Linking volatile and non-volatile compounds to sensory profiles and consumer liking of wild edible Nordic mushrooms

Running title: Linking chemical compounds to sensory properties of Nordic mushrooms

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Abstract

Current information on the links between the chemistry and hedonic liking of edible mushrooms is scarce. In this study, 84 consumers evaluated the appearance, odor, taste, texture and overall liking

of samples of Nordic edible wild mushroom species. Subsequently, multivariate models on the effects of non-volatile, odor-contributing volatile compounds, sensory attributes and hedonic likings were created. The non-volatile compounds were measured with quantitative NMR.

The mushroom species were different in their sugar and acid contents. Three consumer clusters were found with species*cluster interactions. Correlations with sensory attributes and chemical components were found, and the multivariate models also indicated predictor attributes for each consumer cluster.

The results indicate that: the sensory properties could be correlated to both volatile and non-volatile compounds, there are consumer clusters with differing likings as regards on mushrooms, and these clusters are heterogenic groups with no simple factors such as age explaining their liking scores.

Keywords: mushrooms; hedonic testing; NMR; external preference mapping; PLS

1. Introduction

Edible mushrooms are a valued delicacy in many cultures. They are a wide group with variety of different flavors as well as cooking and consuming methods. In our generic descriptive analysis of five cooked mushroom species (Aisala et al., 2018), the samples had characteristic odors, tastes, chemosensory and textural properties and were easily distinguishable. The flavor of mushrooms results from a vast variety of volatile and non-volatile compounds. Pyysalo et al. (1976) studied the concentrations of volatile aroma compounds from seven Finnish edible mushroom species and concluded that the aroma of these species mainly originated from volatiles with eight carbon atoms, such as 1-octen-3-ol and 1-octen-3-one. Results from later studies have indicated that 1-octen-3-one is the major compound causing the mushroom-like odor, while the identifiable odors of different species are caused by other compounds such as fatty acid degradation products, 3-

(methylthio)propanal, terpenoids and N-heterocyclic compounds (Cho, Lee, et al., 2007; de Pinho et al., 2008; Grosshauser & Schieberle, 2013; Zhang et al., 2018). In our gas chromatography–olfactometry measurements (Aisala, Sola, Hopia, Linderborg, & Sandell, 2019), the fatty acid degradation products especially contributed to the odor of wild edible mushrooms. As to the non-volatile components, the flavor of mushrooms is generated by free amino acids and nucleotides, as well as various other compounds such as organic acids, soluble sugars and polyols (Beluhan & Ranogajec, 2011; Rotzoll, Dunkel, & Hofmann, 2006).

Several mushroom species are described as having an especially rich umami taste (Phat, Moon, & Lee, 2016). Umami is the fifth taste modality originating from the sodium salts of amino acids, mainly glutamic and aspartic acid, binding to T1R1 + T1R3 receptors (Nelson et al., 2002). This taste sensation is intensified by 5'-nucleotides, 5'-guanosine monophosphate, 5'-inosine monophosphate, 5'-xanthosine monophosphate and 5'-adenosine monophosphate (Yamaguchi, Yoshikawa, Ikeda, & Ninomiya, 1971). This synergy is typically calculated as an equivalent umami concentration value (EUC) which expresses the synergy in glutamic acid equivalents (Yamaguchi et al., 1971). Free amino acids comprise only 0.4–7% of the total dry matter content and 1–27% of the crude protein in mushrooms (Beluhan & Ranogajec, 2011; Tsai, Tsai, & Mau, 2008; Yang, Lin, & Mau, 2001). However, amino acids generate a great variety of taste perceptions and can be divided into classes based on which taste modality they induce. The taste properties of some amino acids are ambiguous, thus multiple classifications have been used (Kawai, Sekine-Hayakawa, Okiyama, & Ninomiya, 2012; Yang et al., 2001). In the literature (Beluhan & Ranogajec, 2011; Mau, Lin, Ma, & Song, 2001; Yang et al., 2001), amino acids are classified based on their pure tastes: umami-like (glutamic acid and aspartic acid), sweet (serine, glycine, threonine, alanine) and bitter (histidine, arginine, tyrosine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine) and tasteless. In our measurements of the free amino acid and 5'-nucleotides in wild edible mushrooms (Manninen, Rotola-Pukkila, Aisala, Hopia, & Laaksonen, 2018), the contents of each amino acid class and

nucleotides varied between species. Organic acids are usually described as sour (Moskowitz, 1971a) but also astringent (Thomas & Lawless, 1995). In contrast, sugars and polyols are described as sweet (Moskowitz, 1971b). The non-volatile compounds are typically measured by liquid chromatography (Ajlouni, Beelman, Thompson, & Mau, 1995; Heleno et al., 2011; Li et al., 2011), but it has been demonstrated that methods based on NMR can determine the organic acid, sugar and amino acid composition of the sample in a single measurement (Aisala et al., 2016; Cho, Kim, & Choi, 2007).

