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Comparative Study of Fatty Acids Profile of Wild Mushroom Species from Turkey

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ABSTRACT
A comparative study was enhanced on the fatty acids profile of wild mushroom species; namely, *Armillaria tabescens*, *Leucopaxillus gentianeus*, *Pleurotus eryngii* and *Suillus granulatus* growing in Turkey. A total of fifteen fatty acids were identified and quantified by GC-FID and GC-MS. The main fatty acid was linoleic acid in *L. gentianeus*, *P. eryngii*, and *S. granulatus* varied from 40.92 to 68.24% while oleic acid was major fatty acid in *A. tabescens* (35.92%). The other major fatty acids were palmitic and stearic acids in studied mushrooms. The concentration of total saturated, monounsaturated and polyunsaturated fatty acids ranged from 16.71 to 31.90%, 12.04 to 40.41%, 27.64 to 68.24%, respectively. The present study showed that unsaturated fatty acids concentrations were higher than saturated ones. Since unsaturated fatty acids are valuable healthy compounds for human nutrition, mushrooms can be considered as a rich dietary natural source.
Keywords: fatty acid composition, GC-MS, mushroom species, unsaturated fatty acids

INTRODUCTION

Since ancient times, mushrooms have been considered as valuable healthy foods because of their taste, flavour, high nutritional values, and various biological activities such as antioxidant, antifungal, anti-immunomodulatory, antitumor, antibacterial, antidiabetic, anticholesterol, anti-coagulant, antiviral, anti-inflammatory and cytotoxic [1-4]. Mushrooms are nutritionally rich in vitamins, proteins, minerals, fibers and moisture as well as comprise considerable levels of essential fatty acids and phenolic compounds. In addition, they are low in calories and fats [5]. Fatty acids are important constituents of mushrooms. It is known that fatty acids, especially polyunsaturated ones the omega-3, and -6 series are necessary human health for preventing and treatment of hypertension, coronary artery disease, diabetes,

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Table 1. Collection localities and dates, family, edibility and fungarium numbers of studied mushroom species

No	Mushroom	Collection localities and dates	Family	Edibilty	Fungarium numbers
1	*Armillaria tabescens* (Scop.) Emel	Uşak-Sivaslı, 22 November 2008	Physalacriaceae	Inedible	AT-917
2	*Leucopaxillus gentianeus* (Quél.) Kotl.	Uşak-Banaz, 10 December 2008	Tricholomataceae	Inedible	AT-1075
3	*Pleurotus eryngii* var. *eryngii* (DC.) Quél	Uşak-Banaz, 23 November 2008	Pleurotaceae	Edible	AT-934
4	*Suillus granulatus* (L.) Roussel	Uşak-Banaz, 5 May 2008	Suillaceae	Edible	AT-825

osteoporosis, arthritis, cancer, other inflammatory and autoimmune disorders [6]. The research of the fatty acid compositions of mushrooms has interested by the researchers due to having various useful effects on health. Especially, the linoleic acid (18:2) and linolenic acid (18:3) are polyunsaturated acids that are fundamental in human diets [7]. Additionally, the linoleic acid is the precursor of arachidonic acid and it has a role in prostaglandins biosynthesis [8]. So far, some studies are reported on the fatty acids of mushrooms [5, 9-14]. Because of important properties of polyunsaturated fatty acids, scientific studies about mushrooms including polyunsaturated fatty acids have increased with each passing day. The aim of this work was to investigate the fatty acid compositions of four mushrooms namely; *Armillaria tabescens* (Scop.) Emel, *Leucopaxillus gentianeus* (Quél.) Kotl, *Pleurotus eryngii* var. *eryngii* (DC.) Quél and *Suillus granulatus* (L.) Roussel growing in Turkey using GC-FID and GC-MS.

MATERIALS AND METHODS

Mushroom materials

Mushroom species were collected from Uşak, province of Turkey and were identified by Dr. Aziz Türkoğlu, Muğla Sıtkı Koçman University. They were deposited in the Fungarium of Department of Biology, Muğla Sıtkı Koçman University and were stored at −18 °C until they were used. The species names, collection localities and dates, family, edibility and fungarium numbers of four mushroom species are given in **Table 1**.

