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Investigation of Chemical Composition and Nutritional Value of Truffle Mushroom (*Tuber nitidum* Vittad.)

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Abstract: Truffles are very precious and expensive mushrooms owing to their distinctive aroma and great flavor. The present study was carried out to survey the nutritional value of *Tuber nitidum*, a wild edible mushroom belonging to *Tuber* genus. It was analyzed for fatty acid content, phenolic and volatile compounds and amino acid profile. The chemical composition of the studied mushroom species showed that this wild edible fungus is one of a rich phenolic and amino acid source. The characteristic mushroom odor compounds, 1-octen-3-ol (14.81%) and 1-octen-3-one (11.19%), have been also detected. However, it has been reported that *p*-hydroxy benzoic acid, gentisic acid, vanillic acid were found in ethyl acetate extract as phenolic ingredient. In the lipidic extract, it has been determined that 76.94% linoleic acid (C18:2n6c), 12.38% palmitic acid (C16:0), 6.38% oleic acid (C18:1n9c), 2.54% stearic acid (C18:0). The wild edible mushroom, *Tuber nitidum*, a wild edible mushroom, may be of value and importance in the food and pharmaceutical industry as a natural healthy product source.

Trüf Mantarının (*Tuber nitidum* Vittad.) Kimyasal Bileşimi ve Besinsel Değerinin İncelenmesi

Anahtar Kelimeler
Fenolik bileşikler,
Yağ asitleri,
Uçucu bileşikler,
Tuber nitidum,
Headspace-GC/MSD,
UPLC-ESI-MS/MS

Özet: Trüfler, karakteristik aromaları ve leziz tadı nedeniyle çok değerli ve pahalı mantarlardır. Bu çalışma Tuber cinsine ait yabani yenilebilir bir mantar olan *Tuber nitidum*'un besin değerini araştırmak için yürütülmüştür. Yağ asidi içeriği, fenolik ve uçucu bileşikleri ve amino asit profili analiz edildi. Çalışılan mantarın kimyasal bileşimi, bu yabani yenilebilir mantarın zengin fenolik ve amino asit kaynağı olduğunu gösterdi. Karakteristik mantar koku bileşikleri, 1-okten-3-ol (%14.81) ve 1-okten-3-on (%11.19) da tespit edilmiştir. Bununla birlikte, fenolik bileşen olarak etil asetat ekstraktında *p*-hidroksi benzoik asit, gentisik asit, vanilik asit bulunmuştur. Lipid özütünde, %76.94 linoleik asit (C18:2n6c), %12.38 palmitik asit (C16:0), %6.38 oleik asit (C18:1n9c), %2.54 stearik asit (C18:0) olduğu tespit edilmiştir. Yabani yenilebilir mantar olan *Tuber nitidum*, doğal sağlıklı bir ürün kaynağı olarak gıda ve ilaç sektöründe bir değer olarak önemlidir.

1. Introduction

As mushrooms were consumed due to their unique and delightful flavor in the past, however, present-day usage of those in human diet may also be helpful to fight diseases, cancer, cardiovascular and neurodegenerative disorders. Mushrooms symbolize humanizing heritage from the time of old as food and medical substance in line with traditional information transmitted along generations. Mushrooms may not constitute a significant portion of the human diet; however, they have long been considered as highly tasty and nutritive foods [1,2] and their consumption continues to increase in many countries due to a huge diversity of biomolecules with nutritional significance [3]. The functional benefits and presence of compounds with bioactive properties, proteins, trace minerals [1,2,4-7] make the mushrooms very valuable. Some of those compounds can be found in the phenolic, polysaccharidic, and lipidic fractions of mushrooms [1]. Mushrooms are known as functional foods. They

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have phenolic structure with antioxidant and antimicrobial activity, and used as natural drug and nutraceuticals [8].

Its distinctive aroma and marvelous flavor, truffles are the valuable and high-prized tastes that are commonly used in the reputable world kitchen [9]. The summer truffle, *Tuber aestivum*, is the most common European truffle species that has environmental and economic values [10, 11].

