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Mushroom flavour

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ABSTRACT

Mushrooms and fungi not only present a fascinating world of shapes, both macro- and microscopic, but they are also an interesting source of flavours, fragrances and odours, e.g. garlic, coconut, flour-like, cucumber or fruit-like, as well as the most characteristic for this kingdom of living organisms mushroom-like flavour and aroma. Fungi can possess many different and interesting flavours and fragrances – starting from nice anise-like, fruit-like, cucumber, garlic, to cheese-garlic, and ending with potato or flour-like smells. Some mushrooms emit carbide or distinctly faecal-like odour. The taste of mushrooms is frequently correlated with their aroma. What components does the core of a mushroom flavour consist of? Chemical analysis of specimens reveals compounds responsible for characteristic flavour and odour. It was found that the most characteristic flavour compound is defined mainly by C_8 volatiles. Between all C_8 compounds the most important for mushroom flavour are oct-1-en-3-ol, octan-3-ol, octan-3-on and oct-1-en-3-on. Fungi and mushrooms can enable biotechnological production of some flavour components, for instance the *Nidula niveotomentosa* produces a characteristic raspberries compound – raspberry ketone in submerged cultures; the biotechnological production can also provide rare and tasty forest mushroom biomass e.g. edible boletus.

KEY WORDS: odour, fungi, aroma

Introduction

Flavours and special odours are nonconventional tool in mycology. Which of mycologists does not know a garlic like odour of *Mortierella* (Domsch *et al.* 1980) or a coconut-like fragrance of *Trichoderma* (Rifai 1969)? The same odour characterises *Hypocrea caerulescens* Jaklitsh & Voglmayr (Jaklitsh *et al.* 2012). Those who work with basidiomycete fungi as well as ordinary fans of pizza or more sophisticated dishes with mushrooms

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easily recognise the mushroom flavour. But what is the essence of the mushroom flavour? What components does it consist of? The aim of the manuscript is to show the diversity of both fungal and mushroom flavours and aromas as well as their chemical origin and importance.

Overview of aromatic fungi and compounds responsible for their flavours

Flavour industry has been developing for over 160 years. It uses the flowers and whole plants or herbal roots, spices, vegetables and fruits processing as well as essential oils for distillation (flavours and fragrances). As W. A. Poucher (1991) defines in 'The raw materials of perfumery', mushrooms may have an unpleasant odour. However, some odours can be nice and interesting and have been mentioned as a potential source of fragrances, e.g. *Cortinarius suaveolens* Bataille & Joachim has a strong orange blossom scent, which remains after drying (Poucher 1991), *Hygrophora agathosmus* – cherry laurel (Poucher 1991) or rather *Hygrophorus agathosmus* (Fr.) Fr. – an almond/marzipan odour (California Fungi 2014), *Tricholoma aurantium* (Schaeff.) Ricken – cucumber aroma (Poucher 1991); *Lacatry glyciosmos* – bergamot (probably *Lactarius glyciosmus* (Fr.) Fr.); *Lactory camphoratus* – melilot (possibly *Lactarius camphoratus* (Bull.) Fr.) or *Hygrophora hyacenthinus* – jasmine (probably *Hygrophorus hyacinthinus* Quél), *Clitocybe geotrope* – lavender (probably *Clitocybe geotropa* (Bull.) Quél), *Psatella arvensis* (probably *Pratella arvensis* (Schaeff.) Gillet = *Agaricus arvensis* Schaeff.) – aniseed. Most of the mushrooms mentioned by Poucher (1991) cannot be found in the Index Fungorum database, probably due to the errors he made in the spelling of the names. My conjectures of the proper names of those fungi might be incorrect. Although the above-mentioned fungi have not been well recognized by Poucher, it is worth pointing out that they were for the first time treated as useful sources of fragrances in cosmetic and perfumery industries.

