

Determination of fatty acid profile and mineral contents of *Tricholomopsis rutilans* collected from Yozgat

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Yozgat'tan toplanan *Tricholomopsis rutilans* örneklerinin yağ asidi profili ve mineral içeriğinin belirlenmesi

Abstract: This study was carried out to determine the content of some minerals and fatty acids in *Tricholomopsis rutilans* (Schaeff.) Singer samples. *Tricholomopsis rutilans* is a saprophytic mushroom growing on or around conifer stumps and characterized by white spore print, yellow gills, a large yellow cap and yellow stipe entirely covered with red to purplish red scale and fibrils. It is an edible mushroom, although the taste is not nice. The analysed mushroom samples, were collected from different localities of Yozgat province during field trips between 2012-2016 years. Mineral analysis were performed by ICP-MS, and fatty acids were tested by Gas Chromatography-Mass Spectrometry system (GC-MS). Seven different fatty acids (miristic, pentadecanoic, palmitic, palmitoleic, stearic, oleic, and linoleic acid) and six different minerals (Cu, Mn, Zn, Fe, Mg, Na) have been determined in mushroom samples. Oleic and linoleic acid were the major fatty acids with proportions 39.04% and 37.09%, respectively. Na and Mg were found to be the most abundant minerals with 126.895 mgkg⁻¹ and 754.605 mgkg⁻¹, respectively among the determined minerals.

Key words: *Tricholomopsis rutilans*, fatty acids, mineral content, ICP-MS, GC-MS

Özet: Bu çalışma *Tricholomopsis rutilans* (Schaeff.) Singer örneklerinde bazı mineral ve yağ asitlerinin içeriğini belirlemek amacıyla yapılmıştır. *Tricholomopsis rutilans* konifer kütüklerinin üzerinde veya çevresinde gelişen saprofit bir mantardır ve beyaz spor baskısı, sarı jiller ve üzerleri tamamiyle kırmızı-morumsu kırmızı pullar ve fibriller ile kaplı büyük sarı şapka ve sarı sapla karakterize edilir. Tadı güzel olmasa da, yenilebilir bir mantardır. Analiz edilen mantar örnekleri 2012-2016 yılları arasında yapılan gezilerde Yozgat ilinin farklı bölgelerinden toplanmıştır. Mineral analizleri ICP-MS ile yağ asitleri ise gaz kromatografi-kütle spektrometre sistemi (GC-MS) ile test edildi. Analiz sonuçlarına göre mantar örneklerinden yedi farklı yağ asidi (miristik, pentadekanoik, palmitik, palmitoleik, stearik, oleik ve linoleik asit) ve altı farklı mineral (Cu, Mn, Zn, Fe, Mg, Na) tespit edilmiştir. Oleik ve linoleik asit, 39.04% ve 37.09% oranlarla major yağ asitleri olarak belirlenmiştir. Tespit edilen mineraller arasında Na ve Mg'un, 126.895 mgkg⁻¹ ve 754.605 mgkg⁻¹lik oranlarla en bol bulunan mineral mineraller olduğu belirlenmiştir.

Anahtar Kelimeler: *Tricholomopsis rutilans*, yağ asitleri, mineral içeriği, ICP-MS, GC-MS

1. Introduction

The role of mushrooms in nutrition and complementary treatment methods is rapidly increasing. The mushrooms (especially belonging to ascomycota and basidiomycota) have been used for a long time because of their medicinal properties (antioxidant, antimicrobial, anticancer, immunostimulatory and anti-inflammatory activity etc.) in folk medicine of China, Japan and other countries from the Far East. It is believed from researchers that biomacromolecules (polysaccharides or polysaccharide-protein complexes etc.) produced by mushrooms can inhibit tumor growth. Also they can prevent carcinogenesis and tumour metastasis. In accordance with this information, it has been reported that polysaccharide extracts of *Tricholomopsis rutilans* have anticancerogenic, antioxidant and antiinflammatory effects (Hilszczańska, 2012; Rahi and Malik, 2016). Wild edible mushrooms are regarded as a healthy food source due to their high mineral, protein, fiber, unsaturated fatty acids and vitamins contents, and low-fat and calorie levels. They are an important option for vegetarian diets and people who want to feed with a protein rich diet (Orsine et al., 2012; Valverde et al., 2015).

