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# Complex chemical evaluation of the summer truffle (*Tuber aestivum* Vittadini) fruit bodies

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## Summary

Summer truffle (*Tuber aestivum* Vittadini) is one of the most important mycorrhizal mushrooms with underground fruit bodies. Formerly the scientific investigations were focused mainly on its specific fragrant constituents. Our work was concentrated on complex chemical characterization including different organic and inorganic components. Summer truffle has middle crude protein, low fat-, and relatively high fiber and chitin contents; its energy level is low. Distribution of protein fractions is characteristic (in % of crude protein): 40.98; 5.91; 3.85; 19.28 and 29.98 % for albumins, globulins, prolamins, glutelins and NPN (non protein nitrogen), respectively.

We determined soluble oligo- and polysaccharides (9.00 mg/g DM and 49.9 mg/g DM, respectively), as well as the contents of phenoloids and flavonoids (2.8 mg/g DM and 0.093 mg/g DM, respectively). Mineral composition is similar to other mushrooms; four macroelements (K, P, Ca and Mg) give 97.94 % of the all mineral content; occurrence of poisonous elements (as As, Cd, V) is not characteristic.

Chemical nature of *Tuber aestivum* (summer truffle) fruit bodies is very characteristic, regarding not only the occurrence of fragrant components but the classical, "usual" components, too. This rare and highly appreciated hypogaeous mycorrhizal fungus belongs to mushrooms of valuable, specific chemical composition.


## Introduction

Truffles mushrooms are specific mycorrhizal fungi with hypogaeous (underground) fruit bodies (ascocarps, basidiomes). Their occurrence and life was (and partly is today also) slightly unknown, "mysterious", caused by their life-type. Use of these extreme mushrooms has long history. First of all the characteristic fragrance substances and properties were and are appreciated on the market. Mass of gathered truffles (wild growing and cultivated) has been showing decreasing tendency caused by different ecological, environmental etc. reasons.

Summer truffle (*Tuber aestivum* Vittadini) is the most frequent truffle species (or one of these) of Middle Europe (of Carpathian Basin). Its habitat is wide-spreading, we have data of occurrence from different parts of Europe (from Great Britain, Russia, Sweden up to Spain) and from North-Africa (Morocco). There are data on occurrence up to 1300-1600 m in the mountains. *T. aestivum* occurs on different habitats of Hungary mainly in the central chain of mountains but in Middle and South Hungary on the lowlands, too. Soil requirements of summer truffle are relatively wide: best are the loamy soils with the following chemical parameters; pH: 6.1-7.4; content of organic substances 3.1-9.1 %; available $P_2O_5$ content is 200 ppm, available $K_2O$ 500 ppm (Bratek, 2010). The host (mycorrhizal) partners are mostly the *Quercus* (oak) species (*Q. pubescens, Q. robur*, *Q. petraea*, *Q. cerris*), beech (*Fagus sylvatica*), birch (*Betula pendula*), lime species (*T. cordata, T. plathyphyllos*), poplar (*Populus)* species, willow (*Salix)* species, different pines (*Pinus nigra, P. sylvestris* etc.), hornbeam (*Carpinus betulus*), chestnut (*Castanea sativa),* hazelnut (*Corylus avellana*), or other trees and shrubs. The underground fruit bodies of this truffle have 2-10 cm diameter, the color is brown to black, the outer surface (peridium) is ornamented with some pyramidal warts and transverse fine marks. Inner part (gleba) has a dark brown color and a white vein structure. The yellow brown, reticulate ascospores have a network of ridges (Wang and Marcone, 2011).

Nutritional value i.e. the chemical composition of these mushrooms is partly unknown because of the non conventional occurrence and of the specific use. Scientific works and investigations were and are concentrated on the volatile (fragrant) components (March et al., 2006; Kiss et al., 2011; Diaz et al., 2002; Diaz et al., 2003; Cullere et al., 2012). Diaz and co-workers (Diaz et al., 2003) analyzed the "aroma" of *T. aestivum* and T. *melanosporum* by a new and susceptible chemical method (head space solid-phase microextraction). Eighty nine constituents were extracted and isolated, some only from *T. aestivum* or *T. melanosporum*, other molecules were found (in different quantities) in both species. Most characteristic specific fragrant molecules of summer truffle are: DMS (dimethylsuphide); DMDS (dimethyldisulphide); methional; 3-methyl-1-butanol; 1-hexen-3-one; 3-ethylphenol (Cullere at al., 2012). Fragrance of *T. aestivum* is a chemically very complicated mix of different molecules. Such works are very useful for the better level of quality control, for different food industrial products having truffles. Nutritional profile (components) were analyzed mainly for desert truffle species (*Terfezia claveryi, Tirmania* species and for others: Hussan and Al-Ruqaie, 1999) and rare for *T. aestivum* (Saltarelli et al., 2008). Number of these exact data on nutritional value is under the required limit.

