -80 °C and was labelled as day 0. At scheduled intervals (1, 2, 4, 7, 14, 25, 46, 91, 177, 273, 365 days) samples were removed from their storage room and were placed in a -80 °C freezer until analysed.

2.2 SPME/GC-MS analysis

Dried mushroom samples removed from storage freezer (-80 °C) were immediately ground (Oster 6805 blender, at max speed for 10 s) than one gram of powder was inserted into a 20 mL screw cap vial, suitable for volatile analysis. Three replicates per each sample were prepared and analysed.

The vials were kept (for no more than 10 hours) at 10 °C in the thermostatic autosampler tray (CTC CombiPAL, CTC Analytics, Zwingen, Switzerland) before the HS-SPME GC–MS analysis. The equilibration time was 30 minutes at 37 °C. Headspace volatile compounds were extracted and concentrated on a 2 cm Solid Phase Microextration fibre coated with divinylbenzene / carboxen / polydimethylsiloxane 50/30 µm (DBV/CAR/PDMS, Supelco, Bellefonte, PA, USA). This kind of fibre was successfully employed in previous works to profile the headspace of truffles[10] and mushrooms[11]. The fibre was exposed to the mushroom headspace for 30 minutes. Volatile compounds adsorbed on the

SPME fibre were desorbed at 250 °C in the injector port of a GC interfaced with a mass detector which operates in electron ionization mode (EI, internal ionization source; 70 eV) with a scan range from m/z 35–300 (GC Clarus 500, PerkinElmer, Norwalk CT, USA). Separation was achieved on a HP-Innowax fused-silica capillary column (30 m, 0.32 mm ID, 0.5 µm film thickness; Agilent Technologies, Palo Alto, CA, USA). The GC oven temperature program consisted in 40 °C for 3 min, 40-160 °C at 2 °C•min^{-1}, 160-250 °C at 7 °C•min^{-1}, stable at 250 °C for 5 min. Helium was used as carrier gas with a constant column flow rate of 1.5 mL•min^{-1}. Compounds identification was based on mass spectra matching with the standard NIST08/Wiley98 libraries. Data are expressed as percentage of total chromatographic area. To test the repeatability of the method, 9 replicates of a reference sample prepared by homogenizing mushrooms from the same bag were consecutively analysed.

2.3 PTR-ToF-MS analysis

For PTR-Tof-MS analysis a distinct set of samples was prepared. Sample preparation was carried out at the same time as GC-MS, and following the same general operational mode. One-gram aliquots were transferred into 40-ml vials with screw caps, suitable for volatile analysis, and stored as described previously. Before headspace analysis, samples were equilibrated for 30 minutes at 30 °C in a water bath. The equilibration temperature was lower than in GC, with the aim to prevent ion peak saturation. Measurements were performed with a commercial PTR-ToF-MS 8000 apparatus from Ionicon Analytik GmbH (Innsbruck, Austria), in its standard configuration (V mode). The ionization conditions in the drift tube were the following: 110 °C drift tube temperature, 2.30 mbar drift pressure, 500 V drift voltage. This led to an E/N ratio of 130 Townsend (1 Td = 10^{-17} cm^2 V^{-1} s^{-1}). The inlet line consisted of a PEEK capillary tube (internal diameter 0.40 mm), heated at 110 °C. The inlet flow was set at 40 sccm. Each sample was analyzed at one spectrum per second (between *m/z* 15 and 230 Th) and concentrations were estimated by averaging mass spectral signals over 30 spectra. Dead time correction, internal calibration of mass spectral data and peak extraction were performed according to a procedure described elsewhere,[12,13] using a modified Gaussian peak shape. Peak intensity in ppb$_V$ was estimated using the

formula described in literature,[14] using a constant value for the reaction rate constant coefficient ($k = 2.10^{-9}$ cm^3 s^{-1}). This introduces a systematic error for the absolute concentration for each compound that is in most cases below 30% and could be accounted for if the actual rate constant coefficient is available.[15]

2.4 Statistical analysis

GC data reported as percentage of the total chromatographic area, were Log transformed than scaled to unit variance[16] prior Principal Component Analysis (PCA) performed by the software package Simca P+ v.12 (Umetrics, Sweden).

