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Nutrient Elements Contained in *Tuber aestivum* (Summer Truffle) Mushroom

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Abstract: Truffles have been known as valuable food for centuries, especially in France and Italy, and they have high monetary value and grow under the soil. They grow naturally in the Mediterranean climate zone, including our country. The aromas emitted by different *Tuber* species are highly varied; these strong, intense, persistent aromas are essential for attracting animals and insects that pick up and disperse the spores. These aromas are also the characteristics that determine the high value of fresh truffles. In recent years, truffles have attracted the attention of a wide range of people for their nutritional value and have become a soughtafter food by chefs, especially in European kitchens. Truffles are more decadent in protein and mineral content than other mushrooms. Its nutritional value contains 53-76% water, 9% protein, 7% carbohydrates and 8% minerals.*Tuber aestivum* samples, were subjected to extraction after being collected in appropriate periods, and their phenolic components, B group vitamins and vitamin C content were determined by UPLC-ESI-MS/MS and Headspace GC/MSD. According to the analysis results, the three most effective phenolic components p-Hydroxy benzoic acid 12.29 ± 0.37%, Gentisic acid 12.25 ± 0.27%, Protocatechuic acid 10.53 ± 0.74%. 74%, Pantothenic acid (vitamin B5) 2.88 ± 0.13%, Ascorbic acid (vitamin C) 1.13 ± 0.23%, Nicotinic acid (vitamin B3) 0.62 ± 0.01%, Nicotinamide (vitamin B3) 0.58 ± 0.03% were determined at high rates. Truffles are marketed as fresh and processed products. In addition to being used as sauces or spices in meals, they have the potential to be processed into truffle oil or truffle butter. Since the aromatic properties of truffles are lost together with the essential oils when cooked at high temperatures, it is preferred to be consumed fresh without cooking. It also has special extraordinary place in the perfumery industry.

Keywords: Truffle, *Tuber aestivum*, Nutrient Elements, Edible mushroom

Tuber aestivum (Yaz Trüfü) Mantarının İçerdiği Besin Elementleri

Öz: Trüf mantarları toprak altında yetişen, ekonomik değeri yüksek, özellikle Fransa ve İtalya'da yüzyıllardır değerli bir gıda olarak bilinmektedir. Ülkemizin de içinde bulunduğu Akdeniz iklim kuşağında doğal olarak yetişmektedir. Farklı *Tuber* türlerinin yaydığı aromalar son derece çeşitlidir; güçlü ve kalıcı özelliklere sahip bu aromalar, sporları alıp dağıtan hayvanlar ile böcekleri çekmek açısından önem taşır. Bu aromalar ayrıca taze yer mantarlarının yüksek değerini belirleyen özellikleridir. Trüf mantarları son yıllarda besin değeri açısından çok farklı kesimden insanın ilgisini çekmekte ve özellikle Avrupa mutfaklarında şefler tarafından aranan bir besin olarak karşımıza çıkmaktadır. Trüf mantarları protein ve mineral madde içerikleri bakımından diğer mantarlara göre daha zengindir. Besin değeri olarak %53-76 su, %9 protein, %7 karbonhidrat ve %8 mineral içermektedir. Araştırma materyalimiz olan *Tuber aestivum* mantarları uygun dönemlerde toplandıktan sonra ekstraksiyonuna tabi tutulmuş ve UPLC-ESI-MS/MS ve Headspace GC/MSD ile fenolik bileşenleri, B grubu Vitaminleri ve C vitamini belirlenmiştir. Elde edilen analiz sonuçlarına göre fenolik bileşenlerden en etkili üç bileşen; p-Hidroksibenzoik asit %12.29 ± 0.37, Gentisik asit %12.25 ± 0.27, Protokateşuik asit %10.53 ± 0.74 olarak, suda çözünebilen vitaminlerden ise Pantotenik asit (B5 vitamini) %2.88 ± 0.13, Askorbik asit (C vitamini) % 1.13 ± 0.23, Nikotinik asit (B3 vitamini) % 0.62 ± 0.01, Nikotinamid (B3 vitamini) %0.58 ± 0.03 yüksek oranlarda belirlenmiştir. Trüf mantarları taze ve işlenmiş ürün olarak pazarlanmaktadırlar. Yemeklerde sos veya baharat olarak kullanılmasının yanında işlenmiş olarak trüf yağı veya trüf tereyağı olarak değerlendirilme potansiyeline sahiptir. Trüflerin aromatik özellikleri yüksek ısıda pişirildiği zaman uçucu yağlarla birlikte kaybolduğu için pişirilmeden taze olarak tüketilmesi tercih edilmektedir. Ayrıca parfümeri endüstrisinde de çok özel bir yere sahiptir.

