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Chemical composition and nutritional value of a wild edible ectomycorrhizal mushroom, *Tricholoma anatolicum*

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Abstract: The chemical composition and nutritional value of a wild edible ectomycorrhizal mushroom from southwestern Anatolia, *Tricholoma anatolicum*, were analyzed. Moisture, crude oil, protein, ash, total carbohydrate content, and mineral composition of the mushrooms studied, including Fe, Na, K, Zn, Cu, Ca, Cd, and Pb, were determined. The energy values of the samples were also calculated. The analyses were conducted during the 3 different growing stages of the mushrooms: mycelium, young fruiting bodies, and mature fruiting bodies. The highest values for moisture and crude oil contents were found to be in the mycelium, ash, and carbohydrate content of young fruiting bodies. In addition, Na content was found to be the highest in mycelium. The highest values for Fe were found in the young fruiting bodies, and K, Zn, Cu, and Ca were at their highest values in mature bodies. None of the samples contained heavy metals Cd or Pb.

Key words: Chemical composition, ectomycorrhizal mushroom, *Tricholoma anatolicum*

Yenilebilir yabani ektomikorizal bir makrofungus olan *Tricholoma anatolicum*'ün kimyasal kompozisyonu ve besinsel değeri

Özet: Bu çalışmada, güneybatı Anadolu'dan toplanan ve yenilebilir yabani bir ektomikorizal makrofungus olan *Tricholoma anatolicum*'ün kimyasal kompozisyonu ve besin değeri analiz edilmiştir. Makrofungusun nem, yağ, protein, kül, toplam karbohidrat içeriği ve mineral kompozisyonu (Fe, Na, K, Zn, Cu, Ca, Cd, Pb) açığa çıkarılmıştır. Mantar örneklerinin enerji değerleri de ayrıca hesaplanmıştır. Analizler makrofungusun üç farklı gelişim evresine (misel, genç şapka ve olgun şapka) ayrı ayrı uygulanmıştır. En yüksek nem ve yağ içerikleri miselde, kül ve karbohidrat içeriği ise genç şapkada tespit edilmiştir. Bunların yanısıra, sodyum miktarı en yüksek olarak miselde ölçülmüştür. Demir en yüksek genç şapkada, çinko, bakır, potasyum ve kalsiyum ise en yüksek miktarlarda olgun şapkada bulunmuştur. Hiçbir örnekte kadmiyum ve kurşun ağır metallerine rastlanmamıştır.

Anahtar sözcükler: Kimyasal kompozisyon, ektomikorizal makrofungus, *Tricholoma anatolicum*

Introduction

More than 2000 species of mushrooms exist in nature, but fewer than 25 species are accepted as food and only a few of them (*Agaricus bisporus*, *Pleurotus* spp., *Lentinula edodes*, *Volvariella volvacea*, etc.) have attained the level of an item of commerce (1). Wild edible ectomycorrhizal mushrooms, however, are seasonally consumed by certain groups of people, such as local residents of mushroom growing areas, enthusiasts, and gourmets. Wild mushrooms are becoming more and more important in our diet for their nutritional, organoleptic, and pharmacological characteristics (2,3).

The edible ectomycorrhizal mushrooms *Tricholoma anatolicum* and *Tricholoma caligatum* are the mushrooms most frequently harvested in southwestern Anatolia, Turkey. *T. anatolicum*, which is an endemic mushroom species in Turkey, is of great economic importance to local communities in western Turkey (4). This mushroom, mycorrhized with *Cedrus libani*, is collected in cedar forests in autumn by villagers and exported to Japan. The popularity of this organism is mainly due to its sensory qualities, in particular the aroma, taste, and rigid texture of the mushroom cap (5).