Many studies have been made to investigate the chemical contents of mushrooms (Akyüz et al., 2011; Barros et al., 2008; Ergönül et al., 2012; Goyal et al., 2015; Bengü, 2019; Bengü et al., 2019). In these studies, saturated, monounsaturated and polyunsaturated fatty acids were determined in mushrooms. The results of the studies revealed that unsaturated fatty acids (UFA) are higher than saturated fatty acids. Accordingly, mushrooms are important nutrient sources for meeting the daily fatty acid needs of humans. The monounsaturated fatty acids (MUFAs) have attracted attentions in recent years due to their protective effects against heart diseases such as atherosclerosis. The nutritional value of fat in foods is determined by the amount of polyunsaturated fatty acids (PUFAs- especially linoleic and linolenic acid that are also called essential fatty acids) in their structure. Essential fatty acids (EFAs) are used in the synthesis of certain hormones, as well as preventing blood clotting and hypertension. They increases the blood circulation and contributes to the suitable distribution of cholesterol in human body (Kaur et al., 2012; Sokoła-Wysoczańska et al., 2018)

Many studies have shown in general that mushrooms are also important nutrients in terms of some major elements

(potassium, phosphorus, calcium, sodium, magnesium etc.) and some trace elements (iron, zinc, copper, manganese, selenium etc.) too (Adejumo and Awosanya, 2005; Bernaś et al., 2006; Mallikarjuna et al., 2013; Mironczuk-Chodakowska et al., 2013). Trace /micro elements are very important for human body and other biological systems. Zinc (Zn) is involved as a cofactor in the structure of approximately 300 enzymes in energy production and metabolism of carbohydrates, proteins and lipids. Manganese (Mn), as a cofactor of various enzymes involved in metabolic processes and manganese superoxide dismutase, is a trace element necessary for the healthy development and growth of the organism. It is essential for bone development, and metabolism of amino acids, carbohydrates and cholesterol. Iron (Fe) is involved in the structure of many organic compounds that have important functions in our body such as myoglobin, hemoglobin, cytochromes. Magnesium (Mg) as major intracellular mineral is involved in many important structural, metabolic and physiological processes in biological systems such as the synthesis of proteins and nucleic acids, cell replication, to be cofactor for many enzymes, energy metabolism, complexing with ATP. Copper (Cu) is used in oxidation reduction processes and removing free radicals from biological systems. Copper is involved in the structure of some important enzymes such as lysyl oxidase, cytochrome c oxidase, superoxide dismutase. These enzymes are called as copper enzymes. Sodium (Na) as major element of extracellular fluid is involved at the formation of osmotic pressure of blood and other body fluids, transport of certain nutrients through the plasma membrane such as amino acids, glucose, galactose, and excitability of nerve and muscle cells (Seo and Park, 2008; Angelova et al., 2011; Zabłocka-Słowińska and Grajeta, 2012; Mallikarjuna et al., 2013; Gupta, 2014; Strazzullo and Leclercq, 2014; Pietrzak-Fiećko et al., 2016; Al-Fartusie and Mohssan, 2017).

This present study aims to reveal the chemical composition of *Tricholomopsis rutilans* in terms of some minerals and fatty acids.

2. Materials and Method

2.1. Collection and Identification of Mushroom Samples

The mushroom samples identified as *Tricholomopsis rutilans* were collected from different localities of Yozgat province during the field trips between 2012-2016. The mushroom samples, photographed in their natural habitat, were brought to the laboratory for further processing. Spore traces of samples were obtained in laboratory and collection numbers were given. The fresh samples were dried and put into polyethylene bags. Their microscopic properties were investigated with the help of some chemical reagents under a light microscope. Using the obtained morphological and ecological characteristics of the samples, they were identified with the help of existing literature such as Phillips (1981), Breitenbach and Kränzlin (1991) and Jordan (1995).

