DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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ABSTRACT

Mushrooms have been part of the human diet for many millennia. They represent an evolutionarily old and large group of edible organisms. Finnish people have a long history of picking and using wild mushrooms for food especially in the eastern regions of the country, but mushrooms are still an underutilized natural resource as only a fraction of the annual natural production is harvested. The sensory properties of mushrooms have been examined globally in several sensory studies, but so far not in Finland. Additionally, there is still a need for further comparative research between species both as regards sensory profiling and hedonic liking assessed by larger consumer panels. The non-volatile and volatile compounds of mushrooms have been studied with instrumental methods without consideration as to human perceptions. The effects of the compounds on sensory properties of mushrooms has so far received limited attention, especially using appropriate statistical models.

The aim of the current work was to determine the sensory properties of wild edible mushroom species and to examine which volatile and non-volatile compounds would explain these properties. Finnish *Agaricus bisporus* (button mushroom), *Cantharellus cibarius* (chanterelle), *Craterellus tubaeformis* (trumpet chanterelle), *Boletus edulis* (porcini) and *Lactarius camphoratus* (curry milk cap) were the main species of interest. The sensory properties were examined with a trained panel using generic descriptive analysis and with consumers using projective mapping. The hedonic liking of mushrooms was studied with consumers. The volatile compounds were studied with an optimized headspace-solid phase microextraction gas chromatography method and their contribution to odor with gas chromatography-olfactometry. The non-volatile compounds were measured with quantitative nuclear magnetic resonance spectroscopy. Univariate statistical methods such as analysis of variance and multivariate analysis methods such as principal component analysis and partial least squares regression were applied in each sub-study. These were used to determine the key compounds and attributes and to link the chemical and sensory datasets together.

Both the consumers and the trained panel separated the mushroom species based on their sensory properties. Specific descriptive sensory attributes were found for each studied species. Among the consumers, clusters with different hedonic likings of each mushroom species were found. The cluster rather than mushroom species was the main source of variation in the hedonic scores. Likewise, the mushroom samples were well differentiated based on volatile compounds, odor-contributing volatile compounds, and water-soluble non-

volatile compounds. This result was seen both with a metabolomics approach and with the quantified contents of identified compounds. Different aliphatic aldehydes, ketones, and alcohols were the most common odor-contributing volatile compounds. Mannitol and trehalose were the main sugars and sugar alcohols, while malic acid was the main organic acid.

The sensory properties of mushrooms could be linked to several chemical compounds. 1-octen-3-one and 1-octen-3-ol were linked to mushroom odor while 3-(methylthio)propanal was linked to the potato mash odor typical in *Boletus edulis*. High equivalent umami concentration (EUC) values and 5'-nucleotides were predictors of umami, while a high sugar-acid ratio predicted sweetness.

The tools and results of this thesis can be further used to develop mushroom products and as a starting point for a wider screening of the sensory properties and underlying chemistry in Finnish edible wild mushroom species.

SUOMENKIELINEN TIIVISTELMÄ

Ruokasienet ovat olleet osa ihmisten ruokavaliota vuosituhansien ajan. Ne ovat evolutiivisesti vanha ja laaja syötävien organismien joukko. Suomalaiset ovat keränneet, käyttäneet ja arvostaneet metsäsieniä kauan etenkin maan itäosissa. Metsäsienet ovat kuitenkin edelleen alikäytetty luonnonvara, sillä vain pieni osa vuosittaisesta sadosta kerätään. Sienten aistinvaraisia ominaisuuksia on tutkittu useissa ulkomaisissa tutkimuksissa, mutta tutkimuksia ei vielä ole tehty Suomessa. Erityisesti lajien välisiä eroja kartoittavia lisätutkimuksia sekä laajemmilla kuluttajaotoksilla tehtyjä sienten miellyttävyystutkimuksia tarvitaan edelleen. Sienten haihtuvia ja ei-haihtuvia yhdisteitä on mitattu instrumentaalisin menetelmin pääosin huomioimatta ihmisen kokemuksen merkitystä. Yhdisteiden osuutta sienten aistinvaraisiin ominaisuuksiin on tarkasteltu rajallisesti ilman soveltuvia tilastollisia malleja.

Tämän työn tavoitteena oli määrittää metsäsienten aistinvaraiset ominaisuudet sekä tarkastella, mitkä haihtuvat ja ei-haihtuvat yhdisteet selittäisivät näitä ominaisuuksia. Suomalaisten *Agaricus bisporus* (herkkusieni), *Cantharellus cibarius* (keltavahvero, kantarelli), *Craterellus tubaeformis* (suppilovahvero), *Boletus edulis* (herkkutatti) ja *Lactarius camphoratus* (sikurirousku) -lajien näytteet olivat päämielenkiinnon kohteena. Aistinvaraisia ominaisuuksia tarkastelivat sekä koulutettu raati käyttäen kuvailevaa menetelmää että kuluttajat käyttäen projective mapping -menetelmää. Sienten miellyttävyyttä tutkittiin samoin kuluttajilla. Sienten haihtuvia yhdisteitä tutkittiin tätä varten optimoidulla ilmatila-kiinteäfaasiuutto-kaasukromatografiamenetelmällä. Hajuun vaikuttavia haihtuvia yhdisteitä selvitettiin kaasukromatografia-olfaktometrialla. Ei-haihtuvat yhdisteet mitattiin kvantitatiivisella ydinmagneettisella resonanssispektroskopialla. Kunkin osatyön tuloksia tarkasteltiin käyttäen sekä yhden muuttujan tilastomenetelmiä kuten varianssianalyysia että monimuuttujamenetelmiä kuten pääkomponenttianalyysiä sekä osittaisten neliösummien regressiota. Näitä malleja käytettiin määrittämään, mitkä olivat näytteiden pääominaisuudet ja yhdisteet ja selvittämään, miten kemialliset ja aistinvaraiset tulokset liittyivät toisiinsa.

Sekä kuluttajat että koulutettu raati erottivat sienilajit toisistaan aistinvaraisten ominaisuuksien perusteella. Kullekin lajille havaittiin niitä kuvaavia haju-, maku-, kemotunto- ja rakenneominaisuuksia. Kuluttajat voitiin jakaa erilaisiin ryhmiin heidän miellyttävyysarvioidensa perusteella. Tämä ryhmä oli määrittävämpi tekijä miellyttävyysarvioissa kuin sienilaji. Sienilajit erosivat toisistaan myös haihtuvien, sienen hajuun vaikuttavien haihtuvien sekä ei-haihtuvien yhdisteiden suhteen. Tämä tulos saatiin sekä metabolomiikkalähestymistavalla että tunnistettujen yhdisteiden pitoisuuksia mitttaamalla.

Erilaiset alifaattiset aldehydit, ketonit ja alkoholit olivat tyypillisimpiä hajuun vaikuttavia haihtuvia yhdisteitä. Mannitoli ja trehaloosi olivat yleisimmät sokerit ja sokerialkoholit, kun taas omenahappo oli tyypillisin orgaaninen happo.

Sienien aistinvaraiset ominaisuudet voitiin liittää useisiin kemiallisiin yhdisteisiin. 1-okten-3-oni ja 1-okten-3-oli liittyivät sienimäiseen hajuun, kun taas 3-metyylitiopropanaali liittyi *Boletus edulis* -lajille tyypilliseen perunamuusin hajuun. Korkeat umamiekvivalenttikonsentraation (EUC) arvot ja 5'-nukleotidien pitoisuudet selittivät umamia, kun taas korkea sokeri-happosuhde selitti makeutta.

Tämän väitöskirjan työkaluja ja tuloksia voidaan käyttää sienituotteiden kehityksessä. Lisäksi väitöskirjaa voi käyttää lähtökohtana laajemmalle suomalaisten metsäsienilajien aistinvaraisia ominaisuuksia sekä taustalla olevaa kemiaa määrittävälle tutkimukselle.

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
AEDA	aroma extract dilution analysis
CATA	check-all-that-apply
DF	detection frequency
DM	dry matter
DNA	deoxyribonucleic acid
EUC	equivalent umami concentration
FD	flavor dilution
FID	flame ionization detector
GC	gas chromatography
GC-MS	gas chromatography–mass spectrometry
GC-O	gas chromatography–olfactometry
GDA	generic descriptive analysis
^{1}H	proton
HS	headspace
HS-SPME	headspace solid phase microextraction
ID	internal diameter
ITS	internal transcribed spacer
LC	liquid chromatography
N/A	not available
nd	not detected
NIF	nasal impact frequency
MSG	monosodium glutamate
OAV	odorant activity value
ODP	olfactory detector port
PC	principal component
PCA	principal component analysis
PCR	principal component regression
PI	posterior intensity
PLS	partial least-squares regression
QDA	quantitative descriptive analysis
qNMR	quantitative nuclear magnetic resonance spectroscopy
Ref	reference
RT-qPCR	real-time quantitive polymerase chain reaction
SAFE	solvent-assisted flavor evaporation
SDE	simultaneous distillation–extraction
SNIF	surface of nasal impact frequency
SPME	solid phase microextraction
UHPLC	ultra high performance liquid chromatography

LIST OF ORIGINAL PUBLICATIONS

I. Aisala, H.; Laaksonen, O.; Manninen, H.; Raittola, A.; Hopia, A.; Sandell, M. Sensory properties of Nordic edible mushrooms. *Food Research International*, **2018**, *109*, 526–536.

II. Aisala, H.; Linderborg, K. M.; Sandell, M. Fiber depth, column coating and extraction time are major contributors in the headspace solid-phase microextraction–gas chromatography analysis of Nordic wild mushrooms. *European Food Research and Technology*, **2018**, *244* (5), 841–850.

III. Aisala, H.; Sola, J.; Hopia, A.; Linderborg, K. M.; Sandell, M. Odor-contributing volatile compounds of wild edible Nordic mushrooms analyzed with HS–SPME–GC–MS and HS–SPME–GC–O/FID. *Food Chemistry*, **2019**, *283*, 566–578.

IV. Aisala, H.; Manninen, H.; Laaksonen, T.; Myoda, T.; Linderborg, K. M.; Hopia, A.; Sandell, M. Linking volatile and non-volatile compounds to sensory profiles and consumer liking of wild edible Nordic mushrooms. *Submitted.*

1 INTRODUCTION

Edible mushrooms have a global history of use spanning several millennia. There are written indications for consumption in China from the Zhou dynasty over 2500 years ago [1]. Mushrooms were a widely appreciated ingredient in the Roman Empire. In the first century A.D, poet Marcus Valerius Martialis said wittingly,

"Argentum atque aurum facile est laenamque togamque mittere, boletos mittere difficile est." [2]

This translates as "It is easy to send silver and gold and a cloak and a gown, but sending mushrooms is difficult." Buller [3] and later Rolfe and Rolfe [4] interpreted the passage as a reference to the deliciousness of *Amanita Caesarea*; other commodities such as gold and clothes will make their way to a friend when brought by a slave, but these mushrooms will definitely be devoured before the destination. The oldest identified illustration of fungi also comes from the Romans; the frescoes of the Pompeii ruins contain a *Lactarius deliciosus* [3].

Likewise, Finland has a long and varied history of enjoying mushrooms coming from two distinct cultures–Russian/Slavic and Swedish–of mushroom usage that meet inside our borders. The Russian/Slavic tradition focuses especially on the usage of the *Lactarius* and *Russula* species [5]. In his ode to mushroom picking (called "The Third Hunt", in Russian "Треть'я охота"), Russian poet Vladimir Soloukhin praises mushroom species such as *Lactarius deliciosus and torminosus, Suillus luteus, Leccinum scabrum, L. versipelle, L. aurantiacum and L. vulpinum* [6].

On the western side of Finland, a more wary attitude to mushrooms was acquired from the Swedes [7] who in turn inherited their viewpoint from the Germans and from the church. German tribes in the early Middle Ages had no interest in edible mushrooms in contrast to the more southern Roman tradition [8,9]. Meanwhile in Europe, the Catholic and the Orthodox Church contributed to the re-emergence of mushroom usage as eating meat was prohibited during fasting times [10] and mushrooms were an acceptable substitute [7]. The Protestants, on the other hand, did not have strict fasting rules and as such had low incentives for mushroom use [8]. Even Carl von Linné–the great Swedish contributor to taxonomy–still had a hostile attitude towards mushrooms in the 18th century [5,11]. This had long-lasting negative implications to the field of mycology in the form of misleading taxonomical classifications [3]. However, the French-born king of Sweden Karl Johan XIV appreciated *Boletus edulis* and thus the interest in *B. edulis* and *Cantharellus cibarius* species made their way to southwestern Finland via the Swedish-speaking aristocracy [5,8].

The oldest Finnish mushroom book is from 1863 [12]. This half novel, half mushroom guide overviews recipes, identification tips and proper blanching

preparation steps for several mushrooms, including *Lactarius* and *Boletus* species, *Gyromitra esculenta, Cantharellus cibarius,* and *Macrolepiota procera.* The first doctoral dissertation on Finnish mushrooms was published in 1947 by Toivo Rautavaara. In his thesis [8], Rautavaara made a thorough estimate of the annual availability of wild mushroom species in Finland, their economic value, as well as suggestions on their use. The classic saying "millions of Marks are rotting in the forest" was coined from the thesis [5] and resulted in extensive campaigns promoting mushroom picking, training of mushroom consultants [13], and more widespread use. The second and also the latest Finnish PhD thesis on the flavor of mushrooms was published by Heikki Pyysalo in 1976 [14]. This research focused on the volatile compounds present in different mushroom species growing in Finland with special reference to identifying the toxic volatile compounds in *Gyromitra esculenta.* The first article included in the thesis [15] is still routinely cited in the latest international publications concerning the flavor of mushrooms.

The more recent Finnish mushroom research has shifted its focus away from flavor research. Some published works include an estimation of the edible wild mushroom harvest [16–18], usage in households [19], and mushroom nutrient composition analyses [20–23]. Aside from a 10-year old Master's thesis work [24], there are no recent Finnish publications focusing on the flavor of domestic wild edible mushroom species. Furthermore, research on the sensory properties of these mushrooms has not been published using modern sensory methods. This is surprising as meanwhile, Finnish berries have received considerable research interest on the same topic [25–32]. At the same time, global research activity on the sensory properties and flavor chemistry of mushrooms has increased [33–42] with for example a complete PhD thesis on the sensory properties and flavor chemistry of matsutake mushrooms (*Tricholoma matsutake* Sing.) was conducted in South Korea 12 years ago [43].

This thesis examines the sensory properties as well as the underlying chemistry in popular Finnish wild edible mushrooms. In the literature review, the wild and cultivated edible mushroom harvest, sensory properties, non-volatile compounds, and aroma compounds are overviewed. Likewise, the typical analysis parameters, challenges, and limitations in sensory science and flavor research of mushrooms are examined. In the experimental part of the research, mushroom samples were studied using both modern sensory science and instrumental methods. A sensory profile of five mushroom species was generated. Holistic sensory evaluation of mushrooms with untrained consumers was studied. The volatile compounds and their contribution to the odor of mushrooms were investigated. The non-volatile water-soluble compounds, mainly sugars, polyols, organic acids, and free amino acids were measured. The consumer acceptability of mushrooms was studied.

2 REVIEW OF THE LITERATURE

2.1 Taxonomy of edible mushrooms

Mushroom is the general term for the very small fraction of species forming macroscopic fruiting bodies in the kingdom of Fungi, a type of eukaryote organism lacking chlorophyll that originated about 710–1060 million years ago [44]. When the word 'mushroom' is used in this dissertation, it refers specifically to the fruiting bodies of these organisms. There are about 820 species of wild edible mushroom species that are used as food globally [45]. In terms of taxonomy, all species studied in this PhD research belong to phylum Basidiomycota, one of the six main phyla which contains about 30 thousand species [44]. All mushroom species that are examined in this PhD are displayed in **Table 1**.

One of the main defining features of Basidiomycota is that they produce their sexual spores (basidiospores) on a specific type of structures called basidia [46]. Furthermore, the sample species in this PhD–like most edible species–are all members of the class Agaricomycetes, which in turn consists of 16 thousand species [44,47]. The ecological diversity within this class is still enormous. It ranges from cultivated saprotrophic (decayed organic matter digesting) *Agaricus bisporus* and *Lentinula edodes* in the order Agaricales to ectomycorrhizal (symbiotic) *Boletus edulis* and *Suillus variegatus* in the order Boletales. Likewise, ectomycorrhizal *Cantharellus cibarius* and *Craterellus tubaeformis* in the order Cantharellales [44,47] and even the wood-decaying polypore *Fomitopsis betulina* that was found on Ötzi, the Ice Man who lived 5300 years ago [48], belong to this class. The morphological range of the fruiting bodies is also remarkable: even within the order Russulales, the appearances of for example *Lactarius rufus* and *Albatrellus ovinus* are very different.

Morphological, biochemical and ecological cues have been used in the taxonomical placement of mushrooms [47]. On the other hand, historical reasons such as the abovementioned illogical classifications by Linné have made this challenging and many family-level classifications were later realized to be incorrect [3]. Another Swedish taxonomist, Elias Magnus Fries and his successors had a major influence on current mycological taxonomy practices [49]. More recently, as DNA sequencing-based methods have become more common, the taxonomic tree of fungi has been reorganized multiple times even at the class level. Roughly the species in the current Agaricomycetes class were included under the obsolete Homobasidiomycetes still two decades ago [50], which in turn was approximately preceded by the Hymenomycetes taxonomic group until only some 25 years ago [46].

Table 1. Edible mushroom species mentioned in this PhD dissertation. All species aside from *Gyromitra esculenta* and *Morchella deliciosa* belong to class Agaricomycetes as these two species are part of phylum Ascomycota.

Binomial name	English name	Finnish name
Cultivated species		
Agaricus bisporus (J.E.Lange) Imbach	button mushroom	herkkusieni
Agaricus bitorquis (Quél.) Sacc.	pavement mushroom	puistoherkkusieni
Flammulina velutipes (Curtis) Singer	enokitake	talvijuurekas
Lentinula edodes (Berk.) Pegler	shiitake	siitake
Lentinus sajor-caju (Fr.) Fr.	No English name	No Finnish name
Pleurotus ostreatus (Jacq.) P. Kumm.	oyster mushroom	osterivinokas
Tricholoma matsutake (S. Ito & S. Imai) Singer	matsutake, pine mushroom	männyntuoksu-valmuska
Volvariella volvacea (Bull.) Singer	straw mushroom	viljelytuppisieni
Wild species		
Albatrellus ovinus (Schaeff.) Kotl. & Pouzar	No English name	lampaankääpä
Amanita caesarea (Scop.) Pers	Caesar's mushroom	keisarikärpässieni
Boletus edulis Bull.	porcini	herkkutatti
Cantharellus cibarius Fr.	chanterelle	keltavahvero, kantarelli
Craterellus cornucopioides (L.) Pers.	horn of plenty, black trumpet	mustatorvisieni
Craterellus tubaeformis (Fr.) Quél.	trumpet chanterelle, funnel chanterelle	suppilovahvero
Gyromitra esculenta (Pers.) Fr.	No English name	korvasieni
Hydnum repandum L.	wood hedgehog	vaaleaorakas
Hydnum rufescens Pers.	terracotta hedgehog	rusko-orakas
Lactarius camphoratus (Bull.) Fr.	curry milk cap	sikurirousku
Lactarius deliciosus (L. ex Fr.) S.F.Gray	red pine mushroom	männynleppärousku
Lactarius rufus (Scop.) Fr.	rufous milk cap	kangasrousku
Lactarius torminosus (Schaeff.) Gray	woolly milkcap	karvarousku
Lactarius trivialis (Fr.) Fr.	No English name	haaparousku
Leccinum aurantiacum (Bull.) Gray	red-capped scaber stalk	haavanpunikkitatti
Leccinum scabrum (Bull.) Gray	scaber stalk	lehmäntatti
Leccinum versipelle (Fr. & Hök) Snell	orange birch bolete	koivunpunikkitatti
Leccinum vulpinum Watling	foxy bolete	männynpunikkitatti
Macroleptiota procera (Scop.) Singer	parasol mushroom	ukonsieni
Morchella deliciosa Fr.	No English name	no Finnish name
Suillus luteus (L.) Roussel	slippery jack	voitatti
Suillus variegatus (Sw.) Kuntze	velvet bolete	kangastatti

As a family-level example, *Craterellus tubaeformis* was moved from the previous *Cantharellus* genus as a result of DNA sequencing data about 20 years ago [51]. Raja et al. [52] recently demonstrated that incorrect identification is still common: wild mushrooms samples sold in supermarkets in the United States had inaccurate labelling at the species level. Raja *et al.* suggested using the internal transcribed spacer (ITS) regions of mushroom DNA to identify mushroom products.

In terms of phylogenetic relationships, the order Cantharellales is the most dissimilar, as it diverged over 100 million years earlier from the rest of the orders in Agaricomycetes according to a recent molecular dating analysis [53]. Compared to the animal kingdom, the Agaricomycetes are remarkably old. Cantharellales diverged about 130 million years earlier from other orders than the oldest known eutherian mammal fossil *Juramaia* [54] and even the most recent order Boletales still diverged about 60 million years earlier than primates from other mammals [53,55]. Likewise, comparing the Agaricomycetes divergence to current vegetable species places the age of the class in the proper context. The Agaricales-Boletales pair diverged from each other approximately the same time as monocots (such as onions, rice and wheat) diverged from eudicots (such as peas, potatoes and carrots): 140–150 million years ago [56,57]. The phylogenetic tree of the fungal orders of interest in this thesis is displayed in **Figure 1**. The genomes of 83 species in the Basidiomycota were recently sequenced [58] which should facilitate detailed comparisons of different edible mushrooms in the near future.

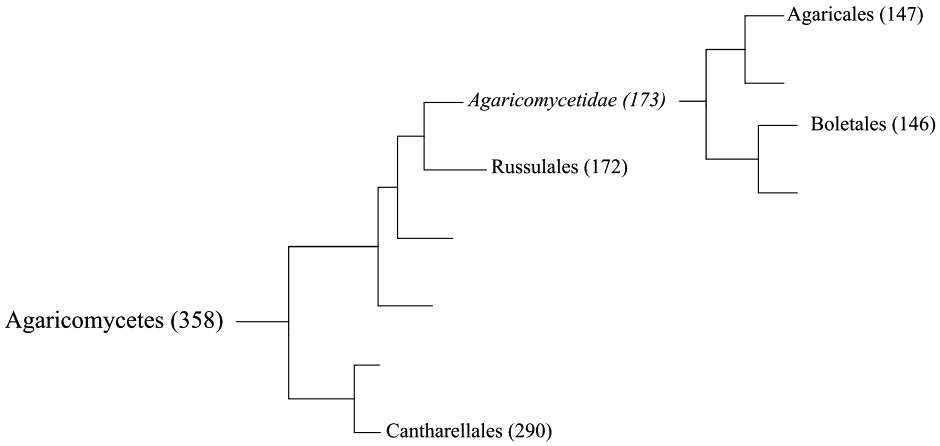

Figure 1. The phylogenetic relations between the mushroom subclasses (in italics) and orders of the class Agaricomycetes overviewed in this PhD. The numbers in brackets are the estimated stem ages in millions of years, signifying the divergence of each mushroom class and order of interest. Adapted from the maximum-clade-credibility tree of Zhao et al. [53].

2.2 Mushroom yield and utilization in Finland

While there are about 200 edible wild mushrooms species in Finland [59], the number of used species is much lower and the appreciation for each species differs between authors. Finnish mushroom guidebooks usually classify mushrooms with a three-star system in which one-star species are considered edible and three stars species delicious. Depending on the publication, three-star species range from 33–38, two-star species 25–37, and 2–3 star species after blanching or other heat treatment range from 8–10, comprising a total of 73–78 species [60–62]. From the early 1980s to 2012 there was national Finnish legislation on allowed marketable mushroom species [63]. These 20 species were selected as they are easily identifiable and recognizable from poisonous or non-edible species and have commercial relevance. After 2012, the Finnish Food Safety Authority has maintained a list of recommended marketed mushroom species which currently contains 31 species [64]. *Boletus edulis*, *Cantharellus cibarius,* and *Craterellus tubaeformis* have been the mainstay species on these lists as 3-star mushrooms.