Chemicals and spectral measurements

Methanol, *n*-hexane and boron trifluoride-methanol solution (BF_3:MeOH) were obtained from E. Merck (Darmstadt, Germany). GC-FID analyses were performed on a Shimadzu GC-17 AAF, V3, 230 V series gas chromatography (Japan) and GC–MS analyses were on Varian Saturn 2100 (USA).

Extraction

Each mushroom was extracted individually with 500 mL *n*-hexane for three times (24 h x 3) at room temperature (298 K), then filtered and evaporated to dryness under vacuum. They stored at refrigerator until they were methylated with 14 % BF_3:MeOH solution.

Derivatization of fatty acids with GC and GC-MS

The *n*-hexane extract (100 mg) was dissolved in 0.5 M NaOH (2 mL) in a 25 mL flask. After the flask was heated in a water bath (50 °C), then 2 mL BF_3:MeOH was added. The mixture was boiled for 2 minutes, and then the mixture was left until it cooled down, and then the volume was completed to 25 mL with saturated NaCl solution. Esters were extracted with *n*-hexane; thus, the organic layer was separated. The hexane layer was washed with a potassium bicarbonate solution (4 mL, 2 %) and dried with anhydrous Na_2SO_4 and filtered. The organic solvent was removed under reduced pressure by a rotary evaporator to give methyl esters.

Gas chromatography (GC)

GC analyses of the methyl derivatives of fatty acids were performed by Shimadzu GC-17 AAF, V3, 230 V series gas chromatography (Japan) coupled with a Flame Ionisation Detector (FID) and a DB-1 fused silica capillary non-polar column (30 m x 0.25 id., film thickness 0.25 μm). Injector and detector temperatures were 250 and 270 °C, respectively, carrier gas was He at a flow rate of 1.4 mL/min; sample size, 1.0 μL; split ratio, 50:1. The initial oven temperature was held at 100 °C for 5 min, then increased up to 238 ^{o}C with 3 ^{o}C/min increments and held at this temperature for 9 min. The relative percentages of separated compounds were calculated by using GC Solution computer program.

Gas chromatography-Mass spectrometry (GC-MS)

GC-MS analyses of the methyl derivatives of fatty acids using Varian Saturn 2100T (USA) coupled with an ion trap mass spectrometer (IT-MSD) and a DB-1 MS fused silica non-polar capillary column (30 m x 0.25 mm ID, film thickness 0.25 μm). For GC–MSD detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium (15 psi) at a flow rate of 1.3 mL/min. Injector and MS transfer line temperatures were set at 250 and 200 °C, respectively. The oven temperature was held at 100 °C for 5 min, then increased up to 238 °C with 3 °C/min increments and held at this temperature for 9 min. Diluted samples (1/25, w/v, in hexane) of 0.2 μL were injected manually in the split mode. Split ratio was 50:1. EI-MS were taken at 70 eV ionization energy. Mass range was from *m/z* 28 to 650 amu. Scan time 0.5 sec with 0.1 inters scan delays. The library search was carried out using NIST and Wiley 2005 (Gas Chromatography-Mass Spectrometry) GC-MS libraries. FAME (Fatty acid Methyl Ester) mixture (Supelco™ 37, Catalog no: 47885-U) were identified by comparing their retention times with those of the pure FAMEs standards.

