Odor-contributing volatile compounds of wild edible Nordic mushrooms analyzed with HS–SPME–GC–MS and HS–SPME–GC–O/FID

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ABSTRACT

Although Nordic wild edible mushrooms offer a wide range of different odors their scientific examination has been scarce. The aim of this study was to characterize the aroma compounds of four Finnish wild mushroom species with trained assessors using gas chromatography–olfactometry as well as gas chromatography–mass spectrometry. Headspace volatiles were extracted from *sous vide* cooked mushroom samples (*Boletus edulis*, *Lactarius camphoratus*, *Cantharellus cibarius* and *Craterellus tubaeformis*) using solid-phase microextraction. Odor-contributing compounds were measured with two columns of differing polarity using the detection frequency method. Compounds were identified based on reference compounds, linear retention indices, odor descriptions, and mass spectrometry. Both the volatile compound profiles and the aromagrams were distinct with characteristic compounds for each species. The results demonstrate that especially saturated and unsaturated aldehydes and ketones contribute to the odor of the studied wild mushrooms. This thorough comparison also indicates compounds linked to the sensory properties of mushrooms.

1. Introduction

Nordic wild edible mushrooms offer a wide range of different odors. For example, Finnish mushroom guidebooks describe odors ranging from floury and turnip-like to fruity and even reminiscent of seafood in fresh mushrooms. Recently, we determined the sensory profiles of four Finnish wild cooked mushrooms with generic descriptive analysis and 18 sensory attributes (Aisala et al., 2018) and found carrot, potato mash, roasted, cardboard, soil, and forest-like odors in addition to the typical mushroom-like odor. Similarly, de Pinho and others reported farm-feed, floral, honey-like, nutty and hay-herb type odors in 11 Portuguese wild mushrooms (de Pinho et al., 2008).

After the classic experiments on mushroom volatiles (Cronin & Ward, 1971; Picardi & Issenberg, 1973; Pyysalo, 1976; Pyysalo & Suihko, 1976; Thomas, 1973), there has been a recent rise in research activity related to the volatile compounds in both cultivated and wild edible mushrooms (Aprea et al., 2015; Cho, Kim, Choi, & Kim, 2006; Cho et al., 2007; Csóka, Geosel, Amtmann, & Korany, 2017; de Pinho et al., 2008; Fons, Rapior, Eyssartier, & Bessière, 2003; Grosshauser & Schieberle, 2013; Kleofas et al., 2015; Misharina, Muhutdinova, Zharikova, Terenina, & Krikunova, 2009; Politowicz, Lech, Sánchez-Rodríguez, Szumny, & Carbonell-Barrachina, 2017; Rapior, Marion,

Pélissier, & Bessière, 1997; Tietel & Masaphy, 2018; Wood et al., 2012; Wood, Brandes, Watson, Jones, & Largent, 1994; Zhang et al., 2018; Zhou, Feng, & Ye, 2015). These publications demonstrate that each mushroom species has a distinct volatile compound profile. However, most of them have only examined the volatile compounds of mushrooms with instrumental methods, earlier especially with SDE–GC–MS (simultaneous distillation and extraction–gas chromatography–mass spectrometry) and more recently typically with HS–SPME–GC–MS (headspace solid-phase microextraction–gas chromatography–mass spectrometry). This has been done without a direct connection to actual odor perception and a human nose. Only a few volatile compounds in mushrooms bind to olfactory receptors and are present at concentrations above their threshold level and thus can influence the odor. Articles that have utilized gas chromatography–olfactometry (Buchbauer, Jirovetz, Wasicky, & Nikiforov, 1993; Cho et al., 2006, 2007; Grosshauser & Schieberle, 2013; Kleofas et al., 2015; Misharina et al., 2009; Zhang et al., 2018) have shown that predicting the odor-contributing volatiles in mushrooms by chemical measurements alone may lead to wrong conclusions, as even the largest peaks often have no odor impact and vice versa.

Among the 150 volatile compounds identified from mushrooms, compounds containing eight carbons have been considered to be

responsible for the mushroom-like odor. 1-octen-3-ol and 1-octen-3-one have been consistently reported to be important, but also 1-octanol, 3-octanol, (*E*)-2-octen-1-ol and 3-octanone are typically present in mushrooms (Cronin & Ward, 1971; Picardi & Issenberg, 1973; Pyysalo, 1976; Pyysalo & Suihko, 1976). These compounds have also been detected in more recent studies and from several mushroom species (Cho et al., 2006; Kleofas et al., 2015; Rapior et al., 1997). Flavor dilution (FD) factors determined *via* aroma extract dilution analyses (Cho et al., 2006; Fischer & Grosch, 1987; Grosshauser & Schieberle, 2013; Kleofas et al., 2015; Zhang et al., 2018) have further specified that while the 1-octen-3-ol content is much higher in mushrooms, 1-octen-3-one makes a bigger contribution to this odor impression. This is due to the considerably lower odor threshold of the ketone form (Cronin & Ward, 1971; Pyysalo & Suihko, 1976) which offsets the major difference in the relative contents of these volatile compounds. The precursors of these 8-carbon volatiles are known to be fatty acids, specifically from linoleic acid *via* a 10-hydroperoxide intermediate and likely involving a fungal-specific pathway (Combet et al., 2006; Dunkel et al., 2014).

Thomas (1973) identified several *N*-heterocycle compounds, mainly pyrazines and pyrroles, from dried porcini (*Boletus edulis*), and Aprea et al. (2015) reported that the pyrazine content increases during storage. Wood et al. (1994) reported that (*E*)-2-nonenal formed from linoleic acid is the main compound of the cucumber-like odor of certain mushrooms. Cho et al. (2007) reported that 3-octanol, methional (3-(methylthio)propanal) and linalool have high FD factors in fresh pine mushrooms (*Tricholoma matsutake*), in addition to 1-octen-3-one and 1-octen-3-ol. Grosshauser and Schieberle (2013) found 3-methylbutanal, methional, 2-acetyl-1-pyrroline, as well as several pyrazines and lactones, contributing to the odor of pan-fried button mushrooms (*Agaricus bisporus*). Zhang et al. (2018) recently specified that in fresh porcini, several unsaturated carbonyls, such as (*E*)-2-octenal and (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal, as well as 3-methylbutanal, contribute to the odor. On the other hand, several pyrazine compounds as well as methional were reported to be important for the odor of dried porcini. These observations were in good agreement with the earlier reports from Misharina et al. (2009).

Comparative gas chromatography–olfactometry studies between mushroom species are still scarce, and since the experiments by Pyysalo (1976) the odor properties of Nordic wild edible mushrooms have not been studied. Most publications examine fresh or dried mushrooms, even though the typical usage of mushrooms is after thermal processing. There is a gap in knowledge on which volatile compounds contribute to the odor of wild edible mushrooms as researched by human senses. The aims of this research were to 1) study the odor-contributing volatile compounds of cooked, wild edible Nordic mushrooms, and 2) to elucidate the differences in the sensory impact and chemical content between selected mushrooms species.

2. Materials and methods

2.1. Samples

Four species of Nordic forest mushrooms; chanterelle (*Cantharellus cibarius*), trumpet chanterelle (*Craterellus tubaeformis*), porcini (*Boletus edulis*) and curry milkcap (*Lactarius camphoratus*); were studied. They were from the same batch as in a previous study (Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018) and processed with the same *sous vide* cooking method (Aisala et al., 2018). Porcini, chanterelle and trumpet chanterelle are all important edible mushroom species with substantial market value. Curry milkcap, on the other hand, has during recent years received high interest among mushroom enthusiasts and is thus a potential candidate as a valued commercial mushroom in the future. The mushroom species studied have only partly overlapping harvest periods in the Nordic forests during the summer and fall. In short, the samples were cleaned with a brush and cut into pieces (width 1 cm) within 36 h of collection. The samples were vacuum packed and

cooked at 80 °C for 10 min. The cooked samples were cooled in water and then frozen at −20 °C for 6 weeks. Frozen samples were cut to 1–2 cm² pieces, pooled, divided into 10 g aliquots and put back in a freezer for 1–2 months until analysis.

2.2. Sample extraction and instrumental analysis

Volatile compounds were extracted from sample headspace using solid-phase microextraction (SPME) with a 1 cm StableFlex divinylbenzene/Carboxen/polydimethylsiloxane fiber from Supelco (Bellefonte, PA) as optimized previously (Aisala, Linderborg, & Sandell, 2017). In short, 10 g of the frozen mushroom sample were weighed in a 90-mL Erlenmeyer flask and 5 μL of 1000 μg/mL ethyl propanoate (aqueous, internal standard) were added. Ethyl propanoate was selected as the internal standard on the basis of the retention time region being devoid of odor in the mushroom samples as well as its differing odor quality from the typical mushroom volatiles. The flask was immersed in a 30 °C water bath and left to stabilize for 30 min. The SPME fiber was exposed to the sample headspace and extracted for 45 min.