All data detected and recorded by the PTR-TOF-MS were processed and analyzed using MATLAB (MathWorks, Natick, MA) and the methodology used for data processing is reported elsewhere.[12-14]

3. Results and discussion

3.1 SPME GC-MS analysis: method repeatability

Repeatability of the method was based on the analyses of 9 consecutive sampling from the same batch. The relative standard deviation expressed as percentage was lower than 12 % for all compounds (data not shown) but for camphene (15%), 1,8-cienole (18.5%), *p*-cymene (14.7%), 2-ethyl-1-hexanol (30%) and valeric acid (15.7%).

3.2 SPME GC-MS analysis: volatile compound profiling

Table 1 reports the 66 volatile compounds identified in the headspace of dried Porcini by GC-MS at the day time 0. Of these, twenty-five were reported in previous works where solvent extraction

techniques were used.[7,17] In the present work Headspace SPME was employed for the first time to profile the volatile component of dried porcini. Monoterpenes represent the widest class of compounds identified comprising 12 molecules. The DBV/CAR/PDMS fibre coating was shown to be particularly suited for the detection of mono- and sesqui- terpenes.[18] All the identified monoterpenes were previously reported in fresh wild mushroom;[19] in particular α-pinene, camphene, limonene, 1,8-cineole, *p*-cymene and linalool were found in Boletus species[19] but the only monoterpene previously reported in dried porcini is linalool.[17]

After monoterpenes, acids and aldehydes are the most represented classes with 11 compounds per each class. Nine of the identified acids belong to the homologous series of straight-chain monocarboxylic acid from C2 to C10, the other 2 acids are isomers of the C4 and C5 monocarboxylic acids.

Of the 11 aldehydes found only 4 were previously reported in dry porcini: hexanal, octanal, nonanal and benzaldehyde.[7,17]

Another well represented group of compounds are the ketones with 8 molecules including the two isomers 1-octen-3-one and 3-octen-2-one that, together the alcohol 1-octen-3-ol from which are derived, are considered key aroma compounds in most of the edible mushroom species.[4,20] Other than 1-octen-3-ol, five further alcohols were identified: 3-methyl-1-butanol, hexanol, 2-ethyl-1-hexanol, 1-octanol and 2-phenylethanol (Table 1).

In the present study several heterocyclic compounds were identified, 6 pyrazines, 4 furans, a pyran and a pyridine. In a previous work Thomas identified in dried porcini 12 pyrazines, 6 furans and 9 pyrroles.[7] These compounds are products of Maillard reaction[21] occurring during drying process and are responsible for typical odour notes of dried mushrooms.[17,22]

3.3 SPME GC-MS analysis: evolution of volatile compounds during storage

To provide a general overview of volatile compounds evolution during shelf life, GC data (66 compounds) were compressed and represented by PCA. In figure 1 the bi-plot of the first 2 principal components, explaining 70 % of the total variance of the data, is reported. Small stars represent the loadings (variables) and each other point represents the mean value of three samples replicates. Volatile profile of dried mushrooms stored at -25 °C (open diamonds) does not change significantly during the whole shelf-life period studied (up to 365 days) indicating the reduced chemical reactivity of the product.

The light grey circles and dark grey triangles represent samples stored up to 365 days at 20 °C and 37 °C respectively. The first component shows how the volatile profile changes during the storage (from right to left) with time. When dried mushrooms are stored at 20 °C, after 1 year, the concentration of volatile profiling is completely different from the product at day 0. Increasing the storage temperature from 20 °C to 37 °C there is a drastic acceleration of these changes. When stored at 37 °C, after 91 days, the volatile profile of dried mushroom is similar to the volatile profile of the same samples stored at 20 °C after 1 year. Thus at 37 °C, the volatile profiling changes 4.5 times faster than at 20 °C.

When stored at -25 °C, as expected, the overall volatile compounds emitted by dried mushrooms changes very little during 1 year of storage and this is true for all volatile compounds (Figure 2).

When samples are stored at 20 °C, the emission of volatile compounds during the first week is almost stable and starts to increase at the second week. The increase continues until the 39th week and only the last sampling time at 52 weeks shows a decline. When the storage temperature is set to 37 °C, during the first week, emission of volatile compounds is comparable with the 20 °C storage conditions. After the first week the increase in volatile compounds is faster reaching a maximum of emission at 10 weeks. After the 10 weeks of storage volatile compounds emission starts to decline until the end of the experiment.

Although the total volatile emission presents the described trends, the different classes of compounds show different behaviours during storage.