Basidiomycetes show a broad profile of smells: from a bitter almond-like one in *Clitocybe gibba* (Pers.) P. Kumm., *C. odora* (Bull.) P. Kumm., an almond-like scent combined with a nut flavour in *Agaricus bitorquis* (Quél.) Sacc., a flour-like one in *Tricholoma equestre* (L.) P. Kumm., *Calocybe gambosa* (Fr.) Singer, *Catathelasma imperiale* (Quél.) Singer, *Entoloma sinuatum* (Bull.) P. Kumm., a carbide-like one – *Tricholoma sulphureum* (Bull.) P. Kumm., a fruit-like one in *Lepista nuda* (Bull.) Cooke, *Inocybe erubescens* A. Blytt, *Russula emetica* (Schaeff.) Pers. and *Lactarius deliciosus* (L.) Gray, *L. deterrimus* Gröger, *Fistulina hepatica* (Schaeff.) With., a cucumber combined with flour-like one in *Lyophyllum connatum* (Schumach.) Singer and in *Mycena galericulata* (Scop.) Gray, a cucumber- or herring-like one in *Macrocystidia cucumis* (Pers.) Joss., a garlic-like one in *Marasmius alliaceus* (Jacq.) Fr., *Micromphale perforans* (Hoffm.) Gray, a radish-like one in *Mycena pura* (Pers.) P. Kumm., *Volvariella speciosa* (Fr.) Singer, *V. bombycina* (Schaeff.) Singer and in *Pluteus cervinus* (Schaeff.) P. Kumm., a mushroom odour combined with an anise-like one in *Leucoagaricus leucothites* (Vittad.) Wasser and more distinct anise-like one in *Agaricus sylvicola* (Vittad.) Peck or anise odour in *Gloeophyllum odoratum* (Wulfen) Imazeki and *Trametes suaveolens* (L.) Fr., an odour of a raw potato in *Amanita citrina* Pers., a dill-like one in *Polyporus* *umbellatus* (Pers.) Fr. (Snowarski 2005, 2010). Chemical analyses reveal many fungal substances which can be sources of different fragrances and flavours (Table 1). *Nidula niveotomentosa* (Henn.) Lloyd (a bird's nest fungus) is an example of a species applied in biotechnology as it is used to produce a characteristic raspberries compound – raspberry ketone [4-(4-hydroxyphenyl)butan-2-one] and betuligenol in submerged cultures (Taupp *et al*. 2008). In *Clitopilus prunulus* (Scop.) P. Kumm., *Catathelasma ventricosum* (Peck.) Singer as well as *Tricholoma virgatum* (Fr.) P. Kumm. for the cucumber odour (*E*)-non-2-enal is responsible. In Greece a high-quality mushroom *Hygrophorus russocoriaceus* Berk. & T.K. Mill. grows, whose unique cedar aroma is attributed to sesquiterpenes found only in this species of the genus *Hygrophorus*. *Lactarius helvus* (Fr.) Fr. possesses a characteristic chicory and fenugreek smell; therefore it is used as a spice mushroom, although raw it is mildly toxic. It is known as a maggi-pilz (maggi-mushroom) (Fraatz & Zorn 2010). *Ceratocystis* species have been studied because of their fruit-like aromas, *C. variospora* (R.W. Davidson) C. Moreau as a source of geraniol (Reineccius 1994). Some fungi show a significant anise odour, in *Clitocybe odora* *p*-anisaldehyde masks other flavour compounds. Anisaldehyde is also responsible for an odour in other *Clitocybe* spp., but in *Agaricus essettei* Bon and *Gyrophragmium dunalii* (Fr.) Zeller a mixture of benzaldehyde and benzyl alcohol makes a profile of its odour (Rapior *et al.* 2002, Fraatz & Zorn 2010). *Lentinellus cochleatus* (Pers.) P. Karst. as well as *C. odora* and *A. essettei* are known for their anise-like scent, but *L. cochleatus* has a different compound profile: *p*-anisaldehyde, methyl *p*-anisate, methyl(*Z*)-*p*-methoxycinnamate and methyl (*E*)-*p*-methoxycinnamate are responsible for an anise-like aroma (Rapior *et al*. 2002).