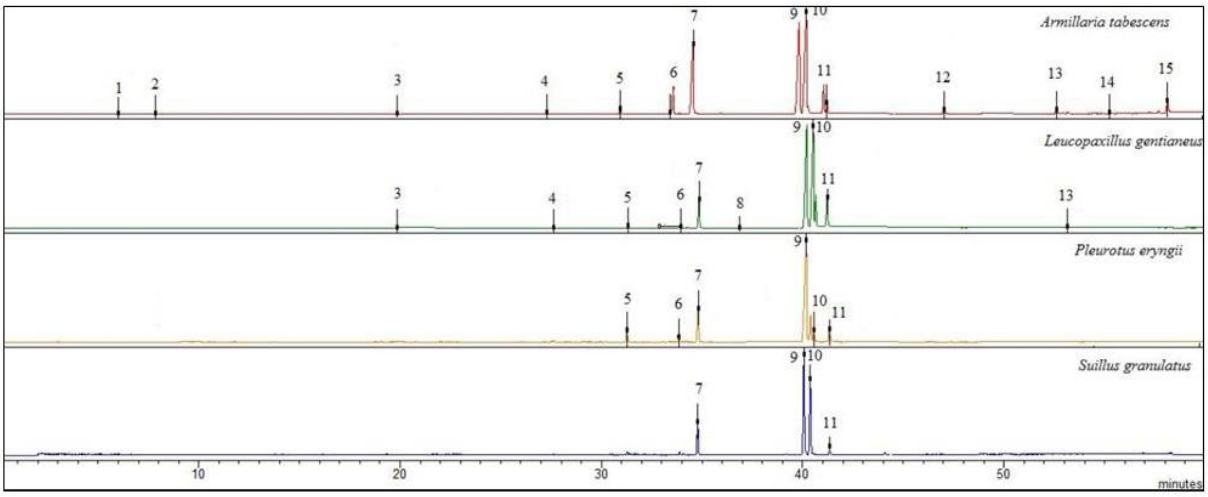

Figure 1. Chromatograms of fatty acids of studied mushroom species

Statistical Analysis

All data on the fatty acid composition were the averages of three parallel sample measurements. The data were recorded as the mean ± S.E.M. Significant differences between the means were determined by student's-*t* test, and *p* values <0.05 were regarded as significant.

RESULTS AND DISCUSSION

The fatty acid compositions of *A. tabescens*, *L. gentianeus*, *P. eryngii* and *S. granulatus* mushroom species revealed the presence of fifteen fatty acids in total using GC and GC-MS (**Table 2**, **Figure 1**). The fatty acid compositions were different among all species. Unsaturated fatty acid amounts were higher than saturated ones in all of them. The carbon chain lengths of fatty acids were ranged from 8 to 24. Linoleic acid ($C_{18:2\ \omega6}$), oleic acid ($C_{18:1\ \omega9}$) and palmitic acid ($C_{16:0}$) were the major fatty acid in all studied species. Similar results were observed for various mushroom species [11-20].

As shown in **Table 2**, linoleic acid, which is the most abundant fatty acid, was determined between 27.64 – 68.24% in the studied mushrooms. This fatty acid is very important for human diet. Likewise, it is known that linoleic acid is the precursor of 1-octen-3-ol, known as the alcohol of mushroom, which is the principal aromatic compound in most mushrooms and might contribute to mushrooms flavour [21]. Oleic acid, which is the main monounsaturated fatty acid, was also major fatty acid ranged from 10.05 to 39.78% in studied mushrooms. Both linoleic and oleic acids decrease the risk of cardiovascular disease; thus, mushrooms are recommended people in the human diet for the high blood cholesterol [22].

In this study, linoleic acid was main fatty acid in *L. gentianeus*, *P. eryngii*, and *S. granulatus* mushrooms species while oleic acid was major fatty acid in *A. tabescens*. In the same way, linoleic acid was previously reported as major fatty acid in *P. eryngii* (68.8%) [19], *S. granulatus* (42.3 and 44.6%) [17, 18]. On the contrary of our results, Reis et al. [20] and Yılmaz et al. [11]

Table 2. The fatty acid compositions (%) of the mushroom species [a]