Anahtar kelimeler: Trüf, *Tuber aestivum*, Besin elementleri, Yemeklik mantar

Introduction

Mushrooms that naturally grow in the wild or cultivated are generally defined as macrofungi, which are of a size that can be seen with the naked eye and collected by hand and have distinctive fruiting bodies (Zhang et al., 2007). These mushrooms have a long history of use both as food and in traditional medicine. Numerous written sources indicate that mushrooms have been used by humans not only as a food source but also for their medicinal properties (Benjamin, 1995; Barroetaveña and Toledo, 2016). In many countries in Central and Eastern Europe, the consumption of wild mushrooms is preferred over cultivated ones (Kalač, 2009). There are several reasons for this. First, wild mushrooms often have, more decadent flavours and different textures. Mushrooms to growing in forests, and other natural regions generally offer more unique and special tastes than cultivated varieties. Another reason is the cultural and historical significance of these mushrooms. Example for example, in countries such as Poland, Hungary, Russia, and the Balkans, the wild mushroom gathering is a tradition passed down through generations and a way of life. Families learn to gather mushrooms from forests, use them in traditional recipes, and preserve them. Moreover, collecting wild mushrooms can contribute to the local economy, as these mushrooms are sometimes traded, providing an opportunity to earn income (Kalač, 2009).

Mushrooms have been collected from nature and consumed for many years to meet human nutritional needs due to their rich content of essential amino acids, carbohydrates, fibre, and important vitamins and minerals. It is suggested that mushrooms, which are commonly consumed as food, are rich in vitamin C and B vitamins, which are essential for the immune system. Additionally, certain mushroom species are widely used in the pharmaceutical and cosmetics industries due to the bioactive compounds they contain (Cheung, 2013; Kalac, 2013; Erdem et al., 2018)

Edible mushrooms are a rich source of protein containing essential amino acids. Due to their nutritional value, healing properties, and unique taste and aroma, they create a distinctive flavour in the foods used they use (Pekşen and Karaca, 2000). Because of their nutritional benefits and delicious taste, mushrooms are widely preferred in food and beverage establishments and by consumers today (Erdem et al., 2018). The most essential nutrient in mushrooms is their protein content, which has a similar amino acid composition to the proteins found in animal-based foods. Because of this similarity, they are considered an alternative to animal products such as meat, milk, and eggs. Wild mushrooms, which grow in natural environments, have a richer protein and lower fat content than cultivated mushrooms (Ravikrishnan et al., 2017).

In addition to being consumed as a food source, mushrooms have been shown to play an essential role in the prevention or slowing down of various diseases, such as cancer, hypertension, hypercholesterolemia, insomnia, allergies, stress, asthma, and diabetes (Feng, 2001; Jiskani, 2001; Guillamón et al., 2010). This diversity highlights the health benefits of the chemical compounds found in edible mushrooms and the importance of bioactive chemical components in medicinal mushrooms (Kumar, 2015). Recently, there has been growing awareness regarding the nutritional value of macrofungi, including truffle mushrooms (Claridge and Trappe, 2005). Truffles are highly valued, especially in gastronomy, and are a sought-after food source. Due to their aroma, flavour, and rarity, several species are commercially valuable. Truffles belong to genus of the *Tuber* Mich. Ex F. H. Wigg.:Fr. (*Ascomycota, Pezizales, Tuberaceae*). They are characterized by the fruiting body of the fungus, which develops underground in symbiosis with a variety of trees and shrubs, such as oaks (*Quercus* L.), pines

