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Investigation of Aroma Components of *Tuber aestivum* and *Tuber borchii* Collected From Different Location in Türkiye

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Abstract: Truffle is an important food source for wild animals and a significant part of the ecosystem, indicating a healthy forest. With their unique aromas, truffles are also a crucial food source for humans. The volatile substances released by *Tuber* not only appeal to our sense of smell but also contribute to various biological activities. Different aromas result in different tastes; therefore, aroma is essential in defining the organoleptic properties and quality of underground fungi. This research aims to investigate according to the percentage the chemical components considering the habitat and mycorrhizal host tree species of naturally occurring summer truffle *Tuber aestivum* Vittad. and white truffle *Tuber borchii* Vittad. in Southwest Anatolia and Marmara regions. For this purpose, the aroma components of *T. aestivum* and *T. borchii* collected from two different regions were analyzed using the HS-SPME-GC/MS system. Accordingly, a total of 59 different compounds (volatile organic compounds) were identified, including 20 compounds in *T. aestivum* from Muğla, 13 compounds in *T. aestivum* from Kırklareli, 44 compounds in *T. borchii* from Muğla, and 33 compounds in *T. borchii* from Kırklareli, belonging to different classes of compounds. While terpenes such as limonene (37.62%), *p*-cymene (4.79%), and *β*-pinene (4.12%) were major compounds in *T. aestivum* collected from Muğla, 2-octen-1-ol (46.78%), 1-octen-3-ol (40.44%), and 3-octanol (2.62%) predominantly constituted the aroma in *T. aestivum* from Kırklareli. The characteristic sulfur compounds commonly found in *Tuber* species were present in 42.19% of *T. borchii* collected from Kırklareli and 12.15% from Muğla. When comparing *T. borchii* grown in Kırklareli and Muğla, 3-methyl-4,5-dihydrothiophene (29.53% and 6.73%) *p*-methyl thiobenzaldehyde (2.75% and 5.42%), and methionine (9.91% in Kırklareli, not detected in Muğla) were found in different percentage rates. Based on the data obtained in this study, the classification of both *Tuber* species, with respect to their geographical origin, was determined using hierarchical cluster analysis (HCA). Accordingly, it is show that of the aroma of *Tuber* species of the chemical components and the aroma components to the formation should be cultivated after optimizing the ecological conditions that contribute to the symbiotic life of the plant species they provide.

Keywords: *Tuber aestivum, Tuber borchii,* Volatile organic compound (VOC), Aroma, Truffle.

Türkiye'nin Farklı Lokasyonlarından Toplanan *Tuber aestivum* ve *Tuber borchii*'nin Aroma Bileşenlerinin Araştırılması