Peak no	Fatty acids	*Armillaria tabescens* (%)	*Leucopaxillus gentianeus* (%)	*Pleurotus eryngii* (%)	*Suillus granulatus* (%)
1	Caprylic acid ($C_{8:0}$)	0.14±0.004	nd[c]	nd	nd
2	Nonanoic acid ($C_{9:0}$)	0.10±0.003	nd	nd	nd
3	Lauric acid ($C_{12:0}$)	0.09±0.001	0.19±0.001	nd	nd
4	Myristic acid ($C_{14:0}$)	0.35±0.004	0.18±0.001	nd	nd
5	Pentadecanoic acid ($C_{15:0}$)	0.91±0.002	0.62±0.002	4.39±0.001	nd
6	Palmitoleic acid ($C_{16:1}$ ω7)	4.49±0.001	0.39±0.001	1.99±0.001	nd
7	Palmitic acid ($C_{16:0}$)	**19.40±0.004**	7.95±0.003	**14.04±0.002**	**14.43±0.002**
8	Margaric acid ($C_{17:0}$)	nd	0.24±0.002	nd	nd
9	Linoleic acid ($C_{18:2}$ ω6)	**27.64±0.004**	**40.92±0.004**	**68.24±0.004**	**47.64±0.004**
10	Oleic acid ($C_{18:1}$ ω9)	**35.92±0.004**	**39.78±0.003**	**10.05±0.002**	**35.62±0.003**
11	Stearic acid ($C_{18:0}$)	5.37±0.002	**8.98±0.002**	1.29±0.002	2.28±0.001
12	Arachidic acid ($C_{20:0}$)	0.82±0.001	nd	nd	nd
13	Behenic acid ($C_{22:0}$)	0.89±0.001	0.75±0.001	nd	nd
14	Tricosanoic acid ($C_{23:0}$)	0.27±0.002	nd	nd	nd
15	Lignoceric acid ($C_{24:0}$)	3.56±0.003	nd	nd	nd
	∑ Saturated fatty acids	31.90	18.91	19.72	16.71
	∑ Monounsaturated fatty acids	40.41	40.17	12.04	35.62
	∑ Polyunsaturated fatty acids	27.64	40.92	68.24	47.64
	∑ Unsaturated fatty acids	68.05	81.09	80.28	83.26
	ω6/ω9[b]	0.77	1.03	6.79	1.34

[a] Values represent the means ± S.E.M. of three parallel measurements ($p<0.05$).
[b] ω6/ω9: linoleic acid-oleic acid ratio
[c] nd: not detected

found a less percentage of linoleic acid in *P. eryngii* (24.71 and 26.6%). Also, Cox et al. [23] reported a lower concentration of oleic acid in *A. tabescens*.

Palmitic and stearic acids, which are among saturated fatty acids, were also found in the studied mushroom species. The percentage of palmitic acid ranged from 7.95 to 19.40% while stearic acid varied from 1.29 to 8.98%. Other fatty acids such as $C_{8:0}$, $C_{9:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:1}$, $C_{17:0}$, $C_{20:0}$, $C_{22:0}$, $C_{23:0}$ and $C_{24:0}$ were found in the mushroom species, but all of them were in small quantity.

The total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and unsaturated fatty acid (UFA) compositions of the studied mushroom species were ranged from 16.71 to 31.90%, 12.04 to 40.41%, 27.64 to 68.24% and 68.05 to 83.26%, respectively. As result of high level of palmitic acid in *A. tabescens*, SFA content (31.90%) was higher than other studied species. PUFA content was present in highest level in *P. eryngii* due to linoleic acid (68.24%). Unsaturated fatty acids increase nutritional values of mushrooms.

As given in **Table 2**, the ratio of linoleic acid to oleic acid (L/O) was found between 0.77-6.79. The linoleic: oleic acid ratio provides an important criterion from a chemotaxonomic viewpoint for the upcoming studies and is useful for the taxonomical differentiation between species of the same genus.

CONCLUSIONS

Mushrooms can be considered as healthy foods because of their low-fat composition, low calories and high essential fatty acid levels especially linoleic acid. Low-fat diets and low calorie are recommended by people with high blood cholesterol. Therefore, mushrooms are perfect food in human diet. The present study indicates that unsaturated fatty acids are higher than saturated ones. Since mushrooms contain high percentage of unsaturated and essential fatty acids, the findings of results support their potential uses as food supplements and nutraceuticals.

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