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Correlation between the Pattern Volatiles and the Overall Aroma of Wild Edible Mushrooms

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Volatile and semivolatile 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 (HS-SPME) and by liquid extraction combined with gas chromatography–mass spectrometry (GC−MS). Fifty volatiles and nonvolatiles components were formally identified and 13 others were tentatively identified. Using sensorial analysis, the descriptors "mushroomlike", "farm-feed", "floral", "honeylike", "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 analyzed species.

KEYWORDS: Wild edible mushrooms; volatile; semivolatile compounds; sensorial analysis; GC−MS; solid-phase microextraction

INTRODUCTION

Wild edible mushrooms are consumed a lot in many countries as a food; they are cooked or eaten in salads. Their culinary and commercial value is mainly due to their organoleptic properties such as their aroma and flavor. In addition, the aroma is very characteristic of each mushroom species, which determines the distinction between them (*1, 2*).

The determination of volatiles profile has been used in chemometrical comparisons of strains or species (*3*), to assess the authenticity of flavoring substances and food products commercially available (*4*), and as a way of authentication of different species (*5*). Distinctive odors have been used as taxonomic markers for mushroom species identification (*6*). Furthermore, several mushrooms have been screened to establish their flavor profiles in order to produce aroma compounds, using mushroom mycelium, through novel biotechnologies (*7*).

The typical flavor and aroma substances of mushrooms are divided into volatile and nonvolatile compounds. The taste of edible mushrooms is primarily attributed to several water-soluble substances, including 5′-nucleotides, free amino acids, and soluble carbohydrates. 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 flavor. 1-Octen-3-ol, described as "mushroom-like flavor" and "raw mushroom" is considered to be the main component responsible for the characteristic flavor of most of the edible mushroom species (*8*).

Other compounds have also been identified, namely esters and terpenes (*9*), indole, and 3-chloroindole (*10*).

Despite the high consumption of mushrooms, few studies concern their aroma. The Trás-os-Montes region (northeastern Portugal) is known for the variety of its soils and diversity of climate conditions. This variability assumes an important role in mushroom production, which is why this region is recognized as one of the richest regions in wild edible species.

Eleven wild edible mushrooms collected in this region were studied in the present work: *Suillus bellini*, *Suillus luteus*, *Suillus granulatus*, *Tricholomopsis rutilans*, *Hygrophorus agathosmus*, *Amanita rubescens*, *Russula cyanoxantha*, *Boletus edulis*, *Tricholoma equestre*, *Fistulina hepatica*, and *Cantharellus cibarius*.

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Table 1. Characterization of Mushroom Samples

sample	species	origin	orchard	date of collection
1	*Suillus bellini*	Bragança	*Pinus pinaster*	November 2004
2	*Tricholomopsis rutilans*	Bragança	*Pinus pinaster*	December 2004
3	*Hygrophorus agathosmus*	Vinhais	*Pinus pinaster* + *Castanea sativa*	December 2005
4	*Amanita rubescens*	Bragança	*Castanea sativa*	June 2005
5	*Russula cyanoxantha*	Bragança	*Quercus pyrenaica*	May 2006
6	*Boletus edulis*	Bragança	*Castanea sativa*	June 2005
7	*Tricholoma equestre*	Carrazeda de Ansiães	*Pinus pinaster*	November 2005
8	*Suillus luteus*	Vinhais	*Pinus pinaster* + *Castanea sativa*	November 2005
9	*Suillus granulatus*	Bragança	*Pinus pinaster*	November 2005
10	*Fistulina hepatica*	Bragança	*Castanea sativa*	September 2004
11	*Cantharellus cibarius*	Bragança	*Castanea sativa*	May 2005

As far as we know, with the exception of *B. edulis*, *F. hepatica*, *C. cibarius*, and *S. luteus*, there is no knowledge of characterization of these species' volatile compounds.

Earlier studies reported the determination of nitrogenous constituents of *B. edulis* and their relation to flavor (*11*), and the quantification of the enantiomeric ratio of 1-octen-3-ol in this species (*4*), the flavor composition of *C. cibarius* (*7*), the identification of the major volatile compounds of *F. hepatica* (*12*), and the formation of volatile compounds from submerged and surface-cultured mushroom (*9*). Studies determining the volatile organic compounds content in roots of *Pinus sylvestris* seedlings inoculated with *S. luteus* have also been performed (*13, 14*).

In this work, different volatile extraction techniques were used in order to get a complete screening of volatile and semivolatile compounds of all these species. The HS-SPME technique was used into the headspace of the mushroom powder and in the headspace of the mushroom dissolved in 10% ethanol. In addition, 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.

MATERIALS AND METHODS

Standards. Reference compounds were purchased from various suppliers: 4-decanol (Acros Organic, USA), butyric, isobutyric, isovaleric, caproic, palmitoleic, myristoleic, stearic, oleic, linoleic, cinnamic, and benzoic acids, caproic acid ethyl ester, caprylic acid ethyl ester, capric acid ethyl ester, eugenol, furfuryl alcohol, *trans*-2-octen-1-ol, 2-phenylethanol, 6-methyl-5-hepten-2-one, β-pinene, pantolactone, valerolactone, limonene, nerolidol, geranylacetone, methional, and ergosterol were from Sigma (St. Louis, MO); phenylacetic acid, hexyl acetate, *trans*-2-hexen-1-ol, 1-hexanol, nicotinamide were from Merck (Darmstadt, Germany); 3-octanol, 1-octen-3-ol, benzylic alcohol, benzaldehyde, phenylacetaldehyde, (*E*)-2-decenal, 3-octanone, β-ionone, and 1-octen-3-one were obtained from SAFC (Steinheim, Germany); 1,4-cineole, *o*-cymene, linalool, α-terpineol, limonene, α-pinene, and eucalyptol were from Extrasynthese (Genay, France); and menthol and (*E,E*)-farnesylacetone from Fluka (Buchs, Switzerland).