After extraction, the volatiles were desorbed in the split/splitless injector of an HP 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector (FID) and an olfactory detector port ODP-1 (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). The column effluent was split 1:1 between the FID and the ODP using deactivated fused silica capillaries (50 cm length, 0.25 mm i.d.). The sniffing port was installed on the side wall of the GC and supplied with humidified air at 65–70 mL/min. Helium was used as a carrier gas at a constant linear gas flow velocity of 34 cm/s. The injector had a 0.75 mm SPME liner from Restek Corporation (Bellefonte, PA). The injector temperature was 240 °C. Splitless mode was used with a 3 min desorption time, after which the fiber was removed and a 20:1 split ratio was used for the rest of the run. A 30 m × 0.25 mm × 1.00 μm RTX-5 Sil MS column by Restek Corporation was used to separate the extracted compounds. The initial oven temperature was 45 °C, followed by a rate of 9 °C/min to 60 °C, then 5 °C/min to 100 °C, 7 °C/min to 150 °C, and 16 °C/min to 280 °C (total run time 24 min). The FID temperature was 290 °C. All peak areas were normalized to the peak area of the internal standard (Viljanen, Heiniö, Juvonen, Kössö, & Puupponen-Pimiä, 2014).

The GC-olfactometry (GC-O) data was collected *via* an in-house hardware system: The evaluator had a pressing device, and pressing the button in the device generated a noise signal in the accompanying amplifier. The device was also equipped with a small light that indicated that the evaluator was pressing the signal button correctly. A microphone mounted next to the sniffing port recorded what the evaluator described throughout the run. These two signals were sent to the stereo line-in connector of the GC control computer and the output was recorded as an MP3 file during each sample run.

2.3. GC–olfactometry panel recruitment and training

The evaluators were recruited *via* an electronic flier sent to university students and personnel. Fifteen people were invited to GC–olfactometry training before the main evaluations. Ten of the assessors were women and five were men and they were 25–70 years old with a median age 30 of years. Eight of them had a high level of experience in sensory evaluation and GC–O and their senses had been tested in a sensory laboratory while the other 7 had less or no previous experience.

The training was adapted from a previously published method (Vene, Seisonen, Koppel, Leitner, & Paalme, 2013) for detection frequency type GC–olfactometry and contained three sessions. The first and second session consisted of training the vocabulary and verbal expression speed using standard compounds in sniffing bottles while the third training was familiarization of the GC–O, using both standard compound mixtures and a blind mushroom sample.

Table 1
Used compounds, dilutions and suggested odor descriptions by the evaluators in the first two training sessions.

Compound	Dilution in 1st session	Dilution in 2nd session	Odor descriptions
Blank (propylene glycol)	1	1	Odorless
Methional	1/150	–	Cooked potato, turnip
1-Octen-3-ol	1/120	–	Dark, mushroom, porcini
Eucalyptol	1/120	–	Eucalyptus, spicy, pepper
Pentanal	1/120	–	Almond, fruity, sweet
(E)-2-Nonenal	1/120	1/180	(Pickled) cucumber, grass, green
Nonanal	1/120	1/180	Citrus, old plastic container, rubber
Benzaldehyde	–	1/180	Almond, sweet
(−)-α-Pinene	–	1/75	Green, resin, forest
Ethyl propanoate (internal standard)	–	1/180	Fruity, sweet, solvent
β-Cyclocitral	–	1/150	Plastic, aniseed, herb-like

2.4. Sniffing bottles training

Candidate compounds to be used in the training were selected based on previous research (Aisala et al., 2017; Aprea et al., 2015; Cho et al., 2007; de Pinho et al., 2008; Pyysalo, 1976), as well as pilot studies on current samples. The goal was to demonstrate as many odor qualities as possible that could be present in the main evaluations and thus orientate evaluators into the mindset that there are no wrong answers in the descriptions.

Altogether 20 compounds were considered for trainings. Six compounds were selected in the first training session and likewise six compounds included for the second session based on their odor descriptions and intensities. Additionally, a blank sample containing only propylene glycol was included in both sessions. The selected compounds, their dilutions and odor descriptions given by evaluators are presented in Table 1.

Each training sample was done by diluting the compound in propylene glycol and pipetting 5 μL of the solution into a 30 mL glass vial containing a 1 cm^2 square of filter paper and 0.3 g cotton. The closed vials were wrapped in tin foil to prevent giving any visual cues, and 3-digit codes were used in referring to samples. Each vial was stabilized for at least 30 min in room temperature before evaluation and served in random order.

Trainings were done in groups of 3−7 people and lasted about 30 min per session. A brief introduction on the aims of the study, equipment used, nature of the main evaluation and contents of the training sessions was given at the beginning of the first session. Each of the evaluators was given a paper, pen, glass of water and a randomized series of coded sniffing bottles. Evaluators sniffed the samples individually and wrote their impressions on the paper. The evaluators were allowed to smell the bottles multiple times. Evaluators were instructed to describe their first impression on the paper as this would be the case also when evaluating by GC–O. After everyone was ready, group discussion about the sample descriptions and intensities was performed.

The second sniffing training session was similar to the first one. However, some samples were switched and all samples were milder. Additionally, less time was given to think about the odor descriptions. This was done to simulate the GC-O evaluation setting more closely.

2.5. Training by GC–O

The assessors were asked to come to the third session and main evaluations about 15 min before the scheduled time and to refrain from excess physical activity right before the evaluation. They were also instructed not to use strong perfumes on the evaluation day and to avoid eating and drinking aside from water for 30 min before the evaluation.

Basic familiarization with the GC–O equipment and evaluation was conducted at the beginning of the third session. This included introducing a proper sitting posture and distance of the nose to the GC–O

glass cone, breathing technique, and demonstration on how to use the pressing device and microphone. Acoustic earmuffs were given to the evaluators to block outside noise. The assessors could not see the FID chromatogram while sniffing.

The first sample of the third training session was a 250 ppm solution of pentanal, methional and benzaldehyde in propylene glycol; 2 μL of the solution were pipetted into a 5-mL glass vial and extracted by SPME. The run lasted about 5 min. The main focus for the assessors of this first run was to detect the compounds and to press the odor signal button from the beginning to the end of the odor sensation (total duration) for each compound; the evaluator was also asked to give descriptions if possible. The experience was reviewed and suggestions were given to improve performance.

The second sample contained 3-methylbutanal, 1-octen-3-ol, eucalyptol and nonanal (250 ppm) and 2-pentylfuran (500 ppm) and was evaluated in about 7 min. This set was considered to be more difficult as 1-octen-3-ol and 2-pentylfuran eluted very close to each other. The evaluators were asked to give more detailed descriptions in addition to pressing the signal button for the correct duration. After a 45-minute break, a third sample was given. This was a chanterelle sample prepared as described in Section 2.2.

2.6. Main GC–O analyses and data treatment

Each assessor took part in 3–4 sessions, each time analyzing different mushroom species. This resulted in 12 analyses from individual assessors for each mushroom species. Evaluation order was randomized without any pre-information about the sample for each assessor to minimize bias. The assessors were given a food product as reward at the end of each visit.

The audio recordings containing the odor descriptions and signal durations were processed with Audacity® 2.1.2 (Mazzoni, 2016). Each detected odor – seen as a noise signal in the right audio track – was transcribed as a description label with the start and end times, and these were in turn exported to Microsoft Excel 2013 (Redmond, WA). Individual odor signals were summed to form nasal impact frequencies (NIFs) for each mushroom (Pollien et al., 1997), with a NIF of 100% corresponding to all assessors detecting an odor at the same time. The aromagram peaks were integrated using Labsolutions 5.57 (Shimadzu Corporation, Kyoto, Japan) and these SNIF (surface of nasal impact frequency) values (calculated as milli-NIF-seconds) were used to assess the importance of each odor compound. SNIF values instead of NIFs were used to account for different durations of odor impressions and to compensate for the slight deviations in detection time. Only peaks that were detected by 3 or more people (NIF ≥ 33%) were integrated and integration limits were set so that the peak shapes approximately conformed to Gaussian distributions.