Although extensive research has been conducted on both volatile and non-volatile compounds of mushrooms, there have only been a few studies comparing the chemical data of flavor compounds with the results from sensory analysis. Cho et al. (2007) performed a 15-attribute descriptive sensory analysis with a trained panel as well as gas chromatography-olfactometry on matsutake mushrooms and correlated these datasets with a multivariate model. Similarly, de Pinho et al. (2008) utilized a trained panel and the chemical data of volatile compounds to compare the odor properties of wild edible mushrooms. Phat et al. (2016) compared the umami taste compound contents in mushroom extracts with the results collected by sensory analysis and electronic tongue system.

To our knowledge, there are no studies combining chemical data of both volatile and non-volatile compounds with sensory profiles of mushroom species. Furthermore, the knowledge on the flavor profile of Nordic mushroom species is scarce. Additionally, the hedonic studies for mushrooms have only been done thus far with cultivated species (Hiraide, Yokoyama, & Miyazaki, 2005; Ren, Pan, Li, Chen, & Duan, 2018). In the hedonic studies, there has been no consumer clustering apart from age groups (Ren et al., 2018).

The objective of this study was to analyze the relationship between the sensory perception attributes of wild edible Nordic mushrooms and volatile and non-volatile flavor components. Partial least squares regression (PLS) was used in order to find correlations with the results of chemical and sensory analysis. A nuclear magnetic resonance (NMR) spectroscopy dataset on mushroom non-

volatile compounds was collected. This was combined with the previously collected data of the free amino acids and 5'-nucleotides (Manninen et al., 2018) and odor-contributing volatile compounds (Aisala et al., 2019) to explain the sensory properties of mushrooms (Aisala et al., 2018). Moreover, a hedonic liking study was conducted on studied mushrooms in order to evaluate correlations between liking and descriptive sensory profiles.

2. Materials and methods

2.1.1. Samples

Four species of Nordic edible wild mushrooms, chanterelle (*Cantharellus cibarius* Fr.), trumpet chanterelle (*Craterellus tubaeformis* (Fr.) Quél.), porcini (*Boletus edulis* Bull.) and curry milkcap (*Lactarius camphoratus* (Bull.) Fr.) as well as cultivated button mushrooms (*Agaricus bisporus* (J.E.Lange) Imbach), were studied. Samples from the same batch were used in previous studies (Aisala et al., 2018; Manninen et al., 2018) and prepared in the same way. In brief, fresh mushrooms were cooked with a *sous vide* process (80 °C) for 10 minutes, frozen at -20 °C, cut to 1–2 cm^3 cubes and pooled while frozen and stored at -20 °C until analysis.

2.1.2. Chemicals

Altogether 31 reference sugars, organic acids and amino acids were used in the nuclear magnetic resonance (NMR) measurements: D-glucose, D-fructose, sucrose, trehalose dihydrate, citric acid, DL-malic acid, formic acid, fumaric acid, maleic acid, γ-aminobutyric acid, L-alanine, L-arginine, L-asparagine monohydrate, L-aspartic acid, L-cysteine, L-glutamine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine hydrochloride, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine and choline chloride. These were all bought from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) except D-fructose which was bought from Kanto Chemical Co, Inc (Tokyo, Japan).

For the phosphate buffer, K_2HPO_4 from Kanto Chemical Co, Inc. and KH_2PO_4 from Chameleon reagent (Osaka, Japan) were diluted in D_2O (99,8% D) from Acros Organics (Geel, Belgium). 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) from Tokyo Chemical Industry Co, Ltd. (Tokyo, Japan) was used as the internal standard.

2.1.3. Sample preparation for NMR spectroscopy

Frozen samples were weighed in 50 mL Falcon tubes in batches of approximately 10 g and freeze-dried in vacuum at -30 °C for 46 hours. Before the NMR measurements, the samples were freeze-dried a second time for 70 hours to eliminate any build-up moisture. Dry matter content was determined based on the weighed masses before and after freeze-drying. The average dry matter contents (and standard deviations) were 81.1 (0.4) g kg^{-1} for button mushrooms, 79.3 (1.1) g kg^{-1} for chanterelle, 83.4 (2.2) g kg^{-1} for trumpet chanterelle, 100.1 (0.5) g kg^{-1} for porcini, and 142.3 (8.2) g kg^{-1} for curry milk cap. These values were in good agreement with our previous measurements of the same batch (Manninen et al., 2018). The freeze-dried samples were ground to a fine powder with a mortar and pestle, pooled by species and stored at -18 °C for one week until extractions.

Sixty milligrams of mushroom powder was measured into 2 mL centrifuge tubes in quadruplicate. 600 µL of 0.1 M phosphate buffer in D_2O (pH 7.0) was added to the powder. The mixture was vortexed for 30 s, sonicated for 10 min at room temperature, incubated for 15 min in a sample shaker and centrifuged (10,000 g, 15 min). The supernatant was removed and the procedure repeated once. The supernatants were combined, centrifuged once more and 600 µL of the combined extract supernatant was used for NMR measurements. Finally, 100 µL of 5 mM DSS in the D_2O-phosphate buffer was added to this aliquot for chemical shift referencing and quantification.