Samples. Samples of 11 different wild edible mushroom species were collected in Trás-os-Montes region (northeast of Portugal), and their characterization is described in **Table 1**. After being harvested, the mushrooms were immediately transferred to the laboratory. Taxonomic identification followed that of several authors (*15–20*) and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança. Samples were dehydrated in a ventilated oven at 30 °C, for 5 days. All materials were kept in the dark, in hermetically sealed bags, and were screened through a 910 μm fine sieve before analysis.

Headspace Solid Phase Microextraction (HS-SPME) Technique. *HS-SPME Fibers.* There are several commercial fibers that can be used to extract volatiles. According to the literature (*21*), three of them are the most adaptable to the mentioned compounds and the matrix in study. The used fibers were coated with different stationary phases and various film thicknesses: black, carboxen/polydimethylsiloxane (CAR/PDMS), 75 μm; orange, carbowax/divinylbenzene (CW/DVB), 65 μm; and gray, divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS), 50/30 μm. They were conditioned by inserting them into the GC injector. The time and temperature conditions were 300 °C for 1 h, 220 °C for 30 min, and finally 270 °C for 1 h.

HS-SPME Powder. Approximately 25 mg of each mushroom powder were hermetically sealed in a 15 mL vial with a polypropylene hole cap and PTFE/silicone septa (Supelco, Bellefonte, PA). The sample was then magnetically stirred at 600 rpm, at 35 °C, for 30 min. Afterward, the fiber was exposed to the headspace for 15 min with an agitation of 800 rpm. Subsequently, it was pulled into the needle sheath and the HS-SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption. After 10 min, the fiber was removed from the injection port and conditioned in another GC for 15 min at 250 °C (*22–24*).

HS-SPME Solution. To quantify volatiles in mushrooms, we dissolved approximately 25 mg of each mushroom powder in 5 mL of 10% ethanol solution in a 15 mL vial, to which 50 μL of internal standard (4-decanol) in alcoholic solution (1.26 mg/L) was added. One-half a gram of anhydrous sodium sulfate was added to promote the release of analytes from the matrix. After this, the vial was sealed with a polypropylene hole cap and PTFE/silicone septa (Supelco, Bellefonte, PA) and the sample was magnetically stirred at 760 rpm, at 35 °C, for 30 min. The fiber was then exposed to the headspace for 30 min with an agitation of 800 rpm. Afterward, it was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption. After 4 min, the fiber was removed from the injection port and conditioned in another GC for 15 min at 250 °C (*25*). Area of each compound was divided by the area of 4-decanol.

Dichloromethane (DCM) Extraction. Approximately 200 mg of each mushroom powder was extracted with 25 mL of dichloromethane, in which 50 μL of 4-decanol alcoholic solution (1.26 mg/L) were added as an internal standard. The sample suspended in dichloromethane was magnetically stirred at 760 rpm for 4 h and then filtered through a Büchner filter under vacuum. Afterward, the extract was dehydrated with 0.5 g of anhydrous sodium sulfate, concentrated under a stream of nitrogen gas to obtain a final volume of 0.3 mL (*8*).

Gas Chromatography–Mass Spectrometry Analysis. Dichloromethane extracts were analyzed using a Varian CP-3800 gas chromatograph (USA) equipped with a VARIAN Saturn 4000 mass selective detector (USA) and a Saturn GC/MS workstation software version 6.8. The column used for samples analysis was VF-5 ms (30 m × 0.25 mm × 0.25 μm) from VARIAN. Stabilwax-DA fused silica column (60 m × 0.25 mm, 0.25 μm) (Restek, USA) was used in order to check the identity of some compounds found in the first column. The injector port was heated to 220 °C. The injections were performed in a split mode, with a ratio of 1/40. The carrier gas was Helium C-60 (Gasin, Portugal), at a constant flow of 1 mL/min. The oven temperature was set at 40 °C for 1 min, then increased at

2 °C/min to 220 °C and held for 30 min. All mass spectra were acquired in the electron impact (EI) mode. Ionization was maintained off during the first 4 min, to avoid solvent overloading. The ion trap detector was set as follows: the transfer line, manifold and trap temperatures were respectively 280, 50, and 180 °C. The mass ranged from 50 to 600 *m/z*, with a scan rate of 6 scan/s. The emission current was 50 μA, and the electron multiplier was set in relative mode to auto tune procedure. The maximum ionization time was 25000 μs, with an ionization storage level of 35 *m/z*. The injection volume for liquid extracts was 1 μL and the analysis was performed in Full Scan mode.

For HS-SPME analysis, the oven temperature conditions were maintained the same way as for the analysis of dichloromethane extracts. The liner was a HS-SPME specific one and injection was done in split-less mode. Ionization was kept off for only 0.5 min. The mass ranged from 33 to 350 *m/z*.