2.2. Fatty acid analysis

The mineral and fatty acid contents of mushrooms were analyzed at Bingol University Central Research Laboratory. Christie (1990) was followed in the preparation of methyl esters of fatty acids, after some

revisions. With some revision Hara and Radin (1978) was followed for lipid extraction. A gas chromatograph instrument with a FID and MS (GC-MS, Agilent 7890 GC/5970 MS Series-Santa Clara, CA, USA), and a high polarity capillary column (HP-88, 100 m × 0.25 mm, 0.20 um film (Part no: 112-88A7, Agilent, Santa Clara, CA, USA) was used for fatty acid analyzes. Helium was used as the carrier gas (helium at 1 mLmin⁻¹. at 120 °C). The injector temperature was set at 250 °C, and the detector temperature at 250 °C. The oven temperature was initially set at 120 °C for 2 min, and then raised to 250 °C at 5 °C min⁻¹. Because hold time is 16 minutes, total analysis is 45 minutes. The detector gas was air set at 350 mLmin⁻¹, and hydrogen gas was set at 35 mLmin⁻¹. The detector make up gas was nitrogen at 35 mLmin⁻¹. Other conditions; split ratio is 1/10, solvent delay time is 12 minutes, injection volume is 1 uL. Injection system with auto sampler was used.

2.3. Mineral analysis

All mineral analyzes were performed using the ICP-MS (PerkinElmer NexION 2000) instrument using the conditions in Table 1.

Table 1. ICP-MS conditions for mineral analysis

Parameter/Component	Description / Value
Nebulizer	MEINHARD® plus Glass Type C
Nebulizer flow	Optimized for < 2% oxides
Nebulizer gas flow rate	0,93 L/min
Spray Chamber	Glass cyclonic (baffled), 2 °C
Injector	2.0 mm i.d.
Deflector voltage	-12 V
Analog stage voltage	-1750 V
RF Power	1600 W
Rinse time	45 second
Dwell time	50 ms
Aerosol Dilution	Set to 2.5x
Sample Delivery Rate	350 µL/min
Discriminator threshold	26
Alternating current (AC) rod offset	-4
Cones	Ni
Replicates	3

The studies have been made in the form of three repetitions and were averaged. The data of mineral analysis were reported as mgkg⁻¹ and the results of fatty acids were reported as percentages.

3. Results

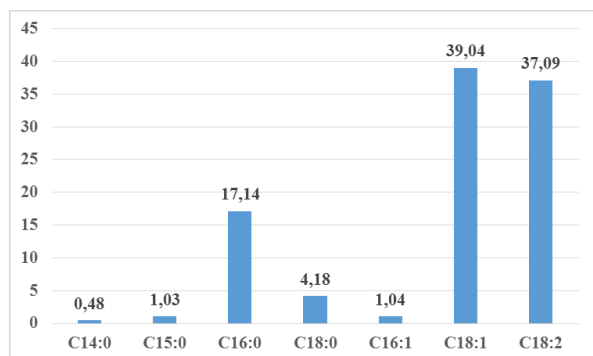
In the present study, fatty acids and mineral composition of *Tricholomopsis rutilans* were analyzed. The fatty acids in the structure of the mushroom samples were tested by GC-MS and different proportions of saturated fatty acids (SFA), MUFAs, and PUFAs were determined. The results for fatty acid composition were shown in Table 2 and Figure 1 as percentages.

In addition, the levels of total saturated, unsaturated, monounsaturated and polyunsaturated fatty acid in the tested samples are given in Table 3 and Figure 2.

Table 2. Fatty acid levels of *T. rutilans* (%)

Myristic acid (C14:0)	0.48
Pentadecanoic acid (C15:0)	1.03
Palmitic acid (C16:0)	17.14
Stearic acid (C18:0)	4.18
ΣSFAs	22.83
Palmitoleic acid (C16:1)	1.04
Oleic acid (C18:1)	39.04
Linoleic acid (C18:2)	37.09
ΣUFAs	77.17