Aims of our studies

- to give exact data on "classical" chemical organic components (crude protein, -fat, -fiber, chitin);
- to produce and present information on protein character based on fractionation by Osborne's method (albumines, globulines, prolamins, glutelins and NPN fraction);
- to extract and measure some components (soluble oligo and polysaccharides, phenoloids, flavonoids) with documented or probable biological effects;
- to control the mineral composition by measurements of twenty two elements.

These data can give a possibility for the complex and better evaluation of this rare and expensive truffle.

## Materials and methods

### Samples

*Tuber aestivum* (summer truffle) samples were gathered from six different habitats of Hungary (sample No. 1: Mountain Mecsek / South Hungary; No. 2: Tápiógyörgye / Hungarian lowland; No. 3: Mt. Bükk / Bükkszentkereszt, North Hungary; No. 4: Szőlösardó / North Hungary; No. 5: Aggtelek / North Hungary; No. 6: Mt. Bükk / Szilvásvárad) in years 2007-2008. The mushrooms were transported for Bot. Dep. of Fac. Vet. Sci., Szent István University (Budapest, Hungary), and were thoroughly cleaned, sliced, carefully dried (at

40 C°) and pulverized. The voucher samples were deposited in the laboratory of Bot. Dep. All analyses were performed from these mushroom powders in triplicate.

**Chemical analyses**

Crude protein, -fiber, -fat and -ash contents were determined according to AOAC official methods (PALAZZOLO et al., 2011), carbohydrate content and the energy level were calculated from the aforementioned data (MATTILA et al., 2002). Determination of chitin content was conducted according to our earlier method (VETTER, 2007), glucose-amine molecules of hydrolyzed samples (20-20 mg) were measured spectrophotometrically in a reaction with 3-methyl-2-benzothiazolone-hydrazone-hydrochloride (MBTH). Fractionation of proteins was carried out according to classical Osborne method, modified and described by Petrovska (PETROVSKA, 2001). Soluble oligo- and polysaccharide fractions were produced by methanol: water (80:20) extraction (for oligosaccharides) and by the following boiling water extraction (for polysaccharides) and were evaluated with anthrone reaction (SALTARELLI et al., 2008). Molecules with phenolic character were extracted with ethanol (80 %) and determined with Folin reagent (DUBOST et al., 2007). Extraction for flavonoids was made in methanol; the concentrations were measured with $AlCl_3$ reagent (SARIKURCKU et al., 2008).

Statistical evaluation of the analytical data was performed by 'Origin 4.0' software.

## Results and discussion

Data on "classical" organic components of summer truffle are summarized in Tab. 1. The average crude protein content (19.11 %) seems to be a middle level among the mushrooms, it is lower than protein of *Agaricus* or *Boletus* species (KALAC, 2009) but significantly higher than those in some wood decaying species (*Laetiporus sulphureus*, *Ganoderma lucidum* etc.). Crude fat level (in average 2.27 % of DM) is low and something lower than fat contents in *Agaricus bisporus, Pleurotus ostreatus* or *Lentinula edodes* (MATTILA et al., 2002). Crude fiber content of summer truffle (22.03 % in average) seems to be higher than normally in common mushrooms (for example: in *Pleurotus* species 11 %: DEL TORO et al., 2006). Its high fiber level can be one of the characteristic chemical properties. Calculated total carbohydrate content (48.9 % DM) and total organic constituent level (92.44 % DM) indicate the dominance of organic components, the average crude ash content (7.55 %) represents the sum of different inorganic constituents. Calculated energy level of *Tuber aestivum* (1224 Kj/100 g DM) is low, similar to other foods of mushroom type (MATTILA et al., 2002).