(*Pinus* L.), and hazels (*Corylus avellana* L.) (Stobbe et al., 2013). The symbiosis between truffles and plants is known as ectomycorrhiza, consisting of a network of branched hyphae attached to plant roots. Ectomycorrhizal symbioses are filamentous structures representing the mutual interactions between fungal mycelium and plant roots. This symbiosis enables truffles to acquire the necessary nutrients from plants, while truffles, in turn, facilitate the absorption of water and minerals from the soil, promoting plant growth (Ori et al., 2019). Truffle species generally grow in regions with Mediterranean climates, such as temperate and subtropical forest habitats, valleys, typically in calcareous soils, depending on the species (Patel et al., 2017; Bonito et al., 2010). According to the Global Biodiversity Information Facility (GBIF.org, 2024), approximately 230 tuber species worldwide are accepted worldwide (GBIF.org, 2024).

Due to its geographical location and climate conditions, Türkiye provides an ideal environment for cultivating many truffle species. Studies have identified 23 genera and 15 families of truffles in Turkey, with 67 truffle species (Şen et al., 2016). Truffle mushrooms are highly prized in gastronomy for their unique tastes and aromas, but their availability is limited due to their specific climate and soil requirements (Allen and Bennett, 2021; Splivallo et al., 2015).

Mushrooms of the *Tuber* genus are renowned flavours for their rich aroma and unique flavours and their significant nutritional value. These truffle mushrooms offer diverse nutrients, including various vitamins, minerals, and other essential dietary components. Truffles are rich in protein, fats, nutritional fibers, ash, crucial amino acids, and metals such as potassium (K), phosphorus (P), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn). Additionally, truffles are abundant in antioxidants like anthocyanins, β-carotene, vitamin C, and phenolic compounds (Patel, 2012). One of the most distinctive characteristics of truffles is their sulfur-containing compounds, which dissipate rapidly when exposed to air. Over time, volatile aromatic compounds, such as 1-octen-3-ol, become more pronounced, contributing to the trufflecharacteristic scent (Pelusio et al., 1995). Ongoing research continues to explore the chemical and biological properties of truffle mushrooms to understand further their nutritional and medicinal potential (Wang and Marcone, 2011).

In Türkiye, *Tuber aestivum* Vittad. (yaz trüfü) is a species that can be grown widely and is one of Central Europe's most commonly found truffle mushrooms (Sesli et al., 2020). It has a broad distribution, ranging from regions across Europe (from Great Britain, Russia, and Sweden to Spain) and North Africa (including Morocco). It has been reported to grow in habitats at altitudes between 1300 and 1600 meters above sea level (Bratek, 2010).

The soil requirements for the summer truffle (*T. aestivum*) are relatively broad compared to other *Tuber* species. The ideal soil conditions for this species include loamy soils with the following chemical parameters: pH between 6.1 and 7.4, organic matter content ranging from 3.1% to 9.1%, and available P_2O_5 content of 200 ppm, with K_2O content around 500 ppm (Bratek, 2010). *Tuber aestivum* predominantly thrives in soils rich in limestone or with high levels of exchangeable calcium (Chevalier and Sourzat, 2013).

Tuber aestivum, known as the summer truffle, is widely recognised for its unique aroma and flavor. Compared to the more intense winter truffles, *T. aestivum* has a milder taste and a more delicate aroma, often described as earthy, nutty, and slightly garlicky (Mustafa et al., 2020). Gourmets and the culinary industry use summer truffles in a variety of dishes, either fresh or in infusions, such as oils, butter, cheeses, and sauces (Oliach et al., 2021). Although not as expensive as other truffle varieties, *T. aestivum* remains a luxury product (Santos-Silva & Brígido, 2024). The market prices for fresh summer truffles can vary significantly between regions, with Spain seeing lower prices (ranging from 25 to 70 EUR per kg), while in Switzerland, prices are much higher (ranging from 200 to 600 EUR per kg) (Oliach et al., 2021). *T. aestivum* holds great gastronomic value due to its unique taste, kitchen versatility, and medicinal properties, making it highly valuable both gastronomically and socio-economically.