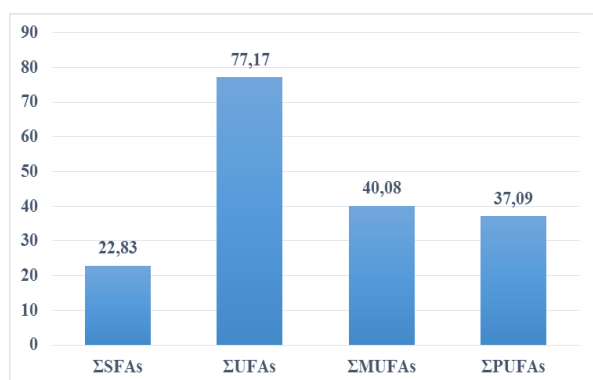
SFA: saturated fatty acid, UFA: unsaturated fatty acid

**Figure 1.** Fatty acid levels of *T. rutilans* (%)

The minerals in the structure of *T. rutilans* samples were analyzed by ICP-MS and six different minerals (Cu, Mn, Zn, Fe, Mg, Na) were determined as mgkg^{-1} at different amounts. The mineral contents in fruiting bodies of *T. rutilans* are shown in Table 4 and Figure 3.

Table 3. Proportion of saturated, unsaturated, monounsaturated and polyunsaturated fatty acids of *T. rutilans* (%)

Total saturated fatty acids (ΣSFAs)	22.83
Total unsaturated fatty acids (ΣUFAs)	77.17
Total monounsaturated fatty acids (ΣMUFAs)	40.08
Total polyunsaturated fatty acids (ΣPUFAs)	37.09

**Figure 2.** Proportion of saturated, unsaturated, monounsaturated and polyunsaturated fatty acids of *T. rutilans* (%)

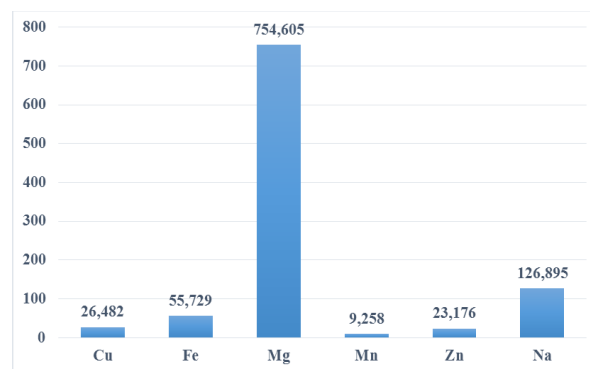
4. Discussions

According to the results, seven different fatty acids (four saturated fatty acids and three unsaturated fatty acids), with carbon chain lengths ranging from 14-18, have been detected in quantities ranging from 0.48% to 39.04% from

T. rutilans samples. Myristic, pentadecanoic, palmitic, stearic, palmitoleic, oleic and linoleic acid were found with proportions 0.48%, 1.03%, 17.14%, 4.18%, 1.04%, 39.04%, 37.09%, respectively (Table 2, Figure 1). Myristic acid was found to be the lowest amount of fatty acid in our samples with proportions 0.48%. Oleic acid was the major fatty acid with 39.04%. Palmitic acid and linoleic acid were the other fatty acids to be at higher amounts with proportions 17.14% and 37.09%, respectively. Linoleic acid which is a PUFA cannot be produced in the human body. So it is called essential fatty acids. ΣUFAs amounts was higher than SFAs amount in our samples (Table 3, Figure 2). Our analysis results are consistent with the similar studies carried out by Yılmaz et al. (2006), Ergönül et al. (2012), Goyal et al. (2015), Doğan (2016) and Bengü (2019). All of them reported the amount of UFAs to be higher than SFAs. Yılmaz et al. (2006) determined the dominant fatty acid as linoleic acid in seven different wild mushroom species. Likewise Ergönül et al. (2012), reported linoleic acid as major fatty acid in *Polyporus squamosus*, *Pleurotus ostreatus*, *Lactarius salmonicolor* and *Flammulina velutipes*, and oleic acid in *Russula anthracina* and *Boletus reticulatus* samples. Goyal et al. (2015) also determined the linoleic acid as the most abundant fatty acid in *Pleurotus sajor caju* and *Agaricus bisporus*. Doğan (2016) reported the fatty acid contents of *Terfezia boudieri* and *Lactarius vellereus*, and presented palmitic, stearic, oleic, linoleic acids as the main fatty acids, among which linoleic acid was found as the major fatty acid. The results of a chemical analysis of some mushroom samples, also yielded linoleic acid as the major fatty acid for *Suillus luteus* and *Coprinus atramentarius* samples while it was oleic acid for *Laetiporus sulphureus*. On the other hand Çınar Yılmaz and Bengü (2018) obtained different results from five different *Lactarius* species, than those discussed above. In their results the saturated fatty acid ratio was higher than the unsaturated fatty acid ratio. Also the dominant fatty acid was stearic acid (C18:0).