Concentrations and percentage distributions of different protein fractions (extractions of which were conducted according to their solubility's) are presented in Tab. 2. First and main component is the albumin fraction (57.8 mg/g DM that corresponds to 41 % of total crude protein content) and logically seems to be the best protein category for consumers. Incidence of globulins is low (8.56 mg/g DM only) but the variability among the different samples is relatively important (lowest and highest values are 5.35 mg/g DM and 12.44 mg/DM, respectively). Albumins and globulins (i.e. the heavily utilizable protein types) give together about the half (~ 47 %) of crude protein content. Prolamines (and prolamine like substances) have only 3.22 and 2.47 mg/g DM contents, which fulfill for 2.27 % and 1.58 % of the crude protein content, respectively. Occurrence of glutelins is remarkably high (25.7 mg/g DM), content of glutelin like substances is low (2.09 mg/g DM). United concentration of these two fractions is 19.28 % of crude protein level. Last but not least: summarized contents of the six protein components present 70.02 % of crude protein. The difference between crude protein level and the sum of the protein fractions (practically the true proteins) is the NPN (non protein nitrogen) rate. Here this fraction is remarkable high, namely 29.98 %. Evaluation and comparison of our data can be conducted based on work of Petrovska (PETROVSKA, 2001) for fifty two basidiomycetous edible mushrooms. Summer truffle has significantly higher albumin, but lower globulin rates, occurrence of glutelins and of NPN fractions are similar than the average data originating from Petrovska's work.

Concentrations of some biologically active compounds are given in Tab. 3. Soluble saccharides (including oligo- and polysaccharides) occur in summer truffle in numerically low concentrations (in average 9.0 mg/g DM for oligo and 49.92 mg/g DM for polysaccharides). The total soluble saccharide fraction (in average 58.92 mg/g DM) is composed mainly of polysaccharides (83.18 % : 16.82 %). Our data can confirm results of Saltarelli (SALTARELLI et al., 2008) i.e. soluble saccharide-content are the highest among different *Tuber* species (*T. magnatum, T. borchii or T. melanosporum*).

Total phenolic and flavonoid contents of *T. aestivum* samples are 2.8 mg/g DM and 0.093 mg/g DM, respectively. Quantity of phenolics of *T. aestivum* seems to be markedly lower than in *Agaricus bisporus* (DUBOST et al., 2007; ELMASTAS et al., 2007) and is slightly lower than those in *Pleurotus ostreatus* or *Lentinula edodes* (DUBOST et al., 2007). Total flavonoid concentration (0.093 mg/g DM) is absolutely lower than those in a desert truffle species (in *Tirmenia*: AL-LAITH, 2010) but similar to these values of *Lentinula edodes* or *Pleurotus* species (YANG et al., 2002).

**Tab. 1:** Different macrocomponents (crude protein, -fat, -fiber, ash, chitin, carbohydrate contents in % of DM, and energy level in Kj/100 g) of *Tuber aestivum* (mean ±SD)

| Samples | Crude protein (% DM) | Crude fat (% DM) | Crude fibre (% DM) | Crude ash (% DM) | Chitin (% DM) | Carbohydrate* (% DM) | Organic constituents* (% DM) | Energy* (Kj/100 g) |
|---|---|---|---|---|---|---|---|---|
| ***Tuber aestivum* 1.** | 20.27±0.08 | 2.64±0.16 | 20.75±0.17 | 8.80±0.03 | 7.51±0.60 | 47.54 | 91.20 | 1231 |
| ***Tuber aestivum* 2.** | 17.86±0.07 | 2.91±0.07 | 22.27±0.8 | 8.03±0.02 | 6.66±0.17 | 48.93 | 91.97 | 1227 |
| ***Tuber aestivum* 3.** | 19.69±0.03 | 2.35±0.01 | 21.62±1.17 | 7.22±0.01 | 12.19±0.35 | 49.12 | 92.78 | 1240 |
| ***Tuber aestivum* 4.** | 19.59±0.23 | 2.01±0.02 | 24.33±0.70 | 7.07±0.08 | 14.07±0.16 | 47.00 | 92.93 | 1189 |
| ***Tuber aestivum* 5.** | 20.10±0.14 | 2.83±0.01 | 23.23±1.17 | 7.08±0.02 | 15.17±0.23 | 46.46 | 92.92 | 1219 |
| ***Tuber aestivum* 6.** | 17.20±0.15 | 0.93±0.08 | 20.03±1.34 | 7.11±0.01 | 8.23±0.28 | 54.73 | 92.89 | 1240 |
| ***Tuber aestivum*, average** | 19.11±1.27 | 2.27±0.73 | 22.03±1.58 | 7.55±0.71 | 10.63±3.6 | 48.9±3.0 | 92.44±0.71 | 1224±19 |