Knowledge of the nutritional value and chemical structure of wild mushrooms has been limited in comparison with other plants. Several studies have been carried out on the chemical composition and nutritional quality of different species of *Tricholoma* mushrooms. The chemical composition of *T. auratum*, *T. batschii*, *T. equestre*, *T. flavovirens*, *T. matsutake*, *T. nudum*, *T. portentosum*, *T. terreum*, *T. stans*, and *T. ustale* have been determined by several authors (6-12). Nutritional values such as protein, fatty acids, carbohydrate, and ash for *T. auratum*, *T. giganteum*, *T. magnivelare*, *T. nudum*, *T. portentosum*, *T. terreum*, and *T. ustale* (1,13,14) and functional properties of *T. giganteum*, *T. acerbum*, *T. portentosum*, and *T. terreum* (2,6,15) were also reported in some studies.

Despite these findings and the economic importance of *T. anatolicum*, to date no information is available about the chemical composition of this organism. In this study, the chemical composition of this ectomycorrhizal mushroom was investigated in order to assess its nutritive potential for the first time. Moreover, the results of chemical analysis might be valuable for chemotaxonomical and cultivation purposes.

Materials and methods

Tricholoma anatolicum was described by Intini et al. (16) as a new species, and the chemical composition of this new species was first analyzed during different developmental stages. *T. anatolicum* caps were collected by villagers in the cedar forests of southwestern Turkey. All collected samples were sorted to reject unsound or bruised materials and cleaned of remaining mycelium and forest cover. After this pretreatment, all samples were kept in an icebox and transferred to the laboratory immediately.

Collected fruit bodies were arranged according to their morphological features, i.e. while young fruit bodies of this macrofungus are covered with a tissue-like partial veil, mature samples do not have a partial veil because of the open cap. For purposes of description, morphological features of the mushroom were common and highly characteristic. For this reason, molecular methods were not used in this study.

T. anatolicum strain MCC11 (Mushroom Culture Collection, Ege University, Department of Bioengineering, Turkey) was cultured on solid Hagem medium [4 g malt extract, 1 g yeast extract, 5 g D-glucose, 0.5 g NH₄Cl, 0.5 g KH₂PO₄, 0.5 g MgSO₄·7H₂O, 0.5 mL FeCl₃ (1% aqueous solution)], 100 mL biotin (50 mg/mL aqueous solution), and 100 mL thiamine (1 mg/mL aqueous solution) in 1 L of double distilled H₂O (17). Liquid media were sealed with cotton plugs and autoclaved at 121 °C for 15 min, and then 3 mycelia plugs (diameter of 6 mm) were transferred to 500-mL Erlenmeyer flasks containing 200 mL of liquid Hagem medium as inoculum. They were kept in a static culture in an incubator at 25 ± 1 °C for at least 75 days. During the incubation period, the static culture was shaken gently once a day to prevent fungal mat formation and anaerobic conditions. After the long incubation period, the mycelium was separated from the cultural liquid by filtration (18).

Mycelia were analyzed after 90 days of inoculation. Both the mycelium and fruiting bodies were analyzed for moisture, crude oil, protein, ash, and mineral content.

Total dry matter, protein, crude oil, and ash content were determined according to AOAC

procedures on a dry basis (19). Analysis of total dry matter content was carried out at 65 °C using a vacuum oven, analysis of protein content was done according to the Kjeldahl method with a conversion factor of 6.25 (20), analysis of crude oil content was by Soxhlet extraction with hexane, and ash content was analyzed by incineration of samples at 550 °C. Total carbohydrates were calculated as the residual difference after subtracting protein, ash, and crude oil content on a dry weight basis of mushroom samples. The total energy of 100 g of mushroom samples was calculated according to the following equations (21) on the basis of edible weight.