Truffle mushrooms are flavour also used as food flavour enhancers and contain functional food components. Due to their unique aroma and high market value, there has been a growing body of research in recent years focusing on the mycology, cultivation, and chemical composition of truffles and their aroma. Recognised Researchers have increasingly recognized the therapeutic properties of truffle mushrooms in recent years. In addition to their culinary uses worldwide, truffles are valued as therapeutic agents due to their bioactive components, including antioxidants, anti-mutagenic, anti-carcinogenic, antimicrobial, antiviral, immune-modulating, anti-inflammatory, antidepressant, and sedative properties (El Enshasy et al., 2013; Hannan et al., 1989).

As a result, interest in and research on truffles have become more diverse in the past decade. In this context, the present study aims to determine the levels of phenolic compounds and the amounts of vitamin B and C in *Tuber aestivum* (The Burgundy truffle), which is widely distributed in Türkiye.

Materials and Methods

Collection of Mushroom Samples

The research material consists of *T. aestivum* specimens collected from the field. These *T.* aestivum mushroom samples were collected in Muğla (Fethiye). During the fieldwork, photographs of the specimens were taken in their natural habitats, and the samples were brought to the laboratory to determine their macroscopic and microscopic characteristics. Associate Professor Dr. Hakan Allı, a faculty member of the Department of Biology at Muğla Sıtkı Koçman University, carried out the identification. Subsequently, a fungarium number was assigned to the specimens.

Extraction of Phenolic Compounds and Instrumental Analysis Conditions

Approximately 3 g of each mushroom sample was subjected to tissue disruption using 200 mL of liquid nitrogen. Then, 30 mL of an acetone (80:20) mixture was added to the sample, and the extraction was carried out at -86 °C for 6 hours. After extraction, the mixture was removed from the cooler and treated in an ultrasonic bath for 15 minutes. Subsequently, the extract was centrifuged at 4000 rpm for 10 minutes at 20°C. The resulting supernatant was filtered through Whatman No. 4 filter paper, and the residue was subjected to two additional extractions with 30 mL acetone mixtures. The combined extracts were concentrated by evaporating the acetone under a reduced vacuum at 40°C using a Rotary Evaporator (Heidolph Basis Hei-VAP ML). The aqueous phase was washed three times with 30 mL n-hexane, and the hexane organic phase was discarded. Any remaining hexane in the aqueous phase was evaporated at 40°C to dryness. The residue was then re-dissolved in a water (80:20) mixture. The solution was filtered through 0.20 µm filters and analysed by UPLC-MS/MS (Ultra Performance Liquid Chromatography - Tandem Mass Spectrometry). This method ensured the efficient extraction of phenolic compounds from the *T. aestivum* samples for subsequent analysis.

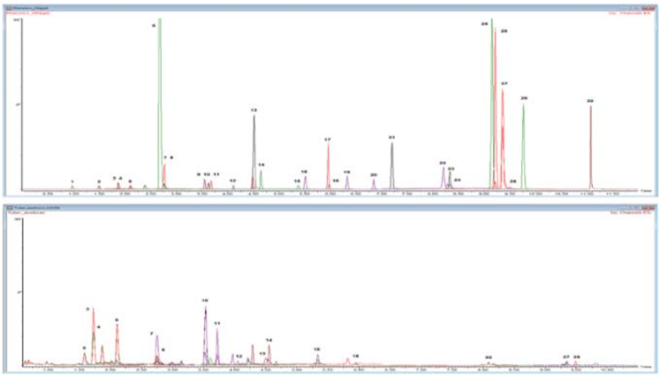

Figure 1. Phenolic Compound Standards and Total Ion Chromatograms from *T. aestivum* Analysis Using UPLC-MS/MS

Standards and reagents

Acetonitrile, n-hexane, petroleum ether, methanol, and ethyl acetate of analytical grade purity were supplied by Merck (Darmstadt, Germany). The fatty acid methyl esters (FAME) standard mixture 37 was purchased from Supelco Analytical Bellefonte (PA, USA). Phenolic standards and amino acids (LAA-21 Kit) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All reagents were of analytical grade.