Öz: Trüf, sağlıklı bir ormanın belirtisi, yabani hayvanların besin kaynağı olmaları yönüyle, ekosistemin önemli bir parçası ve eşsiz aromalarıyla insanlar için önemli bir besin kaynağıdır. *Tuber*'in saldığı uçucu maddeler, sadece koku alma duyumuza hitap etmenin ötesinde çeşitli biyolojik aktivitelere de katkı sağlamaktadır. Farklı aromalar farklı tada sahiptir, dolayısıyla aroma, yer mantarlarının organoleptik özelliklerini ve kalitesini tanımlamada esastır. Bu araştırmada, Güney Batı Anadolu'da ve Marmara'da doğal olarak yetişen yaz trüfü *Tuber aestivum* Vittad. *ve* beyaz trüf *Tuber borchii* Vittad.'nin yayılış gösterdiği habitat, mikorizal olarak yetiştiği ağaç türü dikkate alınarak aromanın yüzdesine göre kimyasal bileşenlerin araştırılması amaçlanmıştır. Bu amaçla, iki farklı bölgeden toplanılan *T. aestivum* ve *T. borchii*'nin aroma bileşenleri HS-SPME-GC/MS sisteminde analiz edildi. Buna göre, farklı sınıflarda *T. aestivum*'da; Muğla'da 20, Kırklareli'de 13, *T. borchii*'de; Muğla'da 44 ve Kırklareli'de 33 aroma bileşeni (uçucu organik bileşik) olmak üzere her iki trüfte birbirinden farklı toplam 59 bileşik belirlendi. Muğla'dan toplanılan *T. aestivum*'da limonen (%37.62), *p*-simen (% 4.79) ve *β*-pinen (%4.12) gibi terpenlerin majör bileşikler olmasına karşılık Kırklareli'ndeki *T. aestivum*'da 2- okten-1-ol (%46.78), 1-okten-3-ol (%40.44) ve 3-oktanol (%2.62) aromanın çok büyük bir kısmını oluşturmaktadır. *Tuber* türlerinde yaygın olarak bulunan karakteristik kükürt bileşikleri; Kırklareli'den toplanılan *T. borchii*'de %42.19 oranında bulunurken, Muğla'da %12.15 oranında bulunmaktadır. Kırklareli ve Muğla'da yetişen *T. borchii* karşılaştırıldığında; 3-metil-4,5-dihidrotiyofen (%29.53 ve %6.73), *p*-metiltiyobenzaldehid (%2.75 ve %5.42) ve metiyonin Kırklareli'de %9.91 oranında iken Muğla'da tespit edilmemiştir. Bu veriler dikkate alınarak her iki *Tuber* türünün coğrafi kökene dayalı sınıflandırılması hiyerarşik küme analizi (HCA) kullanılarak belirlenmiştir. Buna göre *Tuber* türlerinin aromasının kimyasal bileşenlerin oluşumuna katkı sağlayan ekolojik şartların ve simbiyotik yaşam sağladıkları bitkilerin türlerine göre aroma bileşenleri bakımından optimize edildikten sonra kültüre alınması gerektiğini göstermektedir.

Anahtar kelimeler: *Tuber aestivum*, *Tuber borchii*, Uçucu organik bileşik (VOC), Aroma, Trüf

Introduction

Mushrooms are consumed as food because of their nutritional values, aromas and their own smell. Many types of fungi used as a nutritious food are specially collected and used in food in many parts of the world. Edible fungi are important natural sources for healthy eating, considering their low calories, as well as essential fatty acids. Therefore, it is continued to be seen as a popular diet throughout the world (Taş et al., 2021). The "truffle" also known as "hypogeous mushroom" is a complex family, which includes mainly genus *Picoa, Tirmania, Tuber* and *Terfezia*. Truffle is beyond functional food and, it creates the opportunity to develop new products in gastronomy cuisine because of its particular aromas. Truffle grows with mansion plant (pine, linden, oak, nuts) as ectomicorizal mushrooms. They release a very intense aroma and chemical smell signals in their habitat where truffle fungi mature (Trappe & Maser, 1977; Taş et al., 2021). The studies of truffle species to cultivate is more about *Tuber* species because of their commercial interest. Many of the edible *Tuber* spp. (*Tuber magnatum* Pico, *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad., *Tuber borchii* Vittad.) found in the The Mediterranean area have a privileged place in various cuisines in many parts of Europe, America and East Asia.

Aroma is one of the important factors to assess truffle quality (Feng et al., 2019). Truffle species are mushrooms, especially in communities with high socioeconomic levels, which are attractive and provide high added value depending on their aroma. Beyond the functional food aspect of truffle species, they differentiate from other mushrooms and nutrients because of their aroma. Therefore, they create the opportunity to develop new products in terms of gastronomy. With this approach, truffle restaurants serve in developed countries. *Tuber* species' aromas in truffles are standing out in this group because they generate more interest than others in terms of their flavors. The studies of truffle species to cultivate are more about Tuber species due to the interest they receive in the market. The genus *Tuber* breed is located in the Pezizales order of the Ascomycota branch in the *Tuberaceae* (Lee et al., 2020). It contains ectomycorrhizal species of fungi that are economically valuable (Trappe, 1979). In the Northern Hemisphere, approximately 180 species are identified in the group (Zambonelli et al., 2016). The species in *Tuber* are grouped according to their season and morphology, based on where they grow. For example, there are summer, winter, black, and white truffles. Among the major winter truffle species, the winter white truffle (*Tuber magnatum* Pico) is found in Piedmont, Italy, while the winter black truffle (*Tuber melanosporum* Vittad) is found in Perigord. The summer black truffle is known as the most sought-after *Tuber* species in world cuisine, and it grows naturally in various regions in Europe. *Tuber borchii* Vittad. is widely grown in the Bianchetto region of France, and *Tuber aestivum* known as summer truffle is found in the *T. borchii* known as white truffle region of Italy (Zambonelli et al., 2016; Sesli et al., 2020).