The earliest estimates of the mushroom harvest in Finland were from Rautavaara in 1947 [8] who calculated that the total annual wild mushroom crop in a bad year would be 1500 million kg. Of this, the share of collectable, edible species in good condition would be 80 million kg. Ohenoja and Koistinen [65–67] surveyed the yields of larger fungi in northern Finland in the harvest years 1976–1978. In their sample, 68% of all fruiting bodies were edible mushroom species and 37% were in the 20-species marketed mushroom list. The yield of all marketed mushrooms ranged from 9–29 kg fresh mushroom/ha (average 17 kg/ha), depending on the year. The largest yields were for *Suillus 7variegatus, Lactarius rufus* and *Leccinum versipelle*. Ohenoja and Koistinen estimated that in the study period, less than 3% of the local harvest was utilized in northern Finland.

The annual yield for wild mushrooms has been reported to vary greatly (with over one order of magnitude) between harvest locations in recent studies. For example with *Boletus edulis*, the average yield was 5.4 kg/ha but ranged from 0 to 70.8 kg/ha [18]. The main factors influencing the mushroom harvest were the year, temperature, precipitation, and time since the last thinning of the mushroom forest [16,18]. In 1991, it was estimated that about 3% of the collectable edible mushroom crop of 170 million kg was utilized [59]. In the latest collection data from the years 1997–1999 and 2011, the amount of collected mushrooms in Finland ranged from 3–13 million kg per year, depending on the year [17]. Mushrooms can form a significant source of income for a forest owner: the annual harvest from a good mushroom site can yield 25% of the total annual worth of the forest [18].

Based on questionnaires conducted in the late 1970s [19] as well as the late 1990s and 2011 [17], about half of the wild mushrooms picked and used in Finnish households were milk caps (*Lactarius* spp.), with chanterelles (*Cantharellus spp., Craterellus spp.*) and different boletes (*Boletus* spp., *Suillus* spp.) also being commonly used [19]. The national average of used mushrooms was about 8 kg per household per year, but close to 12 kg per household per year in households that picked mushrooms themselves. The average mushroom dish preparation frequency was 2.4 times per month. Overall, Finns have preferred to use wild mushrooms to cultivated mushrooms which is unlike for example Canadians households [19,68].

According to the latest questionnaire on Finnish nature visit activity in 2010 [69], 40% of the population participated in mushroom picking with 7.2 mushroom picking trips on average in a year. Between the evaluation periods of 2000 and 2010, especially the young (15–24 year olds) and elderly (65–74 year olds) [69] as segments of the population have increased their mushroom picking activity. Furthermore, mushroom picking activity has increased over the decade within the 25–34 year cohort to over 30% in the 2010 report; the activity was under 20% in the same cohort population (15–24 year olds) in the study in 2000. Predictors of mushroom picking activity have stayed the same as in the logistic regression model which Sievänen et al. [70] made from the 2000 questionnaire data: Older people were more active in mushroom picking than younger people, women more active than men (odds ratio 1.9), people in eastern (odds ratio 2.38) and southern Finland (odds ratio 2.06) more active than western or northern Finland, and people living in small villages more active than ones living in larger cities (odds ratio 1.25). Similar factors were found by Pekkarinen et al. in the late 1970s [19].

A typical mushroom picking trip lasted 1–2 hours and half of the trips were within 3 kilometers from home [69]. About 76% of the population consider themselves to have the required skills for mushroom picking [69]. An average household picks 1.3–6.2 kg of wild edible mushrooms per year for home use depending on the available harvest [17]. The overall activity of wild mushroom usage has dropped drastically in 60 years, but at the same time the east-west divide has diminished: according to Rautavaara in 1947 [8], 85–90% of households used mushrooms in Karelia and northern Savo but only about 20% in southern Ostrobothnia. By the time Pekkarinen et al. did their research in the late 1970s [19], the corresponding figures had altered to 77–90% and 42%. Part of the reason Finns have a high interest in picking wild edible mushrooms is because of the tradition of everyman's right [71]: everyone is free to pick wild mushrooms as long as they are not a protected species and not in the immediate vicinity of people's homes. Selling unprocessed wild mushrooms is a tax-free income for individuals and there are no limits to the quantity sold [72].

2.3 Sensory properties of mushrooms

2.3.1 Sensory profiling

Descriptive analysis with a trained panel is the golden standard of sensory profiling [73]: it gives detailed information on the important sensory attributes and which of these attributes set the samples apart. This information can be used further to for example identify the effects of processing or to predict consumer acceptance. There are several descriptive analysis techniques available, of which three have been utilized for mushrooms: the trademarked Quantitative Descriptive Analysis (QDA) and Texture Profile methods, and the Generic Descriptive Analysis (GDA) which is partly adapted from QDA [73]. Overall, the literature on the sensory profiling of mushrooms is very limited. Particularly few studies have been published on the sensory characteristics of wild edible mushrooms. The studied species and methodology for the most comprehensive descriptive analyses of mushrooms is overviewed in **Table 2**. These descriptive analyses generally follow the recommended protocol [73]: 8–12 selected panelists are trained using a subset of samples, reference products are introduced and the evaluations are conducted with wide enough scales in multiple sessions.

However, the available information is limited in some aspects. To date, only Cho et al. [34] and Liu et al. [74] have fully specified the reference products they used, but even they did not report the anchored intensities of these products. In addition to the studies listed in the table, there are multiple studies that report only some of the used method parameters. Abbott and Antonio [75] created a texture profile of fried *Agaricus bisporus* and *A. bitorquis* by 6 panelists. They did not specify the texture attributes, training, or scale, but mentioned that there were reference products anchored to the scale as is customary in Szczesniak's method [76]. Liu et al. [77] reported performing a QDA for their *Lentinula edodes* samples. However, they do not specify the training, specific attributes or the scale used, and thus are not included in the table. Hiraide et al. [78] also studied *Lentinula edodes* samples. Eight flavorists evaluated the sulfur, woody, fresh shiitake and soil-like perceptions of extracts using a 7-point category intensity scale, but training or reference samples were not reported.

Rotzoll et al. [79] performed a "taste profile analysis" of a water extract of *Morchella deliciosa* and taste recombinant mixtures. The profile included umami, sour, bitter, sweet and salty taste modalities with mouth-drying astringency. These were evaluated by 14 assessors who were trained with standard compound solutions. The scale was 0–3 and the analyses were done in triplicate. However, the statistical inference testing (for example one-way ANOVA with appropriate post hoc tests) was not specified.

Table 2. Overview of the sensory profiling studies of mushrooms. Consensus training was used in all panels.

Mushroom species	*Tricholoma matsutake*	*Cantharellus cibarius*	*Lentinula edodes*	*Boletus edulis*	Powders from 11 mushroom species [a]	*Lentinula edodes*	*Boletus edulis*	*Lentinus sajor-caju* [b]
Sample treatment	Raw cubes of different quality grades	Different drying methods			Dried mushroom powder in a vial	Different storage times in different packaging	Different preservation methods	Fried, samples from different substrates
Panel size	8	8	9	9	8	10	8	8
Gender ratio[c], age (years)	all female, 21–25	4M + 4F, N/A	5M + 4F, 26–54	5M + 4F, 26–55	N/A	N/A	N/A	4M + 4F, N/A
Method	QDA	Descriptive analysis	Descriptive analysis	Descriptive analysis	Not specified, odor only	Descriptive analysis	QDA for color	Descriptive analysis
Total number of attributes	15	14	11	10	6	7	8	9
Appearance attributes	None	Inner color, piece size	Inner color, outer color, piece size	Inner color, piece size	None	Gill color, gill uniformity, cap surface uniformity, dark zones in the cap, firmness	White, cream, yellow, honey yellow, brown, ashy, grey, pink-violet	Color (light–dark)

Odor attributes	Piny, floral, alcohol, meaty, moldy, wet soil-like, fishy, fermented, metallic	Mushroom, fresh, smoked, spicy, nutty, earthy dry, burnt,	Mushroom, fresh, rancidness, nutty, roasted, bready [b]	Mushroom, fresh, fried, nutty, earth, burnt [b]	Farm-feed, mushroom, floral, honey, nutty, hay-herb	Off-odor	None	Sweet pea, corn, beef broth, mushroom, sweet, bitter [b]
Taste and chemosensory attributes	Sweet, salty, sour, bitter, umami, astringent	woody, sharp and oxidative [b]			None	None	None	
Texture attributes	None	Hardness, sponginess			None	None	None	Tough, fibrous, rubbery
Reference products	Yes, all attributes	Yes, all attributes			No	No	No	Yes, all attributes (anchored)
Scale	15-point category scale	0-10 line scale			Binary-type present/absent	10 cm line scale	5-point category scale	12.6 cm unstructured line scale
Year	2007	2017	2018	2018	2008	2006	2009	2006
Reference	34	33	39	80	41	81	82	74

[a] mushroom species: *Suillus bellini, Suillus luteus, Suillus granulatus, Tricholomopsis rutilans, Hygrophorus agathosmus, Amanita rubescens, Russula cyanoxantha, Boletus edulis, Tricholoma equestre, Fistulina hepatica, Cantharellus cibarius*

[b] reported as *Pleurotus sajor-caju*

[c] M: male, F: female

[d] flavor attributes

Likewise, Mittermeier et al. [40] performed a taste profile analysis of methanol/water extractions and separated fractions of *Cantharellus cibarius* samples. The profile included the taste modalities, astringency, pungency and kokumi and these were evaluated by 17 panelists using a 0–5 line scale. However, the training and reference compounds were not specified and like Rotzoll et al., statistical inference testing was not reported.

Selected observations from these descriptive analyses are: Cho et al. [34] reported that South Korean higher-grade pine mushrooms were sweeter, less sour and bitter, more piny, floral and meaty, and less metallic and astringent than the lower-grade samples. Liu et al. [74] reported that wheat straw and sugar beet pulp with extra buffer type growth substrates produced the most bitter mushrooms, while there were no differences for sweetness between samples. However, the reference products likely affected these evaluations as they were relatively intense compared to the ones used by Cho et al. Even the low intensity bitter reference (anchored at the beginning of the line scale) was still a 1:20 dilution of tea made from 3 tablespoons of tea leaves in 500 ml water. Similarly, the low intensity sweet reference was a 1:20 dilution of 66.7% sugar solution, ie. 3.4% sugar solution. In the Polish-Spanish mushroom studies [33,39,80], *Cantharellus cibarius*, *Lentinula edodes* and *Boletus edulis* behaved similarly in different drying processes. For example, freeze-dried mushrooms always had the most intense mushroom-like and fresh odors, while vacuum-microwave drying and the combined drying procedures produced samples with the most intense hardness. For the *Lentinula edodes* packaged in different materials [81], samples in macroperforated bags had no off-odor and were less brown, had more uniform gills, and less dark zones in the cap compared to other packaging materials.

In addition to the main profiling studies listed in the table, there are some studies focused on one sensory attribute only. Kurkela et al. [83] used an ascending duo-trio test dilution series (Tilgner's dilution index) with seven panelists to study the relative intensity of mushroom-like flavors of mushrooms juices made from different species. The selected threshold dilution ratios were in the range 1/1600–1/100 and in the order *Boletus edulis* < *Agaricus bisporus* < *Cantharellus cibarius* < *Lactarius trivialis* < *Suillus luteus*, meaning that porcini had the lowest mushroom flavor threshold. Phat et al. [42] had 10 panelists evaluate the umami intensity of 17 mushrooms extracts with an 11-point scale after training with monosodium glutamate (MSG) solutions. *Agaricus bisporus* and *Pleurotus ostreatus* were evaluated to have the most intense umami sensation, while *Lentinula edodes* was among the least umami-intense species. Finally, Dermiki et al. [84] compared the umami intensity of two shiitake extracts made with different temperatures using a paired alternative forced choice test and 17 panelists, and additionally created a descriptive

profile of minced meat samples containing these extracts. The sample with a higher extraction temperature was observed as more intense in umami; however, this difference was not observed in the sensory profiles of minced meat samples. Only two studies [75,77] have so far examined cooked samples, both with inadequately reported methods. Likewise, only two studies [41,75] have compared different mushroom species. So far there are no descriptive analyses from Nordic wild edible mushrooms. Overall, there is still a lack of descriptive profiling studies.

Recently, rapid holistic sensory methods that do not require extensive panelist selection and training such as Projective Mapping [85,86] have gained research interest in sensory profiling [87,88]. Several studies have compared the strengths and weaknesses of these modern methods and conventional profiling techniques such as generic descriptive analysis. These studies have used either trained [89] or both trained and consumer panels and various matrices [85,90–92]. While conventional profiling still produces the most repeatable and precise sensory information, modern techniques can be as discriminative, more relatable to consumer preference data, and considerably faster to perform. Recent reviews on the subject [87,88] have thus argued that depending on the research aims, the quantitative and sensitive information that conventional profiling provides can be redundant. However, to date there have been no publications utilizing consumer panels and modern sensory methods such as Check-all-that-apply (CATA) or Projective Mapping on the sensory profiling of mushrooms or mushroom-containing products.

2.3.2 Hedonic liking of mushrooms

Studies that examine the liking of mushrooms or mushroom products are very limited in number but have become more common in the last 5 years. These studies are overviewed in **Table 3**. Only one study has compared species [75], in which the preference of two fried *Agaricus* species was examined. However, the main question in this study was the sensory discrimination via a triangle test; the preference was asked as an additional question. As is the case in sensory profiling studies, the main point of interest has been the effect of different preservation methods. In addition to the hedonic studies listed in the table, the effect of irradiation on the quality of Volvariella volvacea has been studied by six panelists via 5-point quality score cards for color, odor, texture and appearance [93].

Table 3. Overview of the hedonic studies on mushrooms in relation to processing and preservation methods.

Mushroom species	Sample treatment	Panel size	Liking attributes	Scale	Year	Ref
Agaricus bisporus + *A. bitorquis*	Frying for 5 min at 204°C	31	Open question	None: triangle test with additional preference question	1974	[75]
Agaricus bisporus	Raw and cooked, different storage times	20	Mushroom aroma	1–10 hedonic scale (1 = most desirable)	1981	[94]
Pleurotus pulmonarius [a]	Different pretreatment and drying methods	10	Color, aroma, texture	6-point hedonic scale	2007	[95]
Lentinula edodes	Drying, grinding	335, 331	Pre-evaluation acceptance, overall acceptance	7-point hedonic scale (-3 to +3)	2005, 2007	[96,97]
Lentinula edodes	Microwave-puffing	30	Color, flavor, appearance, texture, overall acceptability	7-point hedonic scale	2014	[77]
Lentinula edodes	Vacuum-frying	50	Color, flavor, appearance, texture, oiliness, overall acceptability	9-point hedonic scale	2018	[98]
Pleurotus ostreatus	Different pretreatment and drying methods	10	Appearance, odor, color, texture, overall acceptability	Unspecified, apparently a 20-point quality score	2018	[99]

[a] Reported as Pleurotus sajor caju

On the other hand, there are multiple studies with hedonic test components that examined the effect of adding mushrooms to other food products. In the year 2018 alone, there were four published studies that examine the addition of mushroom to sausage-type, beef patty or noodle products. This demonstrates that the application of mushrooms as part of food products interests the scientific community even if the hedonic studies of mushrooms themselves has been lacking. These studies have been overviewed in **Table 4**. The first

conclusion that can be made after examining these two types of publications is that hedonic testing is often done without following standard sensory practices. Very often the number of participants is very low, nonstandard scales are used, the same panelists do both the descriptive analysis and hedonic ratings of the samples, and neither the use of proper facilities such as a sensory laboratory nor the use of palate cleansers are reported.

Secondly, there is seldom preference mapping or other statistical models reported that would examine the association of hedonic liking and other measured characteristics such as proximate composition or instrumental texture measurements. The hedonic measurement results are thus not fully utilized. Thirdly, only three studies [96,97,100] have used large enough sample sizes for consumer clustering based on factors such as age or product preferences. This limits the application of results. Finally, all the overviewed studies examine cultivated species; there is no information available on the hedonic properties of wild mushrooms. This also means that the hedonic liking of Nordic wild edible mushrooms have not been studied.

Table 4. Overview of the hedonic studies on mushroom-containing foods.

Sample	Mushroom addition	Panel size	Liking attributes	Scale	Year	Ref
Agaricus bisporus in beef taco blend	0–80% of original meat content	147	Apperance, flavor, texture, overall	9-point hedonic scale	2016	[100]
Agaricus bisporus in beef patties	1–2% dried powder	N/A	Saltiness, aroma, acceptability	9-point hedonic scale (anchors 0-9)	2018	[101]
Lentinula edodes in frankfurter	Freeze-dried extract	8 (trained)	Texture, flavor, taste, overall	7-point hedonic scale, (anchors 1-7)	2015	[102]
Flammulina velutipes in sausages	0–1% freeze-dried powder	20	Color, flavor, taste, texture, overall	9-point hedonic scale	2018	[103]
Volvariella volvacea in sausages	0–4% dried powder	12 (trained)	Odor, flavor, color, texture, general	0-10 hedonic scale (dislike-like)	2018	[104]
Pleurotus sapidus mycelia in vegan sausage	1–3% mycelia powder	15 (3 repli-cates)	Flavor, texture	9-point hedonic scale	2018	[105]
Pleurotus ostreatus in noodles	2-10% dried powder	15 (semi-trained)	Taste, color, aroma, texture, flavor, overall	9-point hedonic scale	2018	[106]

2.4 Non-volatile chemical compounds of edible mushrooms explaining sensory properties

While there is a considerable amount of published literature on the non-volatile compounds of edible mushrooms, the major limitations are that the available data is either 1) at least 40 years old [107] or 2) focused on species that are not relevant for Finland [108–116]. The following sections overview publications on non-volatile chemical composition that could be used to predict the sensory properties of edible mushrooms. The sections demonstrate that the reported concentrations of the same species can vary in the same magnitude between studies (harvest locations and years) as between species. Comparative measurements have also demonstrated that the chemical compositions between mushroom stipe and pileus differ significantly [104,117,118].

2.4.1 Dry matter content and proximate chemical composition

The dry matter content and proximate chemical composition of three cultivated and six wild edible mushrooms are presented in **Table 5**. On average, edible mushrooms have about 11% dry matter, which mainly consists of carbohydrates and crude protein. Only some 3% of the dry matter consists of lipid material. The energy content of mushrooms as regards the fresh matter basis is on the same magnitude as carrots, turnips and onions, about 60% of oat porridge made in water, 40% of potatoes and less than 15% of chicken or other meats [119]. In addition, the protein and lipid contents are comparable to these vegetables [119].

2.4.2 Soluble sugars and sugar alcohols

The sugar and sugar alcohol composition of the selected mushroom species is overviewed in **Table 6**. Generally, the most common compounds are mannitol (a six-carbon sugar alcohol), trehalose (α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside) and mannose (an aldohexose), which are displayed in **Figure 2**.

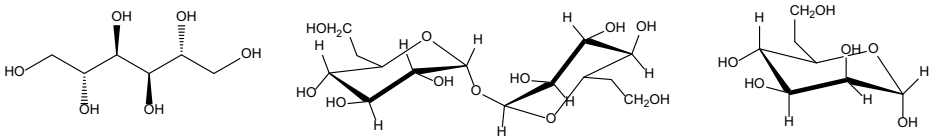

Figure 2. The typical sugars and sugar alcohols in in edible mushrooms. Mannose is presented as α-D-mannopyranose.

Table 5. Proximate chemical compositions of selected cultivated and wild edible mushrooms. Values are on a dry matter basis: for energy as kJ/100 g dry matter, and for protein, carbohydrates, lipids and ash as g/100 g dry matter.

Species (binomial)	**Species** (common)	**Dry matter** (%)	**Energy** (kJ/100 g)	**Protein** [a]	**Carbohydates**	**Lipids**	**Ash**	**Ref**
Agaricus bisporus	button mushroom (white)	7.7	1467	27.1 [b]	58.4	4.3	10.1	20
Agaricus bisporus	button mushroom (white)	8.7	1479	14.1	74.0	2.2	9.7	120
Pleurotus ostreatus	oyster mushroom	8.0	1412	24.6 [b]	62.5	4.4	8.0	20
Pleurotus ostreatus	oyster mushroom	10.8	1517	7.0	85.9	1.4	5.7	120
Lentinula edodes	shiitake	8.4	1395	21.4 [b]	69.0	3.7	5.8	20
Lentinula edodes	shiitake	20.2	1506	4.4	87.1	1.7	6.7	120
Boletus edulis	porcini	10.9	1632	21.1	71.0	2.5	5.5	108
Boletus edulis	porcini	12.2	1488	36.9	64.3	2.9	5.3	121
Cantharellus cibarius	chanterelle	14.2	1488	30.9	52.5	1.9	8.8	121
Cantharellus cibarius	chanterelle	N/A	N/A	35.8 [c]	56.2 [d]	1.5	6.4	122
Cantharellus cibarius	chanterelle	8.6	1477	21.5	53.7	5.0	8.6	107
Craterellus cornucopioides	black trumpet	10.1	1730	47.2	44.5	4.9	10.1	121
Lactarius deliciosus	red pine mushroom	N/A	N/A	20.2 [c]	64.5 [d]	8.0	7.3	122
Albatrellus ovinus	No English name	N/A	N/A	8.4 [c]	N/A	7.1	7.0	123
Suillus variegatus	velvet bolete	9.2	N/A	17.6	63.8	3.3	15.4	109
Macrolepiota procera	parasol mushroom	13.2	1630	24.2	66.8	2.2	5.4	121
Average		10.9	1518	22.7	65.0	3.6	7.9	

[a] Calculated from the N content with the 4.38 conversion factor
[b] Calculated as a sum of individual amino acids
[c] Reported as N×6.25 in the reference, recalculated
[d] Recalculated as 100 – (protein + lipids + ash) because of the corrected protein content

Table 6. Soluble sugars and sugar alcohols of selected cultivated and wild edible mushrooms. Contents are reported as g/100 g dry matter.

Species (binomial)	**Species** (common)	**Fructose**	**Glucose**	**Mannose**	**Trehalose**	**Mannitol**	**Total sugars and polyols**	**Ref**
Agaricus bisporus	button mushroom (white)	0.03	N/A	N/A	0.16	5.6	5.8	[120]
Agaricus bisporus	button mushroom (white) [a]	2.62	N/A	N/A	N/A	23.6	31.9	[124]
Pleurotus ostreatus	oyster mushroom	0.01	N/A	N/A	8.01	0.60	8.7	[120]
Pleurotus ostreatus	oyster mushroom	N/A	1.06	N/A	0.27	0.36	1.8 [c]	[125]
Lentinula edodes	shiitake	0.69	N/A	N/A	3.38	10.0	14.9	[120]
Lentinula edodes	shiitake	N/A	2.86	N/A	2.92	8.4	14.2 [c]	[125]
Boletus edulis	porcini	N/A	N/A	N/A	12.40	2.5	14.9	[108]
Boletus edulis	porcini	N/A	6.28	36.23	9.92	3.7	59.9	[121]
Cantharellus cibarius	chanterelle	N/A	2.78	28.56	6.68	8.6	48.8	[121]
Craterellus cornucopioides	black trumpet	N/A	2.8	nd	0.09	11.2	14.1	[121]
Lactarius deliciosus	red pine mushroom [b]	N/A	N/A	N/A	3.50	13.90	17.4	[126]
Suillus variegatus	velvet bolete	nd	N/A	N/A	4.85	nd	4.85	[109]
Macrolepiota procera	parasol mushroom	N/A	10.8	11.1	0.1	2.4	24.4	[121]
Macrolepiota procera	parasol mushroom [b]	N/A	N/A	N/A	7.6	6.5	14.1	[126]
Average		0.8	4.4	25.3	4.4	7.5	19.7	

[a] day 0 sample
[b] frozen sample
[c] includes also the following compounds that are not detailed in separate columns: sucrose and myo-inositol

2.4.3 Organic acids

The reporting conventions of the organic acids in mushrooms vary; it is not specified whether the contents are on a dry matter or fresh mushroom basis. Thus, it is unclear whether the large variations are due to actual concentration differences. For example, Valentão et al. [127] reported the total organic acid content of *Boletus edulis* as 2.8–4.5 mg/g, while Ribeiro et al. [128] a year later reported the content as 48–58 mg/g dry matter also for a Portuguese *B. edulis*.

