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CHEMICAL COMPOSITION OF SUMMER TRUFFLE (*TUBER AESTIVUM* VITTAD.) FROM BOSNIA AND HERZEGOVINA

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Summary

Ascocarps of summer truffle (*Tuber aestivum* Vittad.) were found in natural oak and beech forests on calcareous soils, at altitude of 840–850 m, near the town of Šipovo in Bosnia and Herzegovina (B&H), with the help of trained dogs.

Until now, no one in B&H has researched the chemical composition of summer truffle. The aim of the work is to analyze nutritional value, elemental composition, and the assortment of fatty acids in the freshly collected ascocarps and to compare achieved results with the results from the literature related to the summer truffle ascocarps found in some other locations, then with the ascomata of other species of truffles, and with the fruiting bodies of some above-ground edible mushrooms.

Chemical analysis showed that *Tuber aestivum* contains about 75.5 % water and about 25.5 % dry matter. The most common group of compounds were carbohydrates, followed by proteins, while the mineral component and fats were much less presented. Among analyzed elements, the most prevalent was potassium with an average concentration of 26,409 mg/kg_{DW} and the least represented was lead with an average concentration of 0.45 mg/kg_{DW}. The main part of the mineral spectrum consisted of three elements: K, P and Ca, with a share of 95.3 %. In ascocarps of summer truffle, 24 fatty acids were detected, of which 13 were saturated and 11 unsaturated. The ratio of saturated to unsaturated fatty acids was 58.34 % to 41.66 %. The most common among saturated fatty acids was palmitic with a share of 25.89 %, while the most common unsaturated fatty acid was elaidic with a share of 28.17 %. No ingredients have been found in the fruiting bodies that would make them unfit for human consumption in any way.

Keywords: summer truffle, nutrition value, elemental concentration, fatty acids.

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1. Introduction

Summer truffle (*Tuber aestivum* Vittad.) is considered the most common truffle in moderate climate of middle European countries [1, 2]. At the same time, only a few of its natural sites have been described in Bosnia and Herzegovina (B&H), while the chemical composition of its ascomata has not been discussed so far [3–6]. Otherwise, chemical analyzes of truffles are often focused on compounds that are responsible for the odour and taste of their fruiting bodies, while less attention is paid to other ingredients [7–10].

Summer truffle is usually consumed fresh, which is justified reason for a better knowledge of its chemical composition and nutritional value. Truffles are hypogeous mushrooms, and as such they have a more direct contact with heavy metals and other harmful substances that have reached the soil under certain circumstances, compared to above-ground mushrooms. These moments should be taken into account when placing these fetish foods on the market, and even when choosing a location for truffle orchard.

Concrete examples show that certain species of truffles have different affinities for different soil elements and compounds. Chemical analysis of the flesh of hog truffle (*Choiromyces meandriformis* Vitt.) found on one location in B&H has showed increased concentration of arsenic. At the same time, chemical analysis of the soil did not show increased concentration of As in comparison with average value for observed soils. This fact leads to the conclusion that hog truffle has particularly emphasized affinity towards arsenic because of its capability to accumulate almost 50 % of arsenic content in the soil, unlike, for example, bolete mushrooms (*Boletus* spp.) which used to accept just around 0.6 % of the available soli arsenic [11]. During the investigation of the absorption capacity of the desert truffle (*Tirmania nivea*) towards heavy metals (Cd, Cr, Ni, Pb), different transfer factors from soil to ascocarp were obtained, including a scenario that the ascocarp contains even higher concentration of some heavy metal (in this case cadmium), than it is in the soil itself [12].

The aim of research is to analyze parameters that determine its nutritional value, its elemental composition, and the assortment of fatty acids in the freshly collected summer truffle ascocarps; a set of fatty acids was processed in detail, considering their importance for organoleptic properties and for the regulation of the fungus/ascocarp – microflora relationships. The achieved results will be compared with the results from the literature related to the summer truffle ascocarps found in some other locations, then with the ascomata of other species of truffles, and with the fruiting bodies of some above-ground edible mushrooms.

2. Materials and Methods

2. 1. Finding truffles and sample formation

Tuber aestivum is a typical hypogeous fungi, so let's hire a trained Laggoto Romagnolo dogs to find it. We collected samples at the beginning of November 2020 in the privately owned oak forest (*Quercetum petraea montanum*) and beech forest (*Fagetum montanum*) on calcareous soil, at altitude of 840-850 m, near the town of Šipovo. According to the ecological and vegetation regionalization of B&H, the study area belongs to the Euro-Siberian-North American region and its Illyrian province [13].