Tuber species detected in studies on hypogeous mushrooms in Türkiye are listed as follows: *Tuber brumale* Vittad. (Öztürk et al., 1997), *Tuber borchii* Vittad. (Kaya, 2009; Elliot et al., 2016), *Tuber aestivum* Vittad. (Gezer et al., 2014), *Tuber excavatum* Vittad. (Castellano

& Türkoğlu, 2012), *Tuber mesentericum* Vittad. (Castellano & Türkoğlu, 2012), *Tuber nitidum* Vittad. (Castellano & Türkoğlu, 2012), *Tuber rufum* Picco (Türkoğlu & Castellano, 2014), *Tuber puberulum* Berk. & Broome (Elliot et al., 2016), *Tuber ferrugineum* Vittad. (Elliot et al., 2016), *Tuber fulgens* Quél (Akata et al., 2020), *Tuber macrosporum* Vittad. (Doğan, 2021).

It is suggested that the aroma components of truffles can be detected with plants in a symbiotic relationship, but the ecological role of essential substances they have in truffle-plant interactions remains speculative (Splivallo et al., 2011). Several literature studies are focused on the origin of the odorants and the identity of the predecessors emitted by truffles (Vahdatzadeh et al., 2015; Vahdatzadeh & Splivallo, 2018).

The aim of this study is to investigate according to the percentage the chemical components of the aroma of naturally occurring summer truffle *Tuber aestivum* Vittad. and white truffle *Tuber borchii* Vittad. in the Southwest Anatolia and Marmara regions of Turkey, taking into consideration the habitat where they are distributed and the tree species they are mycorrhizally associated with.

Material and Methods

Truffle Material

Summer truffle (*Tuber aestivum*) and white truffle (*Tuber borchii*) were collected in Southwest Anatolia and Marmara in Türkiye. Both species are naturally grow in the region (Figure 1). The specimens collected from the field were processed into fungarium material at Muğla Sıtkı Koçman University, Natural Products Research Laboratory. Details about the locations of truffles, the species of trees where they are found mycorrhizally, the Fungarium number, and the time of collection are given in Table 1.

Extraction of the volatile compounds

The volatile compounds of truffle samples collected from different locations were examined using solid-phase microextraction (SPME) and SHIMADZU gas chromatography (GC) and VARIAN 2100 gas chromatography-mass spectrometry (GC-MS) systems. Before analysis, the fiber was preconditioned and thermally cleaned in the injection port of a gas chromatograph according to the instructions provided by the supplier. Analyses were carried out by weighing 5 g of truffle sample in 20 mL vials and adding 5 mL of a 20% sodium chloride solution. The samples were maintained and magnetically stirred for 30 minutes at 50 °C to allow equilibration. Subsequently, the fiber was introduced into the vial and exposed to the headspace above the sample for 30 minutes. Once the extraction step was completed, the SPME fiber was removed from the vial and inserted into the injection port of the GC–MS for thermal desorption of the volatile compounds (Duru et al., 2021).