Chen et al. [117] reported up to 300 mg/g dry weight contents of organic acids in the pileus of *Lentinula edodes*. To circumvent the reporting differences, only the distribution of organic acids in the selected mushroom species is overviewed in **Table 7**. Generally, malic acid and citric acid are the most abundant organic acids aside from *L. edodes*, where succinic acid dominates. The structural formulas of main organic acids are displayed in **Figure 3**.

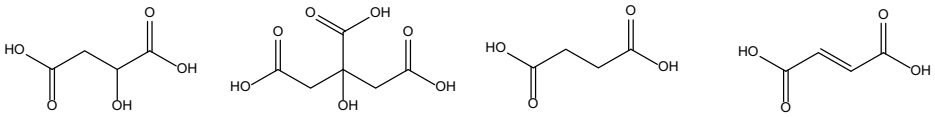

Figure 3. Typical organic acids present in edible mushrooms.

2.4.4 Free amino acids and 5'-nucleotides

Table 8 overviews the free amino acids and 5'-nucleotides that have been reported in cultivated and wild edible mushrooms. All 20 amino acids have been detected, but asparagine, glutamine and proline are seldom reported due to analytical challenges. In general, glutamic acid, alanine, histidine and lysine are the most abundant free amino acids. The distribution of free 5'-nucleotides varies based on the mushroom species, but for example adenosine monophosphate and xanthosine monophosphate are most common in *Boletus edulis*. Equivalent umami concentration (EUC) which calculates the predicted umami intensity of the sample resulting from the synergy of free amino acids and 5'-nucleotides [129] is also presented. However, as EUC values are missing in some reports or are calculated incorrectly based on raw data, they have been recalculated in the table. The largest discrepancies are with *Craterellus cornucopioides* and *Pleurotus ostreatus*: the authors [121] reported the EUC values as 151 mg/100 g and 121 mg/100 g, respectively, while the recalculated values from base data of amino acids and 5'-nucleotides are 1701 mg/100 g and 8351 mg/100 g. The calculations for other samples match the reported values in reference [121].

Table 7. Distribution of organic acids in selected cultivated and wild mushrooms (expressed as % of total organic acids).

Species (binomial)	**Species** (common)	**Identifier** [a]	**Oxalic**	**Citric**	**Malic**	**Quinic**	**Ascorbic**	**Succinic**	**Fumaric**	**Ref**
Lentinula edodes	shiitake, pileus	5	N/A	18	17	N/A	nd	61	0.7	[117]
Lentinula edodes	shiitake, stipe	5	N/A	23	9	N/A	0.3	61	0.8	[117]
Boletus edulis	porcini	11	3	12		78 [c]	nd	nd	3.6	[128]
Boletus edulis	porcini	14	5	15 [b]		80 [c]	nd	0	0.4	[127]
Cantharellus cibarius	chanterelle	1	N/A	13	84	N/A	3.5	N/A	0.1	[130]
Cantharellus cibarius	chanterelle	4	N/A	16	83	N/A	nd	N/A	0.5	[130]
Suillus luteus	slippery jack	16	nd	15		67 [c]	nd	nd	18.0	[128]
Lactarius deliciosus	red pine mushroom	1	0	6 [b]	92	nd	nd	0 %	0.9	[127]

[a] Sample identifier used in each reference
[b] citric+ketoglutaric acid
[c] malic+quinic acid

Table 8. Free L-amino acids and 5'-nucleotides in selected cultivated and wild mushrooms (g/kg dry matter) and the calculated EUC values (g/100 g dry matter).

Species (binomial)	**Species** (common)	**Ala** [b]	**Arg**	**Asp**	**Cys**	**Glu**	**Gly**	**His**	**Ile**	**Leu**	**Lys**	**Met**	**Phe**	**Pro**	**Ref**
Agaricus bisporus	button mushroom (white) [a]	12.05	3.83	6.62	N/A	16.05	3.14	3.01	2.81	3.08	5.89	1.49	6.92	N/A	[124]
Pleurotus ostreatus	oyster mushroom	6.45	0.11	0.17	1.81	41.09	0.34	4.42	nd	0.29	4.65	0.12	0.09	N/A	[121]
Lentinula edodes	shiitake, strain 271	3.47	0.49	0.41	N/A	1.30	0.43	0.43	nd	nd	0.51	1.01	0.22	N/A	[125]
Boletus edulis	porcini	15.1	11.3	19.6	3.08	27.9	8.62	5.54	11.8	16.1	11.6	8.90	8.29	8.52	[110]
Boletus edulis	porcini	8.68	0.59	0.33	2.17	39.09	0.27	2.34	0.12	0.47	5.46	0.74	0.19	N/A	[121]
Boletus edulis	porcini	0.63	0.54	0.65	nd	0.59	0.18	0.44	0.48	0.58	2.17	0.41	1.04	nd	[131]
Cantharellus cibarius	chanterelle	4.98	0.44	0.06	1.99	29.99	0.13	3.15	nd	0.21	5.74	0.41	0.06	N/A	[121]
Cantharellus cibarius	chanterelle	5.39	7.92	8.91	0.79	15.0	3.91	3.15	8.09	8.65	5.29	0.91	4.24	4.25	[110]
Cantharellus cibarius	chanterelle	1.36	0.10	nd	0.19	0.89	0.40	0.34	0.10	0.14	0.36	N/A	0.10	0.25	[132]
Craterellus cornucopioides	black trumpet	2.34	0.46		1.87	45.85	0.17	3.61	nd	1.05	4.69	0.16	0.85	N/A	[121]
Craterellus tubaeformis	trumpet chanterelle	4.92	6.94	9.60	1.25	10.9	3.65	3.99	9.44	8.93	5.64	1.23	4.35	4.29	[110]
Hydnum repandum	wood hedgehog	5.90	6.33	11.60	1.18	13.40	3.99	4.06	8.34	9.51	5.06	1.13	4.60	4.63	[110]
Macrolepiota procera	parasol mushroom	2.21	0.29	0.12	1.45	33.65	0.09	3.29	0.19	0.38	4.11	0.69	0.45	N/A	[121]
Suillus luteus	slippery jack	3.99	0.396	1.58	0.05	nd	0.04	0.13	0.11	0.07	0.15	N/A	0.18	0.31	[132]

Species (binomial)	**Species** (common)	**Ser**	**Thr**	**Trp**	**Tyr**	**Val**	**Total amino acids**	**AMP**	**GMP**	**IMP**	**XMP**	**EUC** (g/100 g)	**Ref**
Agaricus bisporus	button mushroom (white) [a]	4.22	4.67	N/A	1.11	3.03	77.9	2.04	1.52	0.44	2.23	1144	[124]
Pleurotus ostreatus	oyster mushroom	0.03	6.99	0.01	nd	1.21	67.8	1.2	0.6	0.2	2.6	1701 [c]	[121]
Lentinula edodes	shiitake, strain 271	1.05	2.82	nd	nd	0.38	12.5			2.78	8.80	132	[125]
Boletus edulis	porcini	9.77	10.4	3.21	4.09	9.81	194	N/A	N/A	N/A	N/A	N/A	[110]
Boletus edulis	porcini	1.01	9.14	0.03	nd	1.41	72.0	1.65	0.64	0.28	0.71	1186	[121]
Boletus edulis	porcini	0.35	0.19	nd	0.61	nd	8.9	0.09	0.04	0.06	1.91	10.5	[131]
Cantharellus cibarius	chanterelle	0.18	8.98	0.02	nd	1.34	57.7	0.41	0.21	0.03	0.14	249	[121]
Cantharellus cibarius	chanterelle	4.69	4.77	3.41	2.05	4.35	95.8	N/A	N/A	N/A	N/A	N/A	[110]
Cantharellus cibarius	chanterelle	0.25	0.18	0.15	0.30	nd	5.1	N/A	N/A	N/A	N/A	N/A	[132]
Craterellus cornucopioides	black trumpet	0.01	4.56	0.12	0.02	1.72	67.5	0.35	2.88	3.97	7.03	8351 [c]	[121]
Craterellus tubaeformis	trumpet chanterelle	4.62	4.93	1.17	3.67	4.11	93.6	N/A	N/A	N/A	N/A	N/A	[110]
Hydnum repandum	wood hedgehog	5.69	5.46	1.71	2.84	4.64	100.0	N/A	N/A	N/A	N/A	N/A	[110]
Macrolepiota procera	parasol mushroom	0.11	5.78	0.09	0.02	1.39	54.3	1.0	0.1	0.2	0.2	318	[121]
Suillus luteus	slippery jack	0.18	0.14	0.13	0.19	0.54	8.2	N/A	N/A	N/A	N/A	N/A	[132]

[a] day 0 sample

[b] Amino acid abbreviations: Ala: alanine, Arg: arginine, Asp: aspartic acid, Cys: cysteine (reported as cystine in some references), Glu: glutamic acid, His: histidine, Ile: isoleucine, Leu: leucine, Lys: lysine, Met: methionine, Phe: phenylalanine, Pro: proline, Ser: serine, Thr: threonine, Tyr: tyrosine, Val: valine

[c] Recalculated from base values; the reported value in the reference did not match the calculation from amino acids and 5'-nucleotides

2.4.5 Fatty acids

Only four fatty acids–palmitic (16:0), stearic (18:0), oleic (18:1n-9), and linoleic (18:2n-6) acid–comprise 70–99% (average 93%) of the total fatty acid content in both cultivated and wild mushrooms. The distribution of major fatty acids in the selected mushroom species is overviewed in **Table 9**.

2.4.6 Other non-volatile compounds

Other non-volatile compounds in mushrooms that have received research interest include the different phenolic acids and polyphenols [108,109,111,126,128,130,133–138], terpenoids [109,133,139–143], and vitamins, especially tocopherols (vitamin E) and ascorbic acid (vitamin C) [109,111,120,133,134]. A common interest for these and non-volatile compound measurements in general has been their effect on antioxidant activity [108,109,111,122,126,128,133–138] instead of their effect on sensory properties. Additionally, several of these publications have focused on inedible species [137,139,142].

2.4.7 Non-volatile compound interactions in taste perception

Most food matrices such as mushrooms are complex mixtures of compounds. Human sense of taste is neither fully linear nor analytical in the perception of these matrices. There are various mixture interactions that affect taste perception, and concentrations of the compounds further modulate the interactions [144–146]. The shapes of the typical concentration-intensity curves of taste perception are sigmoidal, which means that perception is linear only in moderate tastant intensities [145,146]. At subthreshold levels, there is by definition no taste perception for single-compound solutions, and at high tastant concentrations the taste perception becomes saturated [145,146].

Taste compound mixtures have four main types of interactions: suppression, enhancement, masking, and synergy. In suppression, two or more compounds in suprathreshold concentrations interact in a way that the resulting mixture intensity in a given taste modality is lower than when evaluated separately. Suppression is typical for heterogenous mixtures and in high taste intensities [144]. Some examples are the lowered sourness perception of citric acid in the presence of sucrose and the lowered saltiness perception of NaCl in the presence of sucrose and citric acid [147]. However, this suppression can also be asymmetric: one taste modality is only slightly suppressed while the other is strongly suppressed [145,147]. As an example, sweet compounds have been reported to dominantly suppress bitter and sour compounds, while sweetness was suppressed to a lesser degree by these compounds [147].

Table 9. Contents of major fatty acids and fatty acid groups in selected cultivated and wild mushrooms (expressed as % of total fatty acids).

Species (binomial)	**Species** (common)	**C16:0**	**C18:0**	**C18:1n-9**	**C18:2n-6**	**C18:3n-3**	**SFA** [b]	**MUFA**	**PUFA**	**4 main FAs** [c]	**Ref**
Agaricus bisporus	button mushroom (white)	11.9	3.10	1.1	77.7	0.10	20.3	1.4	78.3	93.8	120
Pleurotus ostreatus	oyster mushroom	11.2	1.60	12.3	68.9	0.10	17.0	13.6	69.4	94.0	120
Lentinula edodes	shiitake	10.3	1.60	2.3	81.1	0.10	15.1	2.9	82.0	95.3	120
Boletus edulis	porcini	17.3	10.1	37.9	24.7	0.54	32.5	41.8	25.7	90.0	148
Boletus edulis	porcini	9.6	3.11	42.1	41.3	0.06	14.8	43.5	41.7	96.1	108
Boletus edulis	porcini	12.7	0.67	8.2	75.6	0.47	13.4	10.5	76.1	97.2	110
Cantharellus cibarius [a]	chanterelle	1.9	0.84	17.8	78.8	nd	3.2	17.9	78.9	99.3	148
Cantharellus cibarius	chanterelle	13.7	3.34	8.4	45.6	nd	17.1	37.3	45.6	71.1	110
Craterellus tubaeformis	trumpet chanterelle	15.2	6.19	57.8	19.8	nd	21.7	58.5	19.8	99.0	110
Hydnum repandum	wood hedgehog	10.9	1.71	42.4	42.5	0.25	12.9	44.4	42.7	97.5	110
Hydnum rufescens [a]	terracotta hedgehog	24.1	9.86	28.6	19.1	nd	39.5	41.2	19.3	81.7	148
Albatrellus ovinus	N/A [d]	6.8	2.0	32.0	52.5	0.4	15.4	32.0	52.9	93.3	123
Lactarius deliciosus	red pine mushroom	12.1	25.3	41.3	17.1	0.3	40.1	42.3	17.6	95.7	149
Suillus variegatus	velvet bolete	12.7	3.47	42.0	37.4	N/A	18.09	44.24	37.67	95.6	109
Suillus luteus [a]	slippery jack	0.6	0.18	61.9	36.7	0.03	1.0	62.2	36.8	99.4	148
Average		11.7	3.5	27.9	49.9	0.2	17.4	32.3	50.3	93.3	

[a] recalculated from mg/kg dry matter values

[b] SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, FAs: fatty acids

[c] Sum of palmitic (16:0), stearic (18:0), oleic (18:1n-9), and linoleic (18:2n-6) acid

[d] No English name available

Enhancement is the counterpart to suppression: the mixture has an increased intensity in a given taste modality. As an example of within-taste enhancement, sweet compounds are generally at least additive in mixtures of moderate concentrations [146,150]. A special case of enhancement is additive mixing; taste compounds that individually are below their thresholds interact additively and the mixture elicits a taste response [144]. The same mixture can display both enhancement and suppression depending on the phase the compounds are at in the sigmoidal response curve [144]. Interestingly, the overall taste intensity of the mixture seems to be roughly the sum of individual taste intensities in low and moderate concentrations, even though there is concurrent suppression or enhancement for each separate taste modality [147,151].

Synergy is an interaction where the taste compounds in a mixture enhance the taste perception and also increase the slope of the sigmoidal concentration-intensity curve [144]. The best known case of synergy is the interaction between certain amino acids and 5'-ribonucleotides [152] first mentioned in section 2.4.4. In low concentrations (<0.1 g/100 ml) of the components especially, the mixture elicits a strong synergistic effect that can be expressed with the following equation of the equivalent umami concentration (EUC) introduced by Yamaguchi et al. [121,129,153]:

EUC (g monosodium glutamate/100 ml solution) = $\Sigma a_i b_i + 1218\ (\Sigma a_i b_i)\ (\Sigma a_j b_j)$

where a_i is the concentration of each umami amino acid, a_j is the concentration of each umami 5'-nucleotide, and b_i and b_j are the relative intensities of these amino acids and 5'-nucleotides. Masking is the counterpart to synergy, but it is not common in compounds that are typical in food matrices [145].

Two further related phenomena are release from suppression and adaptation. Because of the asymmetrical interaction effects, adding further components to a binary taste mixture can modulate the binary system in unexpected ways. An example of this is the addition of sodium acetate to a sucrose-urea mixture [154]. Sucrose and urea suppress the sweetness and bitterness of each roughly as effectively. However, the added sodium acetate suppresses the perception disproportionally to the weak suppression of sucrose, and thus sweetness is perceived much more intensely than in a binary suppressive mixture. Finally, in adaptation, the taste response for a given compound at a steady concentration decreases after continued tasting [155,156]. After adaptation to a single compound, the identification of the additional component in binary suppressive mixture is improved [156].

2.4.8 Linking non-volatile compound data to sensory attributes

There are very limited studies available that have used chemical or instrumental data to explain the sensory properties of mushrooms. Dijkstra and Wiken in 1976 [157] made one of the first combinatory approaches to model the sensory properties of *Agaricus bisporus* extract. They created a base mixture containing certain volatile compounds, free amino acids, 5'-nucleotides, sugars and sugars alcohols as well as urea and ammonia. The contribution of each compound group was tested by omission studies. Dijkstra and Wiken concluded that for amino acids and nucleotides, only glutamic acid, GMP and AMP contributed to the flavor of the mixture, and the included sugars and sugar alcohols were also important.

Phat et al. [42] correlated the measured free Asp and Glu contents, 5'-nucleotide contents, calculated EUC values and electronic tongue measurements to sensory evaluations in 17 mushroom species (**Section 2.3.1**). Their findings were that the electronic tongue measurements correlated only moderately (Spearman correlation coefficient 0.51) with perceived umami intensity, while there was a strong correlation between Asp, Glu and EUC and the sensory results (correlation coefficients 0.87, 0.88 and 0.86, respectively). However, when plotting the EUC and sensory umami intensity results directly (**Figure 4**) it can be seen that apart from the EUC 0–300 mg/g range, the response is quite linear; more so than could be postulated based on the Spearman correlation coefficient.

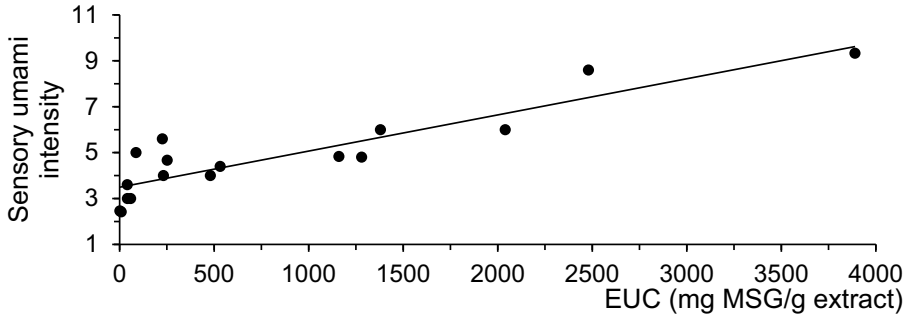

Figure 4. Sensory umami intensities of extracts from 17 mushroom species in relation to the EUC values as reported by Phat et al. [42]. The adjusted R^2 of the linear fit is 0.79. Plotted and linear fitted from raw data in the reference [42].

While Phat et al. [42] do not discuss why there is a high variation in the umami intensity of the low-EUC segment, there are multiple reasons that could account for this. First, the sensory evaluation did not have anchored intensity values for the MSG reference solutions that were used in the training. Second, the scale used in the evaluation was an 11-point category scale anchored with

points 1: very weak, 6: medium, and 11: very strong. The scale has thus limited resolution. Third, the evaluated samples were water extracts of freeze-dried mushrooms that would have various water-soluble components, and thus a possibility for mixture interactions. The electronic tongue measurements [42] of the samples indicated that the samples with low umami scores had high bitterness scores. The interactions of umami have only been examined in a limited number of studies [146]. Kemp and Beauchamp [158] reported that moderate concentrations of MSG suppressed bitterness and Kim et al. similarly reported that umami peptides suppressed bitterness [159]. It could be postulated that bitterness might also suppress umami, but so far there has been a very limited amount of literature in which to base a discussion. Similar to Phat et al., Cho et al. have reported good correlations between the sensory umami intensities [34] and measured EUC values [160] of *Tricholoma matsutake* samples of different quality grades. Dermiki et al. [84] reported a perceptual difference in umami intensity in their *Lentinula edodes* extracts of differing EUC values (9–22.6 vs 0.27–0.73) based on discrimination testing. In contrast, there was no difference in umami intensity for the meat sample with included *Lentinula edodes* extract. However, the change caused in the EUC value by the extract addition was small (0.39 versus 1.68) and not statistically significant. They point out that the EUC equation [129] was established for pure solutions, not the complex heterogenous matrices of food products. Thus it has limited prediction power.

Rotzoll et al. [79,161] created a taste reconstitute model of *Morchella deliciosa* with 33 quantified non-volatile compounds. The model had five major compound groups: 1) umami-like amino acids, 5'-nucleotides and (*S*)-malic acid 1-O-β-D-glucopyranoside, 2) organic acids, 3) sugars, sugar alcohols and sweet amino acids, 4) bitter amino acids, and 5) salty amino acids and inorganic salts. Omitting group 1 resulted in less umami compared to the whole extract and full taste recombinant, group 2 in less sourness and astringency, and groups 3–5 in reduced overall taste intensity and complexity. However, the effect of omitting groups 3–5 was smaller than omitting the first two groups. Mittelmeier et al. [40] compared the taste and chemosensory intensities of different *Cantharellus cibarius* extracts of measured compositions as well taste recombinant mixtures. In the first phase, they report no statistical inference methods, but in the second phase the triangle test results between a full recombinant mixture and partial recombinant mixtures of known compositions were shown. The chemical composition explaining the sensory properties was deduced sequentially using a series of triangle tests and dilution tests. As the result indicates, a series of octadecadien-12-ynoic and octadecadienoic acids were shown to enhance bitter and kokumi sensations.

More typically, instrumental measurements alone, in comparison to the literature without sensory evaluation, are used to predict the sensory properties.

The most typical scenario is dividing the free amino acids into umami, sweet, bitter and tasteless groups and using these ratios for conclusions on the intensities of these taste modalities [113,117,121,124,162,163]. For example, major concentrations of sweet L-alanine and bitter L-histidine have been measured in these studies, and this has been concluded to contribute to the sweetness and bitterness of the mushrooms. While the division of these amino acid taste modality groups generally has been demonstrated as pure solutions in sensory measurements, it should also be noted that the taste perception of individual amino acids changes as a function of chemical concentration [164].

The basic logical assumption that can be made on non-volatile chemical compounds is that sugars and sugar alcohols contribute to sweetness [165]. Thus it has been predicted that the mannitol, trehalose and other soluble sugars would contribute to sweetness in mushrooms [104,113,121,124,125]. Likewise, organic acids typically contribute to sourness [166] and to lesser extent astringency [167,168] and polyphenols to astringency and bitterness [169,170]. Aside from Rotzoll et al. [79,161] these connections have not been made so far in the mushroom-related literature. However, these compounds groups have been measured in multiple studies and sourness and astringency have been separately measured in sensory evaluations of mushrooms [34,79].

Furthermore, different terpenoids such as certain sesquiterpenoids [171] and cucurbitacins [172] that are present in mushrooms are known to be bitter. However, limited conclusions can be currently made from these reports. Often the publications that report these compounds only focus on the extraction and just assume that the end product is relevantly bitter [139,173]. Additionally, the selected species are often so bitter and pungent that they are classified as inedible at least in the Finnish mushroom grading system. Nonetheless, the sensory contribution of terpenoid compounds is relevant to some species especially in the *Russula* and *Lactarius* families [140].