Five fully developed and mature carpophores were isolated from the collected material, which were sent to the laboratory for analysis the very next day. Detailed morphological characteristics of the sample are given in the work of [6].

The original monograph from 1831 was used to identify the taxon [14]. Spores were observed under a microscope and compared with two relevant sources [15, 16]. As a form of additional control, two other sources were consulted [17, 18].

2. 2. Chemical analysis

The water content was determined by drying fresh tissue to constant mass at 105 °C [19]. The levels of total nitrogen were determined by the Kjeldahl method [19], and percent protein was calculated as % N×6.25. Fats content was determined by the Soxhlet method [19]. When determining the chemical elements, 2.5±0.0001 grams of crushed sample was burned in a mixture of nitric and perchloric acid by volume (10 mL of concentrated nitric acid, 10 mL of diluted nitric acid (1:1), 10 mL of concentrated perchloric acid) and evaporated to dryness. The dry matter was dissolved with 10 mL of diluted hydrochloric acid (1:3) [20]. The solution was then filtered into a 50.00 mL volumetric flask and the filtrate was made up with deionized water. The acids were of GR chemical purity. The conductivity of deionized water was 0.045 µS/cm. ICP OES measurements were performed three times for each duplicate using Optima 8000 Optical Emission Spectrophotometer (Perkin Elmer, USA). The sample solutions were pumped by a peristaltic pump from tubes arranged on a Perkin Elmer auto-sampler model 510. Operating conditions of measurement with ICP OES spectrometer OPTIMA 8000, *i.e.* gas flows were as follows: plasma gas (Ar) – 8 L/min, auxillary – 0.2 L/min, nebulizer gas (Ar) – 0.7 L/min, pump flow rate – 1 mL/min, RF power – 1500 W. For the calibration of instrument, a CRM solution proposed by the Instrument Calibration standard 2 was used, with a mineral concentration of 100 mg/L (Pb, Cd, As, Cr, Cu, Zn, Fe, Ca, Na, Mg, P, K; Perkin Elmer, USA). Fatty acid methyl esters of the tested samples were prepared by direct esterification with a saturated solution of methanol with KOH, according to [21–24]. Obtained fatty acid methyl esters were separated in a gas chromatography from Perkin Elmer, model Clarus 680, equipped with flame ionization detector and a capillary column Elite-WAX, 60 m long, with inner diameter 0.32 mm and film thickness 0.50 µm. Initially, the column temperature was set at 60 °C for 2 min, then raised to 200 °C at a rate of 10 °C/min; again, it was raised at 240 °C at a rate of 5 °C/min and maintained at 240 °C for 30 min. Thus, total chromatographic run time was 54 min. The flow rate for the carrier (H_2), auxiliary (N_2) and detector flame (H_2 and synthetic air) gases was 1.5 mL/min. The composition of fatty acid methyl esters and the resulting acids is shown as a percentage of individual fatty acids in the total amount of identified fatty acids (g/100 g of total fatty acids). All samples were studied three times, and obtained results presented as the mean value with standard deviation *i.e.* coefficient of variation (CV).

3. Results

3. 1. Nutritional value

Ascocarp of *Tuber aestivum* from B&H contains about 75.5 % water and about 24.5 % dry matter. The main nutritional constituents of summer truffle are carbohydrates, followed by proteins, while the mineral component and fats are much less present (**Table 1**).

Table 1

Nutritional value of summer truffle from B&H compared to other species of fungi

Species	Origin	Water (%)	Total dry matter (%)	Dry matter components (%)			
				Proteins	Fat	Carbo-hydrates	Ash
Tuber aestivum	B&H	75.49	24.51	6.52	0.69	16.43	0.87
Tuber nitidum	Turkey[22]	82.06	17.94	6.17	0.57	8.60	2.60
Terfezia boudieri	Tunisia[23]	78.23	21.77	5.64	1.72	13.41	1.00
Tirmania nivea	Tunisia[24]	77.63	22.37	6.54	1.54	13.14	1.15
Morchella esculenta	Serbia[25]	89.42	10.58	1.22	0.24	8.29	0.83
Agaricus campestris	Croatia[26]	85.11	14.89	6.25	0.44	7.64	0.56
Boletus edulis	Croatia[26]	87.77	12.23	4.13	0.33	7.18	0.59
Cantharellus cibarius	Croatia[26]	85.76	14.24	4.68	0.29	7.94	1.33
Pleurotus ostreatus	Portugal[27]	89.17	10.83	0.76	0.15	9.30	0.62
Lentinula edodes	Portugal[27]	79.78	20.22	0.89	0.35	17.62	1.36