Identification of compounds was achieved by comparisons of their mass spectra obtained from the sample and those from pure standards injected in the same conditions, and by comparing the Kovat's index with the mass spectra present in the NIST 05 MS Library Database or in the literature.

Sensorial Studies. *Panel.* A panel composed by seven people (university students, professors and laboratory personnel) was engaged in sensorial measurement of reference compounds, in a concentration above their perception limit. Tests were performed using tulip glasses containing 30 mL of each test standard solution, in an adequate room (without sensory odors) at 25 °C. Seven reference compounds were used, based on data obtained from the literature (*26*) and on results previously achieved by GC-MS analysis of the studied mushroom species: *trans*-geranylcetone, *trans*-nerolidol, and limonene representing the "floral/terpenic" aroma; 1-octen-3-one, 3-octanol, and 3-octanone as mushroomlike aroma and isovaleric acid characterizing the volatile acid character.

Descriptors Selection. To the descriptors selection, 0.5 g of each mushroom powder were removed to an empty 15 mL vial, which was immediately sealed with a PTFE-silicone septa (Supelco, Bellefonte, PA) and put in a magnetic plate at 600 rpm for 10 min, at 45 °C. This procedure was performed in order to release the volatile compounds into the vial headspace. Afterward, the container was opened and the panel was asked to give free descriptor terms. The procedure used to select the most important descriptors was the AFNOR NFV-09-021 (*26*). The hedonic and redundant terms, as well as the nonpertinent ones, were then disregarded and a first group of descriptors was thus obtained. The descriptors considered absent by 50% of the panel were eliminated, and a second group was obtained.

Statistical Analysis. Principal component analysis (PCA) and Agglomerative hierarchic cluster analysis (dendogram) (AHC) were carried out using XLSTAT 2007.5 software. PCA method shows similarities between samples projected on a plane and makes it possible to identify which variables determine these similarities and in what way. The dendogram method shows correlations through clusters diagrams.

RESULTS AND DISCUSSION

Sensory Results. The descriptors selected were farm-feed (28%), mushroomlike (24%), floral (18%), honeylike(8%), nutty (8%), hay-herb (7%), and 7% corresponded to other descriptors that were discarded.

The evaluation of the analyzed mushroom species showed several differences among their sensory profiles. The eleven 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 mushroomlike 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* (**Figure 1**).

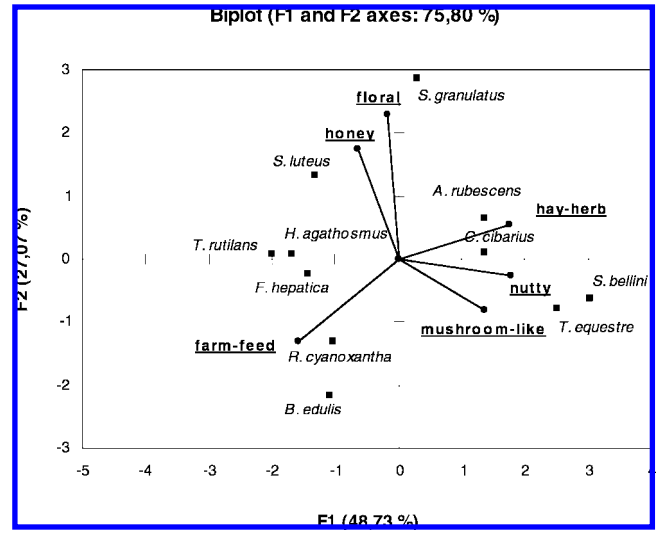

Figure 1. Projection of sensory variables (floral, honeylike, farm-feed, mushroomlike, nutty, and hay-herb) and observation of mushroom samples into the plan composed by the 2 principle axes F1 and F2. The 2 plans contain 75.8% of the total variance.

Analytical Conditions. *Fiber Screening.* After comparing the three fibers, the gray one (divinylbenzene/carboxen/PDMS) was selected for the analysis of all mushroom species, as it was the one that could give the most complete profile of the compounds present in the analyzed species (**Table 2**). In addition, it revealed to be the best and more selective fiber for the identification of aldehydes like methional and phenylacetaldehyde, considered as important to the mushroom flavor (*21, 27*).

Extraction Solvent. To choose the solvent for the volatiles extraction, we tested different organic solvents (hexane, diethyl ether, ethyl acetate, and dichloromethane). By comparing the chromatograms in TIC mode, it has been observed that dichloromethane was the solvent that extracted more compounds.

Some important semivolatile compounds could not be determined using the HS-SPME technique. Therefore, the dichloromethane extraction was chosen to determine those compounds. The volatile compounds are mainly found in HS-SPME extracts, while dichloromethane extracts preferentially present glycerol, fatty acids, phenolic acids (cinnamic and benzoic acids), nicotinamide, ergosterol, and derivatives.