2.1.4. NMR spectroscopy

^1H qNMR spectra were measured with an Agilent 400-MR DD2 spectrometer (Agilent Technologies, Santa Clara, California, US) operating at proton frequency 399.79 MHz. The spectrometer was

equipped with a OneNMR Protune probe and was controlled with VnmrJ 3.2 Revision A. Spectra were recorded at 295 K with sample spinning at 16 Hz in a 5 mm NMR tube (Type S, Wako Pure Chemical Industries, Osaka, Japan) and locked to D_2O. Samples were shimmed to a DSS signal width at half height <0.7 Hz. The NMR parameters were: 30° pulse angle, 16 ppm spectral width and 64k data points (acquisition time 5.11 s), 5 s recycle delay, and 128 scans. The receiver gain was set to 30. The Free induction decays were Fourier transformed with zero-filling to 128 k and with LB = 0.3 Hz in MestReNova version 12.0.3 (Mestrelab Research S.L, Santiago de Compostela, Spain).

2.1.5. NMR spectroscopy validation

Light validation of the extraction method and NMR linearity was performed with two calibration curves, residual extraction and spiking experiments. The first calibration curve contained 0–60 mM of glucose, sucrose, citric acid, malic acid, L-alanine, L-glutamic acid, and L-arginine in six levels. The second curve was created after the sample measurements also in six levels. It contained trehalose (0–50 mM), fumaric acid (0–16 mM), malic acid (0–32 mM), L-glutamine (0–16 mM), L-alanine (0–20 mM), and L-isoleucine (0–16 mM); the ranges were selected based on the sample results. Spiking was carried out by adding known amounts (16–140 mg) of crystalline trehalose, malic acid, fumaric acid, alanine, glutamine and isoleucine to 1800 mg of chanterelle powder before weighing and performing the extraction method as above in triplicate. The recovery coefficient was calculated as the quotient of the experimentally determined and weighed added standard contents. The residual extraction was carried out by continuing with three of the porcini sample precipitates after the main extraction. Three additional extraction rounds were performed and the pooled residual extract measured as above. The residue coefficient was calculated as the quotient of residual extraction and main extraction contents.

2.1.6. NMR compound identifications and quantitation

Each NMR signal was first tentatively assigned by comparing the ^{1}H 1D spectra to published data and reference spectra in the Human Metabolome Database (Aisala et al., 2016; Cho, Kim, et al., 2007; Wishart et al., 2018). Additional composite sample extracts containing all mushroom species were spiked consecutively with all reference compounds listed in the Chemicals section to confirm peak shapes and J values. Chemical shift drift due to pH changes was employed in identification of organic acids. Finally, metabolite identification was confirmed by using gCOSY, HSQCAD and gHMBCAD 2D measurements of the composite samples.

The non-overlapping proton signal areas that were above the limit of quantification were determined. The data analysis protocol of Malz and Jancke (2005) was followed and all concentrations were calculated for fresh weight. Quantification focused on sugars, sugar alcohols and organic acids as the amino acids and 5'-nucleotides had been determined previously (Manninen et al., 2018). However, L-alanine, L-glutamine, L-isoleucine, L-valine, L-glutamic acid, and L-aspartic contents were determined for between-method comparison.

2.1.7. LC-MS analysis of the unknown compound in curry milk cap

Curry milk cap samples without derivatization were prepared as previously reported (Manninen et al., 2018) and measured with a Waters Acquity UHPLC instrument (Waters, Milford, MA) connected to a Waters Xevo Q-TOF MS. The column was a Waters Acquity HSS T3 (2.1 x 100 mm, 1.8 µm). Mobile phases were 0.1 % formic acid in H_2O (A) and acetonitrile (B). The column flow was 0.3 mL/min and the mobile phase was changed to B after 5 minutes. The injection volume was 5 µL. Electrospray ionization on positive mode was used with a 2.5 kV capillary voltage. Full scan mode was used with an m/z 50–1000 range. Data were analyzed with Waters MassLynx V4.1 software.

2.1.8. Sample preparation for the hedonic test

On the sensory evaluation day, frozen samples were thawed in 20–60 g aliquots in *sous vide* bags in a 70 °C water bath for 5 min. However, curry milk cap was not included because of poor sample availability and because this mushroom is typically used as a spice instead of a food ingredient. Representative samples (7–8 g) containing both solid mushroom and dissociated liquid were served in 70 mL transparent glass bowls covered with glass plates. The samples were tempered on a hotplate to 50–60 °C for at least 15 minutes before evaluation. Sample cups were coded with three-digit numbers.

2.1.9. Hedonic test

A total of 84 consumers between 20 and 74 years old (median age 47 years) who used mushrooms or mushroom products at least sometimes participated in the hedonic testing. Volunteer consumers were recruited mainly from the Turku region in Finland. The hedonic test was conducted in a sensory laboratory (ISO 8589, University of Turku). The consumers evaluated the odor, appearance, flavor, texture and overall liking of each of the four mushroom samples. Liking was evaluated using the 9-point hedonic scale labeled with numeric and descriptive anchors in Finnish. Samples were presented monadically and the sample presentation order was randomized among the subjects. The participants were asked to refrain from using strong perfumes on the evaluation day and to refrain from eating or drinking anything aside from water at least 30 minutes before the evaluation. They were instructed to clean their palate with active-carbon filtered water and a piece of low-sodium cracker between the samples.