2.7. Validative GC–O analyses

After tentatively identifying compounds based on odor descriptions,

retention indices and GC–MS analyses (as described below), 4 evaluators were recruited to an additional GC–O experiment to confirm identifications. This experiment utilized aspects of flash profiling and used a different, 30 m × 0.25 mm × 0.25 μm HP-Innowax column (Agilent). The instrumental parameters were as described in Section 2.2 except for the oven program: 45 °C kept for 3 min, then raised by 6 °C/min to 150 °C, and by 10 °C/min to 260 °C. Additionally, pulsed splitless mode with 185 kPa pressure (corresponding to 55 cm/s linear velocity) was used for the 3-min splitless duration, to compensate for broadening effects of the thinner stationary phase.

The assessors were first presented with standard compound mixtures containing a total of 36 volatile compounds over two GC-O sessions and were instructed to describe the odor impressions with terms that were most natural to them. A list of personal odor descriptions and corresponding retention times was given to the assessors for future reference. All four assessors evaluated each of the four samples (prepared without the internal standard) once in random order. The evaluators were instructed to especially look for matching odor descriptions at the previously observed retention times. Data were treated as in Section 2.4 except for the fact that only peaks detected by 2 or more people (NIF ≥ 50%) were integrated.

2.8. Compound identifications

Compounds were identified based on linear retention indices on two columns of different polarities, experimental and literature odor descriptions, mass spectral library (Wiley 275) and reference compounds. Gas chromatography–mass spectrometry analyses were done by an HP 6890 + 5973 GC–MS instrument in duplicate with both RTX-5 Sil MS and HP-Innowax columns. Gas chromatography parameters of the GC–MS analyses were identical to the GC–FID/O ones on the RTX-5 Sil MS column. The GC–MS interface was at 250 °C and the scan range *m/z* 15–400, with 3.75 scans/s. The identified compounds in GC–MS and GC–O/GC–FID datasets were linked by comparing their retention indices and by visual inspection of the peak profiles.

The C5–C20 alkane standard (ASTM-P-0050) from Accustandard (New Haven, CT) was used for building linear retention indices on the RTX-5 column and a C8–C40 alkane standard (DRH-008S-R2), likewise from Accustandard, on the HP-Innowax column. Both alkane standards were supplemented with propane and hexane. Extraction was done as described in Section 2.2 for the GC–O training samples.

A total of 57 solvents and reference compounds were used. Propylene glycol was bought from Amresco (Solon, OH). Benzaldehyde, butyric acid, (+)-carvone, (*E*)-cinnamaldehyde, β-cyclocitral, *p*-cymene, (*E*,*E*)-2,4-decadienal, decanal, ethylbenzene, eugenol, hexanal, 1-hexanol, ethyl 2-methylbutanoate, ethyl propanoate, (*R*)-(+)-limonene, linalool, 3-(methylthio)propanal (methional), 2-methylbutanal, 3-methylbutanal, (*E*)-2-methyl-2-butenal, 6-methyl-5-hepten-2-one, nonanal, (*E*)-2-nonenal, 3-octanol, 3-octanone, 1-octene, 1-octen-3-ol, 1-octen-3-one, (*E*)-2-octenal, (*E*)-2-octen-1-ol, pentanal, 1-pentanol, 2-pentylfuran, (−)-α-pinene, β-pinene, and *o*-xylene were bought from Sigma-Aldrich (St. Louis, MO). 2,3-Butanedione, eucalyptol, β-caryophyllene, heptanal, 2-heptanone, 3-methylbutanol, 2-methylbutanoic acid, 2-methylpropanal, nonane, octane, octanal, and *p*-xylene were bought from Fluka Analytical (Steinheim, Germany). Butanal, (*E*)-2-heptanal, 2-methylpentane and (*E*,*Z*)-2,6-nonadienal were bought from Acros Organics (Geel, Belgium). 2-Methylpentanal and 2,2,4,6,6-pentamethylheptane were bought from Tokyo Chemical Industry Europe (Zwijndrecht, Belgium). Ethyl 2-methylpropanoate was provided by Symrise (Holzminden, Germany). (*Z*)-6-Nonenal was bought from Alfa Aesar (Haverhill, MA). All compounds were of 97% or higher purity except for (*Z*)-6-nonenal, which was of 94% purity and nonanal, which was of 95% purity.

A compound was considered to be unambiguously identified if it had matching retention indices on both columns either with a reference compound or literature data and additionally either matching odor

descriptions (e.g., methional), matching GC–MS identifications (e.g., butanal), or both. With GC–O data, mismatch between odor descriptions and other identification data was considered proof that the identified compound using GC–MS was not in fact the one responsible for the GC–O signal. For closely eluting compounds with similar GC–FID signal intensities (e.g. 2- and 3-methylbutanal), data from reference compound runs and published detection and recognition thresholds (Czerny et al., 2008) were utilized in designating the odor-impact compound.

2.9. Statistical analysis

An unsupervised, initial overview of the volatile compounds was performed for the raw GC–FID chromatogram data from both columns using the ChemoSpec package version 4.4.97 (Hanson, 2017) in RStudio 1.1 (RStudio Team, 2016) running R 3.4.3 (R Core Team, 2017). The chromatograms (*n* = 12 per mushroom species) in the RTX-5 Sil MS were normalized to the peak areas of the internal standard, while no normalization was done to the HP-Innowax chromatograms. Both datasets were binned to a digital resolution of 1.5 s/data point. Principal component analysis (PCA) was performed with mean centering and Pareto scaling to account for the variation of peak intensities within the chromatograms. Classical 95% confidence ellipses were used in determining the sample populations in the scores plots. Aromagrams from the two columns were similarly overviewed by importing the summed aromagrams from each mushroom to RStudio. The data was not normalized, but it was binned to 1 s/data point buckets and no scaling was used in building the PCA models.

The differences in the normalized volatile compound contents between mushrooms on the RTX-5 Sil MS column were examined with IBM SPSS Statistics 24.0 (IBM Corporation, Armonk, NY). Analysis of variance (ANOVA) or the Brown-Forsythe test with either Tukey's HSD or Tamhane's T2 post-hoc test was used after possible data transformations. Alternatively, Kruskal-Wallis and Mann-Whitney's U tests with Bonferroni corrections were used for compounds that did not conform to normality. The criterion for statistical significance in all tests was $p < 0.05$.

Finally, an overview of the volatile compound profiles was made by creating a PCA model with normalized areas of all identified peaks with mean centering and unit variance scaling using The Unscrambler X version 10.4 (CAMO Software As., Oslo, Norway). The association between the total amounts of normalized GC–FID peak areas for each mushroom and odor intensities of the trained sensory panel (Aisala et al., 2018), as well as GC–O total SNIF values and odor intensities, were investigated by calculating squared Pearson product moment correlation coefficients (r^2), using average values for each mushroom in each dataset.

3. Results and discussion

3.1. Volatile compound fingerprints of mushrooms

Unsupervised principal component analysis models on both the GC–FID and GC–O data on both columns reliably separated all four mushroom samples from each other (Supplementary Material, Figs. S2–S9). This is evident by looking at the 95% confidence ellipses on principal components 1–3: all mushroom populations are separate entities. Based on the scores plots, the volatile compound profile of curry milkcap samples is the most distinct of the studied mushrooms. The obtained classification result is in agreement with Zhou et al. (2015), who likewise reported the separation of all 8 studied mushroom species in their multivariate models based on GC–MS and electronic nose sensor data. The limitation of our models is that they are built with GC–FID/O instead of GC–MS data. Thus different co-eluting peaks between mushroom samples do not contribute to the separation in the model. However, the models based on both columns have similar

Table 2
Detected volatile compounds, their retention indices, identification sources and relative contents in relation to the internal standard in the four studied mushrooms.