Although fungi as large-fruited mushrooms are edible for humans, they are not of vital importance for the diet. Boletus King Bolete (*Boletus edulis* Bull.) is appreciated for its special mild taste and nice mushroom scent (tab. 1), and is one of the most popular edible mushrooms in Europe. The other one is a white button mushroom (*Agaricus bisporus* (J.E. Lange) Imbach) - the most commonly cultivated and consumed species in the world (Fraatz &Zorn 2010). Another important but not so famous species is an oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.). Finally, truffles (*Tuber aestivum* (Wulfen) Spreng. – summer truffle, *T. melanosporum* Vittad. – black truffle) are famous for their special aroma and for prices they obtain on the market. Truffles differ strongly in aroma depending on the geographical origin. In order to 'reveal' their distinct mushroom aroma for which oct-1-en-3-ol, octan-3-one, octan-3-ol and oct-1-en-one are responsible, the fruiting bodies with a characteristic sulphurous odour must be left open to the air. The aroma profile of mushrooms changes not only because of the geographical origin but also due to the environmental differences, processing, storing and the age of mushrooms as well as part of fruiting body (caps or gills or hyphae) (Fraatz & Zorn 2010). Some small truffles: *Tuber beyerlei* Trappe, Bonito & Guevara, *T. castilloi* Guevara, Bonito & Trappe, *T. guevarai* Bonito & Trappe, *T. lauryi* Trappe, Bonito & Guevara, *T. mexiusanum* Guevara, Bonito & Cázares, *T. miquihuanense* Guevara, Bonito & Cázares and *T. walker* Healy, Bonito & Guevara grown in Mexico and the USA have a garlic odour (Guevara *et al.* 2013).

Volatile compounds of *A. bisporus* were broadly studied and it was found that the most characteristic flavour compound is defined mainly by C_8 volatiles. Among all volatile compounds, the participation of C_8 volatiles can vary in the case of white button mushroom (from 44% to 98%) and in other mushrooms. It depends on the differences between the species type, conditions of production, time of growth, nitrogen and carbon sources, conditions of post-harvest processing. Among all C_8 compounds, the most important is oct-1-en-3-ol (1-octene-3-ol), but finally over 150 different volatiles have been recovered in the white button mushroom. Besides C_8 volatiles, other aromatic compounds were identified: benzyl alcohol, benzaldehyde, *p*-anisaldehyde (4-methoxybenzaldehyde), benzyl acetate, phenyl ethanol (Dijkstra 1976, Dijkstra & Wikén 1976, Fraatz & Zorn 2010).

The oct-1-en-3-ol occurs in two optically active forms, a predominant in nature (R) – (-)-oct-1-en-3-ol has a fruity mushroom-like odour identified as more intense than (S) – (+)- isomer (note of mould and grass). Additionally the oct-1-en-3-ol was found in black currants, strawberries and potatoes (Dijkstra 1976, Dijkstra & Wikén 1976, Fraatz & Zorn 2010). The other odour is due to oct-1-en-3-one and is described as an odour of boiled mushrooms. With the increase of its concentrations a metallic note appears. In the miscellaneous fungi the predominant components are: oct-1-en-3-ol, oct-1-en-3-one, oct-2-en-1-ol, octan-1-ol, octan-3-ol, octan-3-one, benzaldehyde, limonene, N(2phenyethyl)acetamide, geranyl acetone, farnesyl acetone, (*E,E*)-farnesol (Fraatz & Zorn 2010).

The oct-1-en-3-ol (1-octene-3-ol) was isolated for the first time by Freytag and Ney in 1968 (Dijkstra & Wikén 1976), its typically mushroom aroma is produced by *Aspergillus oryzae* (Ahlb.) Cohn and blue cheese fungus (*Penicillium* spp., *P. roqueforti* Thom). During ripening *P. roqueforti* produces ketones: 2-pentanone, 2-heptanoneand 2-nonanone probably of the prime importance for the typical flavour of Roquefort, Cheddar and other cheeses (Reineccius 1994). Garlic-like and cheese-like aromas with distinct note of mature Camembert were found in *Kalpuya brunnea* M. Trappe, Trappe, & Bonito, a new truffle (Morchellaceae) species described in Oregon, where it is known as an Oregon truffle and is locally collected for commercial use (Trappe *et al.* 2010). Dijkstra and Wikén (1976) found that nucleotides, amino-acids and carbohydrates also contributed significantly to the mushroom flavour of *Agaricus bisporus*, whereas a less significant influence was observed in the case of (the less contribution showed) low-boiling volatiles: benzaldehyde, benzyl alcohol, 1-octen-3-one, *n*-butyric acid and isovaleric acid. No synergistic influence of those components on the flavour was observed by Dijkstra (1976) and Dijkstra and Wikén (1976).

