AROMA COMPOUNDS IN ELEVEN EDIBLE MUSHROOM SPECIES: RELATIONSHIP BETWEEN VOLATILE PROFILE AND SENSORIAL CHARACTERISTICS

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Abstract

Volatile and semi-volatile components of 11 wild edible mushrooms, *Suillus bellini, Suillus luteus, Suillus granulatus, Tricholomopsis rutilans, Hygrophorus agathosmus, Amanita rubescens, Russula cyanoxantha, Boletus edulis, Tricholoma equestre, Fistulina hepatica* and *Cantharellus cibarius*, were determined by headspace solid-phase microextraction and by liquid extraction combined with gas chromatography-mass spectrometry. 51 volatiles were formally identified, based on commercial references, and the 13 others were tentatively identified. Using sensorial analysis, the descriptors “mushroom-like”, “farm-feed”, “floral”, “honey-like”, “hay-herb” and “nutty” were obtained. A correlation between sensory descriptors and volatiles was observed by applying multivariate analysis (principal component analysis and agglomerative hierarchic cluster analysis) to the sensorial and chemical data. The studied edible mushrooms can be divided in three groups. One of them is rich in C8 derivatives, such as 3-octanol, 1-octen-3-ol, trans-2-octen-1-ol, 3-octanone and 1-octen-3-one; another one is rich in terpenic volatile compounds; and the last one is rich in methional. The presence and contents of these compounds give a considerable contribution to the sensory characteristics of the analysed species.

Introduction

Wild edible mushrooms are consumed a lot in many countries as a food. Their culinary and commercial value is mainly due to their organoleptic properties such as their aroma. The aroma of each mushroom species is very characteristic, which determines the distinction between them (1). Among the diverse volatile compounds, a series of aliphatic eight carbon (C8) components, such as 1-octen-3-ol, 2-octen-1-ol, 3-octanol, 1-octanol, 1-octen-3-one, and 3-octanone, have been reported to be the major contributors to the characteristic mushroom flavour. 1-octen-3-ol, described as “mushroom-like flavour” and “raw mushroom” is considered to be the main responsible for the characteristic flavour of most of the edible mushroom species (2). Despite the high consumption of mushrooms, few studies concern their aroma.

In this work, different volatile extraction techniques were used in order to get a complete screening of volatile and semi-volatile compounds of all eleven wild edible mushrooms. HS-SPME technique was used into the headspace of the mushroom and the less volatile compounds were obtained using organic solvents. Finally, a

relationship between the contents of the identified volatiles and the sensorial descriptors was established.

Experimental

Samples. Samples of eleven different wild edible mushroom species (*Suillus bellini, Suillus luteus, Suillus granulatus, Tricholomopsis rutilans, Hygrophorus agathosmus, Amanita rubescens, Russula cyanoxantha, Boletus edulis, Tricholoma equestre, Fistulina hepatica* and *Cantharellus cibarius*) were collected in Trás-os-Montes region.

Sensorial studies. A panel composed by seven people was engaged in sensorial determinations. For the descriptors selection, 0.5 g of each mushroom powder were putted into an empty 15 mL vial, which was immediately sealed with a PTFE-silicone septa and put in a magnetic plate at 600 r.p.m. for 10 min, at 45 ºC. The AFNOR procedure was used to select the most important descriptors (3).

Chemical extraction. 25 mg of each mushroom powder were analysed by HS-SPME (4), for dichloromethane (DCM) extraction, approximately 200 mg of each mushroom powder were used according to (5).

Gas Chromatography - Mass Spectrometry analysis. HS-SPME and dichloromethane extracts were analysed using a Varian CP-3800 gas chromatograph (USA) with a VARIAN Saturn 4000 mass selective detector (USA) and a Saturn GC/MS workstation software 6.8 (4). Identification of compounds was achieved by i) comparing the mass spectra present in the NIST 05 MS Library Database, ii) the Kovats indices and/or iii) by comparisons of the mass spectrum obtained from the sample and those from pure standards injected in the same conditions.

Peak areas were determined by re-constructed FullScan chromatogram using for each compound some specific ions (quantification ions, Table1). The relative area of each peak (%) was determined, considering 100% de sum of all identified peaks.

Statistical analysis. Principal component analysis (PCA) and Agglomerative hierarchic cluster analysis (dendogram) (AHC) were carried out using XLSTAT 2007.5 software.