Finally, it needs to be mentioned that this examination is limited due to scarce sensory data available for mushrooms. For example, several publications [74,113,114,125] employing mostly chemical measurements have speculated that the sweet compounds in mushrooms probably suppress the bitter compounds, leading to low perceived bitterness. While this hypothesis is sound based on general studies of taste mixture interactions, mushroom-specific data is necessary. The closest positive indication to bitter suppression by sweet compounds in mushrooms is the sensory profile of Cho et al. [34] where bitterness correlated negatively with sweetness; the calculated Pearson correlation coefficient from the published sensory data is -0.97. In the future, more research projects would be required to address this question. Specifically, experimental setups that would compare mushroom samples with different non-volatile compositions and sensory profiles are necessary.

2.5 Volatile aroma compounds in mushrooms

2.5.1 Fundamental aspects of food aroma and olfaction

The detailed understanding of our sense of olfaction is still a work in progress. Since the first report of olfactory receptors [174] and the resulting 2004 Nobel prize, there are now about 400 identified olfactory receptors with major individual variation in their sequences [175]. Therefore the olfactory receptors form one of the largest gene families with about 1% share of the mammalian genome [176]. The general requirements for aroma compounds are that 1) they need to be volatile enough to be able to travel to the olfactory epithelium either through the orthonasal or retronasal route, and 2) there is at least one functional olfactory receptor that binds these compounds [176–179]. While over 8000 volatile compounds have been detected in foods, only 227 compounds are needed to explain the odor of most foods [180].

The potency of different aroma compounds for the human olfaction varies remarkably. In terms of detection thresholds–the concentration needed for human olfaction to detect the presence of a compound–there is a 22 million-fold difference between the potencies of weak vinegar-like acetic acid and extremely potent cucumber-like (*E,Z*)-2,6-nonadienal [181]. Therefore, the mere presence of an aroma compound does not dictate the importance of it in a given foodstuff. Typically in aroma chemistry, a so-called odorant activity value (OAV)–a quotient of an aroma compound's concentration in food and its odor threshold–is calculated for each identified compound to assess its importance [179].

However, this relation is a gross simplification. The relationship between the odorant concentration (and other sensory stimulus magnitudes) and its sensory intensity is better explained via the Stevens' power law [182,183]:

$\psi = k\phi^{\theta}$

Where ψ is the sensory intensity, ϕ is the stimulus concentration, θ is the power exponent and k is a stimulus-specific constant. Stevens' Power Law can be used to explain the suprathreshold section of the sigmoidal psychometric function (overviewed in Section 2.4.7) of both the sense of taste and olfaction [184]. Aroma compounds are peculiar among human sense stimuli. While the measured exponents for visual stimuli are in the 0.5–1.7 range [183] and 0.7–1.5 range for taste stimuli [185], the exponents for odorants are considerably smaller than 1. For example, the power exponent of 3-(methylthio)propanal has been determined to be 0.23 and that of vanillin 0.38 [186]. This means that the concentration of 3-(methylthio)propanal has to be over 20-fold and that of vanillin over 6-fold for the sensory intensity to double.

The aroma of food is seldom due to only one key food odorant, but instead a mixture of 3–40 compounds [180]. Furthermore, the mammalian olfaction operates on a combinatorial code: each odorant may activate several odorant receptors and each receptor may bind several different odorants [187–189]. This greatly enhances the functionality of our olfactory system. Using sample odorant mixtures of 10, 20 and 30 components, it has been estimated that human olfaction can discriminate over 10^{10} odors, which is orders of magnitude more than, for example, tones or colors [190]. The contribution of individual compounds to the general aroma is very difficult to study from the base matrix. This is because the human olfactory system can only extract a few components in a complex odor mixture [191,192]. Finally, while odors can trigger powerful memories [193,194], naming odors is typically challenging but improved through training [195–197]. All these features of olfaction and odorants make the analysis of food aroma extremely challenging. In the following subsections, the common findings of volatile and odor-contributing volatile compounds in mushrooms are overviewed.

2.5.2 Volatile compounds in mushrooms

The volatile compounds in mushrooms and mushroom products have received considerable research interest. The following is a short summary of the main studies in the last 50 years of research. Cronin and Ward [198] in 1971 reported 14 different volatile compounds such as 1-octen-3-ol and 1-octen-3-one, 3-methylbutanal, 3-octanol, 3-octanone and benzaldehyde in *Agaricus bisporus*. Similar results were reported by Picardi and Issenberg [199] in 1973. Thomas [200] studied *Boletus edulis* in 1973 and reported several N-heterocyclic compounds such as pyrazines and pyrroles, aromatic compounds such as vanillin and eugenol, and O-heterocyclic compounds such as lactones in addition to the aliphatic carbonyl compounds and alcohols. Pyysalo [15] analyzed the volatiles of seven Finnish edible mushroom species in 1976. 1-octen-3-ol was the most abundant compound with a 33–90% proportion of all volatiles, followed with (*E*)-2-octen-1-ol, 3-methylbutanol, 3-methylbutanal, 3-octanol and (*E*)-2-octenal. By the time Maga [201] published his review on mushroom aroma in 1981, close to 150 volatiles had been identified in mushrooms. Of these, especially the 8-carbon compounds listed above were considered to be the typical volatiles in mushrooms.

Moving forward in time, *Agaricus bisporus* has continued to be widely studied [202–208]. *Boletus edulis* has been featured in six studies in the last 15 years [41,80,205,209–211], *Cantharellus cibarius* in four studies [33,41,205,212], and *Tricholoma matsutake* in three studies [34,37,38] due to a specific South Korean

PhD thesis [43]. *Pleurodotus ostreatus* [204,209,213] and *Lentinula edodes* [39,211,214] have both been examined in three studies. Volatile compound analyses have also been reported of *Craterellus*, *Suillus*, *Lactarius*, *Russula, Hydnum* and other edible mushroom species [41,204,212,215–219] .

Typically the analysis of mushroom volatiles has focused on one or a few species but there are some larger comparative studies as well [41,205,211,212,216,220,221]. In recent studies, the number of detected and identified volatile compounds has ranged from 15–69 per species. While there are major differences in volatile compound profiles between species, the general distribution of volatiles in mushrooms is quite similar. Aliphatic alcohols and carbonyl compounds form the majority of volatiles, indicating that fatty acids are the main precursors for mushroom volatiles [180]. Other major compound groups are monoterpenes and their derivatives, phenyl-substituted carbonyls and alcohols, esters and N-heterocyclic compounds.

Storage conditions and time as well as drying and processing parameters cause major differences in the volatile profiles. Drying resulted in the of severe loss of alcohols, aldehydes and ketones in *Boletus edulis* [36]. During the storage of the same species, alcohol, carbonyl, monoterpene, and sulfur compound contents decreased. At the same time the pyrazine, lactone, and volatile acid contents increase during the first 10 weeks, especially above storage temperature of 20°C [210]. The 1-octen-3-ol level has been reported to decrease down to 20% after 8 storage days of fresh *Agaricus bisporus* [222]. Cooked *Agaricus bisporus* had 2–3 orders of magnitude less of 1-octen-3-ol and 1-octen-3-one than raw samples [202], and similar findings are reported for cooked *Tricholoma matsutake* [38]. Drying methods had similar effects on *Cantharellus cibarius*, *Boletus edulis*, and *Lentinula edodes* [33,39,80]: freeze-drying retained the most volatiles compared to fresh samples, while vacuum microwave dried samples had both lower contents of volatiles and a significantly altered distribution of these compounds.

Finally, it should be mentioned that the volatiles produced in mushroom basidiocarps are generally very similar to the fungal volatiles produced by molds, especially *Aspergillus* species [223–227]. This points to the highly conserved nature of fungal volatile biosynthesis.

2.5.3 Odor-contributing volatile compounds in mushrooms

Only a small subset of all volatile compounds reported in mushrooms have been determined to contribute to the odor. Based on gas chromatography-olfactometry studies, different unsaturated aldehydes, alcohols, and ketones are the most common findings [15,34,36,38,202,203,209,215,228–230]. Additionally pyrazines and other N-heterocyclic compounds as well as certain terpenoids and 3-(methylthio)propanal are often reported. The names and descriptions of typical compounds are presented in **Table 10** and their chemical structures in **Figure 5**. 1-octen-3-ol has been dubbed the "mushroom alcohol" [205] and has received special interest. 1-octen-3-ol is a chiral compound that has two enantiomers, (*R*)-(–)-1-octen-3-ol and (*S*)-(+)-1-octen-3-ol [231]. The (R)-(–) isomer is far more abundant in mushrooms, ranging from a proportion of 82% to over 98% [232].

Table 10. Descriptions of common odor-contributing volatile compounds in mushrooms. The descriptions have been obtained via GC-olfactometry studies of mushrooms.

Compound	Precursor	Descriptions	Ref
1-Octen-3-ol	FA [a]	(Raw) mushroom	34,38,198,202,209,228–230
1-Octen-3-one	FA	Mushroom, metallic	34,36,38,198,202,203,209,228
Hexanal	FA	Green, grass	34,38,203,209,228,229
(*E*)-2-Octenal	FA	Oily, fatty, green, sweet, cucumber	36,38,203,228,229
(*E*)-2-Nonenal	FA	Green, fatty, cucumber	36,221
3-Octanol	FA	(Boiled) mushroom, butter	34,209
(*E,E*)-2,4-Nonadienal	FA	Fatty, green	36,209
(*E,E*)-2,4-Decadienal	FA	Deep-fried, rancid	36,209
3-(Methylthio)propanal	AA	Boiled/cooked potato	34,36,38,202,209,230
Octanal	FA	Cooked mushroom	209
Nonanal	FA	Fat, soap	34
Linalool	ISOP	Citrus, lavender	34,36,38,229
Ethyl 2-methylbutanoate	AA	Floral, sweet	38
3-Methylbutanal	AA	Malt	36,202
2-Acetyl-1-pyrroline	CHO, AA	Popcorn	202
Phenylacetaldehyde	AA	Floral, honey	34,36,38,203,230
2,5-Dimethylpyrazine	CHO, AA	Roasted	36
2,3-Diethyl-5-methylpyrazine	CHO, AA	Earthy, roasted	36,202

[a] FA: fatty acid, AA: amino acid, CHO: carbohydrate, ISOP: isoprenoid. Precursor molecules from references [180,233–236].

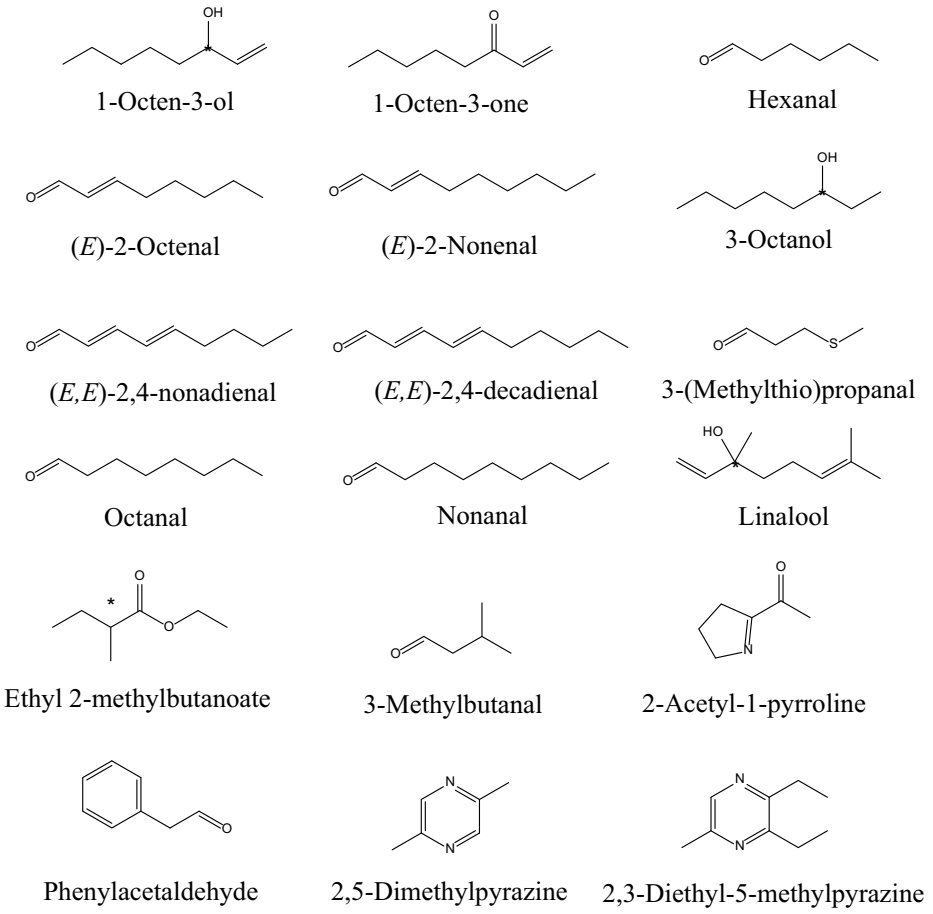

Figure 5. Common odor-contributing volatile compounds in mushrooms. Chiral carbons are marked with an asterisk (*).

As **Table 10** demonstrates, the most typical precursors for the common odor-contributing volatile compounds in mushrooms are fatty acids. More specifically, linoleic acid is the main precursor. As overviewed in section 2.4.5, linoleic acid along with oleic acid is the most common fatty acid in mushrooms. The formation of compounds typically proceeds via enzyme-assisted or auto- and photo-oxidation-mediated lipid oxidation [234,237]. Among the aroma compounds in mushrooms formed from linoleic acid, the pathway for 1-octen-3-ol has been studied the most extensively (see [238] for a review on the topic). First, linoleic acid is cleaved by a lipoxygenase to a fungal-specific 10-hydroperoxide intermediate. The 10-hydroperoxide then undergoes homolytic cleavage by a 10-hydroperoxide lyase into 1-octen-3-ol and 10-oxo-(*E)-8*-decenoic acid [239–241]. This reaction is considered to be part of the fungal injury response system [242], as the levels of 1-octen-3-ol increase as a result of crushing [243] and further increase by the degree of homogenization [206]. The other

cleavage product 10-oxodecanoic acid is also known to induce mushroom basidiocarp growth [238]. In autoxidation and photo-oxidation, linoleic acid first forms mostly 9- and 13-hydroperoxides. These degrade further via β-scission into various compounds depending on the hydroperoxide [234,237]. **Figure 6** overviews some of the pathways of linoleic acid derived aroma compounds.

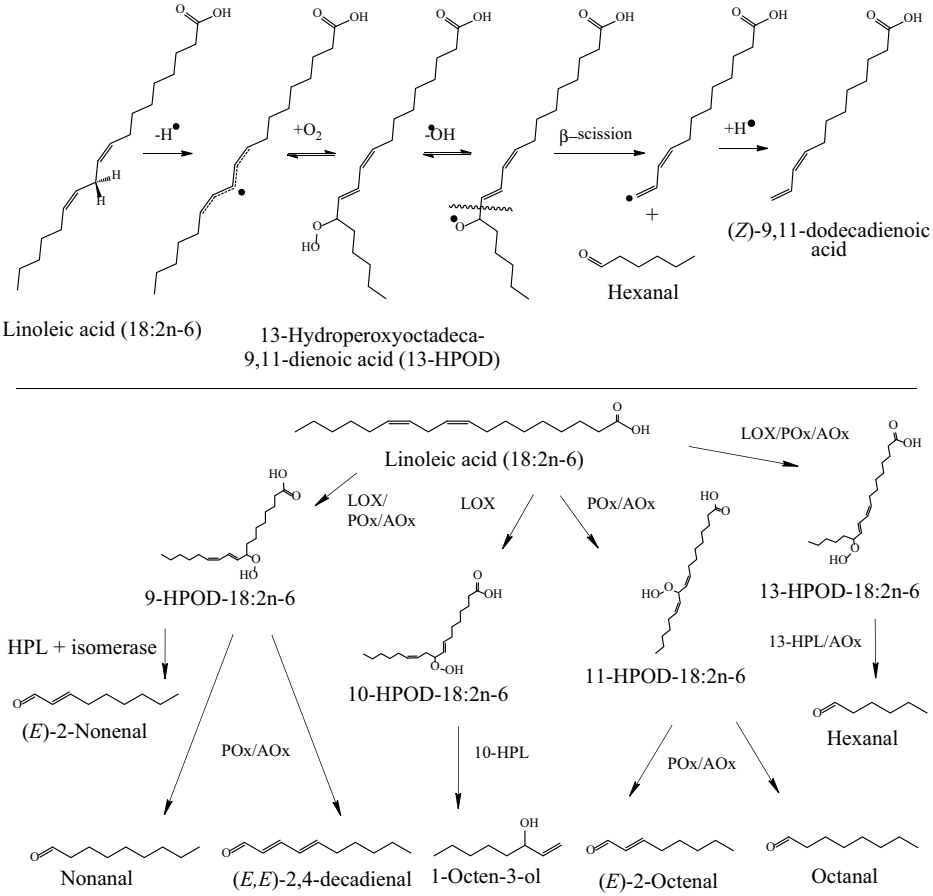

Figure 6. Suggested formation pathways for common odor-contributing volatile compounds present in mushrooms. The autoxidation of linoleic acid via a 13-hydroperoxide intermediate and β-scission into hexanal is shown as an example of the whole pathway. LOX: lipoxygenase, HPOD: hydrogen peroxide, HPL: hydroperoxide lyase, Pox: photo-oxidation, Aox: autoxidation. Adapted and redrawn from references [233–238,240,244].

Aroma compounds from amino acids are typically formed via Strecker degradation. Some of these typical formation pathways are overviewed in **Figure 7**. However, it is important to note that these are not the only possible pathways. For example, 3-methylbutanal can also be also formed enzymatically from L-leucine via an α-ketoisocaproic acid intermediate and from L-valine via

a different pathway [233,245]. The Maillard reaction, on the other hand, can produce various aroma compounds from amino acid and sugar precursors. These include dimethylpyrazines from the reactions between a sugar and glutamine, serine or threonine [246,247], and 2-acetyl-1-pyrroline from a mixture of D-glucose and L-proline [248].

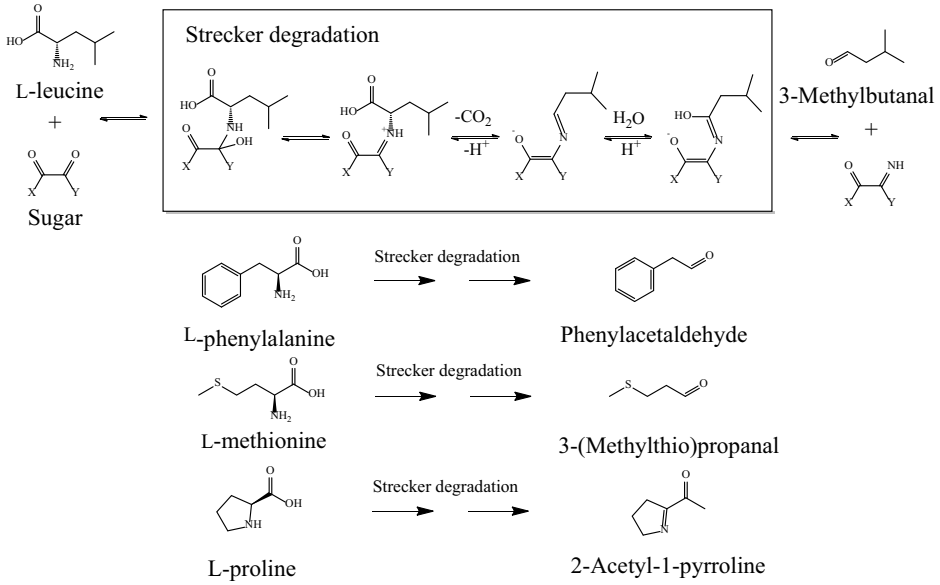

Figure 7. Suggested formation pathways for Strecker degradation-derived common odor-contributing volatile compounds present in mushrooms. The Strecker degradation of L-leucine is shown as a model for the degradation pathway for amino acids. Adapted and redrawn from references [180,236,244,245,249].

1-octen-3-ol and 1-octen-3-one have been studied extensively also in their relative contributions to mushroom aroma. Starting with 1-octen-3-ol, the naturally more common (*R*)-(–)-1-octen-3-ol has about a 30% lower recognition threshold concentration than the (*S*)-(+) isomer [157], which is also more herbaceous in its odor description [231]. However, both Thomas [200] in 1973 and later Pyysalo and Suihko [250] in 1976 reported that 1-octen-3-one in turn has a 2.5–10 times lower threshold than 1-octen-3-ol. This offsets the 10–1000 times higher content of 1-octen-3-ol in mushrooms [94,250]. Fischer and Grosch [228] concluded that it is the ketone, not the alcohol that has the greater contribution to the mushroom-like odor. The odor descriptions of these two compounds additionally depend on their concentration; especially 1-octen-3-one has a metallic side note as the concentration increases [200,250].

Pyysalo [15] and later Buchbauer [251] postulated that while the two compounds above form the typical odor of mushrooms, the other volatile compounds are responsible for the species-specific odors. Some volatiles that have been linked

to species-specific odor attributes in mushrooms are the cucumber-like odor of (*E*)-2-nonenal [221], anise-like *p*-anisate [229], and boiled potato-like 3-(methylthio)-propanal [36]. In aroma recombination studies, the aroma of *Boletus edulis* has been replicated with 12 compounds for raw samples and 20 compounds for cooked samples [36]. Similarly the aroma of cooked *Agaricus bisporus* has been reconstituted with 13 compounds for fried and 10 compounds for the raw sample [202].

The volatile compound profile changes caused by cooking and drying are also reflected in their contribution to the odor of mushrooms. Maga [94] linked the decrease in the 1-octen-3-ol/1-octen-3-one ratio of longer stored samples of *Agaricus bisporus* to lower hedonic liking. He also concluded that 1-octen-3-ol is associated with a desirable mushroom aroma while 1-octen-3-one is associated with undesirable mushroom aroma. Grosshauser and Schieberle [202] concluded in their study that 1-octen-3-ol and 1-octen-3-one make a minimal contribution to the cooked mushroom aroma. On the other hand, 3-methylbutanal, 3-(methylthio)propanal, 2-acetyl-1-pyrroline and different pyrazines contribute to the odor of cooked mushrooms. Cho et al. [38] reported similar changes in cooked *Tricholoma matsutake*: 1-octen-3-one had 75% lower flavor dilution values, while the flavor dilution values of 3-(methylthio)propanal, linalool, and 2-acetylthiazole increased. Zhang et al. [36] found that drying of *Boletus edulis* similarly resulted in the drastically smaller contributions of 1-octen-3-ol and 1-octen3-one, (*E*)-2-octenal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal but major increases in the contributions of 3-(methylthio)propanal and several pyrazines and aromatic compounds.

2.6 Analysis parameters of aroma compounds in mushrooms

According to Molyneux and Schieberle [252], the identification of aroma identification should proceed in five steps. First, the sample should be analyzed with two gas compounds in food is often prone to errors. Their recommendation is that chromatography columns that have different polarities in their stationary phases. This should be supplemented with mass spectrometer analysis. The resulting data of retention indices and mass spectra should be compared to those of reference compounds or published values. If the compounds of interest are chiral, both enantiomers should be analyzed next. Only such compounds that have odor activities as determined with gas chromatography–olfactometry (GC-O) should be the interest of identification. As aroma compounds have typically unique odor descriptions and often several orders of magnitude different odor thresholds, this information can be used as

another source of filtering information. Finally, for new structures, the tentatively identified compounds should be isolated, synthesized and their spectra should be compared with NMR.