The intensity of flavour differs due to the loss of volatiles and some decomposition of flavours. This is important in the production of commercial mushroom concentrates as Hansen and Klingenberg (1983) showed analysing the differences between distillates of twelve mushrooms concentrates obtained from European manufactures. They recognized 1-octen-3-ol, 1-octen-3-one, L-glutamic acid, guanosine-5’-monophosphate as main flavour components. GLC/MS analysis revealed components of mushroom flavour related to 1-octen-3-ol (1-octanol, 3-octanol), aromatic compounds (benzaldehyde, phenylethanol, phenyl acetaldehyde), terpenes (terpinene-4-ol), N-containing components (e.g. pyrazine derivatives). Some of these components were not genuine, e.g. phenylethanol, phenylacetaldehyde, terpinene-4-ol, some of them were recovered only in trace amounts. In some cases C_8 volatiles were not present, although they were described as 'similar to mushroom and yeast'. In the processing as well as cultivation of mushrooms for a natural flavour, it is important that conditions of these processes are shaped with special care, according to the principles suggested for shiitake mushrooms by Yoshii (1980).

The concentration of oct-1-en-3-ol and benzylic acid increases with the maturation of fruiting bodies of mushrooms. These compounds, and broadly C_8 volatiles, are emitted by mushrooms to attract insects to distribute their spores; in some cases oct-1-en-3-ol plays a role as an aggregation hormone for certain beetles. The phenomenon is also discussed with reference to *Fomitopsis pinicola* (Sw.) P. Karst., *Fomes fomentarius* (L.) Fr. as well as in genera *Aspergillus* and *Penicillium* (Fraatz & Zorn 2010). The concentration of oct-1-en-3-ol decreases with an increasing age of the fruiting bodies of shiitake (*Lentinula edodes*), while the amount of octan-3-one increases (Fraatz & Zorn 2010).

Growing fungi in artificial media should be preceded with careful investigation on media composition and conditions of culture (e.g. irradiation). A periodically illuminated culture of *Nidula niveotomentosa* synthesises raspberry compounds from L-phenylalanine. Typically mushroom compounds, oct-1-en-3-ol and octan-3-one, can be produced in submerged cultures by *Penicillium vulpinum* (Cooke & Massee) Seifert & Samson as lipid degradation products. *Pleurotus ostreatus* shows a varied flavour composition in a liquid medium depending on the age and culture conditions, although the predominant compounds oct-1-en-3-ol and octan-3-one were detected. *Pleurotus florida* (=*P. ostreatus* f. *florida* Cetto) obtained in laboratory cultures differed from fruiting bodies and showed a sweet anise and almond scent resulting from the synthesis of p-anisaldehyde and benzaldehyde, whereas the natural compound of *P. florida* oct-1-en-3-ol occurred only in minute amounts (Taupp *et al.* 2008, Fraatz & Zorn 2010).

Woźniak (2007) described a procedure for producing the biomass of mycelium of three varieties of King Bolete designed for consumption. She recommended the procedure for the production of mycelium for dried biomass as a supplement of dried mushrooms, although the concentration of C_8 compounds as well as oct-1-en-3-ol was significantly lower in the mycelium obtained from a submerged liquid culture than in fresh fruiting bodies. The investigations will enable the biotechnological production of mushroom compounds important as flavours and odours. These volatile compounds are commonly known and they are recognized by smell and taste receptors as typical of mushroom or fungal origin. On the other hand, fungi and mushrooms can serve as a source of other compounds and in this way give a possibility of cheaper production than isolation from a natural source.

Table 1. The most popular fungal flavour and smell compounds (according to Nyegue *et al.* 2003, and Fraatz & Zorn 2010).