Alcohols, aldehydes, ketones and monoterpenes diminish during the storage and of course the higher temperature (37 °C) speeds up the process (Figure 2 a,b,c,e respectively). A series of compounds containing eight carbons are considered to be the primary volatiles contributing to mushroom flavour,[6] among these 1-octen-3-ol enzymatically formed from linoleic acid,[23] has been described as imparting a typical raw mushroom odour[24,25] while its oxidation products, 1-octen-3-one and 3-octen-2-one, have been described as fresh[24] or wild mushroom.[25] Depletion of these three compounds is drastic at 37 °C since the first day of storage disappearing almost completely during the first week (Figure 3b,c,d). When stored at 20 °C depletion is slower and the lowest levels of these compounds occur after 10 weeks. The same fate was shown for monoterpenes whose behaviour is similar during storage. Several monoterpenes have been reported in edible wild mushrooms, imparting woody, resinous, balsamic, piny, fresh citrusy odours typical of fresh product.[19] Thus the typical markers of fresh mushroom, still present at the end of drying process, are lost during first weeks of storage at 20 °C and even before when stored at higher temperatures.

On the opposite, carboxylic acids, pyrazines, lactones increase during storage of dried porcini (Figure 2 d,f,h respectively).

Carboxylic acids form from oxidation of alcohols and aldehydes as confirmed by the opposite trend shown by the latter. Lactones, like as carboxylic acids, are products of oxidation and increase as well during storage. The total pyrazine content, formed during drying process of mushroom, increases during storage (Figure 2f). Similar behaviour, during shelf life, was observed in other products.[26,27]

When the storage temperature is 37 °C a decline for both carboxylic acids and pyrazines is observed after 14 weeks. The decline observed in carboxylic acids is due to the depletion of acetic acid (Figure 3a). Acetic acid is a secondary volatile oxidation product formed during lipid oxidation together with hydro peroxides.[28] Its decrease may be due to substrate depletion. Decrease of pyrazines during storage is favoured by the presence of oxygen and exposure to light[27] and thus the decrease observed at 37 °C can be considered an anticipation of what is expected to happen for storage at 20 °C longer than 1 year.

A different behaviour is observed for sulphur compounds (Figure 2g). When samples are stored at 20 °C, their emissions remain stable for 14 weeks after that there is depletion. When the storage temperature is set to 37 °C, sulphur compounds show a fast increase during the first 2 weeks then a fast decrease until 25 weeks, after that a further increase is observed. The observed decrease can be explained with the high volatility of these compounds[29] formed during drying process. Sulphur compounds originate from amino-acid degradation and thus the further increase after 25 weeks may be due to proteolysis, generating more free aminoacids susceptible of degradation.

3.4 PTR-ToF-MS analysis

The analysis of mass spectral data generated 367 mass peaks overall. Data were filtered by eliminating peaks imputable to water chemistry (hydronium ion, water clusters) and to interfering ions (e.g. oxygen, nitrogen monoxide), also generated in minor amounts at the ion source. A second step of filtering was then applied; this consisted in eliminating peaks whose average concentrations were lower than 1 ppb_V: this threshold was determined on empirical basis and the filtering was found to be useful in discarding signals relative to ions present in trace amounts and therefore hard to quantify precisely. After filtering, 201 mass peaks were obtained. Principal component analysis (PCA) was performed on the filtered and original datasets and no major modification in time evolution of the three experimental modes was evidenced (results not shown).

PTR-ToF-MS data were integrated with fragmentation patterns of individual compounds ([31,32], and unpublished data), and with the results of the GC-MS based profiling; this led to a list of 49 mass peaks, that could be associated to one or more compounds (Table 2). Of all 66 compounds detected by GC, only 1-octene could not be linked to any mass peak: this compound is indeed unlikely to be detected by PTR-MS in its conventional ionization mode.[33]

A previous work performed on white truffles[10] had already demonstrated the possibility to establish reliable correlations between gas-chromatographic peaks and PTR-MS mass spectral data; this was confirmed by the present research. Figure 4 depicts the example of monoterpenes (5a), pyrazines

(5b), and 1-octen-3-one and 2-octen-3-one (5c). The good agreement with GC-MS data proves the effectiveness of PTR-ToF-MS in monitoring the time evolution of compounds that are important for the perceived quality of mushrooms. Even though isomers are not separated it is still possible to follow the time evolution of compounds belonging to the same chemical class (e.g. dimethylpyrazines, monoterpenes or octenones) and whose organoleptic properties are generally similar, moreover the lack of separation between isomers is compensated by the extreme rapidity of analysis.