After the end of the hedonic test, the consumers answered a set of background questions related to consumer demographics and mushroom usage. The questionnaire also included the Food Choice Questionnaire (Steptoe, Pollard, & Wardle, 1995) as modified previously (Pohjanheimo & Sandell, 2009) and the 8-question version of the Food Disgust Scale (Hartmann & Siegrist, 2018), both

translated into Finnish. The typical completion time for the whole test was about 30 minutes. Data were collected with the Compusense Cloud version 8.4 (Compusense Inc., Guelph, Ontario, Canada).

2.2. Statistical analysis

2.2.1. Metabolomics approach for NMR data

An unsupervised, initial overview of the NMR data was performed with the ChemoSpec package version 4.4.97 (Hanson, 2017) in RStudio 1.2 running R 3.6.0. First, a correction factor based on dry matter contents and extraction masses was applied to the spectra from 0.3 ppm onwards. Then, the whole spectra were normalized based on the 0.00 ppm DSS signal and binned to 0.02 ppm/point data buckets. Water and DSS peaks as well as redundant spectral regions at <0.7 ppm and >10 ppm were removed. A principal component analysis (PCA) was performed with mean centering and Pareto scaling. Classical 95% confidence ellipses were used in determining the sample populations in the scores plots. In the loadings plot, the binned curry milk cap spectra were used as a reference.

2.2.2. Differences between mushrooms in quantified NMR data

Differences between mushroom samples in each analyzed compound were tested with one-way analysis of variance (ANOVA) or the Brown-Forsythe test with either Tukey's HSD or Tamhane's T2 post-hoc test as directed by the tested homogeneity of variance. The tests were performed with IBM SPSS Statistics 24.0 (IBM, Corporation, Armonk, NY).

2.2.3. Consumer clustering

The consumers were clustered based on their mushroom liking scores. First, the main sources of variation in the 20 hedonic variables (5 hedonic modalities in the 4 mushrooms) were determined with principal component analysis (PCA) using the PCA function of the FactoMineR package in RStudio 1.2. The data was mean centered and no standardization was used. The resulting dataframe was then used as source data for Hierarchical Cluster Analysis (HCA) using the HCPC function of

the FactoMineR package. HCA was performed with Ward's method and Euclidean distances with the algorithm automatically deciding the number of clusters. The algorithm suggested three 20–38 member clusters, and this solution was retained.

Differences in each hedonic modality were examined with a 2-way ANOVA (general linear model, univariate with mushroom species and cluster membership as fixed factors; model included main effects and the interaction term) in SPSS after appropriate data transformations in order to conform to normality. Eta squared values (η^2) were calculated with the recommended procedure (Levine & Hullett, 2002). Post hoc tests were built for cluster differences in each mushroom using simple contrasts with the LMATRIX subcommand and Bonferroni corrections.

The effect of cluster membership on the background variables was studied with either a one-way ANOVA (age, Food Choice Questionnaire variables, Food Disgust Scale results, number of known mushroom species) or with the Kruskal-Wallis test (sample familiarity, mushroom usage frequency). Tukey's HSD or the Mann-Whitney U with Bonferroni corrections were used as post-hoc tests. The effects of gender, education and diet were not examined with statistical tests due to the imbalanced sample.

2.2.4. Combinatory multivariate models

The data retrieved from non-volatile compounds using liquid chromatography (Manninen et al., 2018), odor-contributing volatile compounds using headspace-solid phase microextraction-gas chromatography-olfactometry (Aisala et al., 2019) and sensory properties using generic descriptive analysis (Aisala et al., 2018) including the replicate analyses were first analyzed separately with PCA using The Unscrambler version 10.4.1 (Camo Process AS, Oslo, Norway) with auto scaled data (the readers are referred to these source publications for the methods related to these datasets). EUC values were calculated from free amino acid and 5'-nucleotide contents (Yamaguchi et al., 1971). NMR amino acid data for button mushrooms were used and 5'-nucleotide values from (Li et al., 2011) were

used to calculate the EUC for button mushrooms. Total sugar, total acid and sugar-acid ratios were calculated. Additionally, the total sugars in glucose equivalents, total acids in malic acid equivalents, and equivalent sugar-acid ratios were calculated based on reported relative sweetness and sourness of these compounds (Moskowitz, 1971a, 1971b). After confirming over 75% of the explained variations in both the calibration and validation models in each dataset, the data was averaged over the replicates.

The analysis was continued with a partial least squares regression (PLS) analysis (Unscrambler), using chemical attributes as X-variables (predictors) and sensory properties as Y-variables (responses). The predictors were autoscaled and all data was mean-centered. Separate models for non-volatile and odor-contributing volatile compounds were made before making the final composite model. Sensory drivers of liking were measured with PLS following Guinard et al. (2016). The sensory properties were used as X-variables and the average liking scores for each liking modality and consumer cluster as Y-variables. All data was mean-centered but no scaling was used. The limit for statistical significance for all statistical tests was $p < 0.05$.