Fungal species	Number of detected compounds	Predominant compound of a flavour	Additional flavour compounds
Agaricus bisporus (J.E. Lange) Imbach (white button mushroom)	>150 (also 70 or 22 depending on the method)	C_8: oct-1-en-3-ol, octan-3-ol, octan-3-one, oct-2-en-1-ol	Benzyl alcohol, benzaldehyde, *p*-anisaldehyde, benzyl acetate, phenylethanol
Boletus edulis Bull. (King Bolete)	70 or ~50 depending on the method	C_8: in canned oct-1-en-3-ol, octan-1-ol, octan-3-ol, in boiled octan-3-one; in dried oct-1-en-3-one, γ-octalacton, octa-2,4-dien-1-ol, octanal	--
Calocybe indica Purkay. & A. Chandra (milky mushroom)	--	C_8 predominant	--
Cantharellus cibarius Fr. (chantarelle)	--	α-humulene, α-copaene, β-caryophyllene	--
Hygrophorus spp. (waxy caps)	45	3-methylbutanal, hexanal, p-cymene, octan-3-one, oct-1-en-3-one, octan-3-ol, methyl benzoate,	sesquiterpenes in *H. russocoriaceus*
Lactarius helvus (Fr.) Fr. (Maggi-pilz)	38	capric acid, sotolon, 2-methylbutanoic acid	--
Lentinula edodes (Berk.) Pegler (shiitake)	130; 18 sulphur containing compounds	oct-1-en-3-ol, octan-3-ol, octan-3-one, oct-4-en-3-one, lenthionine (a cyclic sulphur-containing compound)	dimethyl disulphide, dimethyl trisulphide, 1,24-trithiolane
Marasmius alliaceus (Jacq.) Fr. (garlic mushroom)	16 and 27 depending of the method	1,3-dithietane, benzaldehyde, 2,3,5-trithiahexane, 2,3,4,6- tetrathiaheptane, dimethyl disulphide, dimethyl, dimethyl trisulphide, tetrasulphide, 2,4,5,7-tetrathiaoctane,	--
Phallus impudicus L. (common stinkhorn)	--	dimethyl disulphide, dimethyl trisulphide, linalool, (E)-ocimene, phenyl acetetylaldehyde	--
Pleurotus florida (= *P. ostreatus* f. *florida* Cetto) (paddy straw and cotton mushroom; Florida oyster mushroom)	--	C_8: oct-1-en-3-ol	--

Fungal species	Number of detected compounds	Predominant compound of a flavour	Additional flavour compounds
Pleurotus ostreatus (Jacq.) P. Kumm. (oyster mushroom)	28	C_8: oct-1-en-3-ol, octan-3-ol, octan-3-one, octanal, oct-1-en-3-one, (*E*)-oct-2-enal,octan-1-ol octan-3-one (80%), octan-3-ol (14%)	Benzaldehyde (almond odour) Benzyl alcohol (sweet-spicy)2-phenylethanol (rose scent)
Polyporus sulphureus (Bull.) Fr. [*Laetiporus sulphureus* (Bull.) Murrill] (sulphur polypore)	40	in young fruiting bodies: oct-1-en-3-one, oct-1-en-3-ol, 3-methylbutanoic acid, 2-phenylethanol, 2-phenylacetic acid	in aged: 2-methylpropanoic acid, butanoic, acid, 2-phenlylacetic acid,
Termitomyces shimperi Heim. (a mushroom cultivated by termites)	24	oct-1-en-3-ol, 2-phenylethanol, hexanal	--
Tricholoma matsutake (S. Ito & S. Imai) Singer (pine-mushroom)	23	oct-1-en-3-one, oct-1-en-3-ol, octan-3-ol, octan-3-one, (E)-dec-2-enal, α-terpineol, phenylethyl alcohol, ethyl 2-methylbutanoate	2-methylbutyrate, linalool, methional,
Tuber aestivum (Wulfen) Spreng. (summer truffle), *T. melanosporum* Vittad. (black truffle)	72-89 17	dimethyl sulphide, dimethyl trisulphide, oct-1-en-3-ol, octan-3-one, octan-3-ol, oct-1-en-3-one	butan-2-on, butan-2-ol
Volvariella volvacea (Bull.) Singer (padi straw mushroom)	--	oct-1-en-3-ol, oct-2-en-1-ol, limonene, oct-1,5-dien-3-ol, octan-3-ol, octan-1-ol,	--