The high sensitivity of PTR-MS also grants access to compounds whose determination would otherwise require lengthy pre-concentration and/or derivatisation steps: for instance, mass peaks *m/z* 18.034, 46.066, and 60.080, which could be assigned to ammonia and C2 and C3 amines, respectively. Figure 5 shows a marked effect of storage temperature on the headspace concentration of these compounds, with a concentration maximum at 37 °C after 3 and 13 weeks for ammonia (5a) and volatile C2 and C3 amines, respectively (5b and 5c). Ammonia and volatile amines, such as dimethylamine (C2) or trimethylamine (C3), are generated in food through chemical or microbial protein and amino acid degradation and they are considered to be key indicators of freshness in meat and fish products.[34,35] The determination of volatile amines in food is usually performed by means of HPLC, following a pre-derivatisation step.[36]

4. Conclusions

The evolution of volatile compounds emitted by dried porcini was monitored for the first time during commercial shelf life (up to one year). The use of two complementary mass spectrometric techniques allowed the identification of 66 compounds in the headspace of dried porcini by SPME GC-MS, 36 of which reported for the first time, and showed the possibility to follow the volatile emission during shelf life using a direct injection mass spectrometric technique by PTR-ToF-MS. Alcohols, aldehydes, ketones and monoterpenes diminish during the storage while carboxylic acids, pyrazines, lactones and amines increase. Furthermore, PTR-ToF-MS analyses the volatile compounds without any pre concentration or separation in a very fast way, while the resolution achieved and sensibility allows the

profiling of hundreds of mass spectrometric peaks. Additionally PTR-ToF-MS allows the identification and monitoring of compounds not easily achievable by SPME-GC-MS such as ammonia and small volatile ammines. The storage temperature modifies the rate of the observed changes influencing the final quality of the dried porcini. Only generic indications are reported on commercial bags for storage temperatures: "store in a cool, dry place for up to 1 year". The present work suggests that even short periods at temperatures higher than 25 °C should be avoided in order to minimize the drastic changes in the volatile profiling of dried porcini during shelf-life and that lower temperature are better to preserve the initial volatile profiling.

In perspective, the strategy to be adopted to follow time evolution of volatile compounds in food products during shelf-life, could be conveniently based on the identification of compounds by GC-MS and the monitoring in time by rapid and sensitive PTR-ToF-MS measurements in order to maximize the advantages of both techniques.

Acknowledgements

The authors acknowledge the financial support of the Autonomous Province of Trento, Italy (AP 2010/2013) and of Dial Srl Pergine Valsugana (TN) through the project "Studio della shelf-life dei funghi porcini essiccati".

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Table 1. Volatile compounds identified in the headspace of dry porcini at day 0 as measured by SPME GC-MS.

Table 2. Tentative identifications of PTR-MS mass peaks performed on the basis of exact masses, GC-MS data, and fragmentation patterns (when available). For each candidate compound, mass peaks are listed in the expected order of abundance. Only peaks with relative abundance higher than 10% (with respect to the most abundant) were taken into account.

Figure 1. Bi-plot of Principal Component Analysis of the autoscaled GC-MS data. Accounted variance reported in parenthesis in the axes legends. Single experimental points are depicted (◊ = -25°C; ● = 20°C; ▲ = 37°C) with * indicating the loadings. Numbers refer to days of storage.

Figure 2. SPME/GC-MS data. Time evolution of the headspace concentration of: alcohols (a), aldehydes (b), ketones (c), carboxylic acids (d), monoterpenes (e), pyrazines (f), sulfur compounds (g) and lactones (h) at three different storage temperatures (◊ = -25°C; ● = 20°C; ▲ = 37°C).

Figure 3. Time evolution of the headspace concentration of acetic acid (a), 1-octen-3-ol (b), 1-octen-3-one (c), 3-octen-2-one (d) at three different storage temperatures (◊ = -25°C; ● = 20°C; ▲ = 37°C).

Figure 4. Time evolution of the headspace concentration of selected classes of compounds (a - monoterpenes, b - pyrazines, c - octenones) at three different storage temperatures (◊ = -25°C; ● = 20°C; ▲ = 37°C) by PTR-MS. Compound identifications were performed on the basis of exact masses and, when possible, fragmentation patterns and GC-MS data.

Figure 5. Time evolution of the headspace concentration of selected N-compounds at three different storage temperatures (◊ = -25°C; ● = 20°C; ▲ = 37°C) by PTR-MS. Identifications were performed on the basis of exact masses (a - *m/z* 18.034: ammonia; b - *m/z* 46.066: C2 amines; c - *m/z* 60.080: C3 amines).