3. Results and discussion

3.1. NMR spectroscopy

3.1.1. Identified compounds

In total, three sugars and sugar alcohols, four organic acids and 17 amino acids were identified in the qNMR samples (Supplementary material, **Table S1**). Additionally, seven major unidentified compounds were present in the samples. The major unknown peak in curry milk cap was tentatively identified as a dimethylsubstituted pentose with a molecular mass of 147, but more research is needed to unequivocally designate the compound. Therefore, we will only refer to this compound in the following text as “unknown pentose”, with the knowledge that the structure is not fully identified.

3.1.2. Light method validation

The NMR signals in both the first and second calibration curves were linear (R^2>0.999) with the 95% confidence intervals for the slope based on DSS content being 0.96–1.02 and 0.96–1.03, respectively. Residual contents in the porcini extract (**Table S2**) were on average 6.2% and ranged from 5% (L-valine) to 9% (mannitol). However, several compounds were below the limit of quantification in the residual extract which limited the accuracy of these determinations. Recovery was on average 103.5% (Table S2) and ranged from 85% (L-alanine) to 190% (L-isoleucine). The largest source of variation in the recovery experiments were the small absolute reference compound additions. This made the additions more representative of the typical contents in the mushrooms, but resulted in large variations especially in the case of L-alanine, as only a handful of crystal particles comprised the whole standard addition. The free amino acid levels measured with NMR were generally 1–20% higher than the previous UHPLC measurements from samples in the same batch (Table S3).

3.1.3. Separation of species with the metabolomics approach

In the PCA model created with the metabolomics approach, the principal component 1 explained 50% of the variation and principal component 2 explained 32% of the variation (Supplementary material, **Figure S1**). Each sample species separated clearly into their own group in the PCA model (no overlapping 95% confidence ellipses). The loadings plot (Figure S2) indicates that the main regions driving the separation are the sugar regions at 5.2 ppm and 3.6–3.9 ppm, as well as the saturated alkane region at 0.9–1.3 ppm.

3.1.4. Content of sugars and organic acids in mushrooms

The measured sugars, sugar alcohols and organic acids are presented in **Table 1**, with the main compounds in bold. For cooked button mushroom, we found a lower amount of sugars and no fructose compared to that previously reported (Li et al., 2011). The mannitol content was well in agreement with that of fresh button mushrooms (Ajlouni et al., 1995; Tseng & Mau, 1999). On the other hand,

the trehalose content was slightly higher than that reported by Reis et al. (2012) in fresh mushrooms but lower than that reported by Ajlouni et al. (1995). In porcinis, we found smaller amounts of glucose and mannitol, and no mannose, but higher amounts of trehalose compared to the literature (Beluhan & Ranogajec, 2011; Heleno et al., 2011; Tsai et al., 2008). We measured higher concentrations of fumaric and malic acid than that measured from the fresh porcini samples in the literature (Ribeiro et al., 2006; Valentão, Lopes, et al., 2005) but did not, in contrast to the literature, detect citric acid (Ribeiro et al., 2006; Valentão, Lopes, et al., 2005). In chanterelles, we found less glucose and mannitol but more trehalose than reported (Beluhan & Ranogajec, 2011). Concentrations of measured organic acids were higher than reported in the literature for dried chanterelles (Valentão, Andrade, et al., 2005). While NMR as a measuring technique is less sensitive than liquid or gas chromatography for the analysis of sugars, organic acids and amino acids, it yielded a broad overview of the sample across several compound groups. Also in contrast to chromatographic methods, no derivatization steps were needed.

Table 1. Measured contents (with standard deviations, n=4) of sugars and organic acids in the studied mushrooms species expressed in mg g^{-1} fresh mushroom. Significant differences between species are based on one-way ANOVA and Tukey's or the Tamhane T2 post hoc test and are marked with different letters A–E. Major compounds are in bold.

Compound	Trumpet chanterelle			Button mushroom			Curry milk cap			Chanterelle			Porcini		
Glucose	traces [a]		C	traces		C	0.206	(0.048)	B	0.122	(0.022)	B	0.881	(0.070)	A
Mannitol	**18.13**	**(0.17)**	**A**	**19.22**	**(0.23)**	**A**	**19.99**	**(1.43)**	**A**	**4.29**	**(0.18)**	**B**	**0.44**	**(0.17)**	**C**
Trehalose	**0.202**	**(0.008)**	**E**	**0.399**	**(0.010)**	**D**	**2.27**	**(0.032)**	**C**	**14.03**	**(0.181)**	**B**	**33.56**	**(0.455)**	**A**
Citric acid	nd [b]		C	nd		C	0.75	(0.01)	A	0.609	(0.025)	B	nd		C
Fumaric acid	0.284	(0.006)	B	0.25	(0.012)	C	0.563	(0.006)	A	0.294	(0.004)	B	0.108	(0.005)	D
Malic acid	**4.05**	**(0.07)**	**C**	**1.53**	**(0.03)**	**D**	**5.54**	**(0.06)**	**A**	**5.03**	**(0.06)**	**B**	**1.13**	**(0.03)**	**E**
Succinic acid	0.03	(0.001)	C	0.057	(0.002)	B	0.102	(0.002)	A	0.019	(0.000)	E	0.025	(0.001)	D
Unknown pentose	nd		B	nd		B	**5.48**	**(0.06)**	**A**	nd		B	nd		B
Total sugars	18.3	(0.2)	C	19.6	(0.2)	B	22.5	(1.4)	BC	18.5	(0.2)	C	34.9	(0.5)	A
Total acids	4.36	(0.08)	C	1.83	(0.02)	D	6.95	(0.06)	A	5.96	(0.07)	B	1.26	(0.03)	E
Sugar-acid ratio	4.21	(0.04)	C	10.6	(0.1)	B	3.23	(0.21)	D	3.10	(0.02)	D	27.61	(0.95)	A