Energy, kcal = 4 (protein content, g + carbohydrate content, g) + 9 (crude oil content, g)

Energy, kj = 17 (protein content, g + carbohydrate content, g) + 37 (crude oil content, g)

Mineral contents of the samples, including Na, K, Ca, Fe, Zn, Cu, P, Cd, and Pb, were determined by atomic absorption spectrophotometer (22). All plastic and glassware was cleaned by soaking it in 10% nitric acid solution overnight and rinsing it with distilled water prior to use. The element standard solutions and HNO₃ used for digestion were supplied by Merck. A PerkinElmer Analyst 800 atomic absorption spectrometer with a deuterium background corrector was used. The operating parameters for working elements were set as recommended by the manufacturer. Lead and cadmium were determined by HGA graphite furnace using argon as an inert gas. The temperature program for the determination of lead and cadmium was as follows: first drying at

110 °C, second drying at 130 °C, pyrolysis at 450 °C, atomization at 2400 °C, and cleaning-out at 2500 °C. The other elements, namely iron, copper, zinc, calcium, potassium, and sodium, were determined by flame technique. After determination of the ash content of the samples by oven method, ashes of the samples were dissolved in 100 mL of nitric acid and kept in a refrigerator until analysis. The element standard solutions were prepared by diluting stock solutions of 1000 ppm of sodium, cadmium, iron, zinc, copper, lead, potassium, and calcium. The ratio of standard solutions prepared for each element was different.

All determinations were carried out in triplicate. The data were analyzed by Duncan's test with the statistical program MINITAB, Release 13.20.

Results and discussion

The moisture, protein, crude oil, ash, and total carbohydrate contents of samples are given in Table 1. The total dry matter content of the mushrooms decreased during growth or maturation. The moisture content of the mature fruiting body samples was 11.03%. *T. portentosum* and *T. giganteum* contained a greater amount of water, at 93.05% and 86.01%, respectively (1,13). Some mushroom species, namely *Dictyophora indusiata* and *Hericium erinaceus*, had low moisture contents as compared to our findings (13).

The protein content of the mycelium was higher than that of the fruiting bodies in this study. It is

Table 1. Proximate composition on a dry basis and energy contribution of mycelia and fruiting bodies of mushroom *Tricholoma anatolicum*.

	Mycelium	Young fruiting body	Mature fruiting body
Moisture (%)	33.69 ± 2.51 ^a	14.75 ± 0.16 ^b	11.03 ± 0.16 ^c
Ash (%)	0.26 ± 0.06 ^a	2.67 ± 0.12 ^b	1.30 ± 0.08 ^c
Protein (%)	97.02 ± 0.52 ^a	90.24 ± 0.55 ^b	93.95 ± 0.42 ^c
Crude oil (%)	0.97 ± 0.05 ^a	0.56 ± 0.03 ^b	0.53 ± 0.17 ^b
Total carbohydrate (%)	1.74 ± 0.56 ^a	6.71 ± 0.58 ^b	4.40 ± 0.54 ^c
Energy (kcal)	403.8 ± 0.6 ^a	391.2 ± 1.6 ^b	396.6 ± 2.0 ^c
Energy (kJ)	1714.9 ± 2.0 ^a	1668.9 ± 6.4 ^b	1691.6 ± 4.6 ^c

^{a,b,c}: On the same line, mean values followed by different letters are significantly different (P < 0.05).

known that the protein content of mushrooms is affected by a number of factors. The development stage of the mushrooms is a significant factor affecting the protein content. In addition, the type of mushroom, the part sampled, and the location also affect protein content (1). Although changes in the protein content of the fruiting body during development are not yet understood, it was observed that the protein content and its digestibility decreased during the development of oyster mushroom *Pleurotus ostreatus* (23).

Diez and Alvarez (2) reported that *T. portentosum* and *T. terreum* contained 19.6% and 20.1% crude protein, respectively. As a result of amino acid analysis, real protein contents were reported as 15.6% and 15.4% for those samples, respectively. In a departure from the methods used in the present study, they calculated the protein content by multiplying the nitrogen content by the factor of 6.25. Barros et al. (1) reported that *T. portentosum* contained 2.12% crude protein on a fresh basis or 30.5% on a dry basis, based on a conversion factor of 4.38. Yildiz et al. (12) reported a higher amount of protein for some *Tricholoma* species: 29.7% for *T. auratum*, 31.1% for *T. nudum*, 46.5% for *T. terreum*, and 50.5% for *T. ustale*, using the conversion factor of 6.25. In order to calculate the protein content from nitrogen content, different conversion factors, including 4.18, 4.38, or 6.25, were used by researchers (23). Therefore, some differences may occur among the results.