#	Compound[a]	RI (GC–MS) RTX-5 Sil MS	RI (GC–MS) HP-Innowax	RI (GC-FID/O) RTX-5 Sil MS	ID methods[b]	Normalized areas (standard deviations) on RTX-5 Sil MS column, GC-FID[c] Chanterelle	Trumpet chanterelle	Porcini	Curry milk cap
1	Unknown			370		0.22 (0.09) B	0.13 (0.06) B	0.12 (0.02) BC	0.56 (0.17) A
2	Acetone	416	827	413	1-4	1.27 (0.28) C	0.77 (0.24) C	5.95 (1.31) D	7.93 (1.83) A
3	2,3-Butanedione	579	982	578	1-6, 8	0.70 (0.12) A	0.34 (0.14) A	0.08 (0.04) B	0.59 (0.18) A
4	Butanal	596	892	594	1-5	4.25 (1.32) B	6.47 (1.57) A	0.22 (0.05) A	— D
5	3-Methylbutanal	655	924	654	1-7	0.54 (0.07) B	1.04 (0.18) B	0.33 (0.04) A	1.31 (0.29) A
6	1-Butanol	658	1158	657	1-4*	0.54 (0.06) B	0.45 (0.08) B	0.15 (0.02) C	0.67 (0.13) A
7	2-Methylbutanal	665	922	665	1-5	0.27 (0.03) B	1.32 (0.28) B	0.20 (0.03) A	1.46 (0.27) A
8	2-Pentanone[d]	688	982	687	1-4	0.64 (1.21) B	0.62 (0.32) B	— A	— C
9	Pentanal	702	984	701	1, 5-7	5.01 (0.61) A	1.73 (0.44) A	1.37 (0.14) B	1.09 (0.22) C
10	ISTD: Ethyl propanoate			709		100	100	100	100
11	(E)-2-Methyl-2-butenal	745	1107	745	1-5	—	—	—	302 (66) A
12	2-Methylpentanal	751	999	750	1-5	4.77 (0.75) A	1.63 (0.31) A	0.69 (0.07) B	0.76 (0.32) C
13	Ethyl 2-methylpropanoate			757	1, 5, 6*	—	— e	—	—
14	1-Pentanol	764	1258	763	1-5	0.74 (0.10) B	0.17 (0.03) B	0.18 (0.02) C	12.22 (2.87) A
15	Toluene	773	1047	772	1-4	0.30 (0.09) A	0.39 (0.08) A	0.10 (0.04) A	0.36 (0.11) C
16	3-Methyl-2-butenal	788	1211	787	1-4	— B	— B	— B	1.66 (0.44) A
17	1-Octene	791	842	790	1-5	4.21 (0.69) B	19.38 (5.45) B	0.21 (0.07) A	1.02 (0.11) C
18	Octane	800	803	800	1-5	2.72 (0.43) B	21.54 (6.93) B	0.55 (0.18) A	3.26 (0.60) B
19	4-Methyl-3-penten-2-one	802			1-3*	—	—	— e	—
20	Hexanal	804	1090	803	1-8	335 (46) A	39.9 (15.3) A	4.75 (0.68) B	29.9 (6.1) B
21	(E)-2-Octene	814	859	814	1-4	0.87 (0.16) A	0.25 (0.08) A	0.41 (0.22) B	0.67 (0.19) A
22	3-Cyclohepten-1-one	826	1064	826	1-4	12.01 (2.30) B	47.7 (14.9) B	0.49 (0.29) A	0.746 (0.143) C
23	1,3-Octadiene	828	953		1-4	—	—	—	—
24	Ethyl 2-methylbutanoate		1047	851	1,2,4,5*	0.472 (0.083) A	1.23 (0.45) A	—	—
25	(E)-2-Hexenal	857	1226	856	1-4	5.29 (0.70) A	(0.47) A	0.148 (0.037) A	1.53 (0.40) B
26	1-Hexanol	868	1361	867	1-5	0.27 (0.06) B	(0.55) A	—	0.66 (0.16) B
27	p-Xylene	877	1139	880	1-5	0.98 (0.38) A	(0.21) B	0.377 (0.439) A	1.14 (0.37) A
28	Unknown		1359	882		—	—	—	—
29	1-Hepten-3-ol	875			1-4	—	—	—	—
30	Unknown	887		887		—	7.21 (5.44) C	—	0.30 (0.04) B
31	2-Heptanone	892	1187	891	1-5	0.94 (0.15) C	0.70 (0.20) B	7.18 (2.19) C	0.66 (0.18) C
32	2-Butylfuran	895		894	1-3*	0.57 (0.13) B	—	—	—
33	Nonane	900	900	900	1-4	—	—	—	0.74 (0.18) B
34	Heptanal	906	1188	905	1-5	13.1 (2.5) A	1.97 (0.34) A	0.41 (0.16) B	2.36 (0.86) A
35	Methional	917	1461	917	1, 2, 5-8	—	—	— e	— e
36	2-Acetyl-1-pyrroline			931	1, 2, 6*	—	—	—	—
37	α-Pinene	945	1021	944	1-5	0.67 (0.12) B	4.66 (4.07) B	0.18 (0.07) A	1.58 (2.65) C
38	6-Methyl-2-heptanone	958	1239	958	1-4	1.34 (0.27) A	—	—	—
39	(E)-2-Heptenal	964	1329	963	1-5	3.36 (0.67) A	1.39 (0.27) A	0.16 (0.07) B	3.34 (1.10) B
40	1-Heptanol	972	1467	971	1-4	1.39 (0.23) B	0.73 (0.09) B	—	2.51 (1.02) C
41	4-Octanone	976	1227		1-4	— e	3.05 (0.83) B	—	—
42	Benzaldehyde	976	1535	976	1-5	4.13 (0.79) A	—	0.21 (0.03) C	4.54 (1.43) C
43	1-Octen-3-one	984	1306	983	1-8	212 (36) B	172 (15) B	57.4 (17.9) D	383 (104) D
44	1-Octen-3-ol	984	1462		1-8	—	—	—	—
45	2,3-Octanedione	986	1328	986	1-4	4.45 (0.89) A	4.75 (0.87) A	— C	0.87 (0.25) C
46	Unknown	987		986		—	—	— A	—
47	Unknown	987				— e	—	—	— e
48	(E)-6-Methyl-5-hepten-2-one	989	1343	987	1-4	— B	12.94 (1.79) B	0.17 (0.04) B	— e B
49	3-Octanone	990	1256	989	1-7	9.54 (1.87) B	— e C	4.18 (1.59) C	209.2 (46.3) A
50	β-Pinene	992	1104	991	1-5	— B	1.72 (3.81) B	0.08 (0.06) A	— B

(continued on next page)

Table 2 (*continued*)