Table 1. Volatile compounds identified in the headspace of dry porcini at day 0 as measured by SPME GC-MS.

Compound	Mean[1]	CV%	Lit.[2]
acids			
acetic acid	14.181	18	
propanoic acid	0.717	13	
Isobutyric acid	3.220	6	
butyric acid	0.640	6	
isovaleric acid	16.456	10	a
valeric acid	0.069	6	a
hexanoic acid	0.326	21	
heptanoic acid	0.021	11	a
octanoic acid	0.030	16	a
nonanoic acid	0.034	11	a
decanoic acid	0.009	16	a
alcohols			
3-methyl-1-butanol	2.237	8	
hexanol	0.318	9	a
1-octen-3-ol	4.074	22	a,b
2-ethyl-1-hexanol	0.102	15	
1-octanol	0.117	17	
2-phenylethanol	0.167	7	b
aldehydes			
2-methyl propanal	0.422	16	
butanal	0.012	17	
2-methyl butanal	0.904	7	
3-methyl butanal	0.846	12	
pentanal	0.203	8	
hexanal	1.037	9	a,b
heptanal	0.070	14	
phenylacetaldehyde	0.154	13	
octanal	0.116	10	b
nonanal	0.102	4	b
benzaldehyde	0.734	9	a,b
ketones			
2-butanone	0.910	19	
2-hexanone	0.468	11	
2-heptanone	0.640	11	a
3-octanone	1.144	8	

Compound	Mean[1]	CV%	Lit.[2]
hydrocarbons			
1-octene	0.056	6	
ethyl benzene	17.722	10	
xylene	11.489	13	
monoterpenes			
α-pinene	0.617	5	c
camphene	0.212	17	c
β-pinene	0.273	8	
sabinene	0.047	3	
δ-3-carene	0.226	12	
β-myrcene	0.175	5	
limonene	4.100	8	c
1,8-cienole	0.025	16	c
β-terpinene	0.066	10	
p-cymene	0.096	6	c
linalool	0.027	8	b,c
bornyl acetate	0.052	9	
furans and pyrans			
2-penthylfuran	0.759	7	
γ-valerolactone	3.156	10	a
δ-valerolactone	0.715	11	
butyrolactone	1.429	9	a
pantolactone	0.078	10	
pyrazines			
2,3,4,5-tetrahydro pyridine	0.113	6	
methylpyrazine	0.999	5	a,b
2,5-dimethylpyrazine	1.920	8	a,b
2,6-dimethylpyrazine	1.545	7	a,b
2-ethyl-6-methylpyrazine	0.705	6	
2-ethyl-5-methylpyrazine	0.214	5	a
2,5-dimethyl-3-ethylpyrazine	0.355	6	b
sulfur compounds			
dimethyldisulfide	0.014	10	
methional	0.003	24	b
3-(methylthio)-1-propanol	0.035	9	

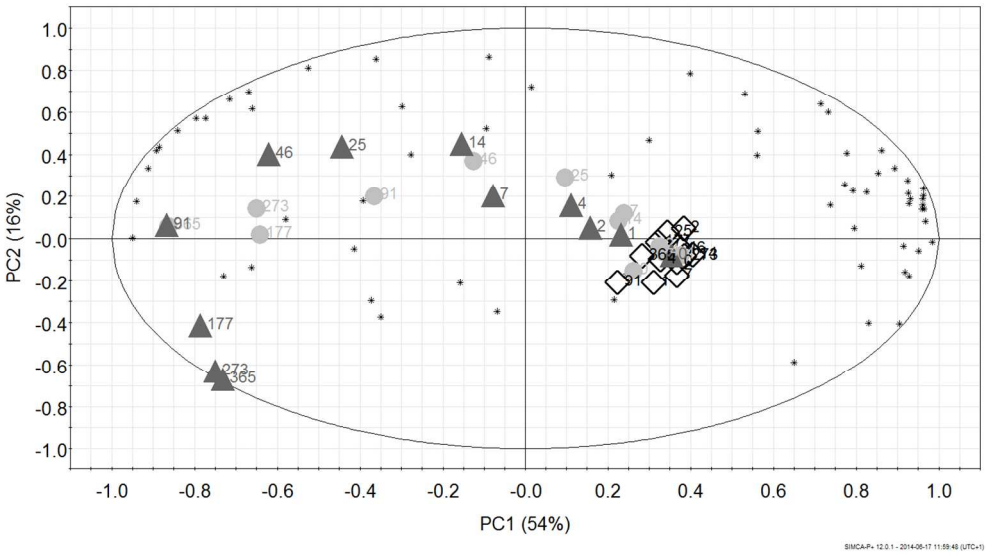
3-hydroxy-2-butanone	0.649	11			
1-octen-3-one	1.409	24	b		
3-octen-2-one	0.098	6	a,b		
acetophenone	0.144	5			