Aroma Composition. Solid-phase microextraction and dichloromethane extraction allowed the identification of 64 volatile and nonvolatile compounds in the analyzed mushroom species (**Table 3**). These include a total of 5 volatile acids (**1**, **2**, **4**, **6**, **14**), 8 nonvolatile acids (**36**, **42**, **48**, **52**, **55**, **58**, **59**, **60**), 7 esters (**20**, **24**, **39**, **46**, **54**, **56**, **57**), 9 alcohols (**5**, **7**, **8**, **21**, **22**, **29**, **32**, **34**, **35**), 7 aldehydes (**3**, **9**, **12**, **13**, **31**, **41**, **43**), 7 ketones (**15**, **17**, **18**, **38**, **49**, **50**, **53**), 11 terpenes (**10**, **19**, **23**, **25**, **26**, **27**, **28**, **33**, **37**, **40**, **51**), 1 volatile phenol (**45**), 2 lactones (**11**, **30**), and 7 other nonvolatile compounds (**16**, **44**, **47**, **61**, **62**, **63**, **64**) (**Table 3**). Thirteen compounds were tentatively identified using the NIST 05 MS Library Database and fifty one were identified by comparison of the kovats index and the MS spectrum of the pure chemical standards as referred in Material and Methods section. In some cases, the same extracts and standards were injected on two different polarity columns.

In what concerns volatile acids, *S. granulatus* and *R. cyanoxantha* were the richest ones, followed by *T. rutilans* and *H. agathosmus*, which also had a considerable percentage.

Table 2. Volatiles Identified by SPME-GC/MS Analysis of a *S. luteus* Mushroom Species, According to the Type of Fiber

number	compd	retention time	fiber		
			DVB/CAR/PDMS	CAR/PDMS	CW/DVB
1	butyric acid	4.223	nd[a]	nd	nd
2	isobutyric acid[b]	4.789	nd	nd	nd
3	hexanal[b]	4.813	48176	132831	31313
4	isovaleric acid	5.452	nd	nd	nd
5	furfuryl alcohol	6.006	nd	nd	nd
6	valeric acid[b]	6.106	nd	nd	nd
7	*trans*-2-hexen-1-ol	6.303	nd	nd	nd
8	1-hexanol	6.428	nd	41842	40699
9	methional	7.516	19727	nd	nd
10	α-pinene	8.111	98626	nd	1627
11	valerolactone	8.725	610643	346258	157101
12	benzaldehyde	9.115	18567	42817	68320
13	3-methyl benzaldehyde[b]	9.215	nd	nd	nd
14	caproic acid	9.327	689777	653220	92046
15	1-octen-3-one	9.411	170197	nd	3775
16	glycerol[b]	9.523	nd	nd	nd
17	6-methyl-5-hepten-2-one	9.658	52819	182812	144273
18	3-octanone	9.726	nd	nd	nd
19	β-pinene	9.739	52819	193605	144011
20	caproic acid ethyl ester	10.018	nd	nd	1896
21	3-octanol	10.051	nd	nd	nd
22	1-octen-3-ol	10.151	nd	9849	nd
23	camphene	10.213	nd	nd	nd
24	hexyl acetate	10.443	169184	44644	8150
25	1,4-cineole	10.548	12586	nd	nd
26	*o*-cymene	10.832	58149	7847	4249
27	limonene	10.966	2655000000	73542	36622
28	eucalyptol	11.087	2216000000	nd	nd
29	benzylic alcohol	11.246	55373	35386	24253
30	pantolactone	11.353	205470	152769	337200
31	phenylacetaldehyde	11.494	1250000000	32686	76194
32	*trans*-2-octen-1-ol	12.124	nd	69932	nd
33	linalool	13.171	92846	385748	151262
34	2-nonen-1-ol[b]	13.376	121188	230920	79600
35	phenylethanol	13.615	108510	32003	54554
36	benzoic acid	14.835	nd	nd	nd
37	menthol	15.533	10606	19342	23367
38	2-piperidone[b]	15.645	nd	nd	nd
39	caprylic acid ethyl ester	15.907	nd	nd	15257
40	α-terpineol	16.062	32963	54125	18130
41	undecanal[b]	16.345	34896	51450	21906
42	phenylacetic acid	17.551	9679	501865	145799
43	(*E*)-2-decenal	17.917	7458	12764	nd
44	indole[b]	18.771	nd	nd	nd
45	eugenol	20.324	3172	nd	4966
46	capric acid ethyl ester	21.328	nd	nd	33638
47	nicotinamide	21.362	nd	nd	nd
48	cinnamic acid	22.207	nd	nd	nd
49	*trans*-geranylcetone	22.767	360797	437857	nd
50	β-ionone	23.627	1578	2510	nd
51	*trans*-nerolidol	25.470	220907	516135	247986
52	myristoleic acid	29.702	nd	nd	nd
53	farnesylacetone	33.008	nd	13228	21884
54	palmitoleic acid methyl ester	33.241	nd	nd	nd
55	palmitoleic acid[b]	33.723	nd	nd	nd
56	linoleic acid methyl ester[b]	36.491	nd	nd	nd
57	elaidic acid methyl ester[b]	36.618	nd	nd	nd
58	stearic acid	37.411	nd	nd	nd
59	oleic acid	37.458	nd	nd	nd
60	linoleic acid	37.821	nd	nd	nd
61	dehydroergosterol[b]	65.608	nd	nd	nd
62	ergosterol	67.519	nd	nd	nd
63	campesterol[b]	70.543	nd	nd	nd
64	lanosterol[b]	74.408	nd	nd	nd
sum of relative areas			6 124 266 713	4 323 144	2 386 446

[a] nd: not detected. [b] Compounds tentatively identified by NIST 05.