Determination of Vitamin B Group and Vitamin C Content by UPLC-MS/MS

Approximately 3 g of *Tuber aestivum* were weighed, and 30 mL of a water H2O: CH3CN (80:20) mixture was added. The mixture was left to extract at +20°C for 6 hours. After this period, the mixture was removed from the cooling system and subjected to ultrasonic bath treatment for 15 minutes. The extract was centrifuged at 4000 rpm at 20°C for 10 minutes.

Table 1. Acquity UPLC Instrument Parameters

Acquity UPLC Device Parameters				
Column	Acquity UPLC BEH C18 column (1.7µm 2.1x100mm)			
Mobil Phase A	H_2O with 0,5% CH_3COOH			
Mobil Phase B	MeCN with 0,5%CH_3COOH			
Column Oven Temp	40°C			
Injection Volume	2µL			
Gradient Program	Time (min.)	Flow (mL/ min.)	% A	% B
	0.00	0.650	99.00	01.00
	1.00	0.650	99.00	01.00
	10.00	0.650	70.00	30.00
	12.00	0.650	05.00	95.00
	12.10	0.650	99.00	01.00
	15.00	0.650	99.00	01.00

The supernatant was filtered through the Whatman No. 4 filter paper, and the remaining residue was further extracted twice with 30 mL of the same H_2O mixture.The combined extracts were concentrated by evaporating the acetonitrile under a reduced vacuum at 40°C. The resulting solution was then filtered through Macherey-Nagel Chromafil Xtra PTFE-20/25 0.20 µm filters and analysed by UPLC-MS/MS.

Result and Discussion Phenolic compounds are essential plant and fungal components due to their ability to scavenge radicals, owing to the hydroxyl groups they contain (Shao et al., 2010). Phenolic acids, in particular, are considered good antioxidants due to their ability to chelate and scavenge free radicals, especially hydroxyl and peroxyl radicals, superoxide anions, and peroxynitrites (Carocho & Ferreira, 2013). Phenolic compounds have been shown to have protective effects in various human diseases, such as cancer, cardiovascular diseases, and inflammatory processes (García-Lafuente et al., 2009).

Truffle mushrooms are rich in bioactive compounds such as ascorbic acid, ergosterol, phenolics, flavonoids, terpenoids, phytosterols, and polysaccharides. In particular, truffle mushrooms contain a significant amount of flavonoids known for their antioxidant, anti-inflammatory, anti-mutagenic, and anticancer activities. These biological activities make truffles a crucial secondary metabolite of natural products.

However, edible mushrooms cannot synthesise these compounds and, therefore, do not possess these valuable metabolites. From this perspective, researchers have shown interest in the biological activities of truffle mushrooms and their potential applications in the medical field (Panche et al., 2016; Gil-Ramírez et al., 2016). In this context, the analysis of *Tuber aestivum* has led to the determination of the Transition, Cone, and Collision Energy values of its phenolic compounds, which are presented in Table 2.

Table 2. Transition, Cone, and Collision Energy Values of Phenolic Compounds Found in *Tuber aestivum*