GC–FID and GC–MS analyses

For GC–FID analyses, a flame ionization detector (FID) and Rxi-5Sil MS (Restek) fused silica non-polar capillary column (30 m × 0.25 mm I.D., film thickness 0.25 μm) were used. The detector and injector temperatures were set to 270°C and 250°C, respectively. Helium was used as a carrier gas at a flow rate of 1.4 mL/min. The initial oven temperature was kept at 60°C for 5 minutes, then increased to 280°C, and maintained at this temperature for 5 minutes. The Class GC10 GC computer program (SHIMADZU) was used to identify the percentage of volatile compounds. The analyses were performed in triplicate, and the data were averaged to obtain the mean values. GC–MS analyses were performed using an ion trap MS spectrometer (VARIAN) and an Rxi-5Sil MS (Restek) fused silica non-polar capillary column (30 m × 0.25 mm I.D., film thickness 0.25 μm). Samples were injected in splitless mode, and helium was used as a carrier gas at a flow rate of 1.4 mL/min. The injector port and MS transfer line temperatures were set to 220°C and 290°C, respectively. The ion source temperature was 200°C, and EI – MS measurements were taken at 70 eV ionization energy. The mass range was m/z 28 to 650 amu. The scan time was 0.5 s with delays between scans of 0.1 s. The oven temperature was initially kept at 60°C for 5 minutes, then increased to 280°C at a rate of 4 °C/min and maintained at this temperature for 5 minutes. The essential compounds were identified based on GC retention indices and computer matching with Wiley, ADAMS, and NIST 08 MS databases. Additionally, models of mass spectra reported in the literature were compared, and injection with reference compounds was done when possible (Adams, 2007).

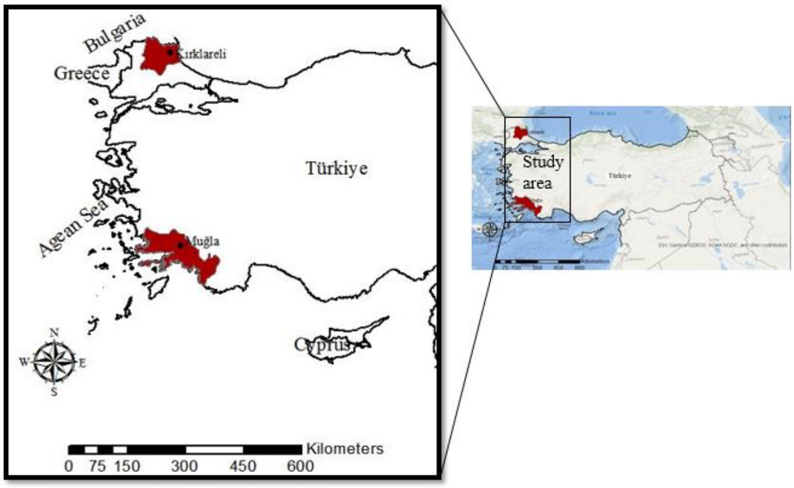

Figure 1. Location map of the *Tuber aestivum* and *Tuber borchii* collected from the field

Table 1. Location map of the *Tuber aestivum (summer truffle)* (a) and *Tuber borchii* (white truffle)

***Tuber* species name**	**Mycorrhizal tree species and altitude**	**Fungarium number**	**Collected season**
 ***a) T. aestivum (summer truffle)***	Muğla/Menteşe, in *Pinus brutia* Ten*. forest,* 660 m.	CK 1014	March 2021
	Kırklareli/Vize, *oak* and *hornbeam mix forest,*165m.	CK 1016	July 2021
 ***b) T. borchii (white truffle)***	Muğla/Menteşe, in *Pinus brutia* Ten. *forest,* 640 m,	CK 1022	April 2021
	Kırklareli/Vize, *oak* and *hornbeam mix forest,*175m.	CK 1029	March 2021

Statistical analysis

MINITAB 16.0 software was used to perform all statistical computations for chemometric investigations of the volatile chemicals in *Tubers* samples using hierarchical cluster analysis (HCA). With the Ward Linkage approach and Euclidean distance, hierarchical relations were obtained using cluster analysis.

Results

Volatile compounds

The aroma components of *T. aestivum* and *T. borchii,* which were gathered at different locations in Türkiye (fluencer organic components), were analyzed using SPME–GC–MS. The chemical analysis results of truffle samples for aroma components are presented in Table 2 as percentages (%). Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$). A total of 59 compounds were identified from the two different *Tuber* species, including alkanes and alkenes, sulfur compounds, alcohols, aldehydes, ketones, esters, aromatic compounds, terpenes, and others. These compounds differ both according to the percentage the chemical components of aromas according to the *Tuber* species and the region where they grew up. In addition to the sulfur-containing compounds characteristic of truffles, compounds such as octanal, [E]-2-octenal and 3-octanone, which are commonly found in the aroma of edible mushrooms, are found in all samples analyzed.

