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Fatty Acid Composition in Ten Mushroom Species Collected from Middle Black Sea Region of Turkey

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The aim of this study was to determine the amount and composition of fatty acids in 10 edible mushroom species, which belongs to *Trichomataceae, Plourotaceae, Clavariaceae, Cantharellaceae, Agaricaceae, Strophariaceae, Lepiotaceae, Russsulaceae* familials grown in the middle black sea region (Tokat, Amasya, Ordu provinces). The amount and composition of fatty acids in dried mushrooms samples were detected by a gas chromatography. The fatty acids were determined using the fatty acids methyl-esters standards. Fatty acids with 9-24 carbons were found in mushrooms samples. Also, fatty acids with a single carbon and single double-bound were found. Linoleic acid (18:2 ω 6c) occurred greater than 50 % in most of the samples studied. Fatty acid analysis of the mushroom in the present study showed that unsaturated fatty acids were higher than the saturated ones.

Key Words: Fatty acid composition, Mushroom, Gas chromatography, Middle black sea region.

INTRODUCTION

Mushrooms, poor in calories and rich in fats, vegetable proteins, chitten, vitamins and minerals, are healthy foods[1,2], composed mainly of unsaturated fatty acids, corresponding to 40 % on dry weight basis[3]. The mushrooms have been used in the orient for medicinal purposes[4].

Considerable experimental evidence suggests that one of the most important food component that helps to reduce serum cholesterols its polyunsaturated fatty acids content[5-8]. Fatty acids can regulate lipid metabolism at three different levels *i.e.*, (1) They interact with enzymes to affect their activity, (2) They interact with nuclear transcription factors to modulate gene expression and (3) They can affect mRNA stability and thus regulate expression of enzymes.

Fatty acid and/or derivates interact with enzymes either directly to affect activity or with the nuclear transcription factors or affect the stability mRNAs encoding proteins involved in lipid metabolism. The knowledge about the effects of fatty acids species on the genetic machinery as a whole could become a starting point for individualization of nutritional needs[9].

Linoleic acid (18:2) and linolenic acid (18:3) are two long-chained fatty acids that are fundamental in to human diets. They are termed essential fatty acids are converted to their respective long-chained polyunsaturated fatty acids (PUFA) *in vivo* by an alternating sequence of desaturation and elongation. These fatty acids function as integral components of membrane phospholipids and as a precursor of prastanoid production and in the regulation of cellular function including endocytosis and ion channel modulation. As such a lack of dietary essential fatty acids and/or their in efficient methabolism has been implicated in the aetiology and progeression of diseas, including cardiovascular disease and diabetes[10].

The aim of this study was to determine the fatty acids composition in 10 edible mushroom species belonging to *Trichomataceae, Plourotaceae, Clavariaceae, Cantharellaceae, Agaricaceae, Strophariaceae, Lepiotaceae, Russsulaceae* families grown in the middle black sea region.

EXPERIMENTAL

Mushroom samples: Edible fungi grow naturally during the rainy season on dead pieces of wood, buried or on exposed roots of trees at different stages of decay. The mushroom species were collected, from Tokat, Amasya, Ordu (in the middle black sea region of Turkey) province in the spring. The colours, odour, other apparent properties, collection sites, dates and vegetation of mushroom samples were noted. The habitat and morphological characteristics for the identification of these species in the different collection localities were

TABLE-1
HABITAT, EDIBILITY AND THE FAMILIES OF MUSHROOMS USED IN THIS STUDY

Class, family and species	Habitat	Edibility
Agaricus biterquis (Quel) Sacc	On manure heaps, garden waste and roadsides	Edible
Macrolepiota procera (Scop.: Fr.) Sing.	In open woods and pastures Season summer and autumn	Edible
Cantharellus cibarius Fr.	In all kinds of woodland, but usually associated with frondose trees	Edible
Craterullus cornucopioides (L.ex Fr.) Pers.	Gregarious or clustered amongst leaf litter of deciduous wood.	Edible
Lepiota excoriata (Schaeff ex. Fr) Kummer	In pasture, heaths and open woodland	Edible
Pleurotus eryngii (D.C. ex Fr) Quel	On roots and decaying remains of umbellifers, especially Eryngium and Heracleun	Edible
Polyporus squamosus Huds. : Fr	Parasitic on deciduous trees, especially elm, beech and sycamore	Edible
Clitoceybe odora (Bull. ex Fr.) Kummer	Singly, scattered, or in groups on leaf litter under hardwoods, especially oak	Edible
Pholiota sp	In tufts on conifer stumps or fallen trunks.	Edible
Tricholoma terreum (Schaff.: Fr.) Kummer	In woods, especially with conifers	Edible