[1] Data expressed as percentage of total area chromatogram.
[2] Lit.: literature. Previously reported: (a) compounds in dry porcini from [7]; (b) compounds in dry porcini from [17]; (c) monoterpenes in raw *Boletus* species from [19].

Table 2. Tentative identifications of PTR-MS mass peaks, performed on the basis of exact masses, GC-MS data, and fragmentation patterns (when available). For each candidate compound, mass peaks are listed in the expected order of abundance. Only peaks with relative abundance higher than 10% (with respect to the most abundant) were taken into account.

Chemical class	Tentative identification	Mass peak (*m/z*)
Carboxylic acids	Acetic acid	61.025, 44.022[a]
	Propanoic acid	75.044
	Isobutyric acid/butyric acid	89.060, 71.086
	Isovaleric acid/valeric acid	103.075, 57.070, 71.086
	Hexanoic acid	117.091, 99.081
	Heptanoic acid	131.107
	Octanoic acid	145.120
	Nonanoic acid	159.133
	Decanoic acid	173.150
Aldehydes/Ketones	3-Hydroxy-2-butanone	89.060
	2-Methylpropanal/butanal/2-butanone	73.063, 56.056[a]
	2-Methylbutanal/3-methylbutanal/pentanal	87.081, 69.069
	2-Hexanone/hexanal	83.086, 56.056[a]
	Benzaldehyde	107.045
	2-Heptanone/heptanal	115.113, 97.101, 56.056[a]
	Phenylacetaldehyde/acetophenone	121.074
	1-Octen-3-one/2-octen-3-one	127.113
	Octanal/3-octanone	129.127, 111.118
	Nonanal	143.147
Alcohols	1-Octen-3-ol	129.127, 111.118, 69.069
	3-Methyl-1-butanol	71.086, 44.056[a]
	Hexanol	44.056[a], 85.098, 57.070
	Phenylethanol	105.070
	2-Ethyl-1-hexanol/1-octanol	57.070, 71.086, 44.056[a]
Lactones	Butyrolactone	87.040
	γ-valerolactone/δ-valerolactone	101.059
	Pantolactone	131.073
Furans	2-Pentylfuran	139.113
Hydrocarbons	Xylene/ethylbenzene	107.086, 79.053
Monoterpenes	*p*-Cymene	93.070, 135.117
	α-pinene/β-pinene/camphene/δ-carene β-myrcene/limonene/sabinene/β-terpinene	81.069, 137.133, 95.086
	1,8-Cineol/linalool	81.069, 137.133
	Bornyl acetate	153.127
N-heterocycles	2,3,4,5-Tetrahydropyridine	84.082
	Methylpyrazine	95.060
	2,5-Dimethylpyrazine/2,6-dimethylpyrazine	109.073
	2-Ethyl-6-methylpyrazine/2-ethyl-5-methylpyrazine	123.091
	2,5-Dimethyl-3-ethylpyrazine	137.108
Sulfur compounds	Dimethyldisulfide	94.999
	Methional	105.031
	3-Methylthio-1-propanol	107.053
Other nitrogen compounds	Ammonia	18.034
	C2 amines	46.066
	C3 amines	60.080

a. Since the main peak is saturated, the corresponding ^{13}C isotopologue is employed.