Table 2. Volatile organic compounds of *Tuber aestivum* and *Tuber borchii* from different regions (%) *

Class of compounds	Compounds	RI	*T.aestivum* (Muğla region) (%)	*T.aestivum* (Kırklareli region) (%)	*T.borchii* (Muğla region) (%)	*T.borchii* (Kırklareli region) (%)
Sulfur compounds	Methional	872	-	-	-	9.91 [c] ± 0.45
	3-Methylthiopropanal	885	5.9 [b] ± 0.15	-	-	-
	3-Methyl-4.5-dihydrothiophene	1125	-	-	6.73 [c] ± 0.20	29.53 [d] ±1.02
	p-(Methylthio) benzaldehyde	1346	-	-	5.42 [c] ± 0.13	2.75 [a] ± 0.08
Alkanes and Alkenes	3-Octene	799	-	-	1.10 [a] ± 0.03	1.88 [a] ± 0.07
	Hexadecane	1612	-	-	8.94 [d] ± 0.38	-
Alcohols	1-Octen-3-ol	961	-	40.44 [d] ± 1.08	1.21 [a] ± 0.04	11.71 [c] ± 0.35
	3-Octanol	992	0.52 [a] ± 0.02	2.62 [b] ± 0.06	-	-
	2-Octen-1-ol	1024	7.05 [b] ± 0.25	46.48 [d] ± 1.15	-	-
	3-Octen-1-ol	1062	0.43 [a] ± 0.01	1.50 [b] ± 0.05	-	-
	Octanol	1068	-	0.59 [a] ± 0.02	0.37 [a] ± 0.01	-
	Nonanol	1089	-	-	4.22 [b] ± 0.12	6.37 [b] ± 0.18
	(E)-3-Decen-1-ol	1232	-	-	2.06 [b] ± 0.09	0.58 [a] ± 0.02
	Hexadecanol	1825	-	-	1.18 [a] ± 0.02	-
Esters	Ethyl. (E)-3-hexenoate	1012	-	-	1.16 [a] ± 0.02	-
	Ethyl phenylacetate	1212	5.04 [b] ± 0.12	-	-	-
	Linalyacetate	1242	-	-	0.41 [a] ± 0.01	0.25 [a] ± 0.01
	Bornylacetate	1275	-	-	1.30 [a] ± 0.03	0.49 [a] ± 0.01
	Methyl caproate	1313	-	-	0.43 [a] ± 0.01	0.32 [a] ± 0.01
	Lauric acid methyl ester	1514	-	-	1.34 [a] ± 0.03	-
	Isopropyllaurate	1621	-	-	4.60 [b] ± 0.15	-
	Methylmyristate	1708	-	-	6.18 [c] ± 0.18	-
	Methylpalmitate	1907	-	-	1.77 [a] ± 0.07	-
Aldehydes	Heptanal	880	-	-	-	2.41 [a] ± 0.10
	2-Methylene-hekzanal	892	1.48 [a] ± 0.05	-	-	-
	cis-2-Heptenal	935	1.04 [a] ± 0.04	-	0.50 [a] ± 0.02	0.52 [a] ± 0.02