The nutritional ingredients of truffles were firstly published in 1892 [12], and last inquiries report that truffles consist of 5-9% (by dry weight) of total lipids and a relatively high amount of unsaturated fatty acids (UFAs) in their *Tuber* fruiting bodies [13]. The major fatty acids (FAs), such as linoleic, oleic, palmitic acids, have been studied mostly in previous studies. However, the minor FAs may not been adequately examined. Free amino acids (FAAs) content of truffles was previously studied using amino acid analyzer [9]. Also, the total phenolic content is in agreement with the antioxidant properties; however, the chemical composition of phenolic content may significantly affect the radical stabilization and, the antioxidant activity, in general.

Thus, the identification of individual phenolic compounds, which find in natural sources, is an important issue of today's world. Therefore, the present study characterizes Turkish wild mushroom, *Tuber nitidum* was examined using GC/MSD and UPLC-ESI-MS/MS instruments in terms of nutrient content and chemical compounds. In addition, phenolic compounds, volatiles, fatty acid and free amino acid contents have been identified and quantified. *Tuber nitidum* has been found to be rich in phenolic compounds with healing and therapeutic properties.

2. Materials and Methods

2.1. Mushroom

Samples of *Tuber nitidum* originating from northwest Turkey were collected in 2016. Samples were lyophilized using freeze dryer.

2.2. Standards and reagents

Acetonitrile, n-hexane, petroleum ether, methanol, and ethyl acetate were 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.

2.3. Chemical composition

2.3.1. Nutritional value

In the determination of chemical composition and nutritional content of *Tuber nitidum*, AOAC procedures (1995) were used [14]. Protein content was calculated using Kjeldahl method; fat content was determined by petroleum ether and soxhlet apparatus; energy was calculated according to [8] Eq. (1).

Energy (kcal)=
4 × (g protein + g carbohydrate)+ 9 × (g fat) (1)

2.3.2. Fatty acid analysis

Samples were made powder using liquid nitrogen and freeze dryer. Fatty acids were then extracted using a mixture of hexane: petroleum ether (50:50). Hundred milligrams of extract was dissolved with 10 mL of *n*-hexane and 100 µL of 2N methanolic potassium hydroxide (KOH). The sample was then centrifuged, and filtered by 0.20 µm LC filter disk. Fatty acid methyl esters were analyzed by gas chromatography mass spectrometry (GC-MS) (Agilent 7890GC - 5975C inert MSD, Agilent Technologies, Wilmington, Delaware, USA) using J&W 112-88, A7 HP-88 (250°C, 60m x 250µm x 0.25µm) column.

2.3.3. Free amino acid analysis

Free amino acids were analyzed according to a fast amino acid method published elsewhere [15]. 1 g of dried sample was mixed with 5 mL of methanolic 0.1 % (v/v) formic acid. The sample was then centrifuged and filtered using LC filter disk. Free amino acids were analyzed by ultra performance liquid chromatography (UPLC) (Waters Acquity Ultra Performance LC, Waters Co., Milford, MA, USA) and tandem mass spectrometry (MS/MS) (Xevo TQ-S MS-MS, Waters Co., Milford, MA, USA). All determinations were carried out in triplicate. Chromatographic separation was achieved on an Acquity UPLC BEH reverse phase C18 column (1.7 µm 2.1 × 100 mm) thermostatted at 40 °C. Chromatographic and mass spectrometric conditions were described previously by Kıvrak et al. [15].

2.3.4. Determination of phenolic compounds

In the analysis of phenolic compounds, in-house extraction technique and UPLC-ESI-MS/MS instrument were used. Three grams of sample were extracted three times with 30 mL of 80% (v/v) acetone. It takes 6 hours at -86°C. The mixture of extracts was evaporated. Then the residue was dissolved with 80% (v/v) methanol, then filtered

using filter disk. Phenolic compounds were analyzed by UPLC-ESI-MS/MS. The chromatographic and mass spectrometry conditions were previous literature [16, 17].