Among the volatile acids, there are 5 fatty acids (myristoleic, palmitoleic, stearic, linoleic and oleic acids) and 3 others: benzoic, cinnamic, and phenylacetic acids. In previous reports, other phenolic compounds have been observed in some of the species studied in this work: *C. cibarius* (*28*), *F. hepatica* (*29*), and *A. rubescens, R. cyanoxantha, T. equestre, S. luteus*, and *S. granulatus* (*30*). *H. agathosmus* presented the highest percentage of myristoleic, palmitoleic, oleic, and cinnamic acids.

Table 3. Relative Percentage (%) of Volatile and Semivolatile Compounds of Mushroom Species Using HS-SPME and by DCM Extraction

			samples[a]												
compd	retention time (min)	quantification ions (*m/z*)	*A. rubescens*	*B. edulis*	*C. cibarius*	*F. hepatica*	*H. agathosmus*	*R. cyanoxantha*	*S. bellini*	*S. granulatus*	*S. luteus*	*T. equestre*	*T. rutilans*	method	sensory descriptors (ref)
(**1**) butyric acid	4.223	60; 73	nd	46.3	nd	nd	61.2	nd	41.9	100	nd	nd	nd	DCM	
(**2**) isobutyric acid[b]	4.789	60	44.7	48.2	nd	nd	33	100	22.9	63	nd	14.8	44.7	HS-SPME	(*39*)
(**3**) hexanal[b]	4.813	56	nd	77.9	5.8	10.1	nd	nd	100	nd	11.8	78.5	7.6	HS-SPME	(*38*)
(**4**) isovaleric acid	5.452	60	22.9	21.4	nd	12.5	45.5	95.1	33	100	9.9	12.8	22.9	DCM	(*39*)
(**5**) furfuryl alcohol	6.006	98	nd	100	nd	nd	nd	nd	nd	nd	nd	nd	nd	HS-SPME	(*37*)
(**6**) valeric acid[b]	6.106	60	nd	nd	nd	nd	22.8	69.1	27.6	78.9	nd	5.8	100	HS-SPME	(*39*)
(**7**) *trans*-2-hexen-1-ol	6.303	57; 82	nd	nd	33.1	nd	75.4	nd	100	nd	nd	nd	nd	HS-SPME	
(**8**) 1-hexanol	6.428	56; 69	nd	12.3	100	4.1	32.9	nd	29.8	21	nd	19.1	8.8	HS-SPME	(*38*)
(**9**) methional	7.516	76; 104	nd	100	nd	nd	nd	nd	nd	8	19	nd	nd	HS-SPME	(*32*)
(**10**) α-pinene	8.111	93	63.6	11.2	4.9	6.7	13.1	31.9	15.8	9.1	100	10.2	13.3	HS-SPME	(*35*)
(**11**) valerolactone	8.725	56; 85	nd	7.3	nd	nd	6.1	nd	6.5	100	34.1	6	nd	DCM	
(**12**) benzaldehyde	9.115	105	39.7	11.5	18	11	22.2	55.2	17.7	13.9	10.7	100	7.2	HS-SPME	
(**13**) 3-methyl benzaldehyde[b]	9.215	120; 105; 133	nd	nd	nd	nd	nd	nd	nd	61.8	100	nd	nd	DCM	
(**14**) caproic acid	9.327	60	33.3	nd	nd	33.4	9.9	57.8	nd	28.2	74.6	100	33.3	HS-SPME	(*39*)
(**15**) 1-octen-3-one	9.411	55; 97	15.7	8.9	100	nd	nd	nd	nd	nd	13	nd	nd	HS-SPME	(*4*)
(**16**) glycerol[b]	9.523	61	nd	7.3	nd	100	22.6	nd	24.2	nd	0.9	nd	nd	DCM	
(**17**) 6-methyl-5-hepten-2-one	9.658	93	nd	nd	nd	nd	nd	13.3	100	nd	40.6	nd	nd	HS-SPME	
(**18**) 3-octanone	9.726	43; 99	100	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	HS-SPME	
(**19**) β-pinene	9.739	93	18.1	nd	nd	21.3	32.8	nd	71.4	100	26.5	nd	nd	HS-SPME	
(**20**) caproic acid ethyl ester	10.018	73; 88	37.3	nd	nd	29.6	100	nd	37.9	nd	nd	nd	69.5	HS-SPME	
(**21**) 3-octanol	10.051	55; 83	100	nd	nd	nd	nd	nd	nd	nd	0.1	nd	nd	HS-SPME	