Phenolic Compounds	Transition (m/z)	Cone (V)	CE (V)	RT (min)
Pyrogallol	125.01 > 69.10, 79.04, 81.02	20	17, 17, 14	0.97
Homogentisic acid	167.03 > 123.03, 122.08, 108.00	10	20, 20, 10	1.47
Protocatechuic acid	153.06 > 108.00, 81.01, 91.01	10	20, 25, 20	1.85
Gentisic acid	153.05 > 109.04, 108.03, 81.00	10	20, 20, 12	1.85
Pyrocatechol	153.06 > 81.01, 108.00, 109.04	8	20, 25, 20	2.38
Galanthamine	288.10 > 198.00, 213.09, 230.95	20	32, 23, 17	2.68
p-Hydroxy benzoic acid	136.98 > 93.03, 65.10	10	25, 14	2.75
3,4-Dihydroxybenzaldehyde	137.00 > 91.93, 107.94, 136.00	8	21, 20, 18	2.76
Catechin hydrate	288.88 > 109.15, 124.99, 245.26	30	25, 20, 15	3.45
Vanillic acid	166.98 > 151.97, 108.03, 123.03	20	18, 12, 14	3.61
Caffeic acid	179.10 > 135.14, 107.10, 133.9	32	23, 23, 24	3.65
Syringic acid	197.20 > 123.00, 167.00, 182.00	15	22, 18, 14	4.11
Vanillin	150.95 > 135.94, 91.90, 107.97	30	20, 20, 14	4.50
Epicatechin	189.18 > 151.00, 203.00, 205.00	20	20, 20, 20	5.50
p-Coumaric acid	163.01 > 119.04, 93.00, 117.01	5	27, 27, 15	4.65
Ferrulic acid	193.03 > 134.06, 178.00, 149.02	20	16, 12,13	5.36
Catechin gallate	441.00 > 168.98, 288.97	30	20, 20	5.91
Rutin	609.00 > 254.99, 270.93, 299.90	17	55, 55, 40	5.95
trans-2-hydroxy cinnamic acid	163.04 > 119.04, 117.01, 93.07	10	25, 22, 13	6.32
Myricetin	316.90 > 107.07, 137.01, 150.97	30	30, 25, 25	6.83
Resveratrol	227.01 > 143.01, 159.05, 185.03	30	25, 18, 18	7.13
trans-Cinnamic acid	146.98 > 103.03, 62.18	30	10, 10	8.19
Luteolin	284.91 > 107.01, 133.05, 151.02	20	30, 33, 30	8.27
Quercetin	303.00 > 137.00, 153.00, 229.00	20	30, 32, 30	8.29
Naringenin	270.98 > 107.00, 119.04, 150.97	20	25, 25, 20	9.07
Genistein	271.00 > 153.00, 215.00, 243.00	20	27, 25, 24	9.22
Apigenin	269.10 > 107.00, 117.00, 149.00	20	30, 30, 25	9.35
Kaempferol	284.90 > 158.97, 117.10, 227.14	10	34, 40, 30	9.50
Hesperetin	301.02 > 108.01, 136.00, 163.99	20	36, 30, 24	9.71
Chrysin	252.99 > 63.05, 107.05, 142.99	20	30, 25, 25	11.06

In this study, 30 phenolic compounds were identified in *T. aestivum*. Among these compounds, p-Hydroxybenzoic acid (12.29 ± 0.37 µg/g), Gentisic acid (12.25 ± 0.27 µg/g), Protocatechuic acid (10.53 ±0.74 µg/g), and Vanillic acid (8.68 ± 0.10 µg/g) were found at high concentrations (Table 3).

Table 3. Analysis Results of Phenolic Compounds in *Tuber aestivum* Using UPLC-ESI-MS/MS (µg/g dry weight ± standard deviation)

Phenolic Compounds	**RT (min)**	***Tuber aestivum***	**Mode**
Pyrogallol	0.97	n.d.	ESI (-)
Homogentisic acid	1.47	6.11 ± 0.26	ESI (-)
Protocatechuic acid	1.85	10.53 ± 0.74	ESI (-)
Gentisic acid	1.86	12.25 ± 0.27	ESI (-)
Pyrocatechol	2.38	0.77 ± 0.03	ESI (-)
Galanthamine	2.68	n.d.	ESI (+)
p-Hydroxy benzoic acid	2.75	12.29 ± 0.37	ESI (-)
3,4-Dihydroxybenzaldehyde	2.76	5.88 ± 0.13	ESI (-)
Catechin hydrate	3.45	n.d.	ESI (-)
Vanillic acid	3.61	8.68 ± 0.10	ESI (-)
Caffeic acid	3.65	0.47 ± 0.04	ESI (-)
Syringic acid	4.11	2.19 ± 0.03	ESI (-)
Vanillin	4.50	1.54 ± 0.01	ESI (-)
p-Coumaric acid	4.65	0.44 ± 0.01	ESI (-)
Ferrulic acid	5.36	0.98 ± 0.05	ESI (-)
Epicatechin	5.50	n.d.	ESI (-)
Catechin gallate	5.91	n.d.	ESI (-)
Rutin	5.95	2.22 ± 0.06	ESI (-)
trans-2-hydroxy cinnamic acid	6.32	n.d.	ESI (-)
Myricetin	6.83	n.d.	ESI (-)
Resveratrol	7.23	n.d.	ESI (-)
trans-Cinnamic acid	8.19	0.08 ± 0.01	ESI (-)
Luteolin	8.27	n.d.	ESI (-)
Quercetin	8.29	n.d.	ESI (+)
Naringenin	9.17	n.d.	ESI (-)
Genistein	9.22	n.d.	ESI (+)
Apigenin	9.35	0.05 ± 0.01	ESI (-)
Kaempferol	9.50	0.07 ± 0.01	ESI (-)
Hesperetin	9.71	n.d.	ESI (-)
Chrysin	11.06	n.d.	ESI (-)

n.d.: Not Detected RT: Retention time All data were presented by means±standard deviation ($p<0.05$)