3. 2. Elemental analysis

Among 12 elements analyzed in this study, the most prevalent was potassium with an average concentration of 26,409 mg/kg_{DW} and the least represented was lead with an average concentration of 0.45 mg/$kg_{DW;}$ only Cd was below the limit of detection in all truffle ascocarps, while as was not detected at all. The main part of the mineral spectrum consists of three elements: K, P and Ca, with a share of 95.3 %, while the other eight elements have 4.7 % of total composition. Similar relationships were found in other samples of summer truffle. In the sample from B&H analyzed in Slovenia, K, P and Ca were represented with 94.25 %, in the samples from Hungary with 95.7 % and in the material from Germany with 94.7 %. These elements also dominate in the samples of *T. brumale* and *T. melanosporum* from Serbia, with a share of 91.8 % and 93.55 %, respectively. It is possible to see that the material originating from Germany separately analyzed the elemental composition of peridium and gleba (**Table 2**, last column), with the explanation that in fact peridium controls the transfer of elements to the host plant and for that reason a higher concentration of elements should be expected. This attitude does not have the force of any law, since the concentrations of K, P and Cd are higher in gleba than in peridium, and the differences in the concentrations of Mg and Zn are less than 15 %. According to the content of potassium, as the most abundant element (26.4 g/kg_{DW}), summer truffle is among average mushrooms, since the potassium content of 410 observed species of wild mushrooms ranged between 1.5 g/kg_{DW} in *Polyporaceae* and 117 g/kg_{DW} in *Coprinaceae* [25–28].

Table 2

Average elemental concentration (mg/kg_{DW}) in summer truffle ascocarps from different areas

No.	Element	B&H		B&H/Slovenia[4]		Hungary[1]		Germany[2]		
		$\bar{X}$	CV (%)	$\bar{X}$	CV (%)	$\bar{X}$	CV (%)	$\bar{X}$	CV (%)	p/g ratio
1	Na	49.90	25.3	110.3	26.5	148.2	27.4	500	64	2.7
2	K	26409	3.0	18885	13.8	25647	9.2	29000	15.5	0.85
3	Ca	3388	12.2	2945.5	7.0	3331	32.4	2300	32.2	3.57
4	Mg	749	2.9	874.5	36.7	1060	16.3	1300	30.5	1.08
5	P	4112	0.7	4785	34.2	7879	7.0	8860	20.3	0.78
6	Zn	338.5	1.4	148.5	34.3	160.5	22.1	166	25.9	1.14
7	Cu	47.3	2.9	100.6	30.6	49.3	26.6	61.6	24.4	1.29
8	Cr	1.1	22.2	2.65	136.2	1.29	27.1	3.56	51.4	2.03
9	Fe	501.2	16.4	381	131.8	230.3	52.2	213	87.8	199
10	Cd	0.0	0.0	5.80	448.7	n.a.	–	2.84	42.3	0.81
11	Pb	0.45	183.6	0.34	123.5	n.a.	–	0.71	121.1	8.62

Notes: n.a. – not analyzed, p/g ratio means element concentration in peridium and in gleba.

Local role book on maximum permitted quantities for certain contaminants in food [29, 30] sets the maximum permitted amounts of Pb≤0.30 mg/kg_{FW} and Cd≤0.20 mg/kg_{FW} in fresh mushrooms: *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*. The role book does not cover truffles because they still do not appear as commercial species on the domestic market. If we were to filter data from the **Table 2** through the stated criteria, it is possible to find out that samples from B&H/Slovenia and Germany have an increased cadmium content and that as such they should not be traded as a food.

When the analyzed chemical elements are arranged in descending array according to their concentration, an identical pattern is obtained for summer truffles from B&H and Hungary, and for *T. melanosporum* from Serbia (**Table 3**). If Cd was not detected in the B&H/Slovenia sample, we could talk about a common pattern for all these localities. In the case of summer truffle samples from Germany, there was a rolling over string (Fe-Zn-Na), in the sense that Na took the first, Fe the second and Zn the third position. In the sample of *T. brumale* from Serbia, there was a change of place between Mg and Fe in relation to the sample of *T. melanosporum*.