#	Compound[a]	RI (GC–MS) RTX-5 Sil MS	RI (GC–MS) HP-Innowax	RI (GC-FID/O) RTX-5 Sil MS	ID methods[b]	Chanterelle	(SD)	grp	Trumpet chanterelle	(SD)	grp	Porcini	(SD)	grp	Curry milk cap	(SD)	grp
51	4-Octanol	993	1388	992	1–4	2.44	(0.55)	A	0.68	(0.44)	B			B			C
52	2,2,4,6,6-Pentamethylheptane	995	953	994	1–5			B	113.5	20.8	A			A	e		B
53	2,5-Dimethyl-5-hexen-3-ol	995	1437		3, 4*												
54	2-Pentylfuran	995	1229	994	1–5	17.87	(3.95)	B			B	29.22	(8.41)	D	3.45	(0.75)	C
55	3-Octanol	998	1401	998	1–5	3.45	(1.24)	B	0.45	(0.16)	C	0.57	(0.30)	C	18.1	(4.6)	A
56	2,3-Dehydro-1,8-cineole	1001	1191	1000	1–4	3.19	(2.60)	A			B			B			B
57	Octanal	1008	1291	1007	1–5	2.00	(0.42)	B	2.37	(0.60)	B	0.70	(0.38)	B	24.1	(8.3)	A
58	δ-3-Carene	1021	1137	1020	1–4	0.19	(0.03)	A			B			B	e		B
59	Unknown	1027		1026				B	8.14	(1.16)	A			A			B
60	2-Ethyl-1-hexanol	1031	1498	1031	1–4	0.68	(0.14)	B	2.19	(0.50)	A	0.68	(0.13)	C	0.72	(0.13)	B
61	2,2,4-Tetramethyloctane	1035	1008	1034	2,3,4*	e		C	9.11	(1.89)	A			A	0.60	(0.18)	B
62	p-Cymene[d]	1036	1265	1034	1–5	0.28	(0.13)	B			C	3.44	(0.80)	C			C
63	An oxygen-containing hydrocarbon	1039	1578	1039		3.43	(0.79)	B	26.01	(4.21)	A			A			C
64	Limonene[d]	1041	1194	1040	1–5	e		C	e		C	0.55	(0.70)	C	1.16	(0.31)	A
65	3,5-Dimethyloctane	1042		1042	3*			D	4.85	(1.03)	A	1.83	(0.43)	A	0.40	(0.07)	C
66	3-Octen-2-one	1045	1414	1044	1–4	2.85	(0.56)	A			B			B			B
67	A hydrocarbon	1045	1422								A						
68	Eucalyptol	1047	1207	1045	1–5			B	e		B	0.14	(0.03)	B			A
69	2,6,6-Trimethylcyclohexanone	1049	1318	1049	1–4	1.29	(0.29)	A			B			B			B
70	Unknown	1059		1055				B			B	1.60	(0.36)	B			B
71	Phenylacetaldehyde	1066	1658	1058	1–4	6.90	(1.64)	B	2.35	(0.42)	B	0.80	(0.14)	B	1.42	(0.29)	A
72	(E)-2-Octenal	1071	1435	1066	1–8	15.91	(3.61)	A	7.62	(0.80)	B	5.87	(1.84)	C	6.30	(2.32)	A
73	(E)-2-Octen-1-ol	1073	1627	1070	1–6			A	9.51	(0.97)	B	0.87	(0.26)	B	7.18	(3.75)	B
74	1-Octanol	1073	1568	1072	1–4			C	0.29	(0.11)	A	0.22	(0.07)	A	13.8	4.9	A
75	2-Nonanone	1095	1393	1094	1–4			C			C	0.14	(0.05)	C	1.11	(1.36)	A
76	Linalool	1104	1558	1104	1–8			C			C	0.15	(0.04)	C	3.62	(1.04)	A
77	(E,E)-2,4-Octadienal	1108	1594	1107	1–4	e		C	11.40	(5.56)	A	2.04	(1.63)	C			C
78	Nonanal	1110	1397	1109	1–7	5.95	(1.37)	B	8.73	(1.54)	A	0.92	(0.18)	A	4.39	(1.21)	B
79	A hydrocarbon	1118		1117	1, 2, 4–8	0.54	(0.19)	C	1.67 e	(0.36)	A			B	0.68	(0.15)	BC
80	(E,Z)-2,6-Nonadienal		1590	1158	1–8												
81	(E)-2-Nonenal	1170	1541	1169	1–8	1.76	(0.46)	A	0.53	(0.14)	B	0.21	(0.04)	B	0.41	(0.10)	B
82	Dodecane	1200		1200	1–5			D	0.55	(0.10)	B	0.43	(0.08)	B	0.76	(0.17)	A
83	Decanal	1215	1503	1215	1–6, 8	4.19	(2.01)	A	3.53	(1.33)	AB	2.25	(1.45)	AB	1.75	(1.09)	C
84	A hydrocarbon	1220		1219				B	0.50	(0.08)	A			A	0.51	(0.11)	A
85	A hydrocarbon	1228		1227				B	1.10	(0.79)	A			A			B
86	(E,E)-2,4-Nonadienal	1233	1712	1233	1–4, 6	0.84	(0.21)	A			C			C	0.40	(0.09)	B
87	β-Cyclocitral	1251	1626	1250	1–5	1.54	(0.38)	A			B			B			B
88	2-Undecanone	1311	1599	1311	1–4	0.74	(0.18)	A			B			B			B
89	(E,Z)-2,4-Decadienal	1319	1775	1319	1–4*	1.44	(0.42)	A			B			B			B
90	(E,E)-2,4-Decadienal	1342	1820	1342	1–7	5.28	(1.71)	A	0.98	(0.31)	B	0.20	(0.05)	B	1.12	(0.34)	B
91	Unknown			1349							A						B
92	Sativene	1379	1443	1378	1, 3–4*	0.50	(0.18)	B			B			B	2.49	(0.65)	A

Normalized areas (standard deviations) on RTX-5 Sil MS column, GC-FID[c]

(continued on next page)

Table 2 (continued)

#	Compound[a]	RI (GC-MS) RTX-5 Sil MS	RI (GC-MS) HP-Innowax	RI (GC-FID/O) RTX-5 Sil MS	ID methods[b]	Normalized areas (standard deviations) on RTX-5 Sil MS column, GC-FID[c] Chanterelle	Trumpet chanterelle	Porcini	Curry milk cap
93	Eugenol	1400		1380	1, 5*	0.29 (0.10) A	C	0.10 (0.03) C	0.40 (0.12) C
94	Tetradecane		1596	1400	1–3, 5*	C	0.15 (0.02) B	0.14 (0.03) B	36.6 (16.1) A
95	β-Caryophyllene	1461		1459	1–5	B	B	B	A
96	Geranyl acetone	1461	1864	1460	1–4	B	0.64 (0.16) A	A	B
97	β-Ionone	1509	1949	1507	1–4	0.88 (0.26) A	B	B	B
98	A caryophyllene isomer	1512	2480	1512	3, 4*	B	B	B	0.88 (0.41) A
99	Diethyl phthalate	1614	2400		1–4	e	e	e	e
	Total sum of peaks					817 (99) B	675 (70) B	239 (22) D	1212 (236) A

[a] For unknown and tentatively identified compounds, the main ions (if available) are listed in a separate Table S3 in Supplementary Materials.

[b] Identification (ID) methods are indexed as follows: 1: Matching retention index to literature on RTX-5 type columns. 2: Matching retention index to literature on Innowax type columns. 3: Matching MS fragmentation profile to mass spectral library on RTX-5. 4: Matching MS fragmentation profile to library on Innowax. 5: Matches with authentic reference compound (retention index, mass fragmentation). 6: Matches with odor description in literature. 7: Matches with odor quality (authentic reference compound) on RTX-5 column. 8: Matches with odor quality (authentic reference compound) on Innowax column. If the standard compound was not detected in GC-O runs it's not included in list items 6–8.

[c] Relative to the peak area of the inner standard (set as 100). Values in the same row followed by different letters (A-D) are significantly different ($p < 0.05$).

[d] Nonparametric Kruskall-Wallis test.

[e] Detected but under the limit of quantitation.

* Tentative identification.

classification, which in part validates the result.

As expected, the loadings plots (Figs. S3, S5, S7, and S97) demonstrate that the regions separating the mushrooms species are quite different between the GC–FID and GC–O data. On the GC–FID model of RTX-5 Sil MS column, especially the 5–7 min and 11–14 min regions (corresponding to retention index ranges 730–820 and 950–1070, respectively) have high loadings on principal components 1–2, while in the GC–O model with the same column almost the whole aromagram has significant loadings on both PCs.

3.2. Volatile compounds in mushrooms

Altogether 99 peaks were detected from the 4 mushroom species, of which 84 were at least tentatively identified (Table 2). Among the compounds, there were 13 alcohols, 21 aldehydes, 17 ketones, 2 esters, 14 hydrocarbons, 7 aromatic ring compounds, 1 sulfur compound, 12 terpenoids and 3 heterocyclic compounds. The contents of all volatile compounds were different between mushroom species as indicated by the ANOVA results. Overall, the relative content of all volatiles was lowest in porcini and highest in curry milkcap with five times the volatile content of porcini samples.

Volatiles common to all species (presented in descending average relative concentration order) included 1-octen-3-ol/1-octen-3-one, hexanal, 3-octanone, 1,3-octadiene/3-cyclohepten-1-one, 2-pentylfuran, (E)-2-octen-1-ol, octanal, octane, 1-octene, 3-octanol, nonanal, heptanal, (E)-2-octenal, acetone, decanal, pentanal, benzaldehyde, (E)-2-heptenal, 2-heptanone, 2-methylpentanal, (E,E)-2,4-decadienal, and 2- and 3-methylbutanal. The most notable variation was in 3-octanone, of which curry milkcap had over 20 times more than the second highest content in chanterelle. Likewise, chanterelle samples had over eight times as much hexanal compared to the second highest content in trumpet chanterelle.

The PCA model created with identified volatile compounds demonstrates the overall volatile profile differences (Fig. 1). Each mushroom had several unique volatile compounds that were not present in other mushrooms. Chanterelle had several unique terpenoids: δ-carene, 2,3-dehydro-1,8-cineole, β-ionone and β-cyclocitral. Chanterelle was also characterized by high amounts of pentanal, 2-methylpentanal, hexanal, 1-hexanol, heptanal, 2,3-octanedione, (E)-2-octen-1-ol, (E)-2-nonenal, and (E,E)-2,4-decadienal. Dried Portuguese (de Pinho et al., 2008) and freeze-dried Polish (Politowicz et al., 2017) chanterelle samples that were extracted with a similar 1-cm divinylbenzene/Carboxen/polydimethylsiloxane SPME fiber both had less hexanal in relation to 1-octen-3-ol but higher amounts of 1-hexanol compared to the chanterelles in this study. Portuguese samples lacked and Polish samples had much lower relative contents of the other characteristic compounds of this study. Fresh French samples extracted with ethyl ether (Fons et al., 2003) contained no hexanal but in contrast had (E)-2-octen-1-ol in comparable concentrations to 1-octen-3-ol.

