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Comparison of Volatile Compounds of Fresh *Boletus edulis* and *B. pinophilus* in Marmara Region of Turkey

Fuat BOZOK[1]*, Mozhgan ZARIFIKHOSROSHAHI[2], Ebru KAFKAS[3], Hatira TAŞKIN[3], Saadet BUYUKALACA[3]

[1]*Department of Biology, Faculty of Science and Arts, Osmaniye Korkut Ata University, Karacaoğlan Campus, 80000, Osmaniye, Turkey; fbozok@osmaniye.edu.tr (*corresponding author)*

[2]*Department of Biotechnology, Institute of Natural and Applied Sciences, Çukurova University, 01330, Adana, Turkey; mn_zarifi@yahoo.com*

[3]*Department of Horticulture, Faculty of Agriculture, Çukurova University, 01330, Adana, Turkey; ebru@cu.edu.tr; hatirataskin1@gmail.com; sbircan@cu.edu.tr*

Abstract

Boletus edulis and *B. pinophilus* are commonly consumed edible species of *Boletus* spp. in Turkey, which are also exported to some European countries. In this study, twenty-three volatile compounds were determined with Headspace Solid-Phase Microextraction / Gas Chromatography / Mass Spectrometry (HS-SPME/GC/MS) in both *B. edulis* and *B. pinophilus*. 1-octen-3-ol (79.75), 2-octen-1-ol (13.18), 1-octen-3-one (2.52), (E)-2-octenal (1.21) in *B. edulis* and 1-octen-3-ol (55.97), 2-octen-1-ol (13.55), 3-octanone (7.43), (E)-2-octenal (6.79), 1-octen-3-one (5.80) and 1,7,7-trimethyl-heptan-2-one (2.04), 2-propenoic acid (1.95) and 1,3-octadiene (1.75) in *B. pinophilus* were identified as main volatile aroma compounds (%), respectively. The present study is the first report on the volatile constituents of *B. edulis* and *B. pinophilus* collected from Turkey.

Keywords: *Boletus,* aroma, mushroom, GC/MS, SPME

Introduction

Boletus edulis Bull: Fr. and *Boletus pinophilus* Pilât (Section Edules), which are also known as Porcini, are edible fungi widespread in the world (De Román and Boa, 2004; Sitta and Floriani, 2008). These fungi are economically significant because of wholesale average value which reached to 200 $ and the worldwide consume is ranging from 20,000 to 100,000 tons (Hall *et al.*, 1998).

There are over a hundred *Boletus* species in the world. The edible *Boletus* spp. are popular and often harvested by European countries because of their taste, aroma and odors (Jaworska and Bernas, 2009). In the genus of *Boletus, B. edulis* and *B. pinophilus* are extremely delicious and consumed in some Asian, American and European countries. In addition to fresh consumption, dried type of *B. edulis* is also consumed (Tsai *et al.*, 2008).

Porcini is an important source of income not only in Europe, but also in several rural areas of the world (Arora, 2008; Beugelsdijk *et al.*, 2008; De Román and Boa, 2004; Leonardi *et al.*, 2005; Sitta and Floriani, 2008). In addition, Turkey has a great potential and is becoming a major exporter of natural and edible mushrooms (Mendil *et al.*, 2004).

Edible *Boletus* species are widespread and especially consumed in Marmara and Western Black Sea regions of Turkey. The quality of mushrooms depends on their aroma, taste, odor and color. Mushrooms have a lot of volatile compounds that are responsible for aroma, taste and odor.

In previous studies, elements content and antioxidant properties of some *Boletus* species in Turkey were reported (Elmastas *et al.*, 2007; Gençcelep *et al.*, 2009; Mendil *et al.*, 2004; Sarikurkcu *et al.*, 2008; Yamac *et al.*, 2007). Volatile compounds of different mushrooms were studied by many researchers (Abraham and Berger, 1994; Breheret *et al.*, 1998; Rapior *et al.*; 1996; Taşkın *et al.*, 2013a; Taşkın *et al.*, 2013b). However, volatile compounds of *B. edulis* and *B. pinophilus* grown in Turkey have not been studied yet. Thus, the aim of this present study was to determine volatile aroma compounds of fresh *B. edulis* and *B. pinophilus* grown in Turkey.