	Octanal	983	2.14 [a] ± 0.08	0.22 [a] ± 0.01	0.45 [a] ± 0.01	2.99 [a] ± 0.10
	[E]-2-Octenal	1039	5.83 [b] ± 0.15	2.43 [b] ± 0.05	0.70 [a] ± 0.03	6.95 [b] ± 0.15
	cis-2-Decenal	1250	-	-	0.44 [a] ± 0.01	0.21 [a] ±0.01
	5-methyl-2-phenyl-2-hexenal	1493	-	-	-	0.27 [a] ± 0.01
	Myristaldehyde	1591	-	-	2.11 [b] ± 0.04	-
Ketones	1-Octen-3-one	959	-	-	2.90 [b] ± 0.05	-
	3-Octanon	988	1.95 [a] ± 0.05	4.78 [c] ± 0.18	10.06 [d] ± 0.45	3.26 [b] ± 0.10
	1-(2.8.8-Trimethyl-5.6.7.8-tetrahydro-4H-cycloheptafuran-5-yle) ethanone	1632	-	-	2.28 [b] ± 0.05	0.17 [a] ±0.01
Aromatic compounds	Styrene	862	-	-	0.50 [a] ± 0.02	0.79 [a] ± 0.03
	Benzaldehyde	956	10.36 [c] ± 0.45	-	5.02 [c] ± 0.12	0.93 [a] ± 0.02
	Phenylacetaldehyde	1043	0.41 [a] ± 0.01	0.2 [b] ± 0.00	-	5.07 [b] ± 0.15
	Acetophenone	1058	-	-	1.68 [a] ± 0.06	0.45 [a] ± 0.01
	Phenylethanol	1081	-	0.12 [a] ± 0.00	1.12 [a] ± 0.05	-
	4-methyl acetophenon	1152	-	0.21 [a] ± 0.01	-	-
	Azulene	1156	-	-	5.25 [c] ± 0.15	-
	Cinnamaldehyde	1262	-	-	0.66 [a] ± 0.02	-
Terpenes	*α*-Pinene	928	0.87 [a] ± 0.02	-	2.61 [b] ± 0.11	0.38 [a] ± 0.01
	β-Pinene	963	4.12 [b] ± 0.15	-	-	-
	p-Cymene	1027	4.79 [b] ± 0.17	-	0.49 [a] ± 0.01	0.4 [a] ± 0.01
	Limonene	1032	37.62 [d] ± 1.40	0.21 [a] ± 0.01	-	0.53 [a] ± 0.02
	Eucalyptol	1035	-	-	1.39 [a] ± 0.05	5.22 [b] ± 0.15
	β-trans-Ocimene	1047	-	-	1.38 [a] ± 0.05	3.01 [b] ± 0.12
	β-Linalool	1083	-	-	3.95 [b] ± 0.12	1.78 [a] ± 0.06
	Borneol	1134	-	-	1.27 [a] ± 0.05	0.23 [a] ± 0.00
	α-Terpineol	1175	-	-	0.90 [a] ± 0.03	-
	Thymol	1278	0.93 [a] ± 0.03[a]	-	1.45 [a] ± 0.05	0.15 [a] ± 0.00
	Carvacrol	1290	7.24 [b] ± 0.30	-	1.44 [a] ± 0.05	0.19 [a] ± 0.01
	α-Terpinylacetate	1334	-	-	0.64 [a] ± 0.02	0.16 [a] ± 0.00
	α-Himachalen	1414	0.55 [a] ± 0.02	0.20 [a] ± 0.01	-	-

	Cedreneoxide	1426	-	-	0.82 [a] ± 0.03	-
	Geranylacetone	1429	-	-	1.17 [a] ± 0.05	0.14 [a] ± 0.00
Others	2-İndanone	1128	1.72 [a] ± 0.07	-	-	-
	γ-Nonalacton	1342	-	-	0.40 [a] ± 0.01	-
Total			100.00	100.00	100.00	100.00

* Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).

The characteristic compounds of truffle species, sulfur compounds, were found to be more abundant in *T. borchii* than in *T. aestivum*. In *T. borchii*, which grows in the Kırklareli region, sulfur compounds account for 42.19% of the aroma, while in the samples collected from Muğla, they represent only 12.15%.

In this study, the aroma of *T. aestivum* was analyzed in Muğla, and 20 volatile organic compounds were identified. In Kırklareli, 13 components were identified. Additionally, the aroma of *T. borchii* was analyzed in Muğla, and 44 volatile organic compounds were identified. In T. *borchii* from Kırklareli, 33 volatile organic compounds were identified. Overall, a total of 59 different compounds were identified between the two truffles studied. The structures of all these compounds were elucidated.In summer truffle *T. aestivum* collected from Muğla and Kırklareli, the major and common compounds are as follows: 2-octen-1-ol (7.05% and 46.48%, respectively), limonene (37.62% and 0.21%), [E]-2-octenal (5.83% and 2.43%), 3-octanone (1.95% and 4.78%), octanal (2.14% and 0.22%), 3-octanol (0.52% and 2.62%), 3-octen-1-ol (0.43% and 1.50%), phenylacetaldehyde (0.41% and 0.20%), and α-himachalen (0.55% and 0.20%).Similarly, in the aroma of white truffle *T. borchii*, which grows in Muğla and Kırklareli, the major and common compounds are as follows: 1-octen-3-ol (1.21% and 11.71%, respectively), 3-octanone (10.06% and 3.26%), benzaldehyde (5.02% and 0.93%), nonanol (4.22% and 6.37%), [E]-2-octenal (0.70% and 6.95%), 3-octene (1.10% and 1.88%), (E)-3-decen-1-ol (2.06% and 0.58%), octanal (0.45% and 2.99%), acetophenone (1.68% and 0.45%), bornyl acetate (1.30% and 0.49%), and linalyl acetate (0.41% and 0.25%), which are the main compounds commonly found in both regions.