Results

Sensory results. The descriptors selected were “farm-feed” (28%), “mushroom-like” (24%), “floral”(18%), “honey-like”(8%), “nutty”(8%), “hay-herb”(7%) and 7% corresponded to other descriptors which were discarded. The evaluation of the analysed mushroom species showed several differences among their sensory profiles. Using PCA with a total variance of 75.8% (F1 and F2 axes), the 11 mushroom species can be divided in three groups. The one with “floral” and “honey” descriptors includes *S. granulatus* and *S. luteus*; the second group, presenting “hay-herb”, “nutty” and “mushroom-like” notes is composed by *A. rubescens*, *C. cibarius*, *S. bellini*, *T. equestre*; and finally the species characterized by “farm-feed” are *H. agathosmus*, *T. rutilans*, *R. cyanoxantha*, *B. edulis*, *F. hepatica*.

Aroma composition. HS-SPME and dichloromethane extractions allowed the identification of 64 volatile compounds in the analysed mushroom species (Table 1). These include a total of 5 volatile acids, 8 non volatile acids, 7 esters, 9 alcohols, 7 aldehydes, 7 ketones, 11 terpenes, 1 volatile phenol, 2 lactones and 7 other compounds. Thirteen compounds were tentatively identified and fifty one were identified by comparison of the Kovats index and the MS spectrum of the pure

chemical standard. In some cases the same extracts and standards were injected on two different polarity columns. Among the volatile acids there are 5 fatty acids (myristoleic, palmitoleic, stearic, linoleic and oleic acids) and 3 others: benzoic, cinnamic and phenylacetic acids. *H. agathosmus* presented the highest percentage of myristoleic, palmitoleic, oleic and cinnamic acids. On the other hand, the species that contained the highest esters percentage – *T. rutilans* and *F. hepatica* – correspond to the ones that presented the lowest percentage of non volatile acids. *S. bellini* was identified as the richest species in alcohols, which are considered to be the main odorants of the "mushroom-like" aroma (2). Among these compounds, *C. cibarius* presented the highest percentage of 1-octen-3-ol, while *A. rubescens* was the one with the highest amount of 3-octanol. *T. equestre* also had a considerable percentage of these two compounds. Statistical results showed that these alcohols have higher correlations with the "nutty" descriptor than with the "mushroom-like" aroma (r=0.897 and r=0.537, respectively). *T. equestre* and *S. luteus* presented the highest levels of aldehydes. Benzaldehyde and phenylacetaldehyde were identified in all of the species. Phenylacetaldehyde is considered to be responsible for "honey" notes (2), *S. luteus* was the specie presenting the highest levels of this aldehyde. *B. edulis* was the richest specie in methional. This compound has a very low olfactive perception limit and its descriptor is "boiled potato" (6). This descriptor was not used by the panel for these mushroom species; however, this species was described with notes of "farm-feed". A very high correlation between methional and "farm-feed" descriptor has been found (r= 0.791), the presence of this compound can explain its aroma characteristics (6, 7), methional has been recently identified in pine-mushroom specie (7).

Among the identified ketones, two different groups emerge: one is constituted by 3-octanone and 1-octen-3-one, while the other one is composed by volatile norisoprenoids, such as β-ionone, 6-methyl-5-hepten-2-one, *trans*-geranylcetone, (*E,E*)-farnesylacetone. These odour-active substances are known to be oxidative by-product or degradation products derived from carotenoids (8, 9). These 4 compounds have never been identified in mushrooms. The *trans*-geranylcetone and the (*E,E*)-farnesylacetone are present in higher levels in the *S. bellini*, *S. granulatus* and *S. luteus* mushroom species, it seems that these compounds can be markers of this mushroom genus. *A. rubescens* was the specie that present the highest contents of 3-octanone, while *C. cibarius* contains the highest amount of 1-octen-3-one. *S. bellini*, *S. granulatus* and *S. luteus*, were the richest species in norisoprenoid compounds. Several terpene compounds have been identified in fresh wild mushrooms before (10), however the *trans*-nerolidol, eucalyptol, menthol and 1,4-cineole have not been found in mushroom species (Table1).

Using an Agglomerative Hierarchic Cluster Analysis (HCA) the eleven studied species were divided in three groups: group 1 was composed by *S. bellini*, *A. rubescens*, *T. equestre* and *C. cibarius*; group 2 comprised *T. rutilans*, *H. agathosmus* and *B. edulis*; and group 3 included *S. luteus*, *S. granulatus*, *R. cyanoxantha* and *F. hepatica*. As far as we know, this work is the first approach to the volatile characterization of these edible mushroom species. The employment of two extraction techniques combined with GC-MS permitted the identification of a large number of compounds in all the studied species. It was possible to distinguish groups of wild edible mushroom species based in their odour properties and their aroma chemical composition.