TABLE-2
SATURATED FATTY ACID, UNSATURATED FATTY ACID, MONOUNSATURATED FATTY ACID, POLYUNSATURATED FATTY ACID, PALMITIC ACID, FATTY ACIDS WITH DIEN, OLEIC ACID, EICOSAPENTANOIC ACID, DOCOSAHEXANOIC ACID, POLYUNSATURATED FATTY ACID/ SATURATED FATTY ACID RATIO, EVEN-C FATTY ACID AND SINGLE-C FATTY ACID PERCENTAGES IN MUSHROOMS STUDIED HERE

	SFA	UFA	MUFA	PUFA	Palmitic acid	Dien	Oleic acid	Linoleic acid	EPA	DHA	PUFA/ SFA	Even–C fatty acids	Single-C Fatty acid
Agaricus biterquis	22.89	3.26	3.26	49.55	18.94	49.55	17.21	49.55	-	-	2.79	95.92	1.67
Polyporus squamasus	3.89	67.71	12.07	54.64	14.07	54.64	9.57	54.64	-	-	2.16	91.63	5.97
Pleurotus eryngii	22.47	75.33	48.73	26.60	9.55	27.37	47.82	26.60	-	-	3.35	91.96	5.84
Lepiota excoriata	68.59	26.58	26.58	-	45.06	-	8.81	-	-	-	0.38	91.12	4.77
Macrolepiota procera	15.90	81.95	19.51	62.44	10.95	62.44	17.40	62.44	-	-	5.15	94.98	2.41
Cantharallus cibarius	20.21	77.69	17.92	59.79	12.81	59.79	13.57	59.79	-	-	3.84	97.92	-
Craterullus cornucopioides	23.79	74.75	22.82	51.93	10.56	51.01	17.68	51.01	-	-	3.14	94.15	4.13
Clitocybe odora	27.37	69.40	6.01	63.39	14.18	63.39	2.24	63.39	-	-	2.53	96.16	0.66
Pholiota sp	22.64	73.47	17.64	55.83	12.13	55.83	14.34	55.83	-	-	3.24	91.43	3.26
Tricholoma terreum	17.04	80.70	18.71	62.00	10.50	60.29	16.61	60.29	-	1.71	4.73	93.49	4.26

recorded and photographed. The mushroom samples were transported to the laboratory. Freshly, mushrooms samples collected different forest and steps areas were completely cleaned before analysis. The samples were dried and stored in laboratory. The habitat of the edibility of mushrooms used is listed in Table-1.

Methylation and GC analysis: All the reagents used for the extractions and derivations were of analytical reagent grade. Sample extraction was performed using methods described by Folch, Lee and Sloane-Stanley[11]. The dried samples were powdered by pounding completely and used for fatty acid analysis. Each mushroom sample was separated, minced in a chloroform/methanol (2:1 v/v) mixture using a high speed blender and filtered through Whatman paper. Extraction solvent (chloroform/methanol: 2:1 v/v) volume was 105 mL for each sample.

The fatty acid methyl esters for gas chromatograph analysis were prepared. Samples were analyzed in triplicate. Extracted samples were methylated in a BF_3-CH_3OH mixture for separation of fatty acids. Briefly, the fatty acids (in the hydrolyzed and derived methyl ester forms) were obtained with 1 mL of NaOH/methanol at 90 ºC for 10 min and then a complete derivation was assured with 1 mL BF_3 at 90 ºC for 10 min. The methyl esters were purified with 1 mL of hexane and 1 mL of water. Individual samples were passed through an anhydrous Na_2SO_4 column and then evaporated to dryness under a steam of nitrogen and redissolved in 100 µL of isooctane. In analysis, the HP-innowax chromatography column (30 m and 0.32 mm, ID 0.25 mm film thickness) and helium as the carrier gas were used. Clarified and methylated samples were run on a GC column containing polyethyleneglycol chromatography medium. The identification and quantitation of fatty acids were performed by gas chromatography using an Agilent 6890 series GC systemors. Detector was FID. The column temperature was held 50 ºC for 1 min, then with the first temperature gradient of 8 ºC/min to 220 ºC for 5 min, the second temperature (finally) gradient was 2 ºC/ min to 250 ºC and held for 7.75 min. Injector temperature was 250 ºC. In analysis, GC gas flow rate was 1.3 mL/min and injection volume was 1 µL.