In white truffle *T. borchii*, which grows in both regions, the following terpenoids were found: *α*-pinene, *p*-cymene, eucalyptol, *β*-trans-ocimene, *β*-linalool, borneol, thymol, carvacrol, *α*-terpinyl acetate, and geranyl acetone. Methional was detected at 9.91% in *T. borchii* collected from the Kırklareli region. Additionally, among other sulfur compounds, 3-methyl-4,5-dihydrothiophene was detected at 6.73% in Muğla and 29.53% in Kırklareli, while p-(methylthio) benzaldehyde was found at 5.42% in Muğla and 2.75% in Kırklareli, and these compounds were common major compounds in both regions. The aromas of both species commonly resemble garlic, spices, and musk smells.

Chemometric analysis

A chemometric analysis was conducted to determine the variability of volatile organic compounds in Tuber species concerning their types and geographical variations. Hierarchical cluster analysis (HCA) was employed using the suggested dendrogram in Table 2, representing data related to sulfur and alcohol compounds, the major compound classes for both truffle species. The data are interconnected and complementary (Figure 2).

When evaluated in terms of sulfur and alcohol compounds, which are the characteristic and main components of aroma in both truffle species, it was observed that the examined species clustered separately in the Hierarchical Clustering Analysis (HCA) *T. aestivum* grown in Kırklareli formed a distinct cluster compared to other specimens, while *T. borchii* and *T. aestivum* grown in Muğla clustered together. In addition, the *T. borchii* sample taken from Kırklareli showed some similarities with other *Tuber* species in Muğla, but still formed a separate cluster.

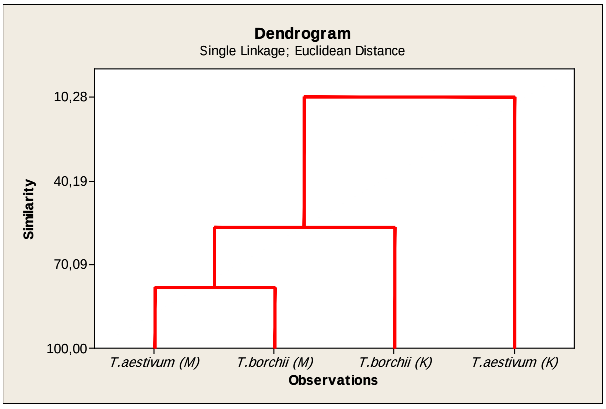

Figure 2. Dendrogram results obtained by Euclidean distance and Ward Linkage method

Discussion

The aromas of *Tuber* species are characterized by sulfur compounds, and they are found at the highest rate

(42.19%) in *T. borchii* collected from Kırklareli, while in *T. borchii* collected from Muğla, they are found at 12.15%. The high content of 3-methyl-4,5-dihydrothiophene and 1-octen-3-ol, identified as predominant in *T. borchii*, align with the results of previous studies reported in the literature (Bellesia et al., 2001; Splivallo & Ebeler, 2015; Lee et al., 2020). In *T. borchii* collected from a forest mixed with oak and hornbeam trees in Kırklareli, the major compounds were 3-methyl-4,5-dihydrothiophene (29.53%), methional (9.91%), and *p-(*methylthio) benzaldehyde (2.75%). It was observed that the sulfur-content aroma has a richer content and strongly varied when viewed sensorially. Among the sulfur compounds, 3-methyl-4,5-dihydrothiophene and *p*-(methylthio) benzaldehyde were detected, and methional was found in both Muğla and Kırklareli samples. Literature studies reported that methional is determined in both *T. borchii* and *T. magnatum* aromas (Vita et al., 2015; Niimi et al., 2021). Some compounds, such as octanal, [E]-2-octenal, and 3-octanone, which are widely found in the aroma of mushrooms, were common in all the analyzed samples (Splivallo et al., 2012; Molinier et al., 2015; Zambonelli et al., 2016; Schmidberger & Schieberle, 2017; Strojnik et al., 2020; Šiškovič et al., 2021).