Many studies have analysed different *Tuber* species and their phenolic compounds. For example, Ding et al. (2010) identified 4,5-Dihydroxy-2-methoxy-benzaldehyde (comatin) in *Tuber melanosporum* samples; Villares et al. (2012) identified homogentisic acid, 4-HBA, and 3,4-dihydroxy benzaldehyde compounds. In their study, phenolic compounds identified in *T. melanosporum* Vittad. (mg/g), *T. aestivum* (mg/g), and *T. indicum* Cooke & Massee (mg/g) included gallic acid, homogentisic acid, protocatechuic acid, p-hydroxybenzoic acid, 3,4-dihydroxy benzaldehyde, p-coumaric acid, and o-coumaric acid. Specifically, the concentrations of these compounds were as follows: for *T. melanosporum* (mg/g), homogentisic acid was 91.06 ±

16.4, p-hydroxybenzoic acid 64.77 ± 7.79, and 3,4-dihydroxy benzaldehyde 54.64 ± 5.15; for *T. aestivum* (mg/g), 3,4-dihydroxy benzaldehyde was 376.59 ± 27.55, homogentisic acid 358.06 ± 24.53, and p-coumaric acid 93.72 ± 38.47; for *T. indicum* (mg/g), homogentisic acid was 189.69 ± 16.95, gallic acid 61.54 ± 9.19, and p-hydroxybenzoic acid 58.42 ± 1.72. Hydroxycinnamic acids were detected only in *T. aestivum*. Similarly, other phenolic compounds, such as gallic acid and protocatechuic acid, were only found in *T. aestivum* and *T. indicum* truffles. *T. aestivum* also showed significantly higher values of homogentisic acid and 3,4-dihydroxy benzaldehyde (Kıvrak and Kıvrak, 2018). In their study, they reported high amounts of phenolic compounds in *T. nitidum* Vittad. including 21.83 ± 0.14 µg/g of p-hydroxybenzoic acid, 19.04 ± 0.12 µg/g of protocatechuic acid, and 16.34 ± 0.10 µg/g of vanillic acid as other high-level phenolic compounds. Another study found the highest levels of gallic acid, p-hydroxybenzoic acid, and p-coumaric acid in *T. melanosporum* (Beara et al., 2014). In this study, the *T. aestivum* samples also showed high levels of these compounds, indicating that *T. aestivum* is rich in phenolic compounds, similar to *T. nitidum* and *T. melanosporum*. Overall, the phenolic acid profile results in the studied truffle species show reasonable alignment with other research studies.

Vitamins are minor compounds in foods that play an essential role in nutrition. Vitamins are divided into two major classes: water-soluble and fat-soluble. Mushrooms have been reported in various studies to be rich in vitamins C, D, and B group vitamins (Anşin et al., 2000; Mattila et al., 2001). The B group vitamins found in mushrooms are adequate in the nervous, digestive, hematopoietic, and wound healing. They also function as coenzymes in biochemical reactions, and deficiencies in these vitamins lead to problems such as polyneuropathy, muscle and nerve system disorders, beriberi, hair loss, conjunctivitis, stomatitis, arthritis, respiratory problems, and anaemia (Calderón-Ospina and Nava-Mesa, 2020). Vitamin B2 plays a role in converting carbohydrates, proteins, and fats into energy and helps prevent cataracts. Vitamin B3 prevents pellagra and regulates blood circulation. Studies have reported that insufficient intake of B vitamins increases the risk of cancer, but consuming them in excess does not prevent cancer formation. Adequate intake of these vitamins is necessary for the body's defence system. The abundant vitamin C in mushrooms is absorbed in the small intestine and reacts quickly with superoxide and hydroxyl radicals, performing an antioxidant role. It also plays a role in the synthesis of bile acids, the absorption of iron, wound healing, and protects foods containing fat, such as fish, margarine, and milk, from oxidative degradation by forming ascorbate radicals and renewing tocopherol radicals (Wolf et al., 1998). Mushrooms have a protective effect due to their significant amounts of vitamins A, C, and β-carotene (Murcia et al., 2002).