In spite of their very high amount of protein, the samples were poor in terms of carbohydrate content. The carbohydrate content of *T. terreum*, *T. portentosum* (2), and *T. portentosum* (1) were reported as 31.1%, 34.6%, and 52.3% on a dry basis, respectively. On the other hand, *T. giganteum* contained a greater amount of carbohydrate (70.1%) than the above species. The lower carbohydrate level may not affect energy contribution due to the compensation from higher protein values in all 3 samples. As shown in Table 1, the carbohydrate contents determined in this study were higher in fruiting bodies than in the mycelium.

It was found that the crude oil content decreased during different developmental stages of the organism, namely the mycelium, young fruiting body, and mature fruiting body. However, there is no rational explanation for the mechanism that produces

this result. While crude oil and protein content were at a maximum level in the mycelium, the ash content and total carbohydrate level were the highest in the young fruiting body. Barros et al. (1) reported that *T. portentosum* contained 0.38% total fat on a fresh basis.

The highest energy contribution was found in the mycelium because it has a relatively higher amount of crude oil and protein content than the fruiting bodies. Barros et al. (1) reported that 100 g of fresh *T. portentosum* gave 26.46 kcal or 111.98 kJ of energy.

The ash content of the fruit bodies was higher than that of the mycelium ($P < 0.05$). Barros et al. (1) reported that *T. portentosum* contained 0.81% and 11.7% ash on a fresh and dry basis, respectively.

As shown in Table 2, the sodium content of the mycelia was found to be much higher than that of the fruiting bodies ($P < 0.05$). The sodium content of *T. terreum* was reported as 92.6-325 mg/kg on a dry basis (8,24). Dursun et al. (9) reported 6773 mg/kg of sodium in *T. terreum* dried at 45 °C for 2 days.

The mycelia contained the maximum level of calcium, 88.13 mg/kg, as compared to the content of the fruiting bodies (Table 2). Demirbaş (8) reported that *T. terreum* contained 68.5 mg/kg of calcium on a dry basis. On the other hand, in the same species dried at 45 °C for 2 days, the calcium level was found to be 4526 mg/kg (9).

Mycelia and young fruiting bodies contained a higher amount of iron than mature fruiting bodies (Table 2). Several researchers studied the iron content of *T. terreum* and reported that this species contains 33.5-632 mg/kg iron (8,11,25). On the other hand, in the same species dried at 45 °C for 2 days, the iron content was reported as 4235 mg/kg (9).

The potassium content increased from 40.6 to 58.4 mg/kg during the development of the mushroom. Edible wild mushroom species have an average potassium content of 34,350 mg/kg on a dry basis, making them an important and valuable potassium source for the human diet. The potassium concentration of mushrooms is relatively constant. An accumulation of potassium in the mushroom samples analyzed was not found (26).

Table 2. Mineral composition of mycelium and body of *Tricholoma anatolicum* on a dry basis (mg/kg).

Minerals	Mycelium	Young body	Mature body
Fe	73.4 ± 5.91 ^a	77.0 ± 4.08 ^a	68.4 ± 0.82 ^b
Na	127.7 ± 6.5 ^a	69.0 ± 2.48 ^b	79.3 ± 0.69 ^c
K	40.6 ± 3.87 ^a	53.2 ± 3.04 ^b	58.3 ± 3.91 ^b
Zn	66.4 ± 0.59 ^a	65.41 ± 0.61 ^a	61.4 ± 0.97 ^b
Cu	38.0 ± 0.17 ^a	38.88 ± 1.34 ^a	46.68 ± 2.77 ^b
Ca	88.13 ± 1.39 ^a	77.86 ± 3.44 ^b	81.86 ± 2.35 ^b
Cd	Nd	Nd	Nd
Pb	Nd	Nd	Nd

^{a,b,c}: On the same line, mean values followed by different letters are significantly different ($P < 0.05$); Nd: not detected.