In the aroma components of *Tuber* species, dimethyl sulfide, dimethyl disulfide, 3-methyl-1-butanol, 1-octen-3-ol, 3-methyl butanal, bis(methylthio)methane are reported to be the most frequently found components (Spanier et al., 2000; Zeppa et al., 2004; Piloni et al., 2005; March et al., 2006; Gioacchini et al., 2008; Cullere et al., 2010; Splivallo et al., 2011; Wang & Marcone, 2011; Beara et al., 2014; Vita et al., 2015; Zhang et al., 2016; Vahdatzadeh & Splivallo, 2018; Feng et al., 2019). As the quantities of sulfur compounds increase, the more distinctive garlic aroma, which is a characteristic of truffles, becomes more pronounced (Costa et al., 2015; Schmidberger & Schieberle, 2017; Mustafa et al., 2020).

The alcohol content is higher in the aroma of *T. aestivum* collected from Kırklareli, while terpenes are more abundant in the samples collected from Muğla. This observation in these two chemical components may be related to the species of the plant with which the truffles grow symbiotically, rather than regional differences in truffle growth. This result supports earlier research reports that truffles differentiate according to the plant species, soil microbiota, and other elements of their habitat (Mello et al., 2006; Vita et al., 2015; Vahdatzadeh et al., 2015; Büntgen et al., 2017; Mustafa et al., 2020; Šiškovič et al., 2021).However, the sample of *T. aestivum* collected from a forest of *Pinus brutia* Ten. in Muğla was richer in this content compared to the samples collected from the Kırklareli province. The major components in this sample included 38.36% limonene, 5.68% *p*-cymene, and 4.12% *β*-pinene terpene compounds.The major compounds in the Muğla sample were 3-methyl thiopropanal and benzaldehyde, in line with results from the literature (Mustafa et al., 2020; Šiškovič et al., 2021).

Hierarchical Clustering Analysis (HCA) was performed in terms of sulfur-containing compounds characteristic of truffles and alcohol compounds, which are the major compound class in the flavor of both truffles. In the dendogram obtained in this way, *T. borchii* specimen grown in Kırklareli clustered differently compared to other specimens, while *T. borchii* and *T. aestivum* in Muğla clustered together, partially similar to other *Tuber* species in Muğla. Appears to be clustered together. These data show that the type of truffle and the region where it grows are the factors that directly affect both the chemical component and the amount of their aroma.

According to the results obtained in this study, it can be concluded that regional differences cause quantities of the aroma changes in the major compound content, and the species of trees in which the ecological environment and/or ectomycorrhizal association can also cause differences in aroma. The aromas of truffle types are factors that directly affect their value in the market. Therefore, scientific studies have been conducted in recent years on truffle cultivation. Factors such as the soil structure of the truffle to be cultured, the ecological conditions, climate, and the tree species where truffles grow can all affect their aroma. These results indicate that after optimizing the aroma components to form preferred compounds in truffles, the ecological conditions and the types of symbiotic life-providing plants need to be considered for the cultivation of summer truffle *T. aestivum* and white truffle *T. borchii.* Recently, there has been promising interest in truffle cultivation in our country. However, we believe that in the future, marketing problems need to be addressed to optimize these mushrooms in terms of aroma components.

Author contributions

Cansu Korkmaz collection of truffle samples, preparation of fungarium, identifications, preparation of samples for analysis, realization of HS-SPME-GC/MS analysis of Mehmet Emin Duru truffles, mass analysis and interpretation of results.

Conflicts of interest

The authors declare no competing interests.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Cansu KORKMAZ, Mehmet Emin DURU).

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