Mushrooms and fungi can also emit unpleasant odours (Pildain *et al.* 2010). *Hygrophorus paupertinus* A.H. Sm. & Hesler, is the one whose odour is defined as ‘’exceedingly strong, penetrating, and disagreeably and distinctly faecal-like’. Volatile compounds responsible for the odour are highly odoriferous 3-chloroindole, indole and 1-octen-3-ol, although the last is known as a compound responsible for a nice mushroom aroma. 3-Chloroindole is known for its faecal-like odour when is concentrated, but simultaneously the same compound becomes pleasant in highly diluted solutions (Poucher 1991). Indole and 3-methylindole were identified in other species with unpleasant odours: *Tricholoma bufonium* (Pers.) Gillet, *T. inamoenum* (Fr.) Gillet, *T. lascivum* (Fr.) Gillet, *T. sulphureum, Lepiota bucknallii* (Berk. & Broome)

Sacc., *Morchella conica* Pers., *Coprinus picaceus* (Bull.) Gray, *Boletus calopus* Pers., *Gyrophragmium dunalii* (Wood *et al.* 2003). Other indole compounds were also found in the fruiting bodies of some mushroom species - *Auricularia polytricha* (Mont.) Sacc., *Suillus bovinus* (L.) Roussel, *Macrolepiota procera* (Scop.) Singer*, Lentinula edodes* (Berk.) Pegler, *Leccinum scabrum* (Bull.) Gray, both before and after thermal processing (Muszyńska *et al.* 2013).

Alcohol (ethanol) is another flavour of fungal origin. Yeasts and *Rhizopus* spp. are responsible for its smell in the case of beer, wine, vodka and exotic fermented beverages such as parakari (fermented cassava) (Henkel 2005). May this popular flavour close the door of fungal world of flavour, odours and aromas as well as fragrances.

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Streszczenie

Grzyby w naszym otoczeniu stanowią nie tylko ciekawy świat kształtów, ale także są nośnikami ciekawych zapachów. Mykolodzy procujący z grzybami mikroskopowymi doskonale wiedzą, że niektóre z nich wydają charakterystyczną woń, jak np. woń czosnku typową dla rodzaju *Mortierella* lub kokosową w przypadku *Trichoderma* spp. W świecie grzybów spotkać można także takie zapachy, którym towarzyszy specyficzny smak. Któż nie zna typowego określenia „smak/aromat grzybowy”? Każdy, nawet osoba niezwiązana zawodowo z grzybami wie, co kryje się pod tym określeniem. Ale co stanowi, że możemy zdefiniować to określenie od strony chemicznej? Kluczową grupą substancji odpowiedzialnych za typowy smak i aromat grzybowy są lotne pochodne związków z grupy C_8, a wśród nich najbardziej istotne okazują się być oct-1-en-3-ol, octan-3-ol, octan-3-on, oct-1-en-3-on.

Grzyby dostarczają rozmaitych wrażeń zapachowych, począwszy od miłych anyżkowych, owocowych, poprzez ogórkowe, czosnkowe, serowo-czosnkowe, następnie przypominające ziemniaki lub mąkę, aż do zapachów nieprzyjemnych, jak zapach karbidu lub fekaliów. Smak niektórych z nich jest skorelowany z zapachem, w wielu przypadkach smaków nie znamy. Za każdym z tych aromatów ukrywają się związki chemiczne pozwalające czasem na uzyskanie wstępnej informacji taksonomicznej.

Bogactwo aromatów w świecie grzybów pozwala na biotechnologiczne wykorzystanie ich do otrzymywania bądź to czystych związków, jak np. w przypadku ketonu malinowego pozyskiwanego z *Nidula niveotomentosa*, bądź np. aromatycznej grzybni mogącej zastąpić rzadkie i pożądane gatunki grzybów leśnych, np. borowików.