RESULTS AND DISCUSSION

In present study, the composition and percentage amount of fatty acids in 10 mushroom species were determined. The habitat, edibility and the families of mushrooms studied are listed in Table-1.

The percentages of the saturated fatty acid, unsaturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, palmitic acid, fatty acids, oleic acid, eicosapentanoic acid, docosahexanoic acid, polyunsaturated fatty acid/saturated fatty acid ratio, even-C fatty acid and single-C fatty acid in mushrooms studied are show in Table-2.

As can be seen from Table-2, carbon chain length of fatty acids is between 9-24 in the mushrooms studied. The most aboundant fatty acids are 15:0, 16:0, 16:1, 18:0, 18:1 and 18:2. The fatty acids 16:0, 18:0, 18:1, 18:2 are detected in all the species, particullarly in *Lepiota excoriata*. However, linoleic acid (18:2 ω6c) is detected in all the species (specifically in *Lepiota excoriata*) and linolenic acid (18:3 ω3c) is not detected in any species studied here. In some mushrooms species, 22:0,

TABLE-3
MUSHROOMS SAMPLES AND THE MOST ABUNDANT FATTY ACIDS

	12:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3	19:0	22:0	24:0
Agaricus bietrqius	+	+	+	+	+	+	+	-	+	-	-
Polyporus squamasus	+	+	+	+	+	+	+	-	-	+	-
Pleurotus eryngii	-	+	+	-	+	+	+	-	+	-	+
Lepiota excoriata	+	+	+	+	+	+	-	-	-	+	+
Macrolepiota procera	-	+	+	+	+	+	+	-	-	-	-
Cantharellus cibarius	+	-	+	-	+	+	+	-	-	+	-
Craterellus cornucopioides	+	+	+	+	+	+	+	+	+	+	+
Clitocybe odora	+	-	+	+	+	+	+	-	-	-	+
Pholiota sp	+	+	+	+	+	+	+	-		+	-
Tricholoma terreum	+	+	+	+	+	+	+	-	-	-	-

24:0 fatty acids are detected. Docohexanoic acid, which is very important for human diet, is obtained in low amount in *Tricholoma terreum*.

Table-3 indicates the widespread fatty acids in the mushrooms studied. The fatty acids and percentages of mushrooms studied are given in Table-4. As shown in Table-4, *Craterellus cornucopioides* contains the highest variations of fatty acids ranging from 12:0 to 24:0, followed by *Pholiota sp, Lepiota excoriata, Polyporus squamasu, Agaricus bietrqius, Tricholoma terreum, Clitocybe odora, Pleurotus eryngii* and *Macrolepiota procera*, respectively.

All the analyzed species are found to be very important food due to linoleic acid content. As seen from unsaturated/ saturated ratios in Table-1, the amount of unsaturated fatty acid is higher than that of saturated fatty acids.

Saturated fatty acids are greater than unsaturated fatty acid only for *Lepiota procera*. However, unsaturated fatty acids are greater in the other species given in Table-3. The most abundant MUFAs percentage (48.73 %) is in *Pleurotus eryngii*. Some samples have single-C fatty acids ranging between 3-5 % and 18-22 %.

The fatty acids (18:2, 18:1 ω9c and 16:1 ω7c) are found to be the most dominant unsaturated fatty acids. However, for all the species, dominant unsaturated fatty acid is linoleic acid. Linoleic acid is determined between 1.68-73.74 % in the mushrooms studied. The other species, except for *Lepiota escoriata,* contains the 18:2 fatty acids as well. This fatty acid is very important for human healty.

The per cent of linoleic acid is found to be 26.60 % in *Pleurotus eryngii*, 54.64 % in *Polyporus squamasus*, 62.44 % in *Macrolepiota procera*, 59.79 % in *Cantharallus cibarius,* 51.01 % in *Craterullus cornucopioides*, 63.39 % in *Clitocybe odora*, 73.74 % in *Pholiota sp*, 60.29 % in *Tricholoma terreum*, 72.73 %. The percentage of linoleic acid in *Lactarious deliciosus* is very low (1.68 %).