The published analyses of mushroom aroma often make shortcuts on these principles especially in the utilization of GC-O or other sensory data. Several publications report the study as an aroma or sensory study even though the actual analysis has only been on the volatiles content [205,213,219,243,253] and optionally with an electronic nose [163,207]. The typical analysis parameters of aroma compounds in mushrooms are examined against this framework in the following subsections.

2.6.1 Extraction and gas chromatography parameters

The published gas chromatography analysis parameters for the volatiles in mushrooms are overviewed in **Table 11**. In terms of extraction methods, different solvent extractions as well as distillation based methods such as simultaneous distillation and extraction (SDE) and solvent assisted flavor evaporation (SAFE) have been the most common extraction methods with a subsequent concentration of the extract. Dichloromethane, diethyl ether, ethyl ether and pentane have been the most common solvents. Dynamic and static headspace extractions have been used as supplementary methods to examine the most volatile compounds. In the last 10 years, headspace-solid phase microextraction (HS-SPME) has become increasingly popular.

In terms of separating columns, mainly three types of stationary phases are used. DB-Wax type (polyethylene glycol) columns are the most typical polar columns, followed with DB-FFAP type (nitroterephthalic acid modified polyethylene glycol) columns. DB-5 type ((5%-Phenyl)-methylpolysiloxane) columns are almost exclusively used as nonpolar columns. 30 and 60 meter lengths are the most typical, with 0.25 mm inner diameter being the most common. The majority of these studies have used both polar and nonpolar columns in their analyses. There are two main benefits of using two columns with different polarities. First, having two detections improves the statistical reliability of the detection, especially when identification relies on retention index windows and authentic standards are not available. Secondly, the risk of coelution is lower. For example, 1-octen-3-ol and 1-octen-3-one have almost complete coelution on DB-5 type columns but separate very well on DB-Wax type column, while the inverse is true for the pair 3-(methylthio)propanal and 2-ethyl-3,6-dimethylpyrazine [36].

Table 11. Analysis parameters in mushroom volatiles research arranged by column type, year and authors. Studies that have used both column types are presented twice in the table.

Year	Species (N) [a]	Treatment	Extraction method [b]	GC column type	Column dimensions	Oven temperature range (°C)	MS range (m/z)	Ref
Polar columns								
1973	a (1)	Fresh and cooked	SDE (hexane), c(N_2)	Carbowax 20M	150×0.5×N/A	110–110	4–400	[199]
1976	b (7)	Fresh, freezing	Steam distillation, ether-pentane extraction, concentration	FFAP	90×N/A×N/A	60–200	-	[15]
1987	a (1)	Fresh	Vacuum distillation, Et_2O-pentane extraction, c(Vigreux)	Supelcowax 10	30×0.32×N/A	35–190	-	[228]
1997	o (2)	Blanching	Et_2O, c(Vigreux) + SHS	DB-FFAP	30×0.32×N/A	32–250	N/A	[215]
1999	a, g, o (3)	Fresh	SDE	PE-Wax	30×0.25×N/A	60–200	-	[204]
2006-2008	d (1)	Freezing, cooking	DCM, HVS, c(Vigreux), fractionation	DB-Wax	60×0.25×0.25	40–200	33–550	[34,37,38,254]
2008	b, c, i, o (11)	Drying	HS-SPME	Stabilwax-DA	60×0.25×0.25	40–220	50–600	[41]
2011	a (1)	Drying, boiling with water	HS-SPME	DB-Wax	30×0.25×0.25	40–230	N/A	[203]
2013	a (1)	Fresh, frying	DCM + SAFE, c(Vigreux)	DB-FFAP	30×0.32×0.25	40–230	-	[202]
2014	n (1)	Fresh, freeze-drying	HS-SPME	DB-FFAP	30×0.25×0.25	50–200	N/A	[253]
2014	o (1)	Freezing	Methanol, pentane-Et_2O + SAFE, c(Vigreux)	VF-Wax MS	30×0.25×0.25	40–240	33–300	[229]

Year	Species (N) [a]	Treatment	Extraction method [b]	GC column type	Column dimensions	Oven temperature range (°C)	MS range (m/z)	Ref
2014	o (1)	Fresh	SDE(Et_2O, DCM) + SAFE, concentration	DB-Wax	30×0.25×0.25	40–260	39–450	230
2015	b (1)	Drying	HS-SPME	HP-Innowax	30×0.32×0.5	40–250	35–300	210
2015	b, f, o (8)	Drying	Ethyl ether extraction, concentration	HP-Innowax	60×0.25×0.25	40–220	40–400	211
2016	f (1)	Drying	HS-SPME	CP Wax 52 CB	50×0.32×1.2	50–230	N/A	214
2017	a, b, c, o (4)	Fresh	SDE (pentane)	AT-Wax	60×0.25×0.25	60–280	N/A	205
2017	n (7)	Fresh	HS-SPME	SP-2560	N/A×0.25×0.2	40–200	33–350	220
Nonpolar columns								
1994	o (7)	Fresh	DCM, c(N_2)	Methyl silicone	12×N/A×N/A	40–250	N/A	221
1997	o (2)	Blanching	Et_2O, c(Vigreux) + static headspace	SE-54	30×0.32×N/A	32–250	N/A	215
1997	i, o (14)	Fresh	DCM	DB-1	25×0.25×0.25	60–200	N/A	216
1997	o (1)	Fresh	DCM + DHS (Tenax)	BP-1	50×0.22×1	50–220	N/A	217
1999	a, g, o (3)	Fresh	SDE	PE-1	30×0.25×N/A	60–200	-	204
2000	f (1)	Fresh, crushing	HS-SPME	DB-1	60×0.25×1.00	40–200	N/A	243
2000	h (1)	Drying	Et_2O, c(N_2)	Optima 5	25×0.2×0.13	50–200	N/A	218
2003	c, e, o (4)	Fresh	Ethyl ether extraction, c(N_2)	Optima 5	25×0.2×0.13	50–200	-	212
2003	m (1)	Fresh	HS-SPME, SDE	MDN-5	30×0.25×0.25	40–280	-	223
2006-2008	d (1)	Freezing, cooking	DCM, HVS, c(Vigreux), fractionation	DB-5 MS	30×0.25×0.25	40–200	33–550	34,38,254
2008	b, c, i, o (11)	Drying	DCM + c(N_2) + HS-SPME	VF-5 MS	30×0.25×0.25	40–220	50–600, 33–350	41

Year	Species (N) [a]	Treatment	Extraction method [b]	GC column type	Column dimensions	Oven temperature range (°C)	MS range (m/z)	Ref
2009	b, g (2)	Fresh, autoclaving	Et_2O, c(Vigreux)	SPB-1	50×0.32×0.25	60–250	-	[209]
2013	a (1)	Fresh	HS-SPME	SLB-5ms	30×0.25×0.25	40–250	40–400	[206]
2013	a (1)	Fresh, frying	DCM + SAFE, c(Vigreux)	DB-5	30×0.32×0.25	40–240	-	[202]
2014	o (1)	Freezing	Methanol, pentane-Et_2O + SAFE, c(Vigreux)	DB-5 MS	30×0.25×0.25	40–240	33–300	[229]
2014	o (1)	Freezing	SDE(Et_2O, DCM) + SAFE, concentration	HP-5 MS	30×0.25×0.25	40–260	39–450	[230]
2016	a (1)	Drying	HS-SPME	DB-5 MS	30×0.25×0.25	N/A–N/A	-	[207]
2017, 2018	c, f (2)	Drying, freeze-drying	HS-SPME	Elite-5 MS	30×0.25×0.25	60–300	35–550	[33,39]
2017	o (1)	Freezing	HS-SPME	HP-5	30×0.25×0.25	50–230	40–280	[219]
2018	g, o (6)	Drying	HS-SPME	HP-5 MS	30×0.25×0.25	40–180	50–550	[213]
Others								
1973	b (1)	Drying	Pentane extract	Self-made	33×0.3×N/A	60–180	-	[200]
2012	h (1)	Drying	HS-SPME	N/A	N/A	N/A	N/A	[255]

[a] a: Agaricus bisporus, b: Boletus edulis, c: Cantharellus cibarius, d: Tricholoma matsutake, e: Craterellus tubaeformis, f: Lentinula edodes, g: Pleurodotus ostreatus, h: Lactarius species, i: Suillus species, o: other wild mushrooms, n: nonedible species, m: fungal microbes

[b] HS-SPME: headspace solid-phase microextraction, SAFE: solvent assisted flavor evaporation, HVS: high vacuum sublimation, SDE: simultaneous distillation and extraction, DCM: dichrolomethane extraction, Et_2O: diethyl ether extraction, DHS: dynamic headspace, c(N_2): concentration under a nitrogen flow, c(Vigreux): concentration on a Vigreux column

2.6.2 HS-SPME parameters

Solid-phase microextraction is an equilibrium method in which several parameters such as fiber coating, headspace volume, and extraction temperature affect the extracted volatile compound profile [256]. This means that while HS-SPME is a very convenient tool for the analysis of volatile compounds, the extracted profile is more of an impression created by the fiber than an accurate representation of actual sample headspace content [257]. Since the creation of the method over 20 years ago [258], there have been numerous studies that have optimized the SPME parameters for various matrices. The general recommendations on the main extraction parameters are overviewed in **Table 12**.

Typically SPME method optimization has been done with univariate experimental designs [256]. In others words, one parameter is varied at a time (see [253] for an optimization example in mushrooms). However, this kind of design eliminates the examination of parameter interactions such as the interaction of extraction temperature and sample headspace volume [259,260]. Thus, a multivariate experimental design such as factorial design [261] and simplex optimization [262] would be faster and more efficient. Another simplification is that often extraction parameters are optimized with small volumes of volatile compound mixtures which evaporate quickly in the sample vial and effectively create a two-phase system (air and the SPME fiber) [259,260]. However, typical mushroom samples have been either dried powders or made into aqueous solutions which results in much more complex extraction kinetics [259,260].

Headspace-solid phase microextraction parameters reported in mushroom volatiles analysis have been overviewed in **Table 13**. The most common fiber type is the divinylbenzene/Carboxen/polydimethylsiloxane fiber. The fiber length is not always specified, but 1 cm and 2 cm versions are used equally often. Extraction and desorption parameters vary significantly between studies. Reported equilibrium times vary from 3 to 60 min while a 30 min extraction time is the most typical. Temperatures over ambient temperature are used for extraction, with the majority of the literature reporting 40–60 °C temperatures. Three studies have added salt in the sample mixture to promote the release of volatiles from the sample matrix. Only two [206,223] of the 12 studies overviewed in **Table 13** specify the used liner type to be a 0.75 mm ID SPME liner. Desorption times range 1–10 min, while a 250 °C desorption temperature in the injector is the most typical.

Table 12. General recommendations for HS-SPME analysis parameters.

Parameter	Recommendation	Ref
Fiber coating	Depends on volatile profile; DVB/Car/PDMS has a wide extraction range and is typical for food aroma	[263]
Agitation	Mixing the sample generally results in faster equilibrium; not feasible for solid or semisolid samples	[256,264]
Ionic strength	Adding salt such as NaCl typically improves sensitivity	[256]
Sample mass	Larger mass increases extracted amount, but limited by fiber capacity and affected by fiber-sample distribution constant K	[256,259,260]
Headspace-sample volume ratio	Less headspace results in higher signal; effect size diminishes as K gets smaller	[259,260]
Vial size	Headspace capacity should be at least 20 times the fiber capacity, which places a lower limit on vial size; interactions with extraction temperature, sample mass	[259]
Equilibrium time	Shortens extraction time, improves repeatability	[256,257]
Extraction temperature	Generally above ambient temperature results in faster extraction; changes extracted profile	[256,259,260]
Extraction time	Longer time increases extracted amount, but has to be repeatable; extracting lower concentration of volatile compounds require more time; interactions with all above parameters	[256,259,260,265]
Fiber depth in the injector	Fiber should be in the middle of the injector; closer proximity to the GC column is beneficial	[256,266]
Desorption temperature	Generally higher is more efficient, limited by compound and fibre thermostability	[256,267,268]
Carrier gas velocity during desorption	Higher linear flow rate is more efficient	[256,266,267]
Liner type	Low-diameter (down to 0.75 mm) is ideal, increases linear flow rate and thus desorption efficiency	[256,266–268]
Desorption time	Depends on above parameters, typically 0.5 to 10 minutes	[267]
Focusing of compounds at the GC column	Low starting oven temperature and a thick-film column are beneficial	[256,257,268,269]

Table 13. SPME extraction conditions in mushroom volatiles research.

Year	Species (N) [a]	Sample size [b]	Vial size (mL)	SPME fiber (length) [c]	Equilibrium + extraction time (min), temperature (°C)	Desorption time (min) and temperature (°C)	Ref
2003	m (1)	20 g inoculated medium	100	Car/PDMS	N/A + 20, 50	260, N/A	223
2008	b, c, i, o (11)	25 mg powder	15	DVB/Car/PDMS	30 + 15, 35	220, 10	41
2011	a (1)	5 mL mushroom soup + istd	15	Car/PDMS	N/A + 30, 50	250, N/A	203
2013	a (1)	100 mg powder:H_2O:peanut oil mixture (2:1:1)	10	PDMS/DVB (1 cm)	N/A + 20, 50	250, 1	206
2014	n (1)	10 mg powder	10	DVB/Car/PDMS	5 + 40, 50	250, 10	253
2015	b (1)	1 g powder	20	DVB/Car/PDMS (2 cm)	30 + 30, 37	250, N/A	210
2016	a (1)	Not specified	20	DVB/Car/PDMS	N/A + 40, 60	250, 5	207
2016	f (1)	3 g of dried mushroom	15	DVB/Car/PDMS	15 + 30, 40	250, 10	214
2017	n (7)	10 g fresh mushroom	100	DVB/Car/PDMS (2 cm)	60 + 30, 25	250, 1	220
2017	o (1)	20 mg powder + NaCl (aq) + istd	20	DVB/Car/PDMS (1 cm)	3 + 30, 45	250, 5	219
2017, 2018	c, f (2)	5 g homogenized mushroom + NaCl + istd	40	DVB/Car/PDMS (1 cm)	10 + 40, 60, 25	250, 10	33,39
2018	g, o (6)	2 g powder, + NaCl + H_2O	40	DVB/Car/PDMS	20 + 35, 60	250, N/A	213

[a] Same species coding as in **Table 11**

[b] istd: internal standard

[c] Car/PDMS: carboxen/polydimethylsiloxane (75 µm thickness), PDMS/DVB: polydimethylsiloxane/divinylbenzene (65 µm thickness), DVB/Car/PDMS: divinylbenzene/carboxen/polydimethylsiloxane (50/30 µm thickness)

2.6.3 Gas chromatography-olfactometry parameters

Gas chromatograpy–olfactometry can infer both qualitative and quantitative information about the odor impact of each volatile compound in a given sample matrix. Different GC-O techniques emphasize different aspects. In general, there are three main method categories: dilution to threshold methods, detection frequency, and direct intensity methods [184,270,271]. In dilution to threshold methods such as the aroma extract dilution analysis (AEDA) the increasingly diluted aroma extract is repeatedly evaluated by trained assessors. As a result, flavor dilution factors that indicate the maximal dilution where the odor is still detectable are obtained for each odor-contributing compound in the extract. As the dilution set results in 5–15 evaluations for a single sample and one assessor, the number of assessors is typically limited to one or two. In detection frequency (DF) methods such as the GC-sniffing method introduced by Pollien et al. [272], a panel of typically 6–12 assessors each evaluate the sample in turn. The nasal impact frequency (NIF) value, in other words the proportion of assessors that detected each compound (0–100%) is used to estimate the odor impact. The individual odor impression durations can further be included in data analysis by integrating the peak areas in the GC-O aromagram; the odor peak areas are called the surface of the nasal impact frequency (SNIF). In direct intensity methods, either the intensity of the eluate is evaluated continuously (the Osme method) or the maximum intensity of each peak is reported (the posterior intensity method, PI). In addition, hybrid methods are used such as the modified frequency method that utilizes aspects of both detection frequency and posterior intensity methods [273]. With all of these methods the individual variation not only in the assessors' sensory sensitivity, but also the differences in their processing of possible signals [274] affects which compounds are detected.

According to the 11 studies of mushroom GC-olfactometry overviewed in **Table 14**, AEDA has been the most typical method for the analysis of odor-contributing volatiles in mushrooms. There are several aspects to be discussed regarding the published studies, the primary aspect being the selection of the GC-O method. While AEDA is the golden standard especially in the German aroma chemistry workflow [275], it has some drawbacks. First, AEDA evaluations only record the retention time and description of the odor impression, but not their duration [184]. Second, the intensity evaluation is based on detection thresholds instead of actual perceived odor intensities [184,276]. Third, the common assumption is that potency increases equally with dilution factors and concentrations for all compounds; this does not take into account the differing Stevens' power law exponents [184,277]. Finally, as the analysis setup is

laborious and only one or two assessors are usually utilized, there is a risk of anosmia or hyposmia for the compounds of interest [184].

Table 14. GC-olfactometry methods used in mushroom aroma research.

Year	Species (N) [a]	GC-O method	GC-O/FID (or MS) split	Assessors	Assessor experience or training	Ref
1976	b (7)	Not mentioned	1:1	4	No	[15]
1987	a (1)	AEDA	1:1	N/A	N/A	[228]
1997	o (1)	Le champ des odeurs (description reference)	7:3	3	N/A	[217]
1999	a, g, o (3)	Not mentioned	1:1	N/A	No	[204]
2006	d (1)	AEDA, 1+2 dilution steps	1:1	2	Over 30 h of experience	[38]
2007	d (1)	AEDA, 1+1 dilution steps	1:1	2	Over 30 h of experience	[34]
2009	b, g (2)	"sniffing analysis"	1:1	3	"trained"	[209]
2011	a (1)	AEDA	1:1	3	trained according to [278]	[203]
2013	a (1)	AEDA	1:1	3	"experienced"	[202]
2014	o (1)	AEDA, 1+1 dilution steps	1:1	N/A	N/A	[230]
2014	o (1)	AEDA, 1+1 dilution steps	1:1	5	N/A	[229]

[a] Same species coding as in **Table 11**

An alternative would be a detection frequency type GC-O. This method is quite robust as it is repeatable [279] even with untrained panels if no specific odor expressions are required [272]. The DF method is also simpler to set up [271], takes into account the differences in sensitivity between the assessors [270], and because of this has been shown to discriminate between concentration levels [272,279]. The drawbacks of DF are that it does not differentiate between compounds that are above the detection thresholds for all assessors in the panel [270,271] and may overestimate the importance of coeluting peaks especially if peak areas (SNIF values) are used [184]. Direct intensity methods have been reported to discriminate between compounds better than DF [279] and as an experimental setup are closer to the evaluation of the actual psychophysical function [184]. On the other hand, the GC-O evaluation with direct intensity methods is more challenging for the assessor as he/she is required to do three things at the same time: to detect the presence, to evaluate the intensity, and to

describe the odor quality of each compound. The results of the direct intensity and DF methods correlate with each other [279,280]. In contrast, Culleré et al. [273] reported that in truffles, there were relevant discrepancies between AEDA and modified frequency; each method emphasized different types of compounds. They postulated that the main reasons are the different sample preparations they used and the selection bias that AEDA has due to the small number of assessors. It seems that the best correlation between sensory perception and instrumental methods would be achieved by a combination of direct intensity-based GC-O methods such as PI or Osme and determination of the Stevens' power law exponents of individual compounds [281]. However, this work is extremely laborious and has not been done with mushrooms so far.

The second aspect is the selection of dilution steps and the number of assessors in AEDA. The studies overviewed in **Table 14** report either 1+1 or 1+2 dilutions and 2–4 assessors, as is typical for AEDA in general. Ferreira et al. [282] argue in their general AEDA optimization paper that this setting has both an inherent bias in the dilution factor and is also very laborious. However, they point out that this nonstatistical examination has only resulted in a small number of consequences as AEDA has been the screening method, not the final result of the study. Ferreira et al. further proposed that based on statistical theory and experimental results, there could be a more economic and precise setup that would also follow standard sensory practices more closely. In this setup, the number of assessors is increased, the dilution steps are increased to e.g. 1+4, and the flavor dilution (FD) value is calculated as a geometric mean of individual FD values. So far however, this recommendation has not been followed in mushroom aroma research employing AEDA.

The third aspect is on training. Information related to training of the assessors is limited or not available at all. Even when training is referenced [203] the source article [278] just covers this with one sentence: "The panelists were trained on a 'flavor language' in several sessions, in which pure reference odorants were evaluated.". Vene et al. [280] have published a GC-O panel training method where they also compared the performance between AEDA, detection frequency (DF) and posterior intensity (PI) type GC-olfactometry. They found the posterior intensity method gave the best results. AEDA had issues of missing detections between dilutions, and the peak widths were difficult to determine accurately in detection frequency. These reasons also indicate the importance of training.

2.7 Summary of the literature review

Edible mushrooms are one of the largest groups of organisms in terms of number of species. A few cultivated species such as *Agaricus bisporus* and *Lentinula edodes* are routinely marketed globally. In Finnish forests, there are dozens of highly appreciated wild species that have a significant yearly harvest. Only a small fraction of this harvest is collected, which makes mushrooms a valuable underutilized natural resource.

The majority of sensory profiling studies for mushrooms have been done in the last 15 years. None of these studies have been made in Finland or even using Nordic mushroom samples. Many of these studies have not fully followed standard sensory practices or at least left out details such as training, reference products or statistical tests. Likewise, hedonic testing of mushrooms or mushroom-containing products has gained research interest mainly in the last 15 years. The main area where the methodology of these studies has been lacking is the number of consumer assessors. This limits the applicability of the results. Additionally, hedonic testing has so far focused on cultivated species. There is a need for further comparative examination of the sensory properties of wild mushrooms using modern sensory methods. More detailed hedonic liking studies where consumers would be segmented based on their liking profiles and drivers of liking are also needed. The available publications have limited information on the sensory properties of mushrooms. They do not specify what are the aspects that explain a like or dislike for edible mushrooms.

The main non-volatile compound groups of mushrooms have been studied in several publications. The proximate chemical composition especially, i.e. the sugars, sugar alcohols, organic acids, fatty acids, free amino acids and 5'-nucleotides have been measured in multiple studies. Additionally the phenolic compounds, terpenoids and vitamins have been studied; however, the research interest for these compounds has been in the nutritional, health, and other medical aspects. Among these groups, the effects of glutamic acid, aspartic acid and the 5'-nucleotides GMP and AMP in mushrooms have been linked to umami via sensory measurements. Additionally the sugars and sugar alcohols have been linked to sweetness and certain terpenoids to the intense bitterness of some mushroom species. The contribution of other non-volatile compound groups on the sensory properties has so far mainly been inferred from instrumental measurements.

The volatile compounds in mushrooms have been measured previously. The main mushroom volatiles 1-octen-3-ol and 1-octen-3-one have received special interest. In general, aliphatic alcohols and carbonyl compounds form the majority of volatiles. The other major compound groups are monoterpenes and their derivatives, phenyl-substituted carbonyls and alcohols, esters and N-

heterocyclic compounds. The volatile compound profiles vary significantly between mushroom species, and storage and processing also have major effects on these profiles. Only 10–20 volatile compounds from the total volatiles have been found to significantly contribute to the odor of mushrooms. Two mushroom volatiles form the basis of mushroom-like odor, but other volatile compounds are responsible for the species-specific odors. The main precursors of these volatile compounds are fatty acids and amino acids. The volatile profile changes caused by cooking and drying are also reflected in their contribution to the odor of mushrooms.