Bi-plot of Principal Component Analysis of the autoscaled GC-MS data. Accounted variance reported in parenthesis in the axes legends. Single experimental points are depicted (◇ = -25°C; ● = 20°C; ▲ = 37°C) with * indicating the loadings. Numbers refer to days of storage.
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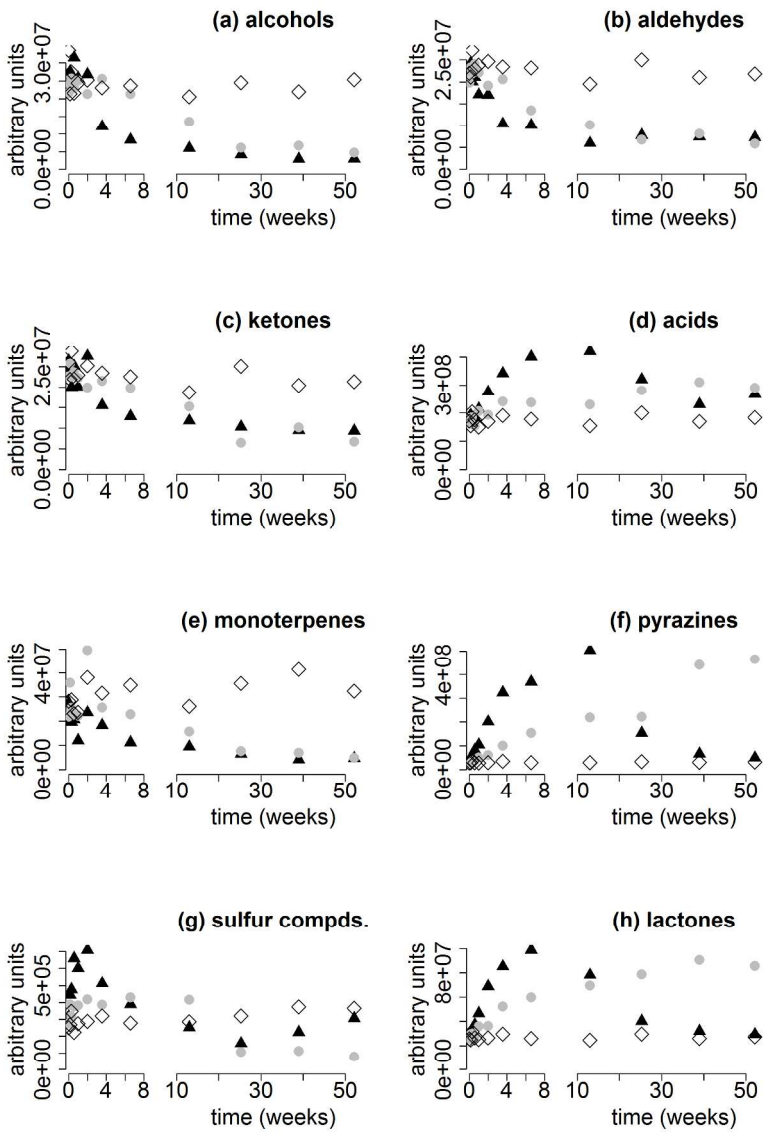
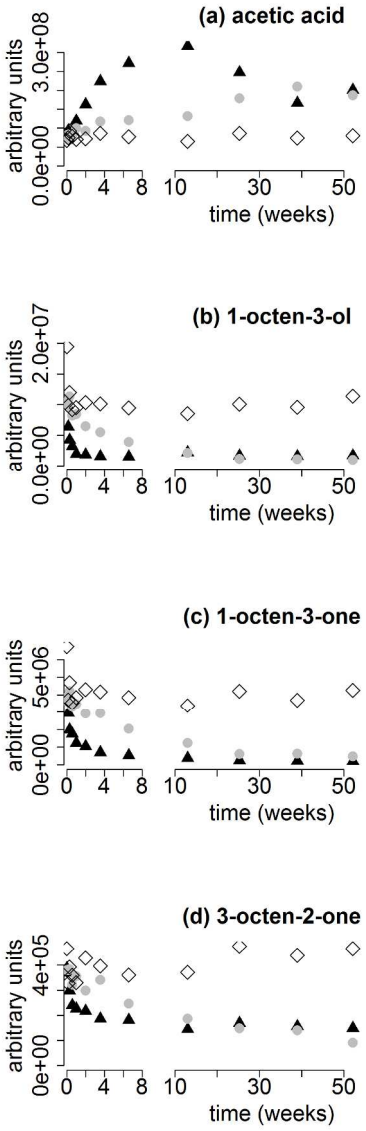


SPME/GC-MS data. Time evolution of the headspace concentration of: alcohols (a), aldehydes (b), ketones (c), carboxylic acids (d), monoterpenes (e), pyrazines (f), sulfur compounds (g) and lactones (h) at three different storage temperatures (◇ = -25°C; ● = 20°C; ▲ = 37°C).
203x304mm (300 x 300 DPI)



