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Volatile Constituents of The Edible *Tricholoma terreum* in Marmara Region of Turkey

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Abstract: *Tricholoma terreum* is a fungal species which is also commonly consumed and widespread in Turkey. Seventeen aroma compounds were identified with Headspace Solid-Phase Microextraction/Gas Chromatography/Mass Spectrometry (HS-SPME/GC-MS). Major aroma compounds in *T. terreum* were as follows; 1-octen-3-ol (37.08 %), (*E*)-2-octen-1-ol (19.68 %), hexanal (16.00 %), 3-octanone (3.36 %), acetic acid (2.63 %) and (E)-2-octenal (2.27 %). This study is the first report on the volatile aroma compounds of *T. terreum* in Turkey.

Keywords: *Tricholoma terreum*, aroma, volatile, GC-MS.

Introduction

Genus *Tricholoma* is an edible and an ectomycorrhizal group which has an economical value. Turkey has a great potential in terms of natural and edible mushrooms [1]. Consumption of mushrooms collected from nature is steadily increasing, as they are rich about proteins, chitin, vitamins and minerals besides having low level of fat and calorie [2, 3].

Popularity of fungi among the people is due to their aromatic compounds. For example, *Tricholoma anatolicum* which has a peculiar smell, is exported to Japan from Turkey and commonly consumed in Turkey [4]. At the same time, *T. terreum*, known as blackgirl or cincile among the people in Turkey, is consumed as a nourishment [5]. There is no information on the volatile compounds of *T. terreum* grown in Turkey, although antioxidant properties, antimicrobial activities, element and nutrient content were studied by different researchers [6-16].

Experimental

Fungi material

In November 2013, fresh *T. terreum* were collected from Demirköy region (41°48'32" N 27°31'13" E, 680 m) of Kirklareli province (Marmara region) in Turkey (Fig. 1). Mushroom samples (10 samples) collected from the field were brought with cloth bags to the laboratory within 24 hours to be prepared properly for analysis. Mushroom samples were deposited in Fungarium of Biology Department of Selçuk University.

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Figure 1. Fruit bodies of *Tricholoma terreum*

SPME analysis of volatile compounds

A supelco fiber holder (Bellefonte, PA-USA) and a 100 µm polydimethylsiloxane (PDMS) coated fused-silica SPME fiber were used for extraction of volatile compounds from *T. terreum* according to Taskin [17] with slight modifications. The fiber was equilibrated before first extraction, for this purpose it was conditioned in the GC injector port at 250°C for 1 h. Headspace Solid-Phase Microextraction (HS-SPME) technique was used for the extraction of volatile compounds of *T. terreum*. Mushroom samples were homogenized with saturated 5 g sodium chloride. 50 g of sample for each extraction was placed into 100 ml glass vial. In HS-SPME analysis, the PDMS fibre was inserted into the headspace of the glass vial and was immersed into the sample for 30 min. duration at 30°C condition using heated magnetic stirrer. During this time, mushroom samples were stirred with a magnetic stirrer. The analyses were thermally desorbed in the injector port of the GC-MS instrument for volatile analysis. Thermal desorption in the injector glass liner at 250°C for 10 min. Analyses were carried out in triplicate.

GC-MS analysis

The identification of volatile compounds of *T. terreum* were performed by using GC-MS. A Perkin Elmer Clarus GC-MS apparatus equipped with CPSil 5CB (25 m x 0.25 mm i.d., 0.4 µm film thickness) fused-silica capillary column was used. The flow rate of helium was 1 ml/min. The injector temperature was 250°C, set for splitless injection. The column temperature was 60°C and increased to 260°C at rate of 5°C/min for 20 min. Mass spectra were taken at 70 eV. Mass range was between m/z 30-425. A library search was carried out by using the Wiley GC-MS Library and NIST Flavor Library of Essential Oil Constituents (program 2008, version 2.2.0. from Perkin Elmer).

The identification was based on comparison of the GC retention times and mass spectra with authentic standards from Sigma-Aldrich when standards were available. When the authentic standards were not available, the identification was based on comparison with the spectral data of the Wiley and NIST Library. Retention indices were calculated using n-alkanes (C8-C17) (Sigma-Aldrich) as reference compounds. The mass spectra were compared with those of reference aroma compounds and also, confirmed with retention indices in published sources. Relative percentages of the separated compounds were

calculated from total ion chromatograms by using the computerized integrator.

Results and discussion

In total, seventeen aroma compounds were determined from *T. terreum* in the present study. Major compounds are as follows; 37.08 % 1-octen-3-ol (mushroom, butter), 19.68 % (*E*)-2-octen-1-ol (fruity), 16.00 % hexanal (freshly cut grass), 3.36 % 3-octanone (fruity, sweet, lavender), 2.63 % acetic acid and 2.27 % (*E*)-2-octenal (sweet) (Table 1). These major compounds consist approximately 81 % of total aroma in *T. terreum*.