The volatile and odor-contributing volatile compounds have been analyzed with various method parameters. Previously, different solvent extractions have been the main sample preparation method, while HS-SPME especially with the DVB/Car/PDMS fiber has recently become more popular. DB-Wax and DB-5 type stationary phase columns are the most common. For GC-O, AEDA has been the most common method. Comparative GC-O studies that use complimentary methods to AEDA are still lacking.

3 AIMS OF THE STUDY

The overall aim of the research was to study the sensory properties of popular Finnish edible wild mushrooms and to examine various compounds that could explain these properties.

The first aim was to develop a sensory lexicon for wild mushrooms and to measure how these mushrooms differ in the intensities of the developed sensory descriptors. (**I)**

The second aim was to optimize a method for the measurement of volatile compounds in wild mushrooms (**II**) and to examine which volatile compounds contribute to the odor of wild mushrooms (**III**).

The third aim was to link the sensory properties to volatile and non-volatile compounds via additional instrumental measurements and to study how the consumer acceptance of mushrooms is linked to both background variables and sensory properties of mushrooms (**IV**).

4 MATERIALS AND METHODS

4.1 Sample material

4.1.1 Preliminary study mushroom samples

Three forest mushrooms and three cultivated mushrooms were used for Projective Mapping, and of these *Cantharellus cibarius* (chanterelle, "keltavahvero") was also used as a sample for solid phase microextraction optimization (**Table 15**).

Table 15. Preliminary mushroom samples in the thesis.

Study	Mushroom species (binomial)	Mushroom species (English)	Location in Finland	Harvest time (year-month)	Fresh mass of the batch (kg)
I, II	*Cantharellus cibarius*	chanterelle	Parainen	2015-09	2.8
I	*Craterellus tubaeformis*	trumpet chanterelle	Salo	2015-10	0.7
I	*Suillus variegatus*	velvet bolete	Turku	2015-09	1.1
I	*Agaricus bisporus* (white)	button mushroom (white)	Mykora Ltd., Eura	2015-11	1.6
I	*Agaricus bisporus* (brown)	button mushroom (brown)			1.6
I	*Lentinula edodes*	shiitake			1.6

The preliminary mushroom samples were blanched by keeping them in boiling water for two minutes with at least a 5:1 water:mushroom ratio (w/w) and drained using a sieve. After cooling down to ambient temperature, the samples were frozen and stored at -20°C until analysis. Study **I** also included fresh (no heat treatment and no freezing) samples of the two *Agaricus bisporus* varieties.

4.1.2 Main mushroom samples

The main experiments of the PhD thesis considered wild edible mushrooms that are popular in Finland. The selected species were chanterelle (*Cantharellus cibarius*, "keltavahvero"), trumpet chanterelle (*Cantharellus tubaeformis*, "suppilovahvero"), porcini (*Boletus edulis*, "herkkutatti") and curry milk cap (*Lactarius camphoratus,* "sikurirousku") and they were collected from different parts of Finland (**Table 16**). The picked mushrooms apart from the

curry milk cap (*Lactarius camphoratus*) used in Study I were stored at +4 °C and processed within 36 hours of picking.

Table 16. Main mushroom samples in the thesis.

Study	Mushroom species (binomial)	Mushroom species (English)	Location in Finland	Harvest time (year-month)	Fresh mass of the batch (kg)
I, III, IV	*Cantharellus cibarius*	chanterelle	Salo	2016-08	3.3
I, III, IV	*Craterellus tubaeformis* [a]	trumpet chanterelle	Kainuu region	2016-09	2.7
			Salo	2016-09	1.0
I, III, IV	*Boletus edulis*	porcini	Köyliö	2016-09	3.4
III, IV	*Lactarius camphoratus*	curry milk cap	Salo	2016-09	0.4
I			Tampere	2016-08	0.14 (DW) [b]
I, IV	*Agaricus bisporus* (white)	button mushroom (white)	Mykora Ltd., Eura	2017-05	2.5

[a] The batches were pooled together
[b] The mushrooms were received as air dried

During the blanching treatment of the preliminary samples it was noted that up to 30% of the mushroom mass was lost as a result of blanching to the cooking medium and air. This loss was also noted as mild flavor properties in the prepared samples. The temperature control was challenging as blanching took place over open pots and the batch size varied. These observations led to the selection of a more controllable heat treatment method for the main samples. *Sous vide* cooking–vacuum packing of food samples in heat-stable food grade plastic pouches and subsequent heat treatment in a typically <100 °C water bath–was determined to fulfil these requirements. The treatment parameters had been optimized for *Agaricus bisporus* samples [162] in a concurrent, separately operated research project "Innovative Technologies and Concepts for Business Growth Based on Finnish Mushrooms" (grant number 3135/31/2015) funded by Business Finland (formerly the Finnish Funding Agency for Innovation). These parameters were adapted for the wild mushroom samples used in this research.

The general scheme of the sample treatment is shown in **Figure 8**. After receiving the samples, the soil material was cleaned off the mushrooms individually with a brush. The mushrooms were manually cut into 1–2 cm slices, and packaged into 16*40 cm size plastic sous vide bags in 200 g aliquots in a single layer. The sous vide bags were vacuum heat sealed with a Supervac

Maschinenbau GmbH (Vienna, Austria) vacuum packaging instrument model GK 113/2. The vacuum level was 7 (range 0−9) and sealing time was 4 (range 0−9). The sealed bags were placed into an 80.0 °C (σ = 0.5 °C) circulating water bath for 10 min (heater P/2 and box 25B, Julabo GmbH, Seelbach, Germany). Each bag was chilled by placing it into a cold water bath (< 20 °C) for 2 min immediately after heat treatment and then into an ice water bath (5–9 °C) for 5 min. After chilling, each bag was immediately frozen at -20 °C.

The mushrooms were cut and pooled after 1–12 weeks of storage. The frozen mushrooms were cut one aliquot at a time at 4 °C with chilled cutting boards and knives to approximately 1–2 cm^3 cubes. Each aliquot was then immediately moved back to -20 °C and pooled. The combined mushroom sample was divided using dimension reduction and cone quartering methods on a large tray and repackaged into plastic bags. The bags were then stored at -20 °C until analysis. During the *sous vide* process, some liquid was dissociated from the mushrooms. This liquid-mushroom volume ratio was retained as well as possible in the sample batches taken from the pooled sample.

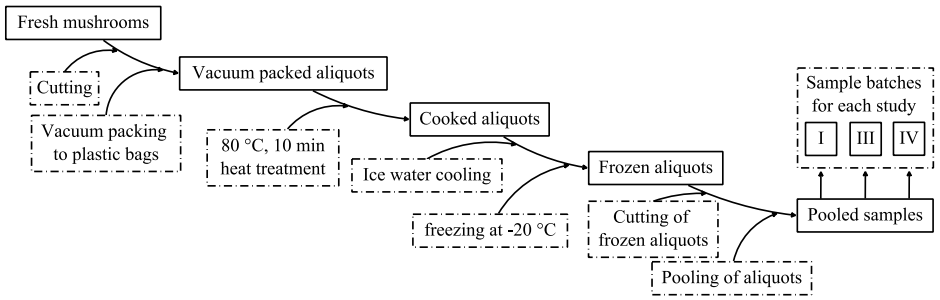

Figure 8. Overall scheme of the sample treatment for the main mushroom samples.

For the *Lactarius camphoratus* samples in Study **I**, the mushrooms were first dried by convectional drying at approximately 36–37 °C for 7–8 hours using an Evermat food dehydrator (Evermat AB, Bjurholm, Sweden) and stored for approximately 10 months at room temperature in an airtight glass jar. After this, the dried mushrooms were rehydrated by adding 700 g of active-carbon filtered water to 100 g dried mushrooms and incubating for 15 minutes at ambient temperature. Finally, the mushrooms were placed in *sous vide* bags and processed further similar to the other samples. This differing preprocessing protocol had to be used due to poor availability of fresh curry milk cap.

4.2 Sensory profiling

All sensory evaluations and the hedonic test (Studies **I** and **IV**) were performed at a sensory laboratory (ISO 8589). Compusense Cloud version 8.4 (Compusense Inc., Guelph, Ontario, Canada) was used for data collection except for Projective Mapping which was done on paper.

4.2.1 Assessors

A total of 52 consumers participated in the Projective Mapping in Study **I**. Only an interest in sensory evaluation was required; no previous experience was needed for participation. The assessors were aged 19–73 (median age 33), and 80% of the assessors were female. The panel size for the Projective Mapping was considered sufficient as it was larger than that typical in similar setups with naïve consumers (19–40) [91,283,284] but smaller than the maximum reported (81–90) [285,286]. The panel for the generic descriptive analysis in Study **I** consisted of 11 voluntary assessors aged 27–49 (median age 38 years). Seven assessors were women and four were men. The assessors were experienced by taking part in several sensory profiling projects. They were known to be able to identify and rank taste solutions, recognize flavor differences, and describe sensory properties of samples with specific sensory terms. Thus the panel size and selection followed the general recommendations for generic descriptive analysis [73].

The panel for the gas chromatography–olfactometry in Study **III** consisted of 15 voluntary assessors aged 25–70 years (median age 30 years). Ten of them had a high level of experience and regular participation in sensory evaluation and the sensory acuity of eight of them had been tested in a sensory laboratory. The other five assessors had less or no previous experience. Four assessors, all with a high level of experience in sensory evaluation further participated in the validative GC-O evalutions.

A total of 84 consumers aged 20–74 years (median age 47 years) participated in the hedonic test in Study **IV**. The participation criteria was the use of mushrooms or mushroom products. Consumers were recruited mainly from the Turku region in Finland. The number of consumers was slightly lower compared to recent sensory studies (41–162 consumers, average 97) conducted with similar setups and various sample matrices [25,28,286–288].

4.2.2 Projective mapping

Projective mapping [86] (Study **I**) coupled with Ultra Flash Profiling was applied based on published methods [25,89,283,289] restricted to one sensory modality at a

time. A3 sized (297 mm × 420 mm) papers were used and the sample order was randomized for each assessor. The evaluation had nine samples in total: the six blanched samples with *Cantharellus cibarius* in duplicate, and fresh samples of the two *Agaricus bisporus* varieties. All samples were presented simultaneously at an ambient temperature in 50 ml covered beakers. The evaluators were asked to create two projective maps. In the first one, the assessors were asked to smell the samples and create the map using odor impressions. If the assessor was also willing to taste the samples, a new A3 paper was given. The assessor was instructed to now taste the samples and use these properties for the second map. Free use of non-hedonic attributes was encouraged in the classification. In both parts, the assessors were asked to write notes on a blank sheet of A5 (for their own use) describing each sample. Instructions for palate cleansing was given according to standard sensory practices. When the assessor was ready with the positioning, they were asked to mark the sample positions with a small cross and the sample code, to mark possible groups, and to write descriptors next to the samples on the sheet. The collected evaluation papers were scanned and the coordinates of each evaluated sample were measured with ImageJ software [290]. The coordinates and descriptor frequencies were determined following published protocols [25,89,283,289].

4.2.3 Generic descriptive analysis

Samples (10–15 g) of the five mushroom species (**Table 16**) containing both solid mushrooms and dissociated liquids in representative mass ratios to the freshly cooked aliquots were used in the evaluations (Study **I**). They were served at 50–60 °C in 70 ml covered glass bowls and coded with three-digit numbers that were changed in each session.

Training for the generic descriptive analysis consisted of four 1½ hour sessions. It was conducted as consensus training with an additional blind training session simulating the actual sensory evaluation. General guidelines for the training of assessors (ISO 8586:2012 [291]) were used. In the first session, assessors were presented with all the mushrooms samples and were asked to describe their appearance, odor, taste, flavor, texture, and chemosensory properties followed by discussion. In further sessions, the verbal descriptions of the lexicon were clarified, and the reference samples and their intensities were agreed on. The final profile had 18 attributes; 8 odor descriptors, 3 taste properties, 3 chemosensory properties and 4 texture descriptors.

All samples were evaluated in triplicate during three sessions. The samples were served monadically straight from the hotplate. Standard sensory practices such as instructions on palate cleansing were followed. A line scale with values

from 0=”none” to 10=”very strong” was used to evaluate the intensities of sensory attributes.

4.2.4 Hedonic test

The consumers evaluated the liking of odor, appearance, flavor, texture and overall liking of four mushroom samples (study **IV**). The same samples apart from the *L. camphoratus* in the generic descriptive analysis were used and served the same way. Liking was evaluated using the 9-point hedonic scale in Finnish. After the hedonic test, the consumers answered a set of background questions related to consumer demographics and mushroom usage. Additionally, they filled the Food Choice Questionnaire [292] as modified previously [293] and the 8-question version of the Food Disgust Scale [294], both translated into Finnish.

4.2.5 Gas chromatography–olfactometry

Gas chromatography-olfactometry was performed with the detection frequency method. The method was selected because of earlier familiarity with the method in the unit [295–297] and due to its suitability for the available instrumentation. The training for the GC-O evaluations was adapted from a previously published method [280] for detection frequency type GC-olfactometry [272] and contained three sessions (Study **III**). The first and second session consisted of training the vocabulary and verbal expression speed using standard compounds in sniffing bottles. The third training was familiarization with the GC-O using both standard compound mixtures and a blind mushroom sample. In the first two sessions, six volatile compounds diluted in propylene glycol and a blank sample containing only the solvent were used as training samples. The compounds were selected based on previous research [15,34,41,210,298] and pilot studies. The samples were presented in closed 30 ml glass vials that were wrapped in tin foil, and 3-digit codes were used when referring to the samples. During the trainings, each assessor in the 3–7 member group were instructed to smell the samples individually and write suitable odor descriptors for each sample on a paper. The evaluators were allowed to smell the bottles multiple times. After everyone was ready, a group discussion was conducted about the sample descriptions and intensities. The second session had different odor compounds, more dilute samples, and less time given to think about odor descriptions.

The third training session was held individually with the GC-O. Basic introduction to the practicalities of the evaluation were given at the start of the session. The training consisted of evaluating a 3-compound mixture, a 5-

compound mixture and the *C. cibarius* sample after a pause. The evaluation experience was discussed after both standard compound mixtures and feedback was given to improve performance.

For the main evaluations, each assessor took part in 3–4 sessions, each time analyzing different mushroom species. Each sample was prepared as described in section 4.3.2. This resulted in 12 analyses from individual assessors for each mushroom species. The evaluation order was randomized without any pre-information about the sample for each assessor to minimize bias. They were instructed to evaluate the duration (start and end times) of each detected odor and to give descriptions of the odors.

In the validative GC-O evaluations, the four assessors were first further trained by being presented with two standard compounds mixtures containing a total of 36 volatile compounds which were candidates for odor-contributing compounds in the main evaluations. The assessors were instructed to describe each odor detected with attributes that were most suitable for 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 once in a random order. The evaluators were instructed to especially look for matching odor descriptions at the previously observed retention times. The audio recordings containing the odor descriptions and signal durations were processed with Audacity® 2.1.2 [299]. Odor signals and their descriptions were transcribed and the sum total of these individual odor signals was used to form nasal impact frequencies (NIFs) for each mushroom. The aromagram peaks were integrated using Labsolutions 5.57 (Shimadzu Corporation, Kyoto, Japan). The integration was done manually for all aromagrams and each peak. Special reference was in the integration limits; they were set in a way that the overall peak shape was influenced minimally by individual odor perception durations and the influence of coeluting peaks was likewise minimized. These SNIF (surface of nasal impact frequency) values (calculated as milli-NIF-seconds)) were used to assess the importance of each odor compound.

4.3 Gas chromatography analysis

4.3.1 Headspace-solid phase microextraction-gas chromatography optimization

Three different aspects of the headspace-solid phase microextraction-gas chromatography (HS-SPME-GC) analysis for mushroom samples were optimized: desorption conditions, gas chromatography separation parameters and extraction parameters.

For the desorption conditions, the effects of the fiber injection depth in the split/splitless injector, the injector temperature and desorption time (corresponding to splitless mode duration) were studied in three experiments using full-factorial experimental designs. The examined parameters studied for the gas chromatography separation were the GC column stationary film thickness, the retention gap presence, the carrier gas (helium) linear velocity, and the oven temperature program. For the extraction parameters, another full-factorial design containing factor levels for three fiber coatings, two sample masses and two extraction times were utilized.

4.3.2 Headspace-solid phase microextraction-gas chromatography–mass spectrometry

The volatile compounds were extracted and desorbed using optimized parameters. Five µL of 1000 µg/mL ethyl propionate (aq), was added as an internal standard. The volatiles were analyzed with 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.). Two GC columns were used to separate the extracted compounds: a 30 m × 0.25 mm × 1.00 µm RTX-5 Sil MS column by Restek Corporation (Bellefonte, PA) was the nonpolar column and a 30 m × 0.25 mm × 0.25 µm HP-Innowax column (Agilent Technologies, Santa Clara, CA) was the polar column. These columns were selected based on earlier research; the most common nonpolar and polar stationary phase columns (**Table 11**) were used. Gas chromatography-mass spectrometry analyses were done using an HP-6890+5973 GC-MS instrument in duplicate with both 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 mass spectrometer scan range was 15-400 m/z.

4.3.3 Volatile and odor-contributing volatile compound identification and quantitation

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. This conformed to the recommended protocol [252]. 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, matching GC-MS identifications, or both. For odor-contributing compounds, nonmatching odor descriptions overrode tentative compound identifications. Additionally, GC-O data from reference compound runs and threshold data [181] were utilized in designating the odor-impacting compound. For quantitation, GC-FID peaks from the RTX-5 Sil MS column were integrated and normalized to the peak area of the internal standard [26].

4.4 ^{1}H quantitative nuclear magnetic resonance spectroscopy

Quantitative proton NMR measurements were made to supplement the previously published ultra high performance liquid chromatography (UHPLC) analyses [300]. These UHPLC analyses measured the free amino acids and 5'-nucleotides in mushroom samples from the same batch as the other analyses and will be included in the PhD thesis of Hanna Manninen.

4.4.1 Sample preparation for NMR spectroscopy

In Study **IV**, samples from the five mushroom species were freeze-dried and ground to a fine powder. Sixty mg of powder was extracted twice with 600 µL aliquots of 0.1 M phosphate buffer in D_2O, centrifuged and 600 µL of the combined extract supernatant was retained for the NMR measurements. To this aliquot, 100 µL of 5 mM DSS in a D_2O-phosphate buffer was added for chemical shift referencing and quantification. Additional composite extracts containing all mushroom species in the same extract were made for compound identifications.

4.4.2 NMR spectroscopy validation

Light validation of the extraction method and NMR linearity was performed with two calibration curves, standard compound recovery and residual extraction experiments. Altogether 11 compounds–3 sugars, 3 organic acids and 5 amino acids–in the concentration range 0–60 mM were included in the calibration curves.

Recovery experiments were carried out by adding known amounts of crystalline trehalose, malic acid, fumaric acid, alanine, glutamine and isoleucine to *C. cibarius* powder and extracting the samples as above. The residual extraction was carried out by extracting *B. edulis* samples three additional times and comparing the compound contents in the residual extract to the main extract.

4.4.3 NMR spectroscopy

^{1}H qNMR spectra were measured with an Agilent 400-MR DD2 spectrometer (Agilent Technologies, Santa Clara, California, US) operating at a proton frequency of 399.79 MHz. The spectrometer was equipped with a OneNMR Protune probe and was controlled with a 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. The following parameters were used: 30° pulse angle, 16 ppm spectral width and 64k data, 5 s recycle delay and 128 scans. The free induction decays were Fourier transformed with zero-filling to 128 k and with LB = 0.3 Hz apodization value in MestReNova version 12.0.3 (Mestrelab Research S.L, Santiago de Compostela, Spain). Additional 2D experiments using gCOSY, HSQCAD and gHMBCAD measurements on composite samples were made to confirm compound identifications.

4.4.4 NMR compound identification and quantitation

The NMR signals were tentatively assigned by comparison to published data and reference spectra in the Human Metabolome Database [35,301–303]. Additional confirmations were made by spiking composite extracts with reference compounds and by the 2D measurements. Non-overlapping proton signal areas that were above the limit of quantification were determined. The data analysis protocol of Malz and Jancke [304] was followed and all concentrations were calculated for fresh weight.

4.5 Statistical analysis

The univariate statistical methods used in the studies are overviewed in **Table 17**. Differences in the normalized concentrations of volatile compounds between samples (Study **III**), as well as in the concentrations of water-soluble compounds between samples (Study **IV**) were analyzed with a one-way analysis of variance (ANOVA). On the other hand, two-way ANOVA was used to analyze the differences between the sensory attributes of the generic descriptive analysis (Study **I**), the peak areas of volatile compounds between extraction and desorption parameters (Study **II**) and hedonic likings (Study **IV**). A linear regression model was created for the peak areas of 1-octen-3-ol and nonanal based on the desorption parameters in Study **II**. Additionally, the panel reproducibility and agreement of the generic descriptive analysis was analyzed with PanelCheck 1.4.2 (Nofima, Tromsø, Norway) following the suggested workflow of univariate and multivariate methods including a 2-way and 3-way

ANOVA as well as PCA [305]. The trained panel data was further re-examined with the MAMCAP package version 1.0 [306–308] in RStudio. If required, appropriate data transformations were used in order to have the sample populations conform to normality. Alternatively, Kruskal-Wallis and Mann-Whitney's U test with Bonferroni corrections were used for compounds that did not conform to normality (study **III**).

The multivariate statistical methods used in the studies are overviewed in **Table 18**. The main methods used were principal component analysis (PCA), principal component regression (PCR) and partial least squares regression (PLS). PCA data included Projective Mapping coordinates and frequencies (Study **I**), generic descriptive analysis intensities (Study **I**), peak areas and/or compound concentrations (Studies **II–IV**) and raw GC-FID and NMR data (Studies **III–IV**). The NMR data was supplemented with UPHLC measurements from the same batch [300]. The same datasets were followed with PLS. Additionally, hierarchical cluster analysis (HCA) was used for consumer clustering.

Statistical analyses and multivariate models were performed using SPSS (IBM Corporation, Armonk, NY) versions 22.0 (Study **I**), 23.0 (Study **II**) and 24.0 (Studies **III**–**IV**), The Unscramber (CAMO Software AS, Oslo, Norway) versions 10.3 (Study **I**), and 10.4 (Studies **II**–**IV**), and the ChemoSpec package version 4.4.97 [309] in RStudio 1.1 [310] running R 3.4.3 [311] (Studies **III**–**IV**). SPSS was used for the ANOVA, linear regression, Pearson moment correlation coefficients and hierarchical cluster analysis; RStudio for the metabolomics principal component analyses of the GC-FID/O and NMR data, and the Unscrambler for all other multivariate models. The criterion for statistical significance in all tests was $p < .05$.

Table 17. Overview of the univariate statistical methods used in the studies.

Study	Method	Variables	Fixed factors	Random factors	Post hoc tests
II	Linear regression	1-octen-3-ol and nonanal peak areas	fiber injection depth, desorption temperature, desorption time		
III	Pearson correlations	Averaged sum of normalized GC-FID peaks, SNIF values, total odor intensities			
III	One-way ANOVA [a]	Normalized concentrations of volatile compounds	sample		Tukey's HSD, Tamhane T2
III	Kruskal-Wallis	Normalized concentrations of volatile compounds	sample		Mann-Whitney's U, bonferroni corrections
IV	One-way ANOVA	Sugars, organic acids and amino acids in the D_2O extract	sample		Tukey's HSD, Tamhane T2
I	Two-way ANOVA	Sensory attributes of the generic descriptive analysis	sample, sample*assessor (mixed assessor model)	assessors, sessions	Tukey's HSD
II	Two-way ANOVA	Total area of all peaks, hexanal area, 1-octen-3-ol area	fiber type, sample mass, extraction time, interactions		Tukey's HSD
IV	Two-way ANOVA	Hedonic likings	sample, cluster, sample*cluster		Custom Lmatrix simple contrasts, bonferroni corrections
IV	One-way ANOVA	Consumer background variables (FDS, FCQ, age, known species)	cluster		Tukey's HSD
IV	Kruskal-Wallis	Consumer background variables (sample familiarity, mushroom usage frequency)	cluster		Mann-Whitney's U, bonferroni corrections

[a] ANOVA: analysis of variance

Table 18. Overview of the multivariate statistical methods used in the studies.