* calculated values

**Tab. 2:** Different protein fractions of *Tuber aestivum* (mg/g DM) (mean ±SD and in percent of crude protein content (%))

| | Protein fractions (mg/g DM) | | | | | |
|---|---|---|---|---|---|---|
| **Sample** | **Albumins** | **Globulins** | **Prolamines** | **Prolamine-like substances** | **Glutelines** | **Gluteline-like substances** |
| ***Tuber aestivum* 1.** (%) | 61.05±0.59<br>36.9 | 12.44±2.15<br>7.5 | 4.65±0.80<br>2.79 | 4.03±0.69<br>2.40 | 14.22±2.46<br>8.58 | 3.57±0.52<br>1.81 |
| ***Tuber aestivum* 2.** (%) | 47.7±9.16<br>32.98 | 9.32±1.79<br>6.44 | 4.06±0.78<br>2.80 | 2.76±0.53<br>1.91 | 33.70±6.47<br>23.3 | 2.34±0.45<br>1.62 |
| ***Tuber aestivum* 3.** (%) | 61.41±15.20<br>46.8 | 8.60±2.12<br>6.52 | 3.15±0.87<br>2.67 | 2.22±0.55<br>1.69 | 21.45±5.31<br>16.3 | 3.14±0.77<br>2.37 |
| ***Tuber aestivum* 4.** (%) | 62.54±17.39<br>47.78 | 5.35±1.48<br>4.06 | 2.73±0.76<br>2.08 | 2.08±0.22<br>0.60 | 25.80±7.17<br>19.75 | 1.47±0.41<br>1.12 |
| ***Tuber aestivum* 5.** (%) | 62.82±18.10<br>48.06 | 6.93±1.99<br>5.28 | 2.60±0.75<br>1.99 | 2.01±0.56<br>1.48 | 24.14±6.95<br>18.45 | 1.56±0.45<br>1.19 |
| ***Tuber aestivum* 6.** (%) | 51.48±12.49<br>33.36 | 8.77±2.12<br>5.66 | 2.14±0.50<br>1.33 | 1.72±0.42<br>1.12 | 35.0±8.49<br>22.68 | 0.49±0.11<br>0.29 |
| ***Tuber aestivum*, average** | 57.8±6.53 | 8.56±2.39 | 3.22±0.95 | 2.47±0.84 | 25.7±7.78 | 2.09±1.28 |

**Tab. 3:** Soluble oligo- and polysaccharides, total phenoloid and flavonoid contents of *Tuber aestivum* (mg/g DM, mean ±SD)

| **Sample** | **Soluble oligosaccharides (mg/g DM)** | **Soluble polysaccharides (mg/g DM)** | **Phenolic content (mg/g DM)** | **Flavonoid content (mg/g DM)** |
|---|---|---|---|---|
| ***Tuber aestivum* 1.** | 8.98±0.89 | 29.88±2.10 | 2.20±0.12 | 0.092±0.006 |
| ***Tuber aestivum* 2.** | 9.08±0.86 | 43.21±1.02 | 2.37±0.08 | 0.094±0.006 |
| ***Tuber aestivum* 3.** | 8.16±0.33 | 32.41±3.77 | 2.64±0.16 | 0.097±0.004 |
| ***Tuber aestivum* 4.** | 8.97±0.16 | 37.32±0.92 | 2.78±0.09 | 0.098±0.003 |
| ***Tuber aestivum* 5.** | 9.30±0.41 | 43.73±0.77 | 3.04±0.11 | 0.109±0.012 |
| ***Tuber aestivum* 6.** | 9.52±0.48 | 107.1±4.12 | 3.77±0.16 | 0.071±0.003 |
| ***Tuber aestivum*, average** | 9.00±0.46 | 49.92±29.0 | 2.8±0.56 | 0.093±0.012 |