The present study shows similarities regards to 1-octen-3-ol as major compound when compared to the previous studies [18-23]. However, in the present study, some differences in major compounds and amounts were observed when compare to Malheiro *et al*.[24]. According to Malheiro *et al*.[24], the volatile aroma compounds of *T. terreum* collected from Bragança municipality (northeast of Portugal) were investigated and 1-octanol was found as major compound, followed by (*E*)-2-octen-1-ol, (*E*)-2-octenal, hexanal, linalool and hexanol while in the present study, 1-octen-3-ol was determined as major compound, followed by (*E*)-2-octen-1-ol, hexanal, 3-octanone, acetic acid and (*E*)-2-octenal. Besides, the volatile aroma compounds of *T. matsutake* and *T. caligatum* (two different species of genus Tricholoma) were investigated by different researchers [25,26]. As results of these studies, the major compounds were methyl cinnamate (85.05), 1-octen-3-ol (9.15 %) in *T. matsutake* [26] and 2,4-dimethyl-1*H*-indole (29.60 %), 5-methoxy-3,4-dimethyl-1*H*-indole (29.50 %), 4-methoxymethyl-2-methyl-1*H*-indole (11.60 %), Methyl-(*E*)-cinnamate (10.50 %) and 4-hydroxymethyl-2-methyl-1*H*-indole (3.90 %) in *T. caligatum* [25]. 1-octen-3-ol was also observed in flavor aroma of *T. matsutake* grown in Korea, however, it was not found in *T. terreum* (Portugal) and *T. caligatum* (France). (*E*)-2-octen-3-ol were 19.68 % and 11.54 % in *T. terreum* grows in Turkey and Portugal, respectively, while it was found 0.52

Table 1. Aroma compounds of *Tricholoma terreum*

No.	RRI[a]	RRI[b]	Compounds	%
1	600	603	Acetic acid	2.63*
2	602	-	Sinapic acid	1.00
3	613	-	Unknown	0.96
4	617	-	Benzofuran-6-ol-3-one, 2-(4-ethoxycarbonyl) benzylidene-	1.15
5	618	690	2-methyl-propanoic acid	0.47
6	685	-	1,7,7-trimethyl-heptane-2-one	0.37
7	732	700	Pentanal	1.58
8	844	880	Hexanal	16.00*
9	927	-	Unknown	1.24
10	957	963	2-heptanal	1.10
11	964	986	6-methyl-5-hepten-2-one	0.30
12	965	990	3-octanone	3.36*
13	976	979	1-octen-3-one	1.36
14	982	978	1-octen-3-ol	37.08*
15	983	-	(*E*)-2-octen-1-ol	19.68*
16	1060	1062	(*E*)-2-octenal	2.27*
17	1957	1949	3-(3,4-dimethoxyphenyl)-2-propenoic acid	0.86

Major compounds are shown by "*"

RRI[a]: Relative retention indices obtained

RRI[b]: Relative retention indices reported in literature

% in *T. matsutake*. Also, it was not observed in *T. caligatum*. Also, hexanal was 16.00 % and 7.73 % in the present study and *T. terreum* in Portugal, besides, it was not observed in *T. matsutake* and *T. caligatum*. Surprisingly, it was observed that 3-octanone content of *T. terreum* in Portugal (16.31 %) was higher than samples which collected from Turkey (3.36 %). These differences in the volatile aroma compounds and amounts in the present and previous studies might be due to location, collecting time and species.

Besides, there are differences in terms of retention indices (RI) values of major compounds between the present and Malheiro *et al*.[24] studies. RI values for (*E*)-2-octen-1-ol, hexanal, (*E*)-2-octenal and 3-octanone compounds are 1046, 780, 1044 and 990 in the study of Malheiro *et al*. [24] and 983, 844, 1060 and 990 in the present study, respectively. It could be say that differences in RI values were due to the use of different column.

In previous studies, it was highlighted that 1-octen-3-ol inhibited the mycelial growth of *Penicillium expansum* PP497A and the conidia germination of *Penicillium paneum* and can be used as natural nutrient preservatives [27, 28]. Besides, it was found that 1-octen-3-ol activated the defence mechanisms of *Arabidopsis thaliana* leaves treated with *Botrytis cinerea* [29]. However, some researchers indicated that 1-octen-3-ol might be used as attractant for insects traps [30, 31], and inhibited the aggregation to nutrients when was sprayed at different concentrations (1, 10, 100 and 1000 ppm) on some insect species [32].

Acetic acid which is the major compound of *T. terreum* has been used in different industries traditionally although it was reported that it has harmful effects on human health [33, 34]. It was concluded that acetic acid caused to chronic bronchitis, pharyngeal edema and irritation of in eye, nose, throat in people who exposed to 50 ppm and more concentrations, likewise, to erosion in teeth and blackening in the skin of hands of five people exposed for 7 to 12 years between 80-120 ppm concentrations [35]. So, over consumption of *T. terreum* might be risky as it contains acetic acid which is a toxic compound.

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