(**22**) 1-octen-3-ol	10.151	57; 99	17.4	31	100	1.6	64.4	16.4	40.3	4.9	nd	58.8	35.5	HS-SPME	
(**23**) camphene[b]	10.213	93	nd	nd	nd	nd	nd	nd	nd	100	nd	nd	nd	HS-SPME	
(**24**) hexyl acetate	10.443	56	11.6	nd	nd	nd	nd	13.3	nd	nd	100	nd	nd	HS-SPME	
(**25**) 1,4-cineole	10.548	111	nd	nd	nd	nd	nd	3.1	nd	100	1.4	nd	1.3	HS-SPME	
(**26**) *o*-cymene	10.832	119	13.5	10.6	5.7	2.8	16.7	19.7	13.9	100	11.6	5.3	16.7	HS-SPME	
(**27**) limonene	10.966	67	11.4	2.6	3.3	6.1	4.1	20.8	2.7	14.5	100	1.5	8.4	HS-SPME	(*35*)
(**28**) eucalyptol	11.087	93	48.6	29.3	39.9	74.6	45.4	42	26.7	63.9	nd	16.6	100	HS-SPME	
(**29**) benzylic alcohol	11.246	79	14.8	10.3	14.7	24.9	16.9	100	11.5	49.8	25	21	8.1	HS-SPME	
(**30**) pantolactone	11.353	71	17.5	76.3	16	23.7	90.5	8.9	100	51.8	30.1	8.6	nd	DCM	
(**31**) phenylacetaldehyde	11.494	91	7.3	2.2	1.5	12.3	5.5	16.2	3.5	30.6	100	19.4	4.2	HS-SPME	(*33*)
(**32**) *trans*-2-octen-1-ol	12.124	57; 81; 95	20.7	nd	nd	3.2	nd	20.6	100	2.2	nd	81.2	82.7	HS-SPME	(*4*)
(**33**) linalool	13.171	93	17	1	nd	1.6	4	22	18.7	100	25.2	nd	nd	HS-SPME	(*35*)
(**34**) 2-nonen-1-ol[b]	13.376	57; 67; 82; 95	31.6	10.2	14.6	6.6	93.9	nd	34.7	20.7	11	88.1	100	HS-SPME	(*39*)
(**35**) 2-phenylethanol	13.615	91	6.6	18.4	1.6	30.8	9.6	13.8	3.8	100	25.9	40.5	7.2	HS-SPME	(*35*)
(**36**) benzoic acid	14.835	77	100	6	nd	nd	11.6	4.3	6.3	nd	nd	nd	nd	DCM	
(**37**) menthol	15.533	81	7.7	31	78.5	21.2	21.4	18.9	100	31.3	8.1	22.7	16.2	HS-SPME	
(**38**) 2-piperidone[b]	15.645	99	22	100	21.1	nd	37.3	15.8	79.9	38.5	21.6	24.7	13.2	DCM	
(**39**) caprylic acid ethyl ester	15.907	73	nd	nd	nd	100	nd	nd	nd	nd	nd	nd	nd	HS-SPME	(*37*)
(**40**) α-terpineol	16.062	59; 121	nd	nd	nd	21.7	nd	100	nd	63.1	50.7	nd	nd	HS-SPME	(*35*)
(**41**) undecanal[b]	16.345	57; 82; 96	66.5	12.9	nd	16.6	69.1	100	nd	nd	7.3	48	48.8	HS-SPME	(*37*)
(**42**) phenylacetic acid	17.551	91	nd	25.2	11.2	nd	59.9	16.9	100	nd	nd	nd	nd	DCM	
(**43**) (*E*)-2-decenal	17.917	70; 83	14.6	nd	16.4	7.3	nd	nd	93.8	nd	3.3	100	nd	HS-SPME	(*37*)
(**44**) indole[b]	18.771	117	nd	nd	nd	nd	16.64	nd	33.1	100	55.9	nd	nd	DCM	(*3*)
(**45**) eugenol	20.324	164	nd	6.3	5.2	12.2	9.4	100	37.8	90.7	nd	nd	14.3	DCM	
(**46**) capric acid ethyl ester	21.328	73	nd	nd	nd	100	nd	nd	nd	nd	nd	nd	nd	HS-SPME	(*39*)
(**47**) nicotinamide	21.362	106	53.3	0.7	nd	1	8.2	1.7	100	10.9	16	8	45.9	DCM	
(**48**) cinnamic acid	22.207	147	nd	nd	nd	nd	100	12.3	nd	nd	nd	nd	nd	DCM	
(**49**) *trans*-geranylcetone	22.767	107	nd	4.5	nd	5.1	7.3	7.3	41.9	100	65.4	2.6	nd	DCM	
(**50**) β-Ionone	23.627	177	nd	59	nd	nd	79.1	nd	37.7	nd	100	87.1	17.4	HS-SPME	(*37*)
(**51**) *trans*-nerolidol	25.470	93	nd	nd	nd	nd	nd	nd	11.3	84.8	100	nd	nd	HS-SPME	
(**52**) myristic acid	29.702	60; 129; 228	nd	nd	nd	nd	100	nd	nd	nd	nd	nd	nd	DCM	
(**53**) farnesylacetone	33.008	69	nd	nd	nd	nd	nd	nd	57.1	100	98.4	nd	nd	DCM	