Materials and methods

Fungi material

In November 2013, fresh *B. edulis* and *B. pinophilus* samples (Fig. 1) were collected from Beypınar village (41°48'35"N, 27°33'33"E, 733 m) of Kırklareli province (Marmara region) in Turkey. Mushroom samples were transferred to the laboratory

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Fig. 1. Fruit bodies of *B. edulis* and *B. pinophilus* (A: *B. edulis*; B: *B. pinophilus*)

in 24 hours after collection time. Identifications of macrofungi samples were made according to Breitenbach and Kränzlin (1991) and Galli (2007). Voucher specimens were deposited in Fungarium of Selçuk University.

Solid-phase microextraction (SPME) analysis of volatile compounds

A supelco fiber holder (Bellefonte, PA-USA) and a 100 µm polydimethylsiloxane (PDMS) coated fused-silica fiber were used for adsorbing volatile compounds from *B. edulis* and *B. pinophilus* according to Taşkın (2013a), with slight modifications. Prior to first extraction, the fiber was conditioned in the GC injector port at 250 °C for 1 h, according to manufacturer's recommendation. HS-SPME technique was used in the extraction of volatile compounds of *B. edulis* and *B. pinophilus*. These fungi were homogenized with saturated 5 g sodium chloride for HS-SPME. Further, 50 g of sample for each extraction was placed into 100 ml glass bottle. In HS-SPME analysis, the PDMS fibre was inserted into the headspace of the glass bottle and was immersed into the sample during 30 min at 30 °C. During this time, mushroom samples were stirred with a magnetic stirrer. After equilibration the fibre was removed from the sample and the analyses were thermally desorbed in the injector port of the GC/MS instrument for analysis. Thermal

Table 1. Volatile compounds of fresh *B. edulis* and *B. pinophilus*

No	RT	RI	Compounds	A %	B %
1	2.147	685	2-Propenoic acid (acrid, tart smell odour)	nd	1.95*
2	2.438	988	1,3-Octadiene (fruity)	0.63	1.75*
3	3.010	847	2-Fluoro-5-trifluoromethylbenzoic acid, propyl ester	0.13	nd
4	3.996	973	4-Penten-2-ol, 4 methyl,	0.05	nd
5	4.008	801	Hexanal	nd	0.47
6	4.656	2731	Tricosanoic acid	nd	0.18
7	6.091	2240	D-Norpropoxyphene	0.18	nd
8	6.190	1250	1-Chloro-decane	nd	0.11
9	6.459	736	3-Methyl-1-butanol	nd	0.70
10	6.511	746	1,2-Ethanedithiol	0.31	nd
11	6.617	983	3-Octanone (fruity, lavender odour)	0.90	7.43*
12	7.200	1075	Octanal	0.08	0.37
13	7.544	956	1-Octen-3-one (mushroom, metallic odour)	2.52*	5.80*
14	7.749	1331	2-Chloro-pentane	nd	0.35
15	8.233	1465	1-Hepten-3-one	0.07	nd
16	8.420	989	Formic acid, ethenyl ester	0.14	nd
17	8.851	1110	Octen-1-ol, acetate	0.08	nd
18	9.178	1106	Nonanal	nd	0.16
19	9.412	1151	(E)-2-Octenal (fatty, nutty odour)	1.21*	6.79*
20	9.452	1053	5-Methyl-1-heptanol	nd	0.30
21	10.048	839	Benzene, 1,4-dichloro	0.09	nd
22	10.106	1026	3-Flouro-benzenamine	nd	0.39
23	10.229	746	Cyclooctanone	nd	0.20
24	10.287	1165	Phosphonic acid, methyl-,dioctyl ester	0.06	nd
25	10.474	1334	Isoprophyl 5,11-dihydroxy-3,7,11 trimethyl-2-dodecenoate	0.04	nd
26	10.777	998	1-Octen-3-ol (fruity, mushroom, sweet odour)	79.75*	55.97*
27	11.378	1031	1,7,7-Trimethyl-heptane-2-one (pungent odour)	0.83	2.04*
28	12.294	1653	2,4- Octadienal	0.07	0.14
29	14.027	1610	2-Octen-1-ol (A green, vegetable-like odour)	13.18*	13.55*
30	15.037		4-(Benzoylmethyl)-6-methyl-2H-1,4-benzoxazin-3-one	0.07	nd
31	15.235	480	4-Hexenoic acid, 6-hydroxy-4-methyl-,methyl ester	0.15	nd
32	15.626		1,1,1,5,5,5-Hexafluoro-4-oxo-2-pentene-2-amine	0.09	nd
33	16.140		1,2-Dimethyl-3,5-diphenyl-1H-Pyrazolium	0.18	nd
34	22.173	744	Cyclohexanemethyl propanoate	nd	0.10
35	22.290	942	3- Methyl-1-octyn-3-ol	nd	0.22
36	22.833	988	3-Methyl-3-hexanethiol	nd	0.46
37	23.195	927	Pentanoic acid	nd	0.16