Study	Method [a]	Replicates [b]	Variables/ X-data	Factors/ Y-data	Data treatment	Scaling
I	PCA	33/3/1	Sensory attributes of the generic descriptive analysis	samples	separate models for raw data, averaging over assessors and averaging over assessors and sessions	autoscaling [c], samples as down-weighted dummy variables
I	PCA	1	Projective Mapping sample coordinates, descriptor frequencies	samples	separate models for each dataset	autoscaling
I	PCR	1	Projective Mapping sample coordinates	descriptor frequencies		autoscaling for X-data
II	PCA	2	All volatile peak parameters	desorption parameters, extraction parameters	separate models for each dataset	autoscaling
II	PLS-DA	2	Selected peak parameters	desorption parameters, extraction parameters	separate models for each dataset	autoscaling
III	PCA	12/4	Raw HS-SPME-GC-FID chromatogram data points on GC two columns	samples	normalization, binning	mean centering, Pareto scaling, classical confidence ellipses
III	PCA	1	Raw GC-O aromagram data points on two GC columns	samples	binning	no scaling
III	PCA	1 (average)	Normalized concentrations of volatile compounds on the RTX-5 Sil MS GC column	samples		autoscaling
III	PCA	1	SNIF values on two GC columns	samples		mean centering

Study	Method [a]	Replicates [b]	Variables/ X-data	Factors/ Y-data	Data treatment	Scaling
IV	PCA	5	Non-volatile compounds measured with HPLC (see reference [300])	samples		autoscaling
IV	PCA	4	Non-volatile compounds measured with NMR	samples		autoscaling
IV	PCA	4	Raw NMR data	samples	normalization, binning	mean centering, Pareto scaling, classical confidence ellipses
IV	PCA	84	Hedonic modalities of the four mushrooms	consumers		mean centering
IV	HCA	84	principal components with eigenvalues >2 from the hedonic liking PCA	consumers	Ward's method	Squared Euclidean distances
IV	PLS	1 (average)	Odor-contributing volatile and non-volatile compounds of the wild mushroom species	attributes of the generic descriptive analysis		autoscaling, samples as down-weighted dummy variables
IV	PLS	1 (average)	sensory attributes	consumer likings by cluster		autoscaling, samples as down-weighted dummy variables

[a] PCA: principal component analysis, PCR: principal component regression, PLS-DA: partial least squares regression discriminant analysis, HCA: hierarchical cluster analysis, PLS: partial least squares regression
[b] number of repetitions for each mushroom species sample in the model
[c] mean-centering and dividing by variable standard deviation

5 RESULTS AND DISCUSSION

5.1 Sensory profiles of mushrooms

5.1.1 Projective mapping

The consumers separated cultivated mushrooms from wild mushrooms both in odor- (**Figure 9**) and flavor-based (**Figure 10**) mapping experiments. The sample configurations are also similar in the combined model (**Figure 11**). The main following observations can be made from the resulting figures: First, based on the distance of the two chanterelle replicates, the consumers perceived only minor differences in the sensory properties of the white and brown button mushrooms.

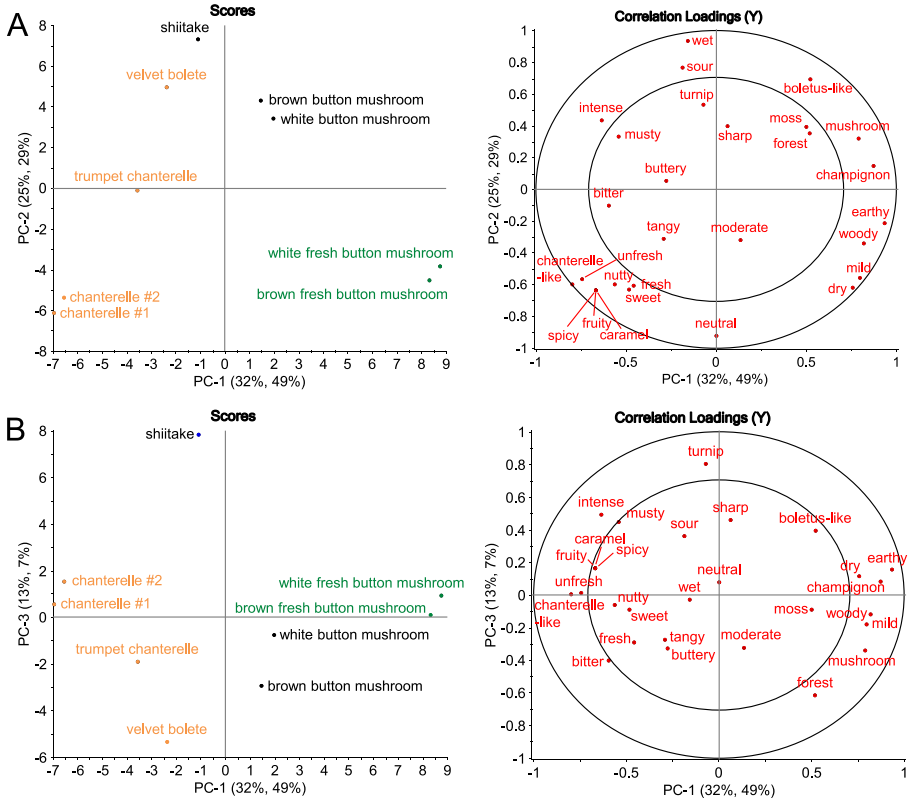

Figure 9. PCR plots of the odor-based Mapping profiles. The model was created using the data from 52 assessors. Left: Scores plots for the samples, right: Correlation loadings of the descriptions. A: PCs 1 and 2, B: PCs 1 and 3. In the scores plot, the samples were classified into three groups: wild mushrooms (orange), fresh cultivated mushrooms (green), and cooked cultivated mushrooms (black).

Second, the most common terms used for describing the samples are related to sensory intensity and mushroom-like odor/flavor. The odor of wild mushrooms was more intense than that of cultivated species, while the inverse was true for flavor intensity. However, other interesting attributes were mentioned as well, such as fruity, earthy and woody in the odor-based map and umami, butter and moss in the flavor-based map. Finally, cooking caused significant changes to the sensory properties as the two types of button mushroom samples were different from each other and therefore separated in the multivariate model.

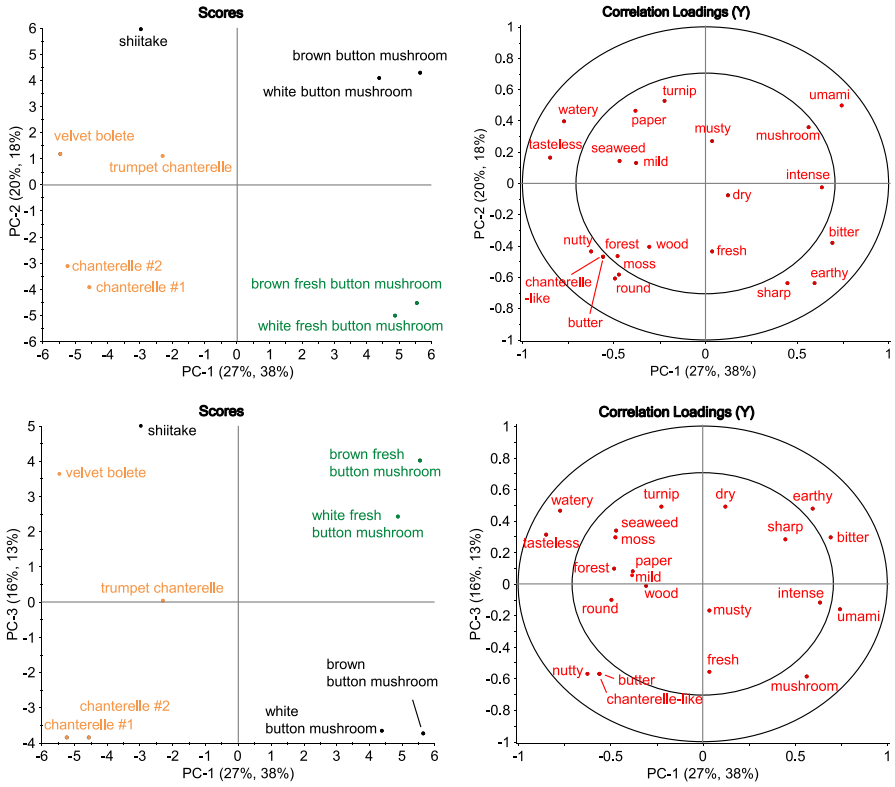

Figure 10. PCR plots of the flavor-based Mapping profile. The model was created with data from the 46 assessors. Left: Scores plots for the samples, right: Correlation loadings of the descriptions. A: PCs 1 and 2, B: PCs 1 and 3. In the scores plot, the samples were classified into three groups: wild mushrooms (orange), fresh cultivated mushrooms (green), and cooked cultivated mushrooms (black).

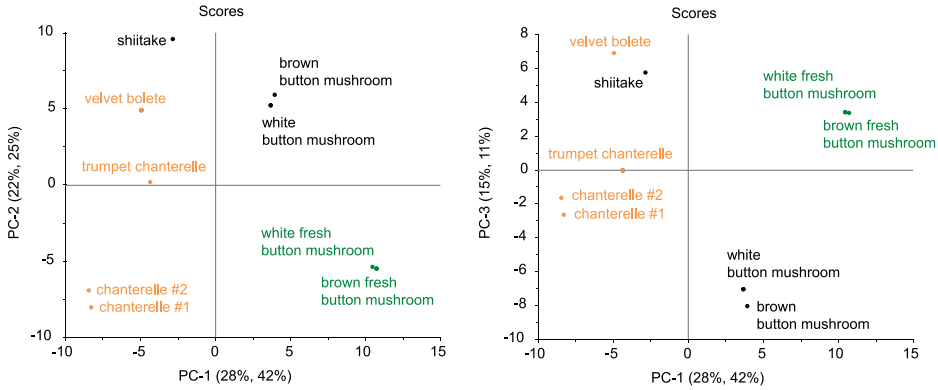

Figure 11. PCR Scores plots of the combined Mapping profile. The model was created by combining the raw data shown in **Figures 7 and 8**. Left: PCs 1 and 2, right: PCs 1 and 3. The samples were classified into three groups: wild mushrooms (orange), fresh cultivated mushrooms (green), and cooked cultivated mushrooms (black).

5.1.2 Generic descriptive analysis

Overall, the trained panel exhibited good discrimination between the samples ($p<0.001$ for all attributes), and there was a significant session effect for only one attribute. However, the assessor effect was significant for most attributes. According to the panel performance tests made in Panelcheck, biting resistance, cardboard odor and a metallic sensation produced the most notable disagreements. On the other hand, the panel was in high agreement concerning the mashed potato odor, roasted odor and bitterness. The MAM-CAP model, which decomposes the assessor effect into scaling and disagreement values, further showed that all attributes aside from squeakiness and cardboard odor had significant disagreement. Scaling issues were most notable for pungency, where 8 out of 11 panelists used the scale differently from the average.

The generic descriptive analysis indicated that the species were different in all 18 attributes in the profile (**Table 19**) and that the overall sensory profiles for each mushroom species are distinct (**Figure 12**). Button mushrooms were most characterized by a mushroom-like odor, were moderately sweet and were intensively umami-like. Chanterelles had an intense cooked carrot odor, moderate intensities of forest and cardboard odors. They also had moderate intensities in taste and chemosensory descriptors, but high levels of squeakiness and toughness. Porcini had the most intense mashed potato odor along with a moderate mushroom-like odor, were the sweetest and close in umami intensity to button mushrooms, and had intermediate values in the texture properties.

Table 19. Mean intensities (n=33) and standard deviations (in brackets) of sensory attributes in the sensory profile of the five mushroom samples. Significant differences between samples are based on a univariate two-way mixed ANOVA. The differences between samples in each attribute are marked separately with superscripts A–D (Tukey's HSD test, $p<0.05$), with the highest value marked with A.

Attribute	Porcini			Chanterelle			Trumpet chanterelle			Curry milk cap			Button mushroom		
Odor															
total intensity of odor	5.4	(1.6)	C	5.9	(1.3)	BC	5.7	(1.4)	C	7.9	(1.0)	A	6.3	(1.6)	B
mushroom	3.5	(2.0)	B	2.9	(1.8)	BC	2.6	(2.2)	C	1.2	(1.6)	D	6.1	(2.6)	A
earth/soil	1.9	(1.9)	BC	2.4	(1.7)	B	3.6	(1.7)	A	3.4	(2.1)	A	1.6	(1.6)	C
forest	1.7	(1.7)	B	3.6	(1.9)	A	4.2	(1.3)	A	1.5	(1.7)	B	2.1	(1.8)	B
cardboard	1.8	(1.6)	C	3.5	(2.5)	A	2.9	(1.9)	AB	3.9	(1.9)	A	2.4	(1.7)	BC
cooked carrot	1.3	(1.5)	B	4.8	(2.5)	A	0.8	(1.0)	BC	0.5	(1.0)	C	1.2	(1.5)	B
mashed potato	5.7	(1.9)	A	0.7	(1.0)	C	0.8	(1.2)	C	0.4	(0.7)	C	2.5	(2.0)	B
roasted odor	0.6	(0.9)	C	1.1	(1.6)	BC	1.7	(1.4)	B	6.4	(1.8)	A	1.1	(1.1)	BC
Taste															
sweetness	6.3	(1.5)	A	4.3	(1.6)	B	3.5	(1.6)	C	1.0	(1.2)	D	5.5	(1.9)	A
umami	5.8	(2.1)	B	5.0	(1.8)	C	4.9	(1.8)	C	3.2	(2.3)	D	6.8	(1.5)	A
bitterness	1.3	(1.3)	D	2.0	(1.5)	BC	2.4	(1.6)	B	7.8	(2.2)	A	1.6	(1.5)	CD
Chemosensory															
astringency	2.0	(1.7)	C	2.8	(2.0)	BC	3.0	(2.2)	B	4.8	(2.0)	A	2.0	(2.0)	C
metallic	1.9	(1.5)	C	3.0	(1.8)	B	3.1	(1.6)	B	3.9	(2.4)	A	2.2	(1.7)	C
pungency	0.5	(0.9)	B	0.6	(1.1)	B	0.8	(1.2)	B	3.4	(2.3)	A	0.4	(0.8)	B
Texture															
toughness	5.0	(1.7)	B	6.9	(1.3)	A	6.3	(1.6)	A	2.9	(2.2)	C	6.4	(1.5)	A
biting resistance	5.5	(1.8)	B	4.6	(2.2)	C	4.2	(2.0)	C	4.3	(1.7)	C	6.5	(1.4)	A
crumbliness	4.4	(1.9)	B	3.1	(2.1)	C	3.6	(1.9)	BC	7.1	(1.4)	A	3.0	(1.8)	C
squeakiness	7.2	(1.6)	A	6.5	(1.7)	AB	6.2	(1.8)	B	4.7	(2.2)	C	7.2	(1.4)	A

Trumpet chanterelles had moderate forest, earthy and cardboard-like odors, and intermediate values in all taste, chemosensory and texture attributes. Curry milk caps were very different from the other samples. They had the most intense overall odor, most intense roasted odor, were intensively bitter, astringent and the only noticeably pungent sample. They had the highest crumbliness but on the other hand low levels of squeakiness and toughness.

Unfortunately, only limited comparisons can be made to literature as the fully reported sensory profiles have been made with different species and especially the odor attributes have used a different lexicon. In terms of profile construction, Cho et al. [34] had the most similar attributes and reference products for their *Tricholoma matsutake* samples. Their wet soil odor attribute corresponded to the earthy attribute used in this profile, and similarly the moldy odor was approximate to the mushroom attribute based on the reference and definition. Sweetness, bitterness, umami, metallic and astringent corresponded well, with even some of the concentrations of reference products being similar. However, while in Cho et al. sour and salty were included in the profile, the trained panel in this study did not consider them to be important. The profile for *Lentinus sajor-caju* shared mushroom odor, sweet, and bitter taste attributes, while the Polish-Spanish mushroom profiling studies [33,39,80] had similar earthy, burnt, woody and mushroom odor attributes.

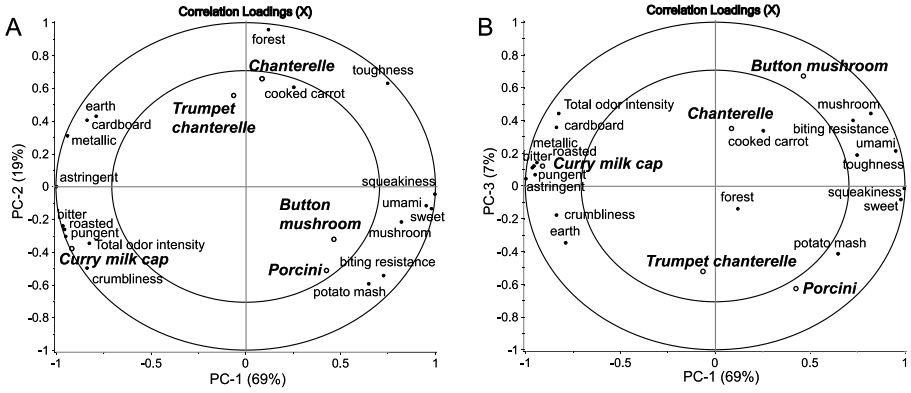

Figure 12. Consensus PCA correlation loadings plots of the descriptive sensory profile. Data is averaged over 11 assessors and 3 sessions. A: principal components 1 and 2; B: PCs 1 and 3. Descriptors are marked with closed circles and evaluated samples with open circles and larger text in bold and italics.

5.2 Metabolomic separation of mushrooms

The mushroom species could be separated in the multivariate models created from both raw volatile compound data (Study **III**) and non-volatile compound data (Study **IV**). As can be seen from the Scores plots of the PCA models (**Figure 13**), the curry milk cap was generally well differentiated from the other mushrooms. Three main conclusions can be made from these models.

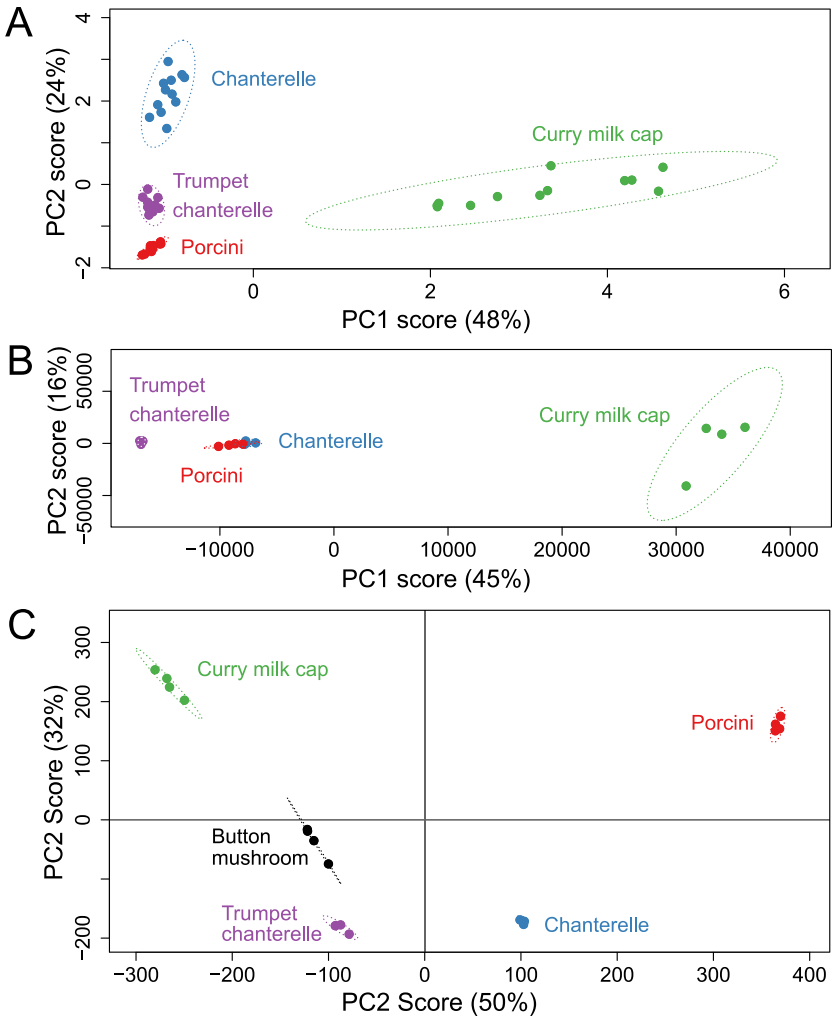

Figure 13. Principal component analysis Scores plots of principal components 1 and 2. The raw data models were created on HS-SPME-GC-FID analysis of volatile compounds analysis on the RTX-5 Sil MS column (A), HP-Innowax column (B), and water-soluble non-volatile compounds analyzed with NMR (C). Mean centering and Pareto scaling was used in the model. Ellipses are 95% confidence limits for each species.

First, the GC-FID data yields similar configurations on both GC columns, which increases the credibility of the analysis. Second, all models similarly demonstrate that the curry milk cap is quite different from the other species in its metabolomics profile. Third, when the approach has been used on different species in the literature, a similar degree of separation has been reported. Volatile compound GC-MS data and principal component analysis has been used to separate eight mushroom species from each other [211], and PCA on raw NMR data has been used to successfully analyze the cultivation region of *Ganoderma lucidum* [312], as well as different quality grades [35] and raw and cooked [313] *Tricholoma matsutake*.

5.3 Odor-contributing volatile compounds of mushrooms

5.3.1 HS-SPME-GC-MS analysis optimization

Based on PLS regression models, the optimal desorption parameters were a maximal fiber depth in the injector, a 240 °C injector temperature and a 3 minute desorption time. The fiber depth results were in line with literature reports. However, this was the first time the effect of fiber injection depth has been commented on mushroom volatiles analysis. All fibers had unique extraction profiles. The divinylbenzene/carboxen/ polydimethylsiloxane (DVB/Car/PDMS) was the most suitable coating for mushroom volatiles. A 45 minute extraction time resulted in larger signals than a 30 minute extraction time and had a greater higher effect than doubling the sample mass from 10 g to 20 g.

5.3.2 Volatile compounds in mushrooms

Altogether 99 peaks were detected from the 4 mushroom species, of which 84 were at least tentatively identified. 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. Using two GC columns in the analysis facilitated the identification of several compounds. For example 1-octen-3-ol and 1-octen-3-one coeluted completely on the RTX-5 Sil MS column but separated well on the HP-Innowax column. Common volatiles to all species 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 contents of all volatile compounds were different between mushrooms species

as indicated by the ANOVA results. Each mushroom species had a unique volatile compound profile: there were both unique compounds in each species and the relative contents of each compound differed as well.

5.3.3 Odor-contributing volatile compounds in mushrooms

Out of the 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. The common odor-contributing volatile compounds for all mushrooms were 1-octen-3-one, 1-octen-3-ol, hexanal, octanal, methional, (*E*)-2-nonenal, 2,3-butanedione, and (*E*)-2-octenal. Each mushroom was also characterized by distinct compounds. The profiles of odor-contributing volatile compounds as detected in the two GC-O datasets are displayed in **Figure 14**.