Table 3. Continued

compd	retention time (min)	quantification ions (*m*/*z*)	samples[a] *A. rubescens*	*B. edulis*	*C. cibarius*	*F. hepatica*	*H. agathosmus*	*R. cyanoxantha*	*S. bellini*	*S. granulatus*	*S. luteus*	*T. equestre*	*T. rutilans*	method	sensory descriptors (ref)
(**54**) palmitoleic acid methyl ester[b]	33.241	87	nd	18.3	33.5	14.6	4	33.4	17	nd	5.4	17	100	DCM	
(**55**) palmitoleic acid	33.723	60; 125; 256	37.2	63.5	2	12.1	100	39.1	90.4	61.5	15	12.8	37.2	DCM	
(**56**) linolelc acid methyl ester[b]	36.491	81	nd	13.3	nd	2.3	nd	4.3	7.3	7.1	nd	5.6	100	DCM	
(**57**) elaidic acid methyl ester[b]	36.618	81	nd	nd	nd	11.9	nd	16.1	14.5	nd	nd	19.1	100	DCM	
(**58**) stearic acid	37.411	60; 129; 284	100	nd	nd	nd	nd	24.2	nd	nd	nd	10.9	nd	DCM	
(**59**) oleic acid	37.458	264; 282	nd	nd	nd	nd	100	12.3	nd	nd	nd	nd	nd	DCM	
(**60**) linoleic acid	37.821	81; 280	nd	100	nd	nd	nd	nd	nd	nd	nd	nd	nd	DCM	
(**61**) dehydroergosterol[b]	65.608	251	0.3	0.7	0.4	0.3	0.6	nd	0.4	0.9	1.2	100	0.2	DCM	
(**62**) ergosterol	67.519	363; 396	nd	100	17.2	31	7.7	nd	7.9	7.5	17.8	1	24.5	DCM	
(**63**) campesterol[b]	70.543	400	48.8	73	30.9	nd	nd	nd	nd	100	46.3	28.3	20.5	DCM	
(**64**) lanosterol[b]	74.408	55	nd	nd	nd	nd	nd	nd	nd	100	nd	nd	nd	DCM	

[a] 100, highest area obtained; nd, not detected. [b] Compounds tentatively identified by NIST 05.

Table 4. Correlations between Sensory and Chemical Variables

chemical variable/sensory variable[a]	correlation between variables
hexanol/hay-herb	0.645
C8OL/mushroomlike	0.537
C8OL/nutty	0.897
phenylethanol/floral	0.775
sum of volatile aldehydes/hay-herb	0.766
methional/farm-feed	0.791
phenylacetaldehyde/honey	0.647
C8One/mushroomlike	0.632
C8One/nutty	0.850
trans-geranylcetone/floral	0.868
sum of terpenes/floral	0.888

[a] C8OL: sum of alcohols with 8 carbon (3-octanol, 1-octen-3-ol, *trans*-2-octen-1-ol). C8One: sum of ketones with 8 carbon atoms (3-octanone, 1-octen-3-one). Sum of terpenes (linalool, terpineol, limonene, α-pinene, menthol, *β*-pinene, camphene, 1,4-cineole, eucalyptol, *o*-cymene, nerolidol).

Palmitoleic acid was the only one that was traced in all the species. *B. edulis* was the only species that presented linoleic acid. These long-chain unsaturated fatty acids show antibacterial activity and are the key ingredients of antimicrobial food additives and some antibacterial herbs (*31*). 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 nonvolatile acids.

S. bellini was identified as the richest species in alcohols, which are considered to be the main odorants of the mushroomlike aroma (*8*). Among these compounds, *C. cibarius* presented the highest percentage of 1-octen-3-ol, whereas *A. rubescens* was the one with the highest amount of 3-octanol. *T. equestre* also had a considerable percentage of these compounds. Statistical results, performed in this study, showed that these alcohols have higher correlations with the "nutty" descriptor than with the mushroomlike aroma (**Table 4**).

It can be seen that *T. equestre* and *S. luteus* presented the highest levels of aldehydes. Benzaldehyde and phenylacetaldehyde were identified in all of the species. The characteristic descriptor of benzaldehyde, in which *T. equestre* was the richest one, is the well-known almondlike aroma. However, this descriptor was not used by the panel. Phenylacetaldehyde is considered to be the compound responsible for honey notes (*8*) and *S. luteus* was the species presenting the highest levels of this aldehyde. This compound was found in pine-mushroom specie (*Tricholoma matsutake* Sing.) (*34*). *B. edulis* was the richest species in methional. This compound has a very low olfactive perception limit and its descriptor is boiled potato (*8, 33*). 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 (**Table 4**), and the presence of this compound can explain its aroma characteristics (*32, 33*). This compound has been recently identified in pine-mushroom species (*34*).

Among the identified ketones, two different groups emerge: one is constituted by 3-octanone and 1-octen-3-one, whereas the other one is composed of volatile norisoprenoids, such as *β*-ionone, 6-methyl-5-hepten-2-one, *trans*-geranylcetone, *(E,E)*-farnesylacetone. These odor-active substances are known to be oxidative byproduct or degradation products derived from carotenoids (*35, 36*). These four compounds have never been identified in mushrooms. 6-Methyl-5-hepten-2-one has been identified as the most abundant ketone some watermelon varieties and it is related to the herbaceous, green,