Note: Major compounds are shown by "*"; RT: Retention Time; A: *B. edulis*; B: *B. pinophilus*; nd: not detected

Table 2. Comparison of the volatile compounds of *Boletus* species

The Present Study				Rapior *et al.* (1997b)				Pysallo (1976)		Misharina *et al.* (2009)	
*B. edulis**	%	*B. pinophilus**	%	*B. aereus**	%	*B. calopus**	%	*B. edulis**	%	*B. edulis***	%
1-octen-3-ol	79.75	1-octen-3-ol	55.97	1-octen-3-ol	75.00	3-octanone	47.00	1-octen-3-ol	49.00	1-octen-3-ol	49.74
2-octen-1-ol	13.18	2-octen-1-ol	13.55	2-octen-1-ol	13.00	3-octanol	27.00	3-methylbutanol	17.00	Octanol	12.40
1-octen-3-one	2.52	(E)-2-octenal	5.80	nd		1-octen-3-ol	15.00	2-octen-1-ol	11.00	3-octanol	9.30
(E)-2-octenal	1.21	1-octen-3-one	6.79	nd		limonene	3.60	1-octen-3-one	8.0	1-octen-3-one	8.43

Note: '*' Fresh, '**' Canned, nd: not detected

desorption was done in the injector glass liner at 250 °C, for 10 min. The analyses were carried out in triplicate.

Gas chromatography/Mass spectrometry (GC/MS) analysis

Aroma compounds of the mushrooms were analysed by GC-MS. A Perkin Elmer Clarus apparatus equipped with CPSil5CB (25 m x 0.25 mm i.d., 0.4 μm film thickness) fused-silica capillary column was used. The flow rate of helium as carrier gas was 1 ml/min. The injector temperature was 250 °C, set for splitless injection. The column temperature was 60°C/5°C/min/260°C for 20 min. Mass spectra were taken at 70 ev. Mass range was between m/z 30-425. A library search was carried out using the Wiley GC-MS Library and Flavor Library of Essential Oil Constituents. The mass spectra were compared with those of reference aroma compounds and also confirmed with retention indices in published sources. Relative percentage amounts of the separated compounds were calculated from total ion chromatograms by the computerized integrator.

Results and discussions

Twenty-three volatile compounds were determined in both *B. edulis* and *B. pinophilus* (Table 1). The major compounds (%) are 1-octen-3-ol (79.75), 2-octen-1-ol (13.18), 1-octen-3-one (2.52), (E)-2-octenal (1.21) in fresh *B. edulis* and 1-octen-3-ol (55.97), 2-octen-1-ol (13.55), 3-octanone (7.43), (E)-2-octenal (6.79), 1-octen-3-one (5.80), 1,7,7-trimethyl-heptan-2-one (2.04), 2-propenoic acid (1.95), 1,3-octadiene (1.75) in fresh *B. pinophilus*. As a results of the present study, it was found that the amount (%) of 1-octen-3-ol in fresh *B. edulis* was higher than that of *B. pinophilus*, while the amounts of 2-octen-1-ol, 3-octanone, (E)-2-octenal, 1-octen-3-one, 1,7,7-trimethyl-heptane-2-one, 2-propenoic acid and 1,3-octadiene in *B. pinophilus* were higher than that of *B. edulis*.