Table 3

Descending array of concentration of elements in truffle's ascocarps

Origin	Rank or position in descending order																				
	1	>	2	>	3	>	4	>	5	>	6	>	7	>	8	>	9	>	10	>	11
Tuber aestivum																					
B&H	K	>	P	>	Ca	>	Mg	>	Fe	>	Zn	>	Na	>	Cu	>	Cr	>	Pb	o.d.o.	
B&H/Sl.[4]	K	>	P	>	Ca	>	Mg	>	Fe	>	Zn	>	Na	>	Cu	>	Cd	>	Cr	>	Pb
Hungary[1]	K	>	P	>	Ca	>	Mg	>	Fe	>	Zn	>	Na	>	Cu	>	Cr		o.d.o.		
Germany[2]	K	>	P	>	Ca	>	Mg	>	Na	>	Fe	>	Zn	>	Cu	>	Cr	>	Cd	>	Pb
Tuber brumale																					
Serbia[30]	K	>	P	>	Ca	>	Fe	>	Mg	>	Zn	>	Na	>	Cu	>	Cr		o.d.o.		
Tuber melanosporum																					
Serbia[30]	K	>	P	>	Ca	>	Mg	>	Fe	>	Zn	>	Na	>	Cu	>	Cr		o.d.o.		

Note: o.d.o. – outside decreasing order.

3. 3. Fatty acids

In ascocarps of summer truffle from B&H, 24 fatty acids were detected, of which 13 were saturated and 11 unsaturated. The ratio of saturated to unsaturated fatty acids was 58.34 % to 41.66 %. The most common among saturated fatty acids was palmitic with a share of 25.89 %, while the most common unsaturated fatty acid was elaidic with a share of 28.17 %. Truffles from B&H differ from other observed truffles in the majority of saturated fatty acids. All other samples were dominated by unsaturated fatty acids, ranging from 61.1 % to 86.24 %; the same is the case with *T. melanosporum* (**Tables 4, 5**). Previous research has shown that unsaturated fatty acids also dominate in most representatives of *Basidiomycetes* [31, 32].

The assortment of fatty acids proved to be a very differential feature, both within the observed species and between them. Most saturated fatty acids were detected in the sample from B&H – a total of 13, and the least in one sample from Italy – only two [33]. When it comes to samples from Italy, it is interesting to note that, despite being the same climate and the same key author, there were not even a minimal cross-section of the sets. Namely, caproic and stearic fatty acids were present in the first sample, and in the second they were absent. Furthermore, a total of 11 saturated fatty acids were detected in three samples from Italy. Of that number, eight appeared in only one sample, while three appeared in two samples; no saturated fatty acids were found in all three observed samples from Italy. The analysis should include some other available sources, for example [34–38], the heterogeneity would be further increased, given that these authors found only 9 % saturated fatty acids and as much as 91 % unsaturated fatty acids in the ascocarp of *T. aestivum*. Palmitic acid can be considered as least common denominator of saturated fatty acids, since it is found in all samples (except one) and has the highest relative frequency (**Table 4**). This saturated fatty acid also occurs in both samples of *T. melanosporum,* and with the highest frequency. Linoleic acid had the highest frequency among unsaturated fatty acids. Also presented in significant amounts in all samples was oleic acid (**Table 5**).

Table 4

Assortment of saturated fatty acids in ascocarps of *T. estivum* from different locations, compared to the *T. melanosporum*

No.	Common name	C:D	***Tuber aestivum* (%)**					***Tuber melanosporum* (%)**	
			B&H	**Ita.**/33/	**Ita.**/35/	**Ita.**/36/	**Fin.**/37/	**Fra.**/38/	**Ita.**/35/
1	Butyrix acid	4:0	0.42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	Caproic acid	6:0	0.39	28.5	n.d.	n.d.	n.d.	n.d.	n.d.
3	Capriylic acid	8:0	0.59	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	Capric acid	10:0	0.70	n.d.	0.15	n.d.	n.d.	n.d.	0.03
5	Lauric acid	12:0	2.49	n.d.	n.d.	0.38	n.d.	n.d.	n.d.
6	Tridecylic acid	13:0	0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	Myristic acid	14:0	3.88	n.d.	1.44	0.27	n.d.	2.9	1.62
8	Pentadecylic a.	15:0	0.73	n.d.	4.00	n.d.	n.d.	n.d.	4.95
9	Palmitic acid	16:0	25.89	n.d.	16.63	12.39	12.0	11.5	17.36
10	Margaric acid	17:0	0.53	n.d.	0.04	n.d.	n.d.	n.d.	0.03
11	Stearic acid	18:0	10.04	10.4	n.d.	5.19	1.03	2.7	n.d.
12	Nonadecylic a.	19:0	n.d.	n.d.	n.d.	n.d.	0.18	n.d.	n.d.
13	Arachidic acid	20:0	11.51	n.d.	8.79	n.d.	0.55	n.d.	7.92
14	Heneicosylic a.	21:0	n.d.	n.d.	6.81	n.d.	n.d.	n.d.	5.83
15	Behenic acid	22:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16	Lignoceric acid	24:0	1.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Σ		58.34	38.9	37.86	18.23	13.76	17.1	37.74

Note: n.d. – not detected.