Trumpet chanterelle uniquely had β-pinene and also several unique, unidentified and tentatively identified peaks (peaks 30, 46, 47, 53, 59, 61, 63, 65, and 85). It was also characterized by high amounts of 1-octene, octane, 3-cyclohepten-1-one/1,3-octadiene, α-pinene, (E)-6-methyl-5-hepten-2-one, 2,2,4,6,6-pentamethylheptane, and (E,E)-2,4-octadienal. Ethyl ether extracts of fresh French trumpet chanterelles (Fons et al., 2003) only contained 1,3-octadiene of these characterizing compounds. Instead, they were reported to contain substantial amounts of phenylacetaldehyde, (E,E)-2,4-nonadienal and (E,E)-2,4-decadienal, which were not major compounds of trumpet chanterelles in this study.

While porcini had no unique compounds present over the limit of quantitation, it was characterized by higher contents of 2-heptanone, 2-pentylfuran, p-cymene, and eucalyptol compared to other mushrooms. Interestingly, none of these compounds were reported to be present in boiled or canned porcini samples from Russia (Misharina et al., 2009), while 2-heptanone and 2-pentylfuran were present in fresh Hungarian samples (Csóka et al., 2017) and notable amounts of eucalyptol but no
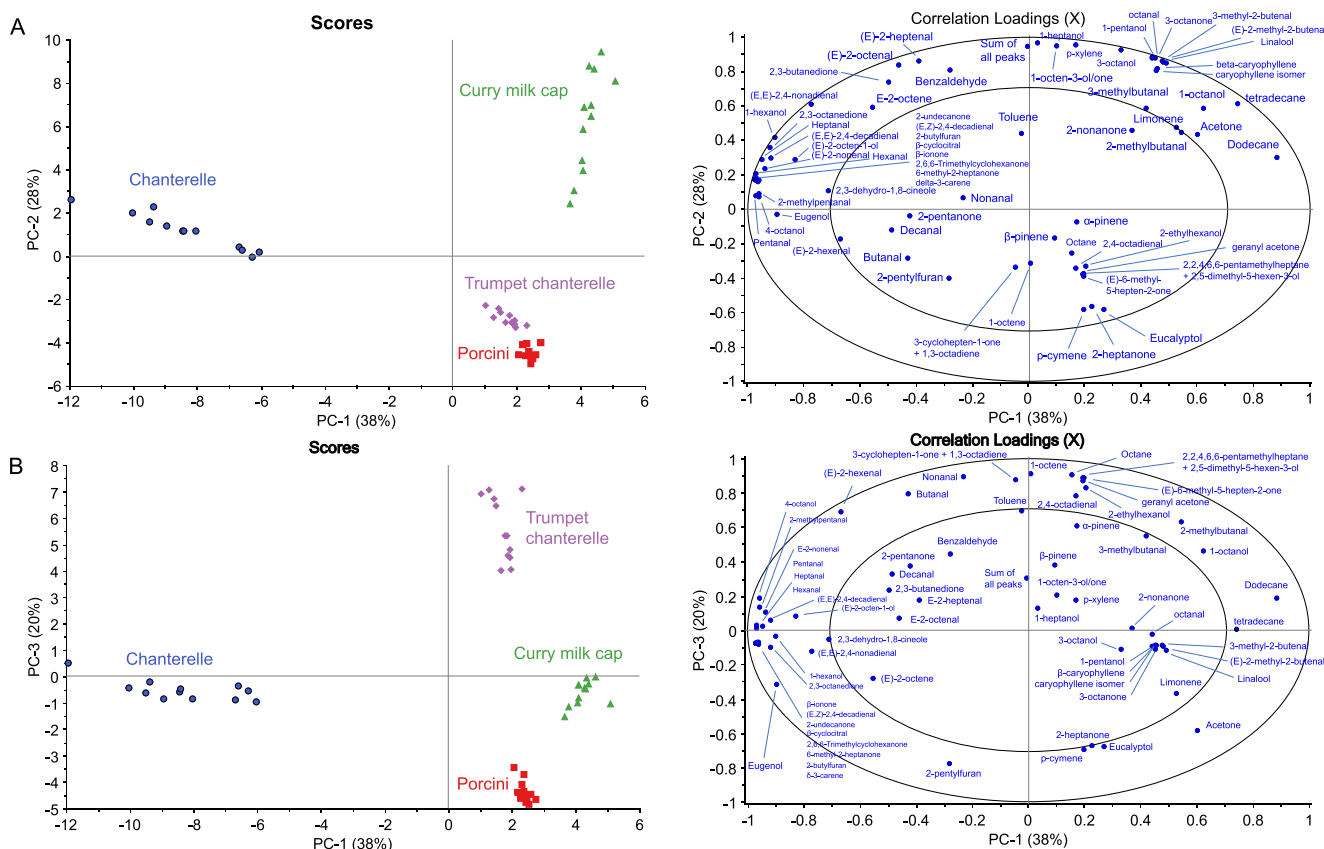
Fig. 1. Principal component analysis Scores (left) and Correlation loadings (right) plot of principal components 1 and 2 (A) and 1 and 3 (B) for the volatile compounds in mushrooms. The model was built using the normalized peak areas of all identified volatile compounds of the HS-SPME-GC-FID data on the RTX-5 Sil MS column.

other compounds in Portuguese dried samples (de Pinho et al., 2008). Instead, the Portuguese samples had substantial contents of furfuryl alcohol, pantolactone and 2-piperidone that were not detected in this study. In relation to 1-octen-3-ol, the fresh Hungarian samples had more 3-octanone, 3-octanol and (*E*)-2-octen-1-ol, while these were absent from Portuguese samples and instead had a lot more hexanal than Finnish samples. On the other hand, Hungarian samples had less hexanal and 1-octanol than the porcini samples in this study.

(*E*)-2-Methyl-2-butenal, 3-methyl-2-butenal, phenylacetaldehyde and β-caryophyllene were the most notable unique peaks for curry milkcap but additionally tentatively identified sativene and an unknown caryophyllene isomer were only present in curry milkcap. It was also characterized by high contents of acetone, 1-pentanol, 3-octanone, 3-octanol, octanal, 1-octanol and linalool compared to other mushrooms. Quabalactone III has been reported to be present in closely related *Lactarius helvus* species (Wood et al., 2012) and 12-hydroxycaryophyllene-4,5-oxide (Daniewski, Grieco, Huffman, Rymkiewicz, & Wawrzun, 1981) has been identified in curry milk cap, but neither of these were detected in this study.

In general, the mushroom samples in this study contained higher relative amounts of fatty acid degradation products, such as hexanal, than in published literature. However, the sample treatment was different, as previous literature has mainly researched freshly frozen or dried mushrooms instead of the heat treatment used in this study, which can explain this difference. Cho et al. (2006) reported higher

contents (shown as higher FD factors) of certain thermal degradation products, such as methional, 3-octanone and phenylacetaldehyde, in pine mushrooms cooked at 190 °C for 2 min compared to raw mushrooms, while there was no difference for other compounds, such as hexanal or (*E*)-2-decenal. This difference between studies is likely due to the more severe thermal treatment process for the pine mushrooms and easier evaporation of volatiles compared to the sample preparation in this study. Other factors that explain the differences to published literature include differences in the growth location, such as latitude, precipitation and forest type and the age of selected basidiocarps.

3.3. Odor-contributing volatile compounds in mushrooms

Overall aromagrams were distinct between mushrooms (Fig. 2). GC–O revealed in total 49 odor-active regions on the RTX-5 Sil MS column and 33 regions on the HP-Innowax column (Table 3, Tables S1–S2 for unknown peaks). Out of 50–57 detected volatile compounds in each mushroom, only 14–23 compounds were also detected *via* GC-O. There were additionally 2–9 odor-active regions on the RTX-5 Sil MS column and 1–5 regions on the HP-Innowax column for each mushroom that did not correspond to any instrumentally detected peak.