Twenty two elements were determined from the inorganic constituents (Tab. 4). The presented mineral composition is similar to the majority of mushrooms (Kalac, 2009; Kalac and Svoboda, 2000; Vetter, 2005; Vetter et al., 2005). First (and main) mineral element is potassium (25647 mg/kg DM), second is the phosphorus (7879 mg/kg DM), third one is the calcium (3331 mg/kg DM). Content of magnesium is about 1000 mg/kg DM; all other elements have lower concentration. Some "microelements" as iron (230 mg/kg DM), zinc (160.5 mg/kg DM), copper (49.3 mg/kg DM) and manganese (17.4 mg/kg DM) have lower contents, but these elements can be important for the metabolism of the consumer. Level of sodium is low (148.2 mg/kg DM), but this tendency is similar to properties of other mushrooms (Vetter, 2003). Concentrations of some other elements (B, Ba, Sr and Ti) are under 20 mg/kg DM level. Considering the poisonous (or known as poisonous) elements: incidence of these (As, Se or V) are under the detectable limits or they have detectable, but low quantities (for Cd 4.11, for Cr: 1.29 mg/kg DM values) i.e. the normal use of truffle has no toxicological risk for us. Interesting question is the aluminum level of *T. aestivum*, the found concentration for total content (166.7mg/kg DM) is relatively high, but we have no information on the form(s) of aluminum in the fruit body, therefore the toxicological importance of this content is doubtful. Mineral spectrum of summer truffle's fruit bodies is characteristic: four elements (K, P, Ca and Mg) give 97.85%, while the all other 18 elements have 2.06% of total composition, only (Fig. 1).

Summer truffle (*T. aestivum*) is not only a historical, rare and expensive mycorrhizal mushroom species, but is has characteristic and specific chemical composition. We analyzed different constituents of organic and inorganic characters (excluding the fragrant molecules). Six mushroom samples were analyzed for the chemical components independently. Average values of certain chemical parameters were used for general characterization. Summer truffle can be characterized by middle crude protein, low crude fat and high crude fiber contents. Last trait seems to be one of the chemical specificities of this mushroom, in general the other (asco- and basidiomycetous) higher mushroom species have lower fiber contents (Kalac, 2009). Chitin level of *T. aestivum* is high, higher than the average of other common cultivated mushrooms (Vetter, 2007). Crude fat level (about 2 % of DM) is absolutely similar to values of other mushroom species. Occurrence and distribution of protein fractions (based on "classical" fractionation according to Osborne's concepts) is characteristic: albumins and globulins represent together about the half of crude proteins, glutelins and glutelin-like substances give together about 20 % of crude protein content and the non protein (NPN) fraction is important (about 30 %). Protein distribution presented here seems to be characteristic for *T. aestivum*, and it is different from the common basidiomycetous mushrooms, as well as from some proteins of plant origin (Petrovska, 2001).

Different substances of biological activity are presented in mushrooms consequently oligo- and polysaccharides of different