5.4 Sugars and acids in mushrooms

The concentrations of all eight sugars and acids were different between the mushroom species. The general profile of these compounds is displayed in **Figure 15**. For the trumpet chanterelle, button mushroom and curry milk cap, mannitol was the major sugar or sugar alcohol. However, for porcini, trehalose was the main sugar, and chanterelle had both compounds. Malic acid was the major organic acid in all mushrooms. Curry milk caps were also characterized by major contents of an unidentified compound, tentatively identified as a cyclic substituted pentose.

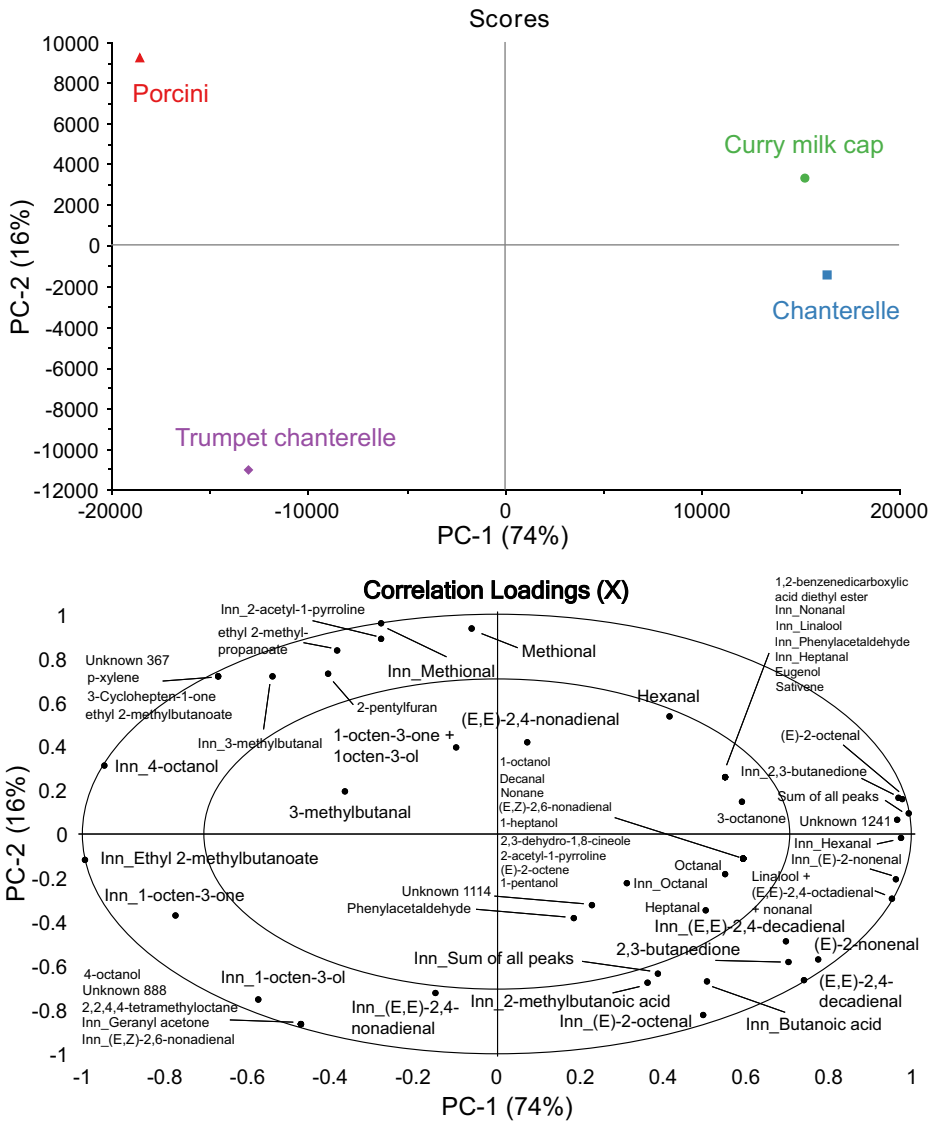

Figure 14. PCA model scores (up) and correlation loadings (down) plots of the odor-contributing volatile compounds. The prefix 'Inn' on the compounds corresponds to HP-Innowax column and numbers in the unknown compounds correspond to retention indices.

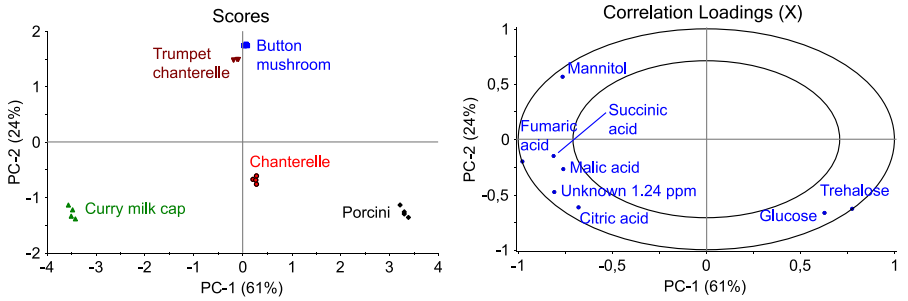

Figure 15. PCA scores (left) and correlation loadings (right) plot of the sugars and acids in mushrooms.

5.5 Hedonic liking of mushrooms

On average, all mushrooms except button mushrooms were at least liked slightly on all liking modalities (average liking across all mushrooms and modalities 6.24). The hierarchical clustering analysis (HCA) indicated that there are two principal clusters with 22 and 62 consumers (**Figure 16**). The larger cluster further splits twice to yield a total of five clusters. Different consumer clusters had differing liking profiles, and the species×consumer cluster interaction was always significant ($p<0.005$). This means that the liking of each mushroom species varied by cluster. However, consumer cluster explained a higher proportion of variation in mushroom liking than mushroom species.

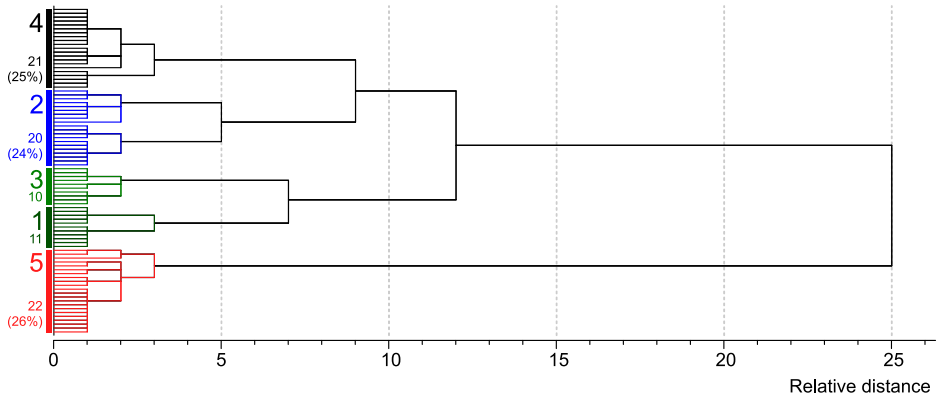

Figure 16. Hierachical cluster analysis (HCA) dendrogram of the consumers. The model was created with data from the 7 principal component loadings of 84 consumers using Ward's method and Squared Euclidean distances. The values on the left indicate clusters and their absolute and relative sizes.

The largest (26% of all study participants) and most different cluster 5 (in terms of Squared Euclidean distance) had high liking scores for all mushrooms and liking modalities, with all liking averages >7 except for the appearance of button mushrooms. In contrast, the smallest cluster 3 (12% of participants) rated all hedonic liking scores the lowest or at least in the lowest score group. The other three clusters variably gave higher ratings to some samples and lower ratings to others.

There were no clear differences in the background variables of different clusters. Cluster 5 had a higher ethical concern than cluster 2 for food and cluster 4 knew more mushroom species than cluster 3. However, age, other food concern categories, food disgust or mushroom usage frequencies were not different between clusters.

5.6 Combining the data

5.6.1 Predicting sensory properties of mushrooms via chemical composition

The PLS model predicting the sensory properties of the wild mushrooms species via non-volatile and odor-contributing volatile compounds is presented in **Figure 17**. All measured organic acids, their total concentrations, as well as several bitter tasting amino acids (histidine, leucine, phenylalanine, methionine) were linked to bitterness and astringency. EUC values, GMP and AMP were closely linked to umami, while glutamic acid and aspartic acid were inversely correlated. This might be due to the high bitterness present in curry milk caps that suppressed the umami perception of glutamic and aspartic acid. Surprisingly, individual sugars, sugar alcohols, and especially their total content expressed in the relative sweetness of glucose were not closely correlated with sweetness. However, the trehalose content, sugar-acid ratios, and some sweet amino acids (alanine and glycine) were closely linked to sweetness. This model demonstrated several taste interactions which were introduced in section 2.4.7: the synergistic function of umami via the EUC value, the suppressive effects of organic acids to sweetness, and the additive effects of sweet amino acids and sugars. The total area sum of odor-active regions (SNIF sum) was a predictor of total odor intensity. Interestingly, the separated 1-octen-3-one and 1-octen-3-ol (peaks Inn_43 and Inn_44) were closely linked to mushroom odor, but their coeluting peak on the RTX-5 Sil MS column (43–44) was inversely correlated and more closely related to the metallic attribute. The unknown compound in curry milk caps tentatively identified as a substituted pentose is closely linked to astringency, pungency and bitterness.

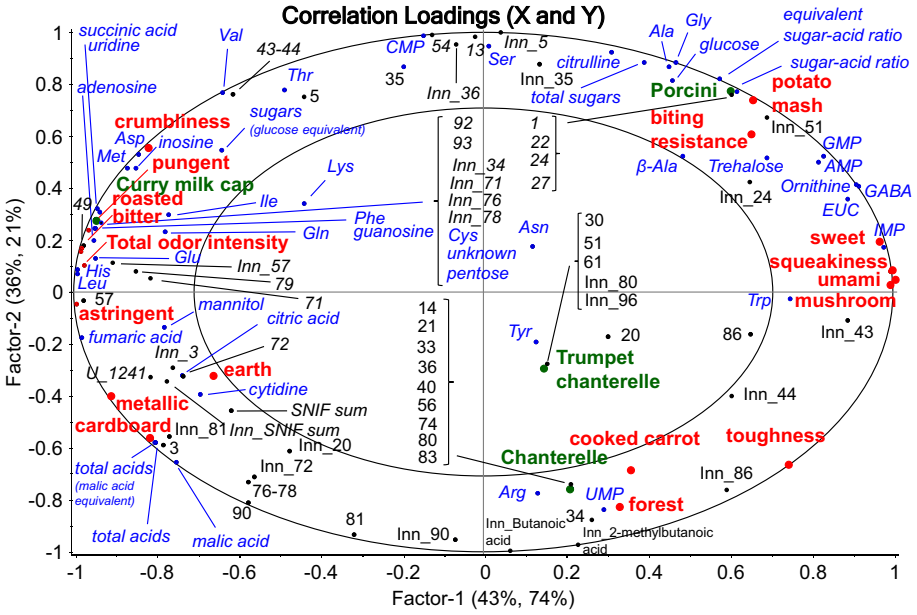

Figure 17. PLS model of the GC-olfactometry (black, numbering from publication III), UHPLC and NMR data (blue) explaining the sensory attributes (red) of mushrooms (green).

5.6.2 Mushroom liking as influenced by sensory properties

Each consumer cluster had links to certain mushroom attributes (**Figure 18**). Cluster 5 (the general likers of mushroom) showed a slight preference for the texture of button mushrooms and an overall liking of porcini. Cluster 1 seemed to dislike the mushroom odor, sweet and umami attributes as well as the squeakiness and biting resistance, but liked earthy, cardboard and forest odors. Cluster 3, on the other hand, liked the sweetness and umami and disliked the metallic and astringent attributes. Cluster 4 liked the crumbliness of porcini and had a minor dislike of toughness and biting resistance. Another, simplified 3-cluster model that was created by merging clusters 1 and 3 as well 2 and 4 is displayed in **Figure 19**. In this model, there is a lower risk of over-interpretation as each cluster has at least 21 members. The three clusters have more systematic groupings on opposite sides of the perceptual space. However, the liking modality-specific information such as the dissimilar overall likings between clusters 2 and 4 for porcini or between clusters 1 and 3 for trumpet chanterelle are lost. Thus, two conclusions can be made based on these results. First, looking only at the average liking score of the sample leaves out a considerable amount of important information. This is in line with the results of consumer studies in general. Second, mushrooms have so variable sensory

properties that disliking one species is not a proper indicator of the dislike of mushrooms in general.

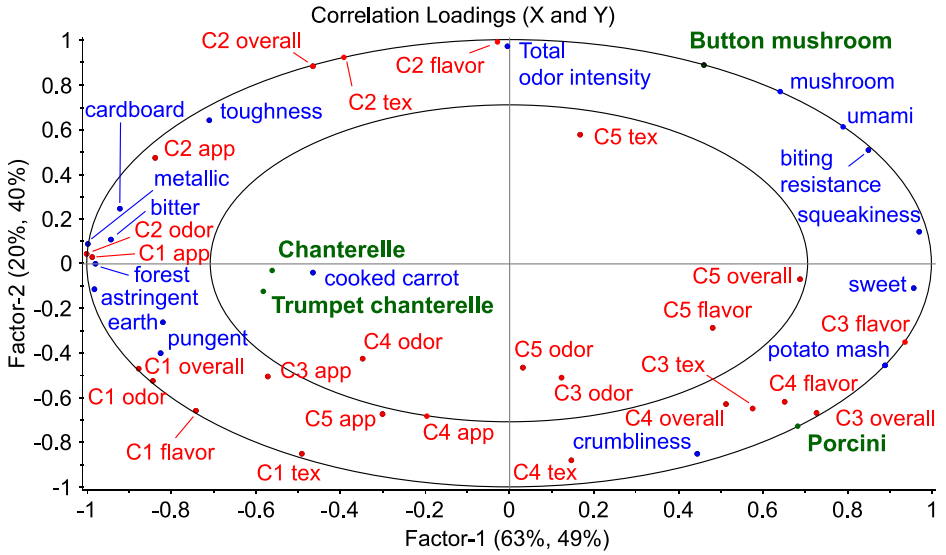

Figure 18. PLS model of the sensory attributes (blue) predicting the overall liking of each liking modality of each consumer cluster (C1–C5, red) for mushrooms (green). Abbreviations: app: appearance, tex: texture.

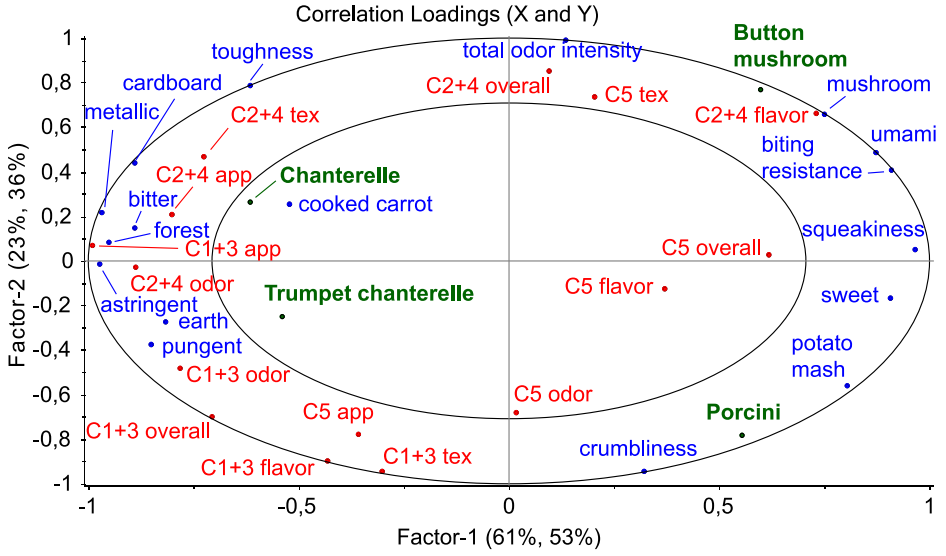

Figure 19. Simplified PLS model of the sensory attributes (blue) predicting the overall liking of each liking modality of three aggregated consumer clusters (C1+3, C2+4, C5, red) for mushrooms (green). Abbreviations: app: appearance, tex: texture.

5.7 General discussion

Mushrooms are a vastly underutilized natural resource with a limited amount of research available. Research is especially limited regarding the wild mushroom species common in the Nordic countries and this prompted the experimental design used in this study. This work was the first time that wild mushrooms grown in Finland were studied using analytical sensory evaluation methods and quantitative consumer studies. In addition, sensory properties were connected to chemical compounds of the same mushroom samples to investigate those molecules that contribute to the chemical sensations such as aroma and taste. This work created a base and a modern set of analysis tools for further mushroom studies.

The *sous vide* process used for processing the mushrooms proved repeatable in terms of temperature control and allowed for a complete recovery of the base mushroom batch. The heat treatment should inactivate most enzymes in the sample aliquots. While the storage at −20 °C likely allowed for some nonenzymatic reactions to occur compared to −80 °C, this storage had consumer and professional kitchen relevance.

The generic descriptive analysis conducted by the trained panel followed standard sensory practices closely and provided a valuable contribution to the limited sensory literature available of wild mushrooms. The developed lexicon and approach formed a working base for extended sensory characterization of other wild mushroom species. The profiles of the four wild mushroom species differed from the cultivated *Agaricus bisporus* samples in the odor, taste, chemosensory and texture properties, which demonstrated that mushrooms are different from one another.

The HS-SPME-GC-MS/FID/O method development was a powerful lesson in the critical points and challenges in aroma chemistry research compared to volatile chemistry research. On one hand, it demonstrated the importance of experimental design in obtaining quality data, and on the other hand the risks in using only instrumental data for method development. In hindsight, it could be hypothesized how the selection of the primary column and the oven program would be different if the method development was supplemented with GC-O data. The inclusion of a second, polar column in the GC-O study proved to be very valuable; the identities of several co-eluting peaks in the nonpolar column were resolved. Additionally, using the peak areas (SNIF values) in the GC-O datasets was beneficial in differentiating compounds with long odor impressions such as methional and 1-octen-3-one. This experience in a way indicates the pilot nature of this work and the iterative nature of science; further research on the same matrix will be much more efficient.

Quantitative NMR was an efficient method for the measurement of the water-soluble, non-volatile compounds present in mushrooms. It showed a good overview of the metabolome of the samples and complemented well the earlier UHPLC free amino acid and 5'-nucleotide analyses done by Hanna Manninen. While the sensitivity of qNMR is orders of magnitude lower than of liquid chromatography based methods, the quantifiable compounds should have sensory perceptional relevance. The qNMR data would be especially useful in comparative analyses such as experiments that examine the geographical or processing based variation in the levels of non-volatile compounds. Due to the simple sample preparation and quick data analysis of the metabolomic profile, qNMR could be used as a screening tool for further studies. The most interesting sample types could be selected for studies employing sensory science and flavor chemistry methods.

5.8 Limitations of the study

Due to practical reasons, the wild mushroom samples were collected from Finland in one harvest year and mainly one location (southwestern Finland) without reference to annual variation in the chemical and sensory properties. The annual and geographical variation was left out of the research plan as the species level was considered more important in this pilot work. In future research, however, the comparison of multiple harvest years and locations would be beneficial.

The selection of sample species was also limited due to the poor availability of sample material in the forest during the harvest year. Relatively large batches of fresh material were required with complete collection information, and despite consultation of the local mycological associations and mushroom distributors in several parts of Finland, the material was not available except for the most popular species. This in turn limited the systematic comparison of differences between mushroom families such as between Cantharellales and Boletales. All samples in the main studies were heat treated. This was done with regards to the typical consumption type of mushrooms and the specific cooking method when considering professional kitchens. The results are thus not applicable to fresh mushrooms in the forest and no systematic comparisons could be done on the effects of cooking, although some pilot testing was carried out. On the other hand, mushrooms are seldom eaten raw.

In terms of research design and methodology, the following are the main limitations: The trained panel had a relatively high variation in some attributes which indicates that they would have benefited from further training and further formulation of the reference products. However, the panel as a whole

discriminated well between the samples. HS-SPME as an equilibrium method limited the quantitative comparison of the results. The detection frequency type GC-O allowed only limited conclusions on the sensory impact of odor-contributing volatile compounds. The relatively modest number of consumers taking part in the hedonic test limited the analysis of consumer clusters although the number of consumers was enough for a general consumer study.

5.9 Further research and practical applications

The results of this thesis raise several new research questions. First, the developed sensory and flavor chemistry tools should be used for a wider selection of wild edible Finnish mushroom species in the future. This work demonstrated that various sensory attributes and underlying chemical compounds are found in even the common wild edible mushroom species. It would be very interesting to see what kind of sensory profiles and odor-contributing volatile compounds are typical for species that are less known to the general public. The different odor profiles and the high variation in the non-volatile precursor compound compositions point to major differences in the biosynthesis of mushroom volatiles. Second, the systematic examination of differences between mushroom families and similarities within the families would form an attractive research project. This could be implemented with a combination of sensory and flavor chemistry methods that are supplemented with molecular biology methods such as real-time quantitative polymerase chain reaction (RT-qPCR). After confirming differences in the underlying chemistries of the sensory properties it would be possible, for instance, to look for the expression pattern of the related genes in the aroma compound formation pathways.

Third, the effects of preservation and cooking processes such as drying, salting, and frying on the sensory properties warrant further study. Due to the short time for picking most species it is imperative to see what differences there are between preservation methods such as freezing and drying, and which method would best preserve the sensory properties for each application. This would also allow for a more detailed understanding of the related reaction pathways behind the perceived sensory properties. Fourth, the consumer clustering of Finnish mushroom users should be examined further. In this study, only volunteer consumers that use and like mushrooms were recruited for the hedonic liking test due to ethical reasons. However, it would be interesting to expand this examination to consumers that dislike and avoid mushrooms. Finally, it would be interesting to study the differences in consumer clustering of different cultures.

6 SUMMARY AND CONCLUSION

The sensory profiles of four wild edible Finnish mushrooms (*Cantharellus cibarius*, *Craterellus tubaeformis*, *Boletus edulis,* and *Lactarius camphoratus*) as well as cultivated *Agaricus bisporus* as a reference were established with generic descriptive analysis. Additionally, the sensory properties for a selection of mushrooms were evaluated by consumers. The results presented in this thesis demonstrate that wild mushrooms have different sensory properties than cultivated mushrooms and that specific attributes can be used to describe each mushroom species.

The parameters of the volatile compound analysis method of mushrooms based on HS-SPME-GC-FID/MS were optimized. The desorption parameters had a small but significant effect that is usually not reported in the literature. For extraction parameters, longer extraction time had a greater effect than larger sample mass. Ninety-nine volatile compounds were detected in the four wild edible mushroom species. There were major differences both in the contents of individual volatile compounds and also in the overall volatile compound profiles between the species. As expected, only a small set of these volatiles were also detectable with GC-olfactometry, and the GC-FID responses and SNIF values were poorly correlated. Different aliphatic aldehydes, ketones, and alcohols were the most common odor-contributing volatile compounds.

The mushroom species were also well separated in the NMR measurements of water-based extracts. Differences in the contents of trehalose, mannitol, malic acid, and fumaric acid were the main sources of variation. The sensory properties of mushrooms were linked to several chemical compounds. High EUC values and 5'-nucleotides were predictors of umami, while a high sugar-acid ratio predicted sweetness. The species was not the main source of variation in the liking of mushrooms for consumers. Instead, consumer clusters with significantly different liking profiles for the selected mushroom species were found. The individuals in these clusters were heterogeneous in their background with no simple well-explaining factors such as age, mushroom usage frequency or differences in their food choice motives.

It is important to understand which sensory attributes and underlying compounds negatively affect the hedonic liking of mushrooms for consumers. The consumers participating in hedonic testing were Finns but the hedonic liking scores of participants still differed significantly from one another. This research brings new information on why mushrooms are not liked by everyone. This information is useful also for product development and individual marketing of mushroom products.

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