Despite the GC–O training, there was notable variation between the perceived odor impacts of compounds between assessors. This was evident in the duration of odor signals: for the same odor description and retention time, the pressing time was 0.5–5 s. The olfactory
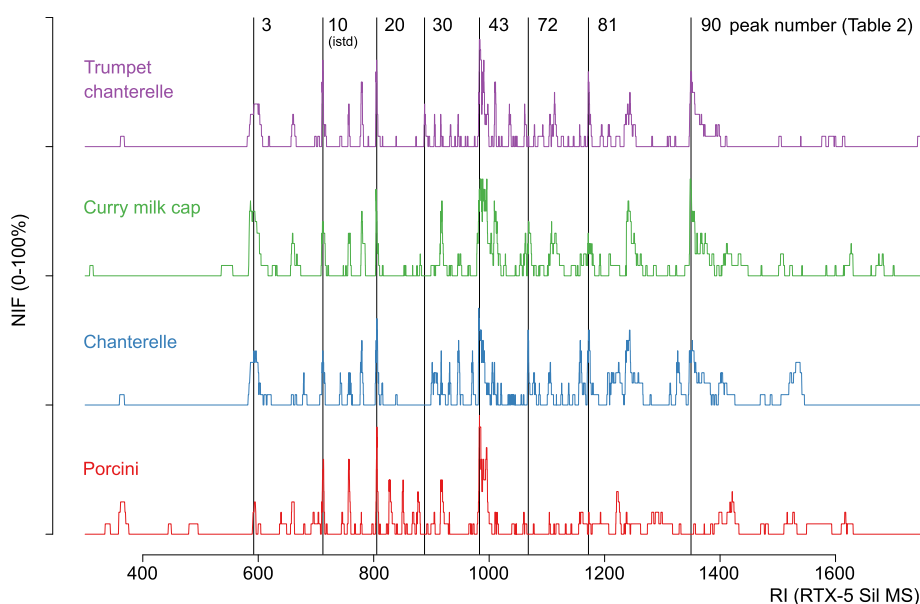
Fig. 2. HS-SPME-GC-O aromagrams of the four studied mushroom species run on the RTX-5 Sil MS column. The aromagrams are built based on data from 12 assessors for each mushroom. Peak numbers on the top of the figure refer to the numbering in Table 2.

sensitivity both within and between subjects has been reported to vary widely (Hoppu, Knaapila, Laaksonen, & Sandell, 2016), but it cannot be determined how much of the perceived difference in this study was due to limited training and how much due to actual differences in sensory acuity. This source of variation could have been examined, if the assessors had made repeated measurements of the same mushroom. However, a conscious decision was made in this study to instead maximize the number of assessors for each mushroom and therefore minimize the risk of specific anosmia for the odor-contributing compounds of interest.

The validative GC-O measurements on the HP-Innowax column helped to confirm the odor impact of several compounds. Butanal, 2-methylbutanal, pentanal, 2-heptanone, 3-octanol, 2-pentylfuran, and α-pinene were not detected with GC–O in standard mixtures and all had much smaller peaks in mushroom samples compared to standards. Thus it is highly unlikely that they contribute to the odor of the examined mushroom species. Additionally, these measurements demonstrated that 1-octen-3-one has consistently higher SNIF values than 1-octen-3-ol in all mushrooms. The SNIF values indicate that the odor impression of the co-eluting RI 984 peak on the RTX-5 Sil MS column is mainly due to the ketone form as reported previously (Cho et al., 2006; Fischer & Grosch, 1987; Grosshauser & Schieberle, 2013; Kleofas et al., 2015; Zhang et al., 2018). It is also likely that since neither 3-octanone nor 2-pentylfuran was detected in the validative GC–O, the SNIF values on the main experiment at their elution sites are either due to the persisting odor of 1-octen-3-one or a synergy between these compounds.

Common odor-contributing volatile compounds for all mushrooms (presented in descending average SNIF value order) were 1-octen-3-one, 1-octen-3-ol, hexanal, octanal, methional, (*E*)-2-nonenal, 2,3-butanedione, and (*E*)-2-octenal. The PCA models built from the SNIF values show that each mushroom is also characterized by distinct compounds (Fig. 3). Porcini was best characterized by the odor impact of methional. Other compounds with unique or bigger odor impacts were ethyl 2-methylpropanoate (RTX-5 column), ethyl 2-methylbutanoate, 3-cyclohepten-1-one/1,3-octadiene (RTX-5 column), 3-methylbutanal, 4-octanol (HP-Innowax column), and 2-acetyl-1-pyrroline (HP-Innowax column). It was also characterized by the missing odor impact of (*E,E*)-

2,4-decadienal and smaller odor impacts of (*E*)-2-octenal and (*E*)-2-nonenal, compared to other mushrooms. Curry milkcap was characterized by the unique odor impacts of octanal, phenylacetaldehyde, linalool, nonanal and the unknown RI 1241 peak on the RTX-5 column. It also had the second largest SNIF values of methional after porcini. On the other hand, curry milkcap had low SNIF values of 1-octen-3-ol and (*E,E*)-2,4-nonadienal compared to other mushrooms.

Trumpet chanterelle was characterized by the 1-octen-3-one and 1-octen-3-ol SNIF values especially on the HP-Innowax column. Unknown peak 30 on the RTX-5 column was unique to trumpet chanterelle as well as the tentatively identified 2,2,4,4-tetramethyloctane, (*E,Z*)-2,6-nonadienal, geranyl acetone, and unknown peaks with retention indices 1861, 1011 and 888 on the HP-Innowax column. On the other hand, methional was only faintly detected in trumpet chanterelle by the assessors compared to other mushrooms. Chanterelle was characterized by the large SNIF values of (*E,E*)-2,4-decadienal, hexanal and (*E*)-2-nonenal. Several odor-active compounds were only detected on the RTX-5 column but not on the HP-Innowax column. These were nonane, heptanal, 2,3-dehydro-1,8-cineole, 2-acetyl-1-pyrroline, (*E,Z*)-2,6-nonadienal, and 1-octanol. Additionally several unknown major peaks: peaks with retention indices 1241, 1535, 1326 and 947 eluted from the RTX-5 column and an unknown peak with RI 1511 from the HP-Innowax column. On the other hand, 3-methylbutanal was not detected from chanterelle with GC-O.

3.4. Comparison of sensory odor profiles and aroma compounds

There was a moderately strong positive correlation between the overall odor intensities of mushrooms in the sensory profile and the total amount of volatiles (Pearson $r^2 = 0.63$). This would explain the highest odor intensity of curry milkcap as it had also clearly the highest content of volatiles. However, many of the biggest peaks in the GC–FID data of curry milkcap, such as (*E*)-2-methyl-2-butenal, were not odor active, which makes this comparison of limited value. There also was a moderate positive correlation between GC–O SNIF values and odor intensities (Pearson $r^2 = 0.43$). The lower association is shown in the fact that SNIF values for curry milkcap were not considerably higher

Table 3

Odor-contributing volatile compounds, retention indices as detected by GC-olfactometry, descriptions and surface of nasal impact frequencies (SNIF) on two columns. Peak numbering corresponds to numbering in Table 2. Unknown, GC-O-only peaks are listed separately in Tables S1 and S2 in Supplementary Material.

#	Compound	RI (RTX-5 Sil MS)	Descriptions (RTX-5)	SNIF RTX-5 Sil MS: Chanterelle	Trumpet chanterelle	Porcini	Curry milk cap	RI (HP-Innowax)	Descriptions (HP-Innowax)	SNIF HP-Innowax: Chanterelle	Trumpet chanterelle	Porcini	Curry milk cap
1	Unknown	367	rotten, stuffy			817							
3	2,3-Butanedione	595	toffee, butter, candy	2333	2483	300	3050	980	caramel, sweet, toffee	3850	3200	3200	4050
5	3-Methylbutanal	660	green, spicy, nutty, chlorine		1133	1167	1517	922	roasted, stuffy, solvent-like		700	2250	1450
10	ISTD: Ethyl propanoate	712	sweet, fruity, liquor	2333	2400	2550	3317						
13	Ethyl 2-methylpropanoate*	757	sweet, fruity, berry	750	967	2450	1900						
14	1-Pentanol	761	sweet, menthol, toffee	633									
20	Hexanal	805	green, grass	3900	2867	3550	3250	1076	green, grass	7650	5100	4800	6850
21	(E)-2-Octene	815	potato, mushroom	450									
22	3-Cyclohepten-1-one	828	stuffy, moss, forest			3500							
24	Ethyl 2-methylbutanoate*	851	essence, fruity, sweet			1833		1036	sweet, berry-like, fruity		2950	3000	
27	p-Xylene	877	rotten, onion, batter			2083							
30	Unknown	888	solvent, plastic, paint		1283								
33	Nonane	901	plastic, pungent	1467									
34	Heptanal	906	solvent, pen	2666	667			1186	wet sawdust, plastic			12,850	2300
35	Methional	917	potato, mushroom	1400	567	4500	4050	1465	(mashed) potato	2650		1200	7150
36	2-Acetyl-1-pyrroline*	931	basmati rice, sweet, spicy	1517				1342	popcorn, sweat, basmati rice				850
40	1-Heptanol	971	cellar, cucumber, sweet, fruity	2100									
43	1-Octen-3-one	983	mushroom	5467	6800	7350	8467	1305	mushroom, forest	13,250	15,300	14,500	10,100
44	1-Octen-3-ol							1460	mushroom	3500	6300	3200	950
49	3-Octanone	990	mushroom, soil, potato	4000	4067	3767	6117						
51	4-Octanol	993	mushroom, soil, earth		683			1379	cellar, dark, solvent, burned	750	2950	5200	850
54	2-Pentylfuran	996	mushroom, menthol	1379	1433	4783	3833						
56	2,3-Dehydro-1,8-cineole	1005	eucalyptus, pepper, citrus	983									
57	Octanal	1010	cucumber, citrus, plastic		1967		4317	1288	citrus, solvent	2700	4600	2650	6200
61	2,2,4-Tetramethyloctane*	1035	mushroom, plastic, burned		1300								
71	Phenylacetaldehyde	1062	cucumber, sweet, liquor		1283		1683	1655	honey-like, flowery, urine, sweet				4150
72	(E)-2-Octenal	1068	sawdust, plastic, chemical, pungent	2600			3183	1433	wax-like, fat, sawdust, plastic	3500	4400	650	3800
74	1-octanol	1077	paint jar, soil	1933									
76	Linalool	1005	sweet, citrus, plastic, solvent, burned	2283	967		1883	1557	herb-like, flowery, sweet, fruity cucumber				3200
77	(E,E)-2,4-Octadienal												
78	Nonanal							1395	sawdust, citrus, plastic				2050
79	Unknown	1114	plastic, sawdust, pickled cucumber		2033		2983						
80	(E,Z)-2,6-Nonadienal	1158	cucumber, fresh, citrus	2883				1590	cucumber, forest clearing	6050	2500		
81	(E)-2-Nonenal	1173	cucumber, air mattress, paint, old books, cardboard	3848	2267		2250	1540	leather, old books, detergent		4400	3000	6450
83	Decanal	1213	sweet, flower, lemon	1233									
86	(E,E)-2,4-Nonadienal	1223	soil, matchbox, forest	2433		1867		1712	sawdust, cucumber, plastic	3250	3300	1900	1050
90	(E,E)-2,4-Decadienal	1351	solvent, wood, cucumber, wood, citrus, paint, fat	5467	4700	4983	4983	1828	sawdust, plastic, sweet	7000	3100		2450
92	Sativene*	1366	sawdust, plants, pastry			1783							
93	Eugenol*	1374	sawdust, pastry			1767							