The major compounds in *B. edulis* and *B. pinophilus*, such as unsaturated alcohols including 1-octen-3-ol and 2-octen-1-ol and ketones including 1-octen-3-one, 1,3-octadiene and 3-octanone are responsible for the characteristic odor of mushrooms (Misharina *et al.*, 2009).

Results of the present study are similar to previous studies with regard to volatile compounds in mushrooms (Dijkstra, 1976; Dijkstra and Wikên, 1976; Pinho *et al.*, 2008; Rapior *et al.*, 1997a; Rapior *et al.*, 1997b; Zawirska-Wojtasiak, 2004). 1-octen-3-ol was found as major compound in fresh *A. bisporus* (4.30 and 3.30 μl/L) (Dijkstra 1976; Dijkstra and Wikên, 1976) and in *Calvatia gigantea* (190.00 μl/L), *Pholiota squarrosa* (22.00 μl/L), *A. bitorquis* (18.00 μl/L), *Pleurotus ostreatus* (17.00 μl/L); also, it was showed that 1-octen-3-ol compound was found in *B. aereus* (75%), *B. calopus* (15%), *Leccinum aurantiacum* (8.7%), *L. quercinum* (52%), *Suillus bovinus* (89%), *S. collinitus* (92%), *S. granulatus* (75%), *S. variegatus* (75%), *Chroogomphus rutilus* (20%), while it was not found in *Xerocomus subtomentosus* (Rapior *et al.*, 1997a; Rapior, *et al.*, 1997b). Besides, high levels (%) of 1-octen-3-ol compound were determined in cultivated and wild mushrooms: *P. ostreatus* (97%), *Lentinus edodes* (95.5%), *P. nameco* (92.5%), *X. badius* (83.3%), *B. edulis* (96.7%), *Macrolepiota procera* (98.3%) (Zawirska-Wojtasiak, 2004).

However, differences in the major compounds (%) were observed when compared with the findings of Pyysalo (1976) and Misharina *et al.* (2009) (Table 2). In the Northern Finland, it was found that the amount of 1-octen-3-ol as major compound in *B. edulis* was 49.0% (Pyysalo, 1976). Also, 1-octen-3-ol (1.94), octanol (0.69), 1-octen-3-one (0.64), 1,5-octadien-3-one (0.25) and 1-octen-3-ol (6.43), octanol (1.60), 3-octanol (1.20), 1-octen-3-one (1.09) were found as major compounds (mg/100g) in boiled and canned *B. edulis*, collected from The Tver and Smolensk Oblast in Russia, respectively (Misharina *et al.*, 2009). Differences in mushroom flavor amounts in the present and previous studies might be due to location and collection time.

As shown in Table 1, major compounds in *B. edulis* and *B. pinophilus* consisted of approximately 95-96% of total aroma, respectively. 1-octen-3-ol, found as a major compound in *B. edulis* and *B. pinophilus*, is considered responsible for the characteristic aroma of many edible mushrooms (Abraham and Berger, 1994; Cho, 2006). However, it could be said that other major and minor components also contributed to taste, odor and aroma.

Conclusions

B. edulis and *B. pinophilus* are common mushrooms and often consumed in Marmara region of Turkey. HS-SPME / GC / MS results show clearly that these mushrooms are quite rich in terms of aroma compounds. This is why *Boletus* species are presumably preferred by consumers. Similar studies which will be held in the future, will help us to have more information about aromas of mushroom and to have more benefit from mushrooms in Turkey.

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