**Tab. 4:** Inorganic constituents of summer truffle fruit bodies (mg/kg DM), mean ±SD

| | ***Tuber aestivum* 1** | ***Tuber aestivum* 2** | ***Tuber aestivum* 3** | ***Tuber aestivum* 4** | ***Tuber aestivum* 5** | ***Tuber aestivum* 6** | ***Tuber aestivum* average** |
|---|---|---|---|---|---|---|---|
| **Al** | 311.3±13.6 | 140.4±13 | 133.9±6.6 | 139.7±17.7 | 164.8±0.35 | 111±8.96 | 166.7±72 |
| **As** | <d.l.* | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. |
| **B** | 7.19±0.57 | 17.22±1.32 | 2.43±0.03 | 2.68±0.54 | 23.76±0.49 | 61.91±4.0 | 19.2±22 |
| **Ba** | 8.89±0.58 | 5.99±0.62 | 6.38±0.11 | 11.32±0.84 | 14.79±0.11 | 3.56±0.2 | 8.48±0.07 |
| **Ca** | 3436±170 | 3535±348 | 3256±87 | 3890±336 | 4547±322 | 1324±70.4 | 3331±1080 |
| **Cd** | 2.80±0.13 | 1.72±0.06 | 10.48±0.20 | 2.94±0.17 | 4.58±0.12 | 2.18±0.13 | 4.11±3.26 |
| **Co** | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. |
| **Cr** | 1.87±0.30 | 1.51±0.13 | 1.16±0.35 | 0.97±0.22 | 0.95±0.06 | 1.31±0.07 | 1.29±0.35 |
| **Cu** | 43.8±3.37 | 46.6±3.56 | 50.4±0.6 | 38.6±1.1 | 74.69±0.83 | 41.7±2.79 | 49.3±13.1 |
| **Fe** | 344.7±6.8 | 168.1±13.9 | 158.3±4.3 | 153.6±13.5 | 419.2±1.6 | 138.4±13.4 | 230.3±120.1 |
| **K** | 28956±801 | 28113±1837 | 24944±108 | 23620±995 | 23321±141 | 24932±1656 | 25647±2347 |
| **Mg** | 1352±48.8 | 1181±97.5 | 992±9.8 | 964±51.9 | 887.8±11.4 | 985.3±80.1 | 1060±173 |
| **Mn** | 17.35±1.04 | 11.47±0.988 | 17.64±0.13 | 12.81±1.0 | 33.47±0.79 | 11.92±0.84 | 17.4±8.30 |
| **Mo** | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. |
| **Na** | 193.3±11.9 | 127.3±17 | 145.7±3.0 | 115.4±3.5 | 201.9±8.5 | 105.7±5.11 | 148.2±40.6 |
| **Ni** | 2.16±0.56 | 1.20±0.09 | 1.54±0.89 | 1.91±0.61 | 2.12±0.47 | 1.15±0.09 | 1.68±0.44 |
| **P** | 8192±265 | 7203±554 | 7963±100 | 7366±245 | 8714±31.7 | 7837±516 | 7879±552 |
| **Se** | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. |
| **Sr** | 11.79±0.91 | 12.99±0.40 | 7.56±0.23 | 9.69±0.80 | 13.26±0.70 | 8.23±0.76 | 10.58±2.44 |
| **Ti** | 12.2±0.19 | 3.30±0.28 | 3.05±0.04 | 2,49±0.30 | 4.30±0.08 | 2.52±0.21 | 4.64±3.76 |
| **V** | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. | <d.l. |
| **Zn** | 156.3±6.85 | 131.4±2.12 | 173.6±2.8 | 155.8±6.5 | 222.6±3.05 | 123.5±0.27 | 160.5±35.4 |

*<d.l. : under detection's limit

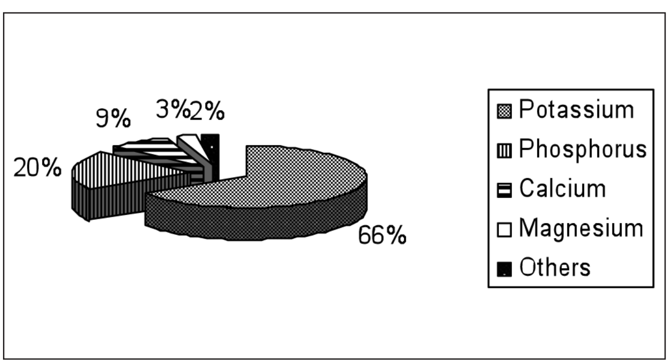


**Fig. 1:** Percentage distribution of the mineral elements in summer truffle (*T. aestivum*) fruit bodies.

solubility. Such water soluble molecules have a probable essential role in health promoting (anti carcinogen, immune stimulating, anti viral etc.) effects of mushrooms. According to the found distribution, the main soluble saccharide component is the polysaccharide fraction (83 %), which fact supports earlier results of Saltarelli (SALTARELLI et al., 2008). Phenolic derivatives represent important group of biologically active molecules, although total their concentration in truffle is lower than those in most common mushroom species (*Agaricus, Pleurotus* etc.).
Mineral spectrum of truffle fruit bodies is similar to composition of other edible mushrooms, i.e. they have high potassium and phosphorus contents, lower but important level from calcium, magnesium and some microelements. Accumulation (occurrence) of the problematic, "poisonous" elements (as Cd, As, Cr or V) was not established in truffle fruit bodies.

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