Table 3 (*continued*)

#	Compound	RI (RTX-5 Sil MS)	Descriptions (RTX-5)	SNIF RTX-5 Sil MS				RI (HP-Innowax)	Descriptions (HP-Innowax)	SNIF HP-Innowax			
				Chanterelle	Trumpet chanterelle	Porcini	Curry milk cap			Chanterelle	Trumpet chanterelle	Porcini	Curry milk cap
96	Geranyl acetone							1844	moldy leaves, plastic		2350		
99	Diethyl phthalate	1625											1400
	Butanoic acid						583	1641	button mushroom, feces-like	5450	3400		
	2-Methylbutanoic acid							1679	drawer, protein powder	3500	3100	2400	2500
	Sum of all odor-active regions (including unknowns)			73,726	44,183	43,200	72,016			68,850	74,550	63,950	75,250

* Tentative identification.

than in other mushrooms. These associations should be interpreted as tentative due to the reduced datasets used in calculations.

As mentioned in the Introduction, Zhang et al. (2018) reported that the cooked potato-smelling methional was the most important contributor to the aroma of dry porcini. Compared to fresh porcini, the mushroom-like and especially the grass-like descriptors diminished in the dry porcini, which were linked to major losses in carbonyl compounds. However, the high content of methional alone does not explain the mashed potato like odor that was characterized earlier in porcini samples used in this study by the trained sensory panel (Aisala et al., 2018). On the other hand, porcini shares a number of other odor-active compounds aside from methional with both raw and boiled potatoes (Petersen, Poll, & Larsen, 1998) and the specific mixture of these compounds could be behind the overall odor impression.

In the trained sensory panel the characterizing odors for chanterelle were cooked carrot, cardboard and forest. No single compound was described as carrot-like in GC-O, but chanterelle does share several compounds that were reported to be important for the odor of cooked carrots (Buttery & Takeoka, 2013). These include heptanal, (E)-2-nonenal, octanal and (E,E)-2,4-decadienal. Hexanal and the RI 1511 compound on the HP-Innowax column likely contribute to the forest-like odor perception with their grass/green and green/dried hay-like odor qualities. It is likely that many of the unknown compounds, such as the fat- and herb-like RI 1241 compound and fruity and smoke-like RI 1535 compounds on the RTX-5 Sil MS column, further contribute to these odor descriptors.

For trumpet chanterelle, the typical odor descriptors in the trained sensory panel were forest, cardboard and earthy/soil. There are several compounds that are linked to these, such as the forest clearing type (E,Z)-2,6-nonadienal and the sawdust and wood type compounds such as (E,E)-2,4-decadienal and the green unknown RI 1011 compound on the HP-Innowax column. Likewise, the soil odor qualities of 1-octen-3-ol/one, 4-octanol and the unknown potato peel like RI 1861 compound on the HP-Innowax column point to their contribution to the earthy odor descriptor.

Curry milkcap is an interesting mushroom as it had almost no mushroom-like odor in the trained sensory panel but still the biggest contents of 1-octen-3-ol/one and comparable SNIF values compared to other mushrooms. The characteristic odor descriptors, roasted, earthy, and cardboard, had no single compounds with matching descriptions. However, the combined effect of methional, 1-octen-3-ol/one, and 3-octanone might contribute to the earthy descriptor. Likewise, several sawdust and plastic-like descriptions of fatty acid degradation products might contribute to the roasted odor descriptor.

4. Conclusions

This study provided a thorough comparison of the volatile compound and odor-contributing volatile compound profiles of Nordic edible wild mushrooms. The results demonstrated that while these mushrooms share a number of volatile compounds, their profiles are distinct with major differences in the relative proportions of compounds. Only a subset of volatile compounds was detected via GC-O. The characteristic compounds for each species were very different between instrumental measurements and as detected by human senses, especially in their order of importance. The simple sample preparation of HS–SPME facilitated finding the similarities and characterizing compounds for each species, as the study design eliminated the systematic error caused by the extraction profile of the SPME fiber. Furthermore, several links between these compounds and the odor descriptors in the sensory panel were found. These results form an important basis for future research to understand the flavor chemistry of mushrooms, the biological processes that determine their sensory properties, as well as the factors related to the liking of mushroom aroma.
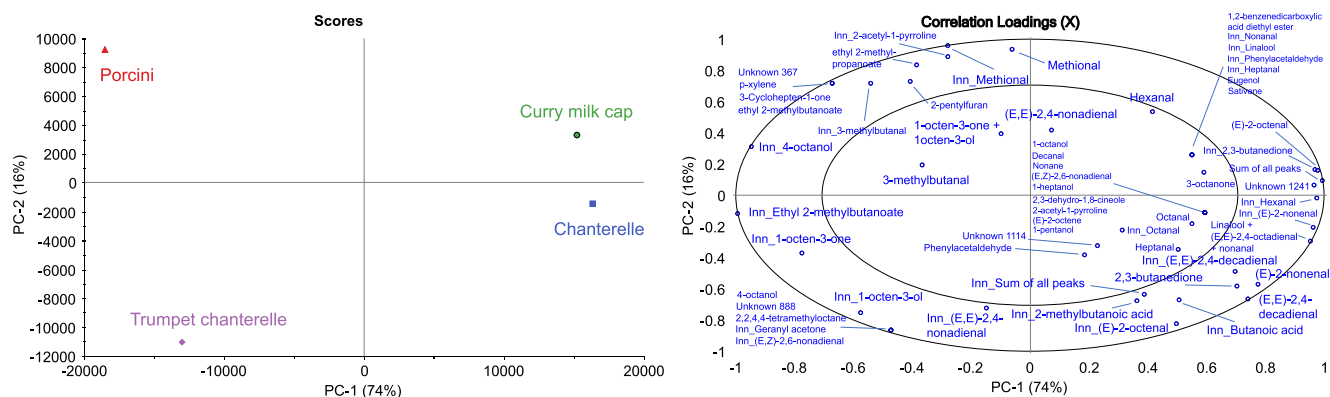
Fig. 3. Principal component analysis Scores (left) and Correlation loadings (right) plot of principal components 1 and 2 for the odor-contributing compounds in mushrooms. The model was built using the SNIF values of identified and major unidentified compounds (numbers refer to retention indices) of the HS-SPME-GC-O data on both the RTX-5 Sil MS and HP-Innowax (prefix 'Inn_') columns.

Declaration of interests

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.01.053.

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