Table 4. Mineral content of fruiting bodies of *T. rutilans* (mgkg^{-1} dry weight)

Copper (Cu)	26.482
Iron (Fe)	55.729
Magnesium (Mg)	754.605
Manganese (Mn)	9.258
Zinc (Zn)	23.176
Sodium (Na)	126.895

**Figure 3.** Mineral content of fruiting bodies of *T. rutilans* (mgkg^{-1} dry weight)

Mushrooms can contain large amounts of both macro and micro elements, in their fruiting bodies, that people should take from foods. We have determined some macro elements (Na, Mg) and micro elements (Fe, Zn, Cu, Mn) in *T. rutilans* samples (Table 4 and Figure 3). We found Cu, Fe, Mn and Zn at lower amounts with the concentrations of 26.482, 55.729, 9.258, 23.176 mgkg⁻¹, respectively, and Mg and Na were at higher amounts with the concentration of 754.605 and 126.895 mgkg⁻¹, respectively. In our mushroom samples analyzed, the lowest mineral was measured as Mn with 9.258 mgkg⁻¹ while Mg was the highest with 754.605 mgkg⁻¹. Sesli and Tüzen (1999) reported the trace elements (Pb, Hg, Cd, Fe, Zn, Mn, Cu, As and Co), contents of two cultivated and 109 wild macrofungi specimens, and presented the Fe, Zn, Mn, Cu amounts in *T. rutilans* as 67.5, 28.5, 19.1 and 82.1 µg/g, respectively. Regarding these four minerals, the highest mineral was Cu, while Fe was measured as the highest one in our study. In a study carried out by Bengü (2019) on mineral values of *Suillus luteus*, *Laetiporus sulphureus* and *Coprinus atramentarius*, Zn, Fe, Cu, Mn were determined in different amounts. In this study, the highest and lowest values of Zn were found in *Coprinus atramentarius* and *Laetiporus sulphureus* samples with 288.4 mgkg⁻¹, 28.36 mgkg⁻¹ respectively. For the same mushrooms, the Fe values were 1183.6 mgkg⁻¹, 162.92 mgkg⁻¹ respectively, the Cu values were 57.12 mgkg⁻¹, 5.0 mgkg⁻¹ respectively, the Mn values were 64.2 mgkg⁻¹, 19.36 mgkg⁻¹ respectively. The highest values for these minerals were determined in *Coprinus atramentarius* samples. Kaya et al. (2017) determined the mineral

content of eleven wild edible mushroom species, and observed the minimum and maximum values of Cu in *Pleurotus ostreatus* and *Psathyrella candolleana* with 15.57 mgkg⁻¹ and 60.43 mgkg⁻¹ respectively. The same values for Zn were reported to be 58.69 mgkg⁻¹ and 110.9 mgkg⁻¹ in *Coprinus comatus* and *Pleurotus ostreatus*, for Mn with 7.115 mgkg⁻¹ and 138.2 mgkg⁻¹ in *Cyclocybe cylindracea* and *Volvopluteus gloiocephalus*, for Mg with 61.03 mgkg⁻¹ and 67.23 mgkg⁻¹ in *Leucoagaricus leucothites* and *Lycoperdon molle*, for Fe with 59.42 mgkg⁻¹ and 585.3 mgkg⁻¹ in *Leucoagaricus leucothites* and *Lycoperdon molle*.

Wild edible mushrooms can be preferred due to their nutritional values as well as ease of accessibility and cheapness. However, the existence of poisonous mushrooms and the fact that most of the edible mushrooms in a region are not known by the local people, is an important problem. Mushrooms consumed in one region are not recognized in another region. Systematic studies and an increase in public awareness about edible fungi may increase the consumption of these valuable nutrient sources.

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