[a] detected, but under the limit of quantification

[b] not detected

3.2. Hedonic liking of mushrooms

3.2.1. Consumer clusters based on mushroom liking scores

The individual liking scores for each liking modality and mushroom species were used to segment the consumers into clusters. This was done to see whether there are groups of consumers with similar liking profiles. The hierarchical cluster analysis indicated that among the participants of this study, there are three different consumer clusters. On average, all mushrooms, except button mushroom, were at least slightly liked in all liking modalities. However, different consumer clusters had differing liking profiles and the cluster effect size was 2–30 times larger than that of the mushroom species (**Table 2**).

The overview of mushroom preferences and drivers of liking related to each cluster can be seen in the PLS model in **Figure 1**. The largest and (in terms of Squared Euclidean distance) most different was cluster 5, which had high liking scores for all mushrooms and liking modalities, with all liking averages >7 except for the appearance of button mushroom. Therefore all liking modalities are in the center part of the PLS model. Cluster 2 was quite similar to cluster 5 as it did not have statistically different scores (albeit with a trend to lower scores) for any mushrooms in odor liking and for half of the mushrooms in other liking modalities. However, cluster 2 had lower average values of up to 3 liking units than cluster 5 for porcini and also significantly lower values for chanterelle as regards taste, texture and overall liking; this is well demonstrated in the PLS model. The sensory attributes associated with cluster 2 are total odor intensity, lack of mushroom-type smell, higher toughness and lower crumbliness.

Cluster 4 had a comparable liking to cluster 2 in most liking modalities. However, cluster 4 was characterized with higher (not different from cluster 5) liking scores for porcini except for odor liking,

and generally lower liking for odor. The liking for chanterelle except for overall liking was statistically indistinguishable from cluster 5. Clusters 1 and 3 had similarly low likings for porcinis and button mushrooms. These clusters had significantly lower average of scores for most of the modalities for porcinis than clusters 4 and 5 whereas the likings were indistinguishable from cluster 2. Furthermore, for champignons, these clusters had significantly lower likings than clusters 5 and 2 in most modalities but most of the modalities were indistinguishable from cluster 4.

353 **Table 2. Liking score two-way ANOVA p values and effect sizes expressed as η^2 by factors, and liking scores (average and standard deviations) for each cluster.**
354 **Significant differences between clusters are based on Bonferroni-corrected simple contrasts in the two-way ANOVA ($p<0.05$) and are marked with letters A-D.**

Attributes and samples	ANOVA p values (η^2)			Averages (standard deviations)									
Odor	Species	Consumer cluster	Interaction	Overall		1			2			3		
Button mushroom	0.001	0.001	0.132	6.1	(1.6)	4.7	(1.3)	b	6.2	(1.5)	a	6.9	(1.3)	a
Chanterelle	(0.049)	(0.177)	(0.023)	7.0	(1.5)	6.5	(1.2)	b	6.6	(1.7)	b	7.7	(1.1)	a
Trumpet chanterelle				6.8	(1.7)	5.7	(1.9)	b	6.5	(1.4)	b	7.7	(1.4)	a
Porcini				6.5	(1.6)	6.1	(1.5)	b	6.0	(1.7)	b	7.5	(1.0)	a
Appearance														
Button mushroom				5.5	(1.7)	3.9	(1.3)	b	5.9	(1.5)	a	6.1	(1.5)	a
Chanterelle	0.001	0.001	0.011	6.5	(1.6)	5.7	(1.6)	b	6.5	(1.5)	ab	7.1	(1.5)	a
Trumpet chanterelle	(0.065)	(0.208)	(0.037)	6.1	(1.5)	5.3	(1.4)	b	5.7	(1.4)	b	7.2	(1.1)	a
Porcini				5.6	(1.8)	4.4	(1.3)	b	5.2	(1.7)	b	6.9	(1.3)	a
Taste														
Button mushroom				6.2	(1.9)	3.9	(1.6)	b	6.7	(1.1)	a	7.3	(1.3)	a
Chanterelle	0.39	0.001	0.001	6.3	(1.8)	4.9	(1.6)	c	6.1	(1.4)	b	7.5	(1.4)	a
Trumpet chanterelle	(0.006)	(0.288)	(0.062)	6.2	(1.7)	5.7	(2.0)	b	5.5	(1.4)	b	7.2	(1.3)	a
Porcini				6.4	(1.6)	5.8	(1.5)	b	5.9	(1.5)	b	7.5	(1.3)	a
Texture														
Button mushroom				5.8	(1.9)	3.6	(0.9)	c	6.0	(1.5)	b	7.2	(1.5)	a
Chanterelle	0.17	0.001	0.001	6.2	(1.7)	4.6	(1.4)	c	6.2	(1.4)	b	7.3	(1.2)	a
Trumpet chanterelle	(0.010)	(0.345)	(0.062)	6.1	(1.6)	5.6	(1.7)	b	5.6	(1.5)	b	7.1	(1.2)	a
Porcini				6.1	(1.8)	5.4	(1.3)	b	5.1	(1.7)	b	7.5	(1.3)	a
Overall liking														
Button mushroom				6.2	(1.8)	3.8	(1.1)	b	6.7	(1.0)	a	7.3	(1.2)	a
Chanterelle	0.13	0.001	0.001	6.5	(1.4)	5.2	(1.4)	c	6.4	(0.9)	b	7.5	(0.9)	a
Trumpet chanterelle	(0.010)	(0.340)	(0.093)	6.3	(1.5)	5.9	(1.5)	b	5.6	(1.4)	b	7.3	(1.1)	a
Porcini				6.3	(1.6)	5.7	(1.4)	b	5.6	(1.4)	b	7.6	(1.1)	a