It should be emphasized that the presented results are based on material collected from one locality. As new summer truffle sites are discovered in B&H, the variability of the newly collected material will need to be analyzed. Also, it is necessary to expand the field of interest to some other interesting ingredients, such as volatile organic compounds, exudates etc.

Table 5
Assortment of unsaturated fatty acids in ascocarps of *T. aestivum* from different locations compared to the *T. melanosporum*

No	Common name	C:D	***Tuber aestivum* (%)**					***Tuber melanosporum* (%)**	
			B&H	Ita./33/	Ita./35/	Ita./36/	Fin./37/	Fra./38/	Ita./35/
1	Myristoleic a.	14:1	0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	Pentadecenoic a. (cis-10)	15:1	0.56	n.d.	n.d.	n.d.	0.18	n.d.	n.d.
3	Palmitoleic a.	16:1	0.82	n.d.	n.d.	n.d.	0.25	n.d.	n.d.
4	Heptadecanoic a. (cis-10)	17:1	0.14	n.d.	0.30	n.d.	0.12	n.d.	0.29
5	Oleic a.	18:1n9c	15.9	15.9	13.53	28.41	31.21	22.7	17.13
6	Elaidic a.	18:1n9t	28.17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	Linoleic a.	18:2	4.99	45.2	39.95	53.37	53.80	57.8	37.58
8	α-Linolenic a.	18:3n3	4.21	n.d.	n.d.	n.d.	0.45	2.3	n.d.
9	γ-Linolenic a.	18:3n6	0.54	n.d.	n.d.	n.d.	0.23	n.d.	n.d.
10	Eicosaenoic a.	20:1	n.d.	n.d.	4.40	n.d.	n.d.	n.d.	3.42
11	Eicosadienoic (cis,cis-11,14)	20:2	0.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12	Dihomolinoleic	20:3	n.d.	n.d.	3.96	n.d.	n.d.	n.d.	3.84
13	Timnodoic a.	20:5	1.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Σ		41.66	61.1	62.14	81.78	86.24	82.8	62.26

Notes: n.d. – not detected; No 10, eicosaenoic acid takes form of gadoleic acid.

4. Conclusions

Specimens of *T. aestivum* were found in oak and beech forests on calcareous soils of B&H. Ascocarp of summer truffle from B&H contains about 75.5 % water and about 25.5 % dry matter. The most common group of compounds are carbohydrates, followed by proteins, while the mineral component and fats are much less present. Among analyzed elements, the most common was potassium with an average concentration of 26,409 mg/kg_{DW} and the least represented was lead with an average concentration of 0.45 mg/kg_{DW}. The main part of the mineral spectrum consists of three elements: K, P and Ca, with a share of 95.3 %, while the other eight elements have 4.7 % of total composition. In the case of commercial harvesting, it should be taken into account that truffles, as hypogeous fungi, may have an increased content of heavy metals and other harmful substances.

In the ascocarp, 24 fatty acids were detected, of which 13 were saturated and 11 unsaturated. The ratio of saturated to unsaturated fatty acids was 58.34 % to 41.66 %. The most common among saturated fatty acids was palmitic with a share of 25.89 %, while the most common unsaturated fatty acid was elaidic with a share of 28.17 %.

The assortment of fatty acids proved to be a very differential feature, both within the observed species and between them. Truffles are definitely capable of synthesizing a full range of saturated and unsaturated fatty acids, and what they will actually produce in the given circumstances depends on the specific situation *in situ*, *i.e.* on the need to keep competitive microorganisms away and to attract beneficial ones. Therefore, it is possible to believe that linking a set of fatty acids with a fine taxonomic differentiation makes no sense.

No ingredients have been found in the fruiting bodies that would make them unfit for human consumption in any way.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper, as well as the published research results, including the financial aspects of conducting the research, obtaining and using its results, as well as any non-financial personal relationships.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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