Table 1. Relative percentage (%) of some of the 64 volatile compounds of mushroom species using HS-SPME and by DCM extraction.

Compound	Kovats index	Quantification Ions (m/z)	Samples (RA%)											Method
			A. rubescens	*B. edulis*	*C. cibarius*	*F. hepatica*	*H. agathosmus*	*R. cyanoxantha*	*S. bellini*	*S. granulatus*	*S. luteus*	*T. equestre*	*T. rutilans*	
trans-2-Hexen-1-ol [a,c]	814	57;82	nd	nd	33.1	nd	75.4	nd	100	nd	nd	nd	nd	**HS-SPME**
1-Hexanol [a,c]	841	56;69	nd	12.3	100	4.1	32.9	nd	29.8	21	nd	19.1	8.8	**HS-SPME**
Methional [a,c]	858	76;104	nd	100	nd	nd	nd	nd	nd	8	19	nd	nd	**HS-SPME**
α-Pinene [a,b]	948	93	63.6	11.2	4.9	6.7	13.1	31.9	15.8	9.1	100	10.2	13.3	**HS-SPME**
1-Octen-3-one [a,c]	956	55;97	15.7	8.9	100	nd	nd	nd	nd	nd	13	nd	nd	**HS-SPME**
6-Methyl-5-hepten-2-one [a,b]	962	93	nd	nd	nd	nd	nd	13.3	100	nd	40.6	nd	nd	**HS-SPME**
3-Octanone [a,c]	970	43;99	100	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**HS-SPME**
β-Pinene [a,b]	978	93	18.1	nd	nd	21.3	32.8	nd	71.4	100	26.5	nd	nd	**HS-SPME**
3-Octanol [a,c]	979	55;83	100	nd	nd	nd	nd	nd	nd	nd	0.1	nd	nd	**HS-SPME**
1-Octen-3-ol [a,c]	998	57;99	17.4	31	100	1.6	64.4	16.4	40.3	4.9	nd	58.8	35.5	**HS-SPME**
1.4-Cyneole [a,b]	1012	111	nd	nd	nd	nd	nd	3.1	nd	100	1.4	nd	1.3	**HS-SPME**
Limonene [a,c]	1018	67	11.4	2.6	3.3	6.1	4.1	20.8	2.7	14.5	100	1.5	8.4	**HS-SPME**
Eucalyptol [a,c]	1059	93	48.6	29.3	39.9	74.6	45.4	42	26.7	63.9	nd	16.6	100	**HS-SPME**
Phenylacetaldehyde [a,c]	1081	91	7.3	2.2	1.5	12.3	5.5	16.2	3.5	30.6	100	19.4	4.2	**HS-SPME**
trans-2-Octen-1-ol [a,c]	1064	57;81;95	20.7	nd	nd	3.2	nd	20.6	100	2.2	nd	81.2	82.7	**HS-SPME**
Linalool [a,c]	1082	93	17	1	nd	1.6	4	22	18.7	100	25.2	nd	nd	**HS-SPME**
2-Phenylethanol [a,c]	1091	91	6.6	18.4	1.6	30.8	9.6	13.8	3.8	100	25.9	40.5	7.2	**HS-SPME**
Menthol [a,c]	1164	81	7.7	31	78.5	21.2	21.4	18.9	100	31.3	8.1	22.7	16.2	**HS-SPME**
α-Terpineol [a,c]	1175	59;121	nd	nd	nd	21.7	nd	100	nd	63.1	50.7	nd	nd	**HS-SPME**
trans-Geranylcetone [a,c]	1383	107	nd	4.5	nd	5.1	7.3	7.3	41.9	100	65.4	2.6	nd	**DCM**
β-Ionone [a,c]	1457	177	nd	59	nd	nd	79.1	nd	37.7	nd	100	87.1	17.4	**HS-SPME**
trans-Nerolidol [a,c]	1564	93	nd	nd	nd	nd	nd	nd	11.3	84.8	100	nd	nd	**HS-SPME**
Farnesylacetone [a,b]	1902	69	nd	nd	nd	nd	nd	nd	57.1	100	98.4	nd	nd	**DCM**

[a] MS, identified by NIST05; [b] RI, identified by retention indices; [c] S, identified by comparison with reference compound; 100: highest area obtained; RA (%): relative areas in percentage; nd: not detected

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