2.3.5. Determination of volatile compounds

In the analysis of volatile compounds of mushroom samples, a rapid and reliable technique, Headspace (HS)-GC/MSD (Agilent 7890 GC/5975C MSD) was used. Five grams of sample was weighted in vial (20 mL), and then anhydrous magnesium sulfate was added. The vial was closed with crimper. And then the vial was incubated at 90°C. It is analyzed for the volatile content using HS-GC/MSD. A HP-5MS capillary column (15 m, 0.25 mm i.d., 0.25 µm film thickness) was used. The Wiley 2007, NIST 2008 and Flavor2 libraries were applied for the qualitative analysis of compounds.

2.4. Statistical analysis

The average values were calculated by Microsoft Excel 2010 (Microsoft Corporation, USA) and stated as mean ± standard deviation (SD).

3. Results

The data of the mycochemical constitution and obtained energetic value of a wild edible mushroom, *Tuber nitidum*, are displayed in Table 1. *Tuber nitidum* mushroom has abundant of carbohydrate and protein. Carbohydrates were the most abundant macro nutritional compounds and the record grades were determined in many mushrooms [18]. Edible mushrooms have a sufficient substitution for the proteins of animal origin [19], which fulfills them as protein foods for the future, due to high protein ingredient in most species, which is higher than in most other vegetables [20].

The results were similar in previously studied mushrooms [8, 17, 18]. In *T. nitidum*, it had been determined low fat content (3.24%) and high energetic value (367.08 kcal/100 g). The moisture ingredient was 82.06 g/100g. The reported moisture content value here is in close agreement with those reported by other researcher groups [21, 22].

The results of the main fatty acid composition of mushrooms are important in order to elucidate nutritional values. The main fatty acids detected in the studied wild mushroom are displayed in Table 1. The fatty acids determined using GC/MSD in higher percentages, *T. nitidum* consisted of 76.94% linoleic acid (C18:2n6c), 12.38% palmitic acid (C16:0), 6.38% oleic acid (C18:1n9c), 2.54% stearic acid (C18:0), 0.58% *cis*-11,14-eicosadienoic acid (C20:2), 0.44% palmitoleic acid (C16:1), 0.26% heptadecanoic acid (C17:0), 0.25% *cis*-11-eicosenoic acid (C20:1), 0.22% linolenic acid (C18:3n3). Notably, the essential fatty acid α-linolenic acid (C18:3n3) (0.22%) was also detected. The results of up to 37 fatty acids were analyzed, and 9 fatty acids were identified and quantified among them.

Table 1. Mycochemical composition and obtained energetic value and main fatty acids of *Tuber nitidum*.

Parameter	*T. nitidum*
Moisture (g/100 g fw)	82.06±0.98
Ash (g/100 g dw)	14.87±0.20
Carbohydrate (g/100 g dw)	49.21±0.33
Proteins (g/100 g dw)	35.27±0.38
Fat (g/100 g dw)	3.24±0.12
Energy (kcal/100 g dw)	367.08±0.20
Palmitic acid (C16:0) (%)	12.38±0.10
Palmitoleic acid (C16:1) (%)	0.44±0.05
Heptadecanoic acid (C17:0) (%)	0.26±0.03
Stearic acid (C18:0) (%)	2.54±0.05
Oleic acid (C18:1n9c) (%)	6.38±0.10
Linoleic acid (C18:2n6c) (%)	76.94±0.18
Linolenic acid (C18:3n3) (%)	0.22±0.02
cis-11-eicosenoic acid (C20:1) (%)	0.25±0.02
cis-11,14-eicosadienoic acid (C20:2) (%)	0.58±0.03

dw: dry weight, fw: fresh weight.