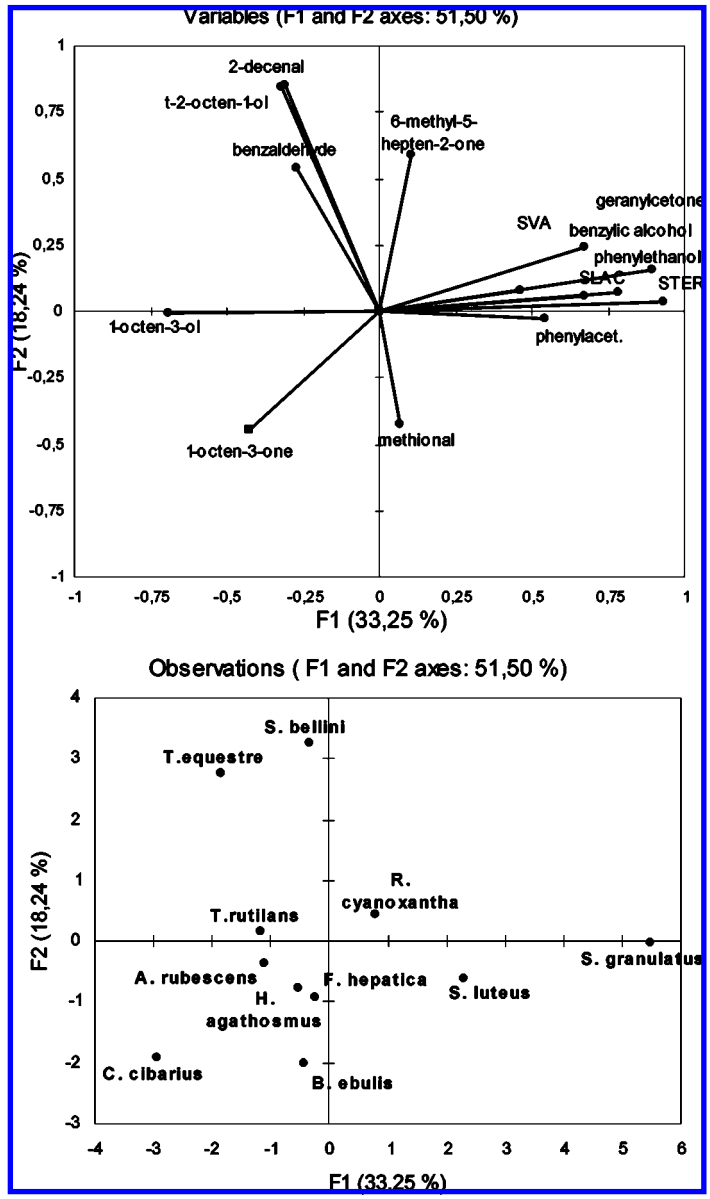

Figure 2. Principal components diagram of the volatile compounds (variables) with different mushroom species. SLAC, sum of lactones (**11**, **30**); STER, sum of terpenes (**10**, **19**, **23**, **25**, **26**, **27**, **28**, **33**, **37**, **40**, **51**); SVA, sum of volatile acids (**1**, **2**, **4**, **6**, **14**); phenylacet, phenylacetaldehyde.

oily and pungent sensory characteristics (*24*). β-Ionone is important flavor in some wine varieties (*37*). *trans*-Geranylcetone and *(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. It is interesting to note that three species from the same genus, *S. bellini*, *S. granulatus*, and *S. luteus*, were the richest species in norisoprenoid compounds. *A. rubescens* was the species that present the highest contents of 3-octanone, whereas *C. cibarius* contains the highest amount of 1-octen-3-one.

Another important chemical class to the flavor characterization includes terpenic compounds. *S. granulatus* and *S. luteus* presented the highest levels of the identified terpenic compounds. Several terpene compounds have been identified in fresh wild mushrooms (*38*) before; however, the *trans*-nerolidol, eucalyptol, menthol, and 1,4-cineole have not been found in mushroom species. All these compounds have been formally identified by using commercial standards.

It can be seen that eugenol, lactones, indole (an alkaloid), 2-piperidone, sterols, and nicotinamide composition is different, depending on the mushroom specie. Among species herein analyzed, *S. granulatus*, *B. edulis*, and *T. equestre* can be distinguished from the rest, since they had very high amounts of sterols. In what concerns nicotinamide (pyridine-3-carboxamide or vitamin B3), the distribution among the mushrooms is quite different: *S. bellini* presented the highest amounts, followed by *A. rubescens* and *T. rutilans*.

To assemble the different mushroom species according to the identified compounds, a principal component analysis (PCA) was performed. **Figure 2** shows the projection of chemical variables into the plans F1 and F2. Moreover, taking sensory and chemical variables into account, an agglomerative hierarchic cluster analysis (HCA) was performed (**Figure 3**). The eleven studied species were divided in three groups: group 1 was composed of *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

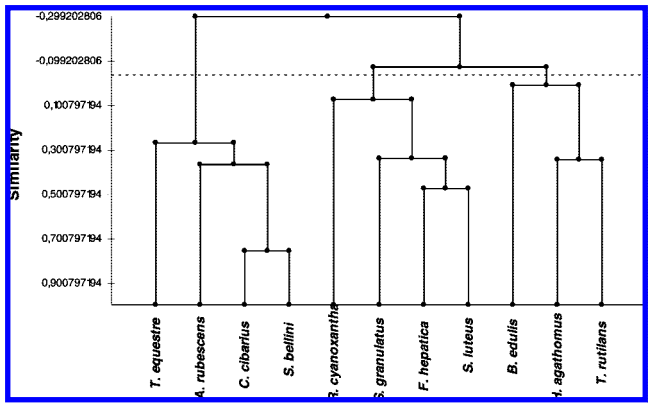

Figure 3. Dendogram of edible mushroom species with volatile compounds contents and sensory analysis values.

GC−MS permitted the identification of a large number of compounds in all the studied species. HS-SPME technique was better to extract the volatile compounds, while dichloromethane extraction took more advantage for the semivolatile ones. This study can be useful to the chemical description of the studied mushrooms.

In addition, it was possible to distinguish groups of wild edible mushroom species on the basis of their odor properties and aroma chemical composition.

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