The zinc content of the mycelia and young fruiting bodies was found to be lower than the content of the mature fruiting bodies. Kalac (23) reported that the fruiting bodies of some wild mushroom growing in unpolluted regions in Europe contained 30-150 mg/kg zinc on a dry basis. Falandysz et al. (27) found that the cap of the parasol mushroom in Poland contained higher amounts of zinc than the stalk.

The copper content of macrofungi is higher than that of green plants. For example, Hungarian fungi samples contained 44-48 mg/kg copper (8). Kalac and Svoboda (28) reported that copper levels in the accumulating species are usually 100-300 mg/kg on a dry basis, which is not considered to be a health risk. Falandysz et al. (27) reported that the copper content of the cap and stalk of parasol mushrooms was not different.

Cadmium and lead are known as principal toxic elements since they inhibit many vital processes. They can be taken up directly from water and, to some extent, from air and dietary food. These elements also have a tendency to accumulate in both plants and animals (8). No heavy metals (Cd or Pb) were detected in any of the mycelia or young and mature fruiting bodies studied. Therefore, the samples used in this study were acceptable according to FAO/WHO (29) standards for Cd and Pb levels. Tüzen et al. (25) reported Pb and Cd levels in *Tricholoma terreum* as 0.697 and 0.785 mg/kg, respectively. Heavy metals, including Cd, Pb, and Hg, were also

found to be higher in the cap than in the stalk of parasol mushrooms (26).

According to FAO/WHO (30) standards, acceptable intakes of Cd and Pb for an adult are 0.42-0.49 and 1.5-1.75 mg/week, respectively. The trace element concentrations in mushrooms are generally species-dependent (28) and are hardly affected by the pH or organic matter content of the soil (31). Falandysz et al. (27) reported that Cd and Pb could be considered limiting metals in edible mushrooms. As shown in Table 2, mycelia or fruiting bodies contained Cd and Pb below detection limits and are thus marked as "Nd" ("not detected") in the Table. In other words, the Cd and Pb concentrations of the samples were below the tolerance limits established by the FAO/WHO.

The results showed that changes in nutritional value depend on the developmental stage of *T. anatolicum*. Although there are no prior published data on changes in nutritional value of *T. anatolicum*, de Andrade et al. (32) reported that the raw protein, ethereal extract, ashes, and raw fiber value of *Lentinula edodes* (Berk.) Pegler varies according to strain, processing after harvesting, development stage of the basidiomata, and type of substrate used. Ayodele and Okhuoya (33) performed a nutritional and phytochemical evaluation of cultivated *Psathyrella atroumbonata* Pegler at the immature and mature stages of the mushroom. They found that nutrient composition was dependent upon the

stage of development; nutrient content was at its maximum during the immature stage and decreased during further development. Alkaloids were detected in the mature fruit body, but not in the immature stage. However, saponins and tannins were present in both the immature and mature stages. Flavonoids and anthraquinones were absent in the mushroom. Apart from nutritional value, phenoloxidase activity (34), Ca²⁺/calmodulin-dependent protein kinase involvement (35), lignocellulolytic enzyme profiles (36), and transcriptional regulation of laccase and cellulase genes (37) were studied by previous researchers.

The ash and carbohydrate contents were highest in the young fruiting bodies of *T. anatolicum*. The proximate and mineral composition of the mycelia, young fruiting bodies, and mature fruiting bodies

were different. In the individualized study of the data, some surprising features were observed, such as the appearance of higher levels of Zn and Ca, which are very important for human health, as compared to Cu and K. The reason for these changes is unclear. Further investigation into the changes in the composition of mushrooms during maturation should be undertaken.

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