However, *T. nitidum* might be a good source of essential fatty acids it has content of crude fat, which was 3.24 g/100 g dried weight, thus it could be regarded as a healthy food to consume. Furthermore, linoleic acid and oleic acid are very important constituent of diet. Having linoleic acid as the major constituent, which is a polyunsaturated fatty acid, *T. nitidum* becomes very essential to human nutrition. This increases nutritional importance of this mushroom.

Phenolic compounds have substantial effects on appearance, taste and savory of food which are important quality parameters in consumption. Phenolic compounds as a natural antioxidant are considered to have physiological benefits for human health [1]. Kim et al. [24] and Palacios et al. [25] have previously reported the existence of phenolic compounds in medicinal mushrooms; however, Villares et al. in 2012 studied the determination and evaluation of phenolic compounds in truffle species.

T. nitidum used in this study contained 21.83 ± 0.14 µg/g *p*-hydroxybenzoic acid as an abundant ingredient; rests were 19.04 ± 0.12 µg/g protocatechuic acid, 16.34 ± 0.10 µg/g vanillic acid (Table 2). These results are correlated with a previous study, although some differences can be considered [23]. This may be due to area of harvest and/or the analysis method, technique, etc.

Table 2. Phenolic ingredients (µg/g dry weigth ± SD) of *T. nitidum* mushroom.

Phenolic Compounds	RT (min)	Concentration (µg/g)
Pyrogallol	0.97	ND
Homogentisic acid	1.92	2.36±0.10
Protocatechuic acid	2.39	19.04±0.12
Gentisic acid	2.39	9.17±0.10
Pyrocatechol	2.96	1.03±0.08
Galanthamine	4.91	ND
p-Hydroxybenzoic acid	4.51	21.83±0.14
3,4-Dihydroxybenzaldehyde	4.64	3.36±0.10
Catechin hydrate	5.55	ND
Vanillic acid	5.61	16.34±0.10
Caffeic acid	5.64	2.41±0.07
Syringic acid	6.11	5.09±0.15
Vanillin	6.50	4.51±0.11
Epicatechin	6.42	ND
p-Coumaric acid	6.59	4.37±0.20
Ferulic acid	7.35	3.35±0.10
Catechin gallate	7.88	ND
Rutin	7.86	0.48±0.05
trans-2-hydroxycinnamic acid	6.61	ND
Myricetin	10.23	ND
Resveratrol	9.16	ND
trans-Cinnamic acid	10.22	0.64±0.08
Luteolin	10.28	0.27±0.09
Quercetin	10.97	ND
Naringenin	10.8	ND
Genistein	10.87	ND
Apigenin	10.86	ND
Kaempferol	10.9	2.32±0.09
Hesperetin	10.96	ND
Chlorogenic acid	5.52	ND
Gallic acid	1.10	2.07±0.10
Chrysin	11.26	ND

ND: Not detected, RT: Retention time

A total of 20 free amino acids were detected in *T. nitidum* in this study displayed in Table 3.

The total content of free amino acids was 444.70 mg of edible weight in *T. nitidum*, the total amount of essential amino acids was 311.11 mg/100g, and constitutes approximately 70% of total free amino acids. It is also important that valine (61.29mg/100g), leucine (60.38mg/100g) and phenylalanine (52.09mg/100g) were found in high amounts. From the results of this study and previous studies [9,26], it is understood that *T. nitidum* has many amino acid components, and this wild edible mushroom species has the potential to be a source for supplementation of essential amino acids. Liu et al. in 2012 [9] conducted the study for the free amino acid content of truffles, and their results were also in accordance with the present study. However, there was difference in the methionine amount this can be due to analysis method.

Table 3. Free amino acid content (mg/100g dry weight ± standard deviation) of *Tuber nitidum* mushroom.