Time evolution of the headspace concentration of acetic acid (a), 1-octen-3-ol (b), 1-octen-3-one (c), 3-octen-2-one (d) at three different storage temperatures (◇ = -25°C; ● = 20°C; ▲ = 37°C).
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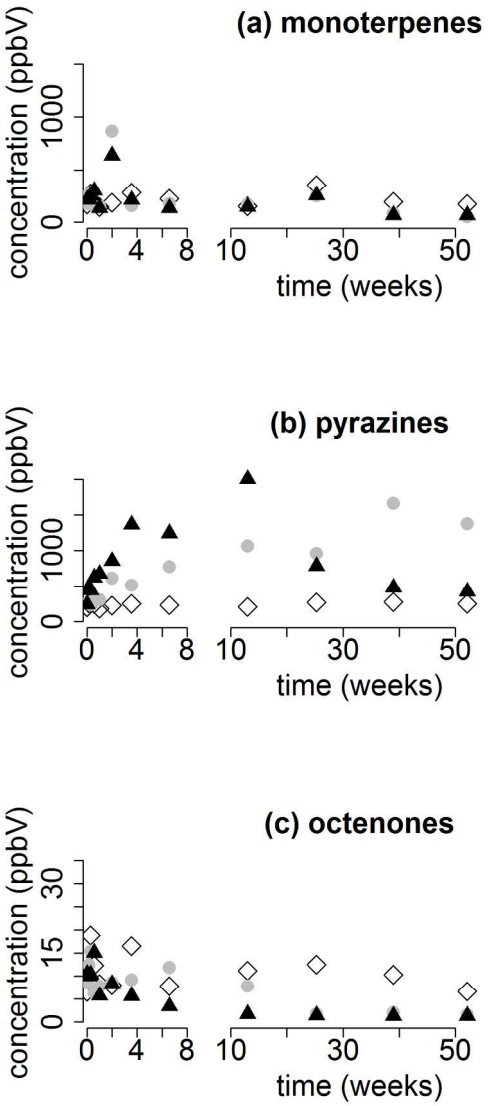

Time evolution of the headspace concentration of selected classes of compounds (a - monoterpenes, b - pyrazines, c - octenones) at three different storage temperatures (◇ = -25°C; ● = 20°C; ▲ = 37°C) by PTR-MS. Compound identifications were performed on the basis of exact masses and, when possible, fragmentation patterns and GC-MS data.
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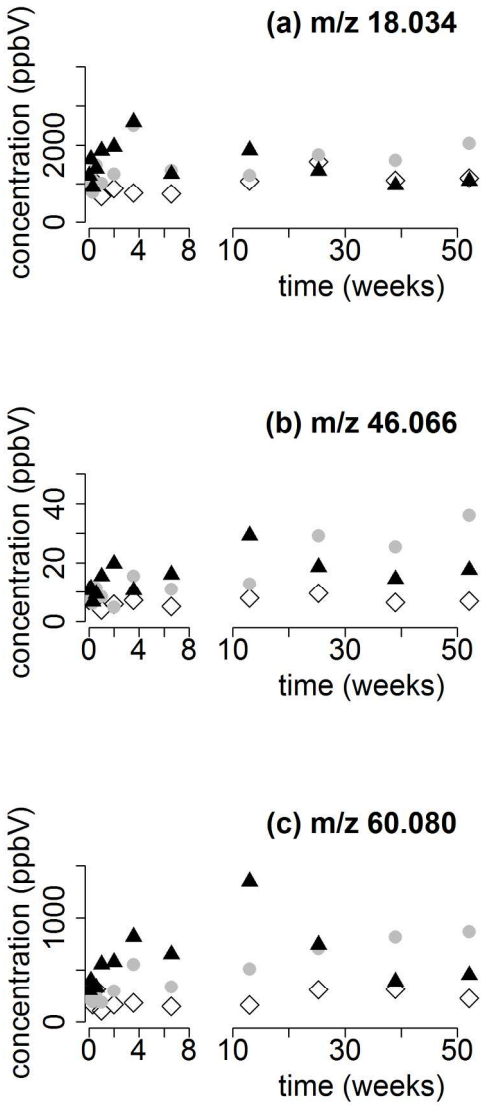

Time evolution of the headspace concentration of selected N-compounds at three different storage temperatures (◇ = -25°C; ● = 20°C; ▲ = 37°C) by PTR-MS. Identifications were performed on the basis of exact masses (a - m/z 18.034: ammonia; b - m/z 46.066: C2 amines; c - m/z 60.080: C3 amines).
101x228mm (300 x 300 DPI)