355

Cluster 3 gave significantly lower liking scores than other clusters for taste and overall liking of chanterelles. Furthermore, cluster 3 gave significantly lower scores to chanterelles for appearance and structure than clusters 2, 4 and 5 in all modalities except in liking for odor. For trumpet chanterelles, the average scores of cluster 3 were lower than scores given by other clusters but there was no significant difference in likings on odor, appearance and structure with cluster 4 and in liking for structure with cluster 1. For chanterelles and trumpet chanterelle cluster 1 had liking scores indistinguishable from clusters 5, 4 and 2 (albeit for the structure of chanterelles). In the PLS model, cluster 1 is negatively correlated with the button mushroom sample. Cluster 1 is also negatively correlated with mushroom odor, sweet and umami attributes and squeakiness and biting resistance, but positively correlated with earthy, cardboard and forest odors. On the other hand, the lesser dislike of porcinis in cluster 3 can also be seen well in the PLS model. The consumer clustering was successful as different liking profiles for the mushroom species could be determined. These profiles indicated that, for example, the largest cluster 5 consisted of consumers with a general liking for all sample species.

3.2.2. Background variables for consumers

The volunteer consumers were predominantly female, represented multiple age groups, were predominantly highly trained and followed an omnivore diet. Fourteen males (17% of total participants) and 70 females (83%) completed the evaluation. There were 26 (31%) 20–35 year olds, 29 (35%) 36–50 year olds and 29 (35%) 50 year old or older participants. Thirteen (15%) had a high school diploma or equivalent, 18 (21%) had an undergraduate degree and 53 (63%) had a higher university degree. Sixty consumers (71%) followed an omnivore diet, 18 (21%) a plant-based omnivore diet and 6 (7%) were vegetarians or vegans.

Of all the background variables, four FCQ categories (health, sensory appeal, natural content, ethical concern) and the number of known mushrooms species were statistically significantly different

($p<0.05$, Table 3). Their η^2 was 2–7%, signifying that only a small part of the variation was explained by cluster membership. There were no statistically significant differences between clusters in other Food Choice Questionnaire categories (mood, convenience, price, weight control, familiarity), Food Disgust Scale, age, sample familiarity, or mushroom usage frequency. The consumers mostly (48% of consumers) used mushrooms a two or three times a month. Thirty-seven percent of participants reported mostly using cultivated mushrooms while 54% mostly used self-picked wild mushrooms. The most common types of mushroom use were as a slightly seasoned ingredient in cooked dishes (selected by 80% of consumers in the top three usage scenarios) and cooked with onion and cream (85% of consumers).

Table 3. The statistically significantly different background variables (averages and standard deviations) related to consumer clusters. Significant differences between clusters are based on Tukey's HSD post hoc test in the one-way ANOVA ($p<0.05$) and are marked with letters a-b. FCQ: Food Choice Questionnaire.

Cluster	1 (n=20)			2 (n=38)			3 (n=26)		
FCQ health	30.8	(4.6)	ab	27.9	(6.0)	b	31.5	(5.7)	a
FCQ sensory appeal	22.5	(3.6)	ab	20.6	(5.2)	b	23.2	(3.0)	a
FCQ natural content	14.0	(4.0)	ab	12.7	(4.0)	b	15.5	(2.9)	a
FCQ ethical concern	14.3	(4.9)	ab	12.9	(4.2)	b	15.8	(3.4)	a
Known mushroom species	6.3	(3.6)	ab	5.8	(3.7)	b	8.7	(4.0)	a

3.3. The correlations between chemical data and the results from sensory analysis

In the PLS model (**Figure 2**) 79% of the variation in the measured odor-contributing volatile compounds and non-volatile compounds explained 95% of the variation in the sensory profile. The curry milk cap has a major negative loading on factor 1, while porcini has a major positive loading on factor 1. Chanterelle has a negative loading on factor 2 and curry milk cap and porcini positive loadings on factor 2. Trumpet chanterelle has a small positive loading on factor 1 and small negative loading on factor 2. Similar configurations were found in the separate PLS models for odor-

400 contributing volatile compounds and non-volatile compounds (Supplementary materials, **Figures S3–**
401 **S4**).