In this study, the amounts of vitamin C and B group vitamins in *Tuber aestivum* are given in Table 4. According to the results, ascorbic acid and pantothenic acid (vitamin B5) was found in high amounts at 2.88 ± 0.13 mg/100g, and vitamin C (ascorbic acid) at 1.13 ± 0.23 mg/100g. Following these, nicotinic acid (vitamin B3) was 0.62 ± 0.01 mg/100g, nicotinamide (vitamin B3) 0.58 ± 0.03 mg/100g, pyridoxine (vitamin B6) 0.39 ± 0.03 mg/100g, riboflavin (vitamin B2) 0.21 ± 0.03 mg/100g, and thiamine (vitamin B1) 0.15 ± 0.01 mg/100g (Table 4).

Table 4. UPLC-ESI MS/MS Analysis Results of Water-Soluble Vitamins Found in *Tuber aestivum* (mg/100g dry weight ± standard deviation)

Vitamin compounds	**R.T. (min)**	***Tuber aestivum***
Thiamine (B_1 vitamin)	0.480	0.15 ± 0.01
Riboflavin (B_2 vitamin)	3.040	0.21 ± 0.03
Nicotinamide (B_3 vitamin)	0.860	0.58 ± 0.03
Nicotinic acid (B_3 vitamin)	0.710	0.62 ± 0.01
Pantothenic acid (B_5 vitamin)	2.860	2.88 ± 0.13
Pyridoxine (B_6 vitamin)	0.830	0.39 ± 0.03
Biotin (B_7 vitamin)	3.070	n.d.
Folic acid (B_9 vitamin)	2.930	n.d.
Cyanocobalamine (B_{12} vitamin)	2.950	n.d.
Ascorbic acid (C vitamin)	0.560	1.13 ± 0.23

n.d.: Not Detected RT: Retention time All data were presented by means±standard deviation.

Thanks to their high content of vitamins, such as thiamine (vitamin B1), riboflavin (B2), niacin (B3), biotin (B7), and ascorbic acid (vitamin C), as well as minerals, mushrooms are a necessary and beneficial meal. (Hee-Gyeong et al., 2022) their study determined the Vitamin C content in *T. melanosporum* samples as 1.65 ± 0.09 mg/100 g, *T. aestivum* as 1.90 ± 0.11 mg/100 g, and *T. magnatum* as 10.15 ± 0.33 mg/100 g. As a result, these mushrooms could likely be a good source of B and C vitamins, depending on the species. The differences in vitamin content among mushroom species are believed to be attributed to factors such as the growing environment, climate, and environmental conditions. The similarities or differences in the results between this study and others can be attributed to factors such as the type of mushroom species used, the habitat in which the species was grown, and the analytical methods and equipment used in the analysis. Therefore, the results obtained in this study are consistent with the literature.

Conclusion

Due to its diverse climate and plant cover, Türkiye hosts many different mushroom species, particularly truffle mushrooms. With their rich nutrient profiles, *Tuber* species stand out, being abundant in proteins, fats, fibre, vitamins, and minerals, making truffles a valuable part of a healthy diet. Along with their gastronomic importance, awareness of their nutritional value is growing. Truffle mushrooms make an essential contribution to nutrition by providing both delicious and healthy nutrients. According to the results of this study, *T. aestivum* is rich in various essential phytochemicals, including phenolic compounds and B and C vitamins. Based on the findings of this study, *T. aestivum* could be of significance for use in nutraceuticals or the pharmaceutical industry, due to these beneficial properties.

Author contributions

All authors have equal contribution

Conflict of Interest

The authors declare no conflict of interest

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 (Sevgin ÖZDERİN, Hakan ALLI)

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