Amino acid	Essential/ Nonessential	*T. nitidum* (mg/100g)
Glycine	Nonessential	1.37 ± 0.10
Alanine	Nonessential	5.46 ± 0.10
Serine	Nonessential	5.07 ± 0.12
Proline	Nonessential	9.87 ± 0.20
Valine	Essential	61.29 ± 0.24
Threonine	Essential	10.72 ± 0.18
4-hydroxy proline	Nonessential	5.44 ± 0.17
Leucine	Essential	60.38 ± 0.47
Isoleucine	Essential	48.06 ± 0.20
Asparagine	Nonessential	10.11 ± 0.15
Aspartic acid	Nonessential	5.81 ± 0.13
Lysine	Essential	12.20 ± 0.20
Glutamine	Nonessential	21.13 ± 0.28
Glutamic acid	Nonessential	19.08 ± 0.10
Methionine	Essential	34.32 ± 0.50
Histidine	Nonessential	1.27 ± 0.08
Phenylalanine	Essential	52.09 ± 0.32
Arginine	Nonessential	29.34 ± 0.41
Tyrosine	Nonessential	19.64 ± 0.50
Tryptophan	Essential	32.05 ± 0.10

Among the volatile compounds identified in the present work (Table 4), 1-octen-3-ol and 3-methyl-1-butanol have been previously detected by other studies [27, 28] in the aroma of *T. aestivum*.

The major compounds of *T. nitidum* are 1-octen-3-ol (14.81%), hexanal (14.21%) and 1-octen-3-one (11.19%); in the present work, other compounds such as benzaldehyde (1.10%), *o*-cymene (0.67%), 2-heptanone (0.87%), 1-hexanol (1.14%) and limonene (0.99%) have also been detected in scant amounts.

Most of edible mushrooms produce matsutake alcohol (1-octen-3-ol), a volatile alcohol [38]. Mushroom like odor is derived from C_8 ($C_8H_{16}O$), and today it is considered the most important mushroom volatile compound [30].

Two of them, 1-octen-3-ol and 1-octen-3-one, have been described as responsible of the characteristic mushroom odor of such fungi.

Table 4. Volatile compounds of *Tuber nitidum* mushroom (p<0.05).

Compounds	RT (min)	Volatile Compound (%)
2-Pentanone	1.027	8.16
3-Methyl-2-butanol	1.079	6.37
Valeraldehyde	1.108	10.48
3-Methyl-1-butanol	1.243	2.63
Butanimidamide	1.291	2.12
1-Pentanol	1.383	4.56
Hexanal	1.527	14.21
1-Hexanol	2.071	1.14
2-Heptanone	2.226	0.87
Heptanal	2.327	1.01
Benzaldehyde	2.880	1.10
1-Octen-3-one	3.246	11.19
1-Octen-3-ol	3.352	14.81
2-Pentyl-furan	3.574	2.84
o-Cymene	4.089	0.67
Limonene	4.195	0.99
2-Octenal	4.364	8.37
Nonanal	5.211	3.21
Citronellal	6.039	5.27

RT: Retention time

4. Discussion and Conclusion

In this study, highly accurate and reliable devices such as Headspace-GC/MSD and UPLC-ESI-MS/MS were used for analysis of mushroom content. It is known that, due to the presence of secondary metabolites and other natural agents in their structure, those food are in a focus of attention as a nutraceutical product [31]. As a result; valine, leucine and phenylalanine as essential amino acids; linoleic acid (C18:2n6c), palmitic acid (C16:0), oleic acid (C18:1n9c), stearic acid (C18:0), cis-11,14-eicosadienoic acid (C20:2) as fatty acids, *p*-hydroxybenzoic acid, protocatechuic acid, vanilic acid, gentisic acid and syringic acid as phenolic compounds; 1-octen-3-ol, 1-octen-3-one, hexanal, benzaldehyde, 1-hexanol, valeraldehyde and limonene as volatile compounds constituents were determined in the fruiting body of *T. nitidum*. These bioactive ingredients play an important role in maintaining human health and metabolism, and delicacy and food additives in terms of nutritional content and bioactive compounds. The contribution of *Tuber nitidum* into the daily portion of a diet may satisfy the wellness, on account of its antioxidant feature and nutrients. Furthermore, different fractions of mushrooms could be used in food or pharmaceutical industries through extraction, separation and purification.

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