402 **3.3.1. Odor-contributing volatile compounds explaining the odor attributes**

403 Potato mash and mushroom attributes have very positive loadings on factor 1, while total odor
404 intensity, earthy, cardboard and roasted attributes have highly negative loadings. Forest and cooked
405 carrot attributes have mildly positive loadings on factor 1 and highly negative loadings on factor 2,
406 while potato mash has a positive loading on factor 2. The odor activities of 1-octen-3-one and
407 1-octen-3-ol on the HP-Innowax column correlate well with the mushroom attribute. However, the
408 coeluting peak of these two compounds inversely correlates with the same attribute. This is explained
409 by our previous publications: curry milk cap has high SNIF values (surface of nasal impact frequency,
410 the area of the GC-olfactometry signal) for these compounds (Aisala et al., 2019), but low perceived
411 mushroom-like odor in the descriptive analysis (Aisala et al., 2018). Total odor intensity correlates
412 strongly with the sum of all SNIF values. Methional, which had high SNIF values in both curry milk
413 cap and porcini, correlates with the potato mash attribute but also somewhat with the roasted attribute.
414 Regarding the roasted attribute that is mostly present in curry milk cap, 2-acetyl-1-pyrroline likely
415 contributes to this perception due to its popcorn-like and roasted odor quality (Grosshauser &
416 Schieberle, 2013) even though the correlation is more via factor 2. Interestingly, while different
417 pyrazines with earthy and roasted odor descriptions have been found from mushrooms (Grosshauser
418 & Schieberle, 2013; Zhang et al., 2018), no compounds with matching retention indices or odor
419 descriptions were found in curry milk cap samples. Compounds explaining cooked carrot attribute
420 include several fatty acid degradation products such as (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal,
421 heptanal and (*E*)-2-nonenal. While the forest attribute is also closely correlated with the
422 abovementioned compounds, their contribution to forest-like perception is less likely. Instead,
423 compounds such as hexanal and several unidentified compounds might cause the odor perception as
424 hypothesized earlier (Aisala et al., 2019).

3.3.2. Non-volatile compounds explaining the taste and chemosensory attributes

The three taste modalities as well as the three chemosensory attributes are mainly explained with factor 1. Umami and sweet had with highly positive loadings while bitter, pungent, astringent and metallic had negative loadings. Curry milk cap correlates with bitterness, astringency, pungency and metallic attributes. Porcini mostly correlates with sweetness and umami. Chanterelle had a secondary link to the metallic attribute, while trumpet chanterelle was not clearly linked to any taste or chemosensory attribute. The results of the PLS-analysis are in line with the PCA presented earlier (Aisala et al., 2018). All the studied organic acids, including their total concentration, several of the bitter tasting amino acids (histidine, leucine, phenylalanine, methionine), four out of the five studied 5'-nucleosides (uridine, cytidine, adenosine, and guanosine), cysteine, and the major unknown compound in curry milk cap had negative loadings on factor 1. Thus, they correlated strongly with pungency, bitterness, astringency and metallicity.

Umami 5'-nucleotides (GMP, AMP, and IMP) had high positive loadings with factor 1, while surprisingly, glutamic and aspartic acid have negative loadings on factor 1. EUC, on the other hand, had positive loadings on both on factor 1 and factor 2. This result conforms well to the established theory of EUC values as a predictor of an umami taste, taking into account both levels of umami amino acids and nucleotides. As reported previously, curry milk cap had the highest concentrations of Glu and Asp, but the lowest concentrations of GMP, AMP, and IMP (Manninen et al., 2018). On the other hand, porcini had moderate levels of Asp and Glu, but relatively high levels of GMP, AMP, and IMP among the four species studied (Manninen et al., 2018). The two chanterelles had intermediate levels of these compounds. Thus in our model, EUC was a stronger predictor of umami intensity than Asp and Glu levels alone. It is therefore similar in effectiveness to GMP, IMP and AMP levels, which is dissimilar to that reported by Phat et al. (2016). It likely that the other sensory attributes in curry milk cap can mask or suppress the umami intensity.

Mannitol and total sugars have limited predictor values on the model. However, the trehalose content, low levels of individual and total acids and especially the sugar-acid ratios have good correlations with sweetness. Moreover, the sweet amino acids Ala and Gly correlate with sweetness, but it is not predicted by Thr and Ser. On the other hand, the unknown pentose-type compound in curry milk cap correlates mostly with bitterness rather than sweetness.

4. Conclusions

Edible mushroom species can be separated based on both metabolomic approaches as well as targeted methods. They are different as regards both their non-volatile and odor contributing volatile profiles, as well as sensory properties. When taking the sensory properties, the non-volatile, and the volatile chemical compounds into account at the same time it is clear that trumpet chanterelle is more similar to chanterelle than to porcini and curry milk cap.

Surprisingly, it was the consumer cluster instead of the mushroom species which was the main source of variation as regards the liking of mushrooms for consumers. The consumer clusters were significantly different in their liking profiles for the selected mushroom species. The individuals in these clusters were heterogeneous in their background: neither age, mushroom usage frequency, nor differences in their food choice motives provided a good explanation for the liking profiles. This research brings new information as to why mushrooms are not pleasing to everyone. This information is also useful for product development and the individual marketing of mushroom products.

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