The amount of linoleic acid is 49.55 % in *Agaricus biterquis*. These results are in the agreement with the findings reported by Yilmaz *et al.*[12]. The lowest amount of palmitic acid (10 %) is detected in *Pleurotus eryngii*, while the highest amount of palmitic acid (45.06 %) is determined in *Lepiota excoriata*. oleic acid level was 47.82 % in *Pleutorus eryngii* and it was from 2.24 to 17.21 % in the other species studied.

In all the species, except for *Agaricus biterquis*, unsaturated fatty acids were higher than the saturated ones. Generally, in all the species, the amount of palmitic acid, oleic acid and linoleic acid was higher than those of others. Díez and Alvarez realized compositional and nutrional studies on two wild edible mushrooms from the northwest Spain[2].

Pamale *et al.*[1] examined a comparative study for nutrients in edible mushrooms. Anke *et al.*[13] detected cibaric acid and 10-hydroxy-8-decenoic acid as two fatty acid derivatives in *Cantharallus cibarius ve C. tubaeformis*. These fatty acid derivates are used as a response to injury. Ayer *et al.*[14] studied and found that anofinic acid, choman-4-one, 3-hydroxy-acetylindole and fatty acid mixture in *Lactarius delious*. Solomko *et al.*[15] detected lipid content and fatty acid composition of the higher edible fungus- the oyster mushroom *Pleurotus ostrearus*. Sun *et al.*[16] found therapeutic effect of some foods on hyperlipidemia in man. Russo[17], studied lipid content and fatty acid composition.

Noel subervlle *et al.*[18] observed three different aromatic compounds in *Lepista nuda* and they pointed out that the most aboundant fatty acids were converted to these aromatic compounds in mushroom. The cholestrol-lowering effect of *Flammula velutipes* was studied by Fukushima *et al.*[19].

Dimou *et al.*[20] studied the fatty acids of nine Pleourotus species and showed that linoleic acid was the major fatty acid (33-68 %). The amount of this major fatty acid in *P. eryngii* was 43-46 %. The result of Dimou *et al.*[20] is in the agreement with our findings. These researchers determined the oleic acid and palmitic acid in high concentration and the stearic acid in low concentration. The effects on state of plasma, KC, lipid profile and, plasma total antioxidant of *P. ostreatusun* were examined by Hossain *et al.*[21]. They found that the mushroom speceis decreased the total cholesterol by 28 %, the LDL-C by 55 %, the TG by 34 % and the non esterified acid by 30 %. Hossain *et al.*[21] observed as 34 % the decreasing of KC cholesterol level and the increasing of HDL-c level. They found the unsaturation level of plasma fatty acid in either normolipidemic or hipercolesterolemic rats.

Leon-Guzman *et al.*[3] examined the eight edible mushroom species and they found the most aboundant fatty acids as 18:2, 27.64 mg/g; 16:0, 4.39 mg/g; 18:0, 1.274 mg/g; 18:1 and 0.637 mg/g in *Agaricus bisporus*. Also, they determined essansiel fatty acid content as 78 %[3]. Medical, biotechnologic and the environment aplication of *Pleurotus ostraatus* has been done and its antitümor activities and hypoglycemic effect were proven[22,23]. *Agaricus bisporus* decreases the effects of LDL-c [8,24,25]. Russo[17], studied lipid content and fatty acid composition in lemon wax.

The results demonstrated that all of the mushrooms species included in this study have a considerable fatty acid

TABLE-4
FATTY ACIDS AND THEIR PERCENTAGES IN STUDIED MUSHROOMS

Agaricus bitorquis	*Polyporus squamasus*	*Pleurotus eryngii*	*Lepiota excoriata*	*Macrolepiota procera*	*Cantharellus cibarius*	*Craterellus cornucopioides*	*Clitocybe odora*	*Pholiata sp*	*Tricholoma Terreum*
FA (%)	FA (%)	FA (%)	FA (%)	FA (%)	FA (%)	FA (%)	FA (%)	FA (%)	FA (%)
11:0 (0.33)	C_9 Dicarboxylic acid (1.02)	C_9 Dicarboxylic acid (1.16)	C_{12} Dicarboxylic acid (0.50)	15:0 (2.41)	C 12:0 Primary alcohol (1.31)	C_9 Dicarbo-xylic acid (0.16)	10:0 30 H 0.34	C_{12}:0 Primary alcohol 1.48	10: 0 20H (0.18)
11:0 Antesio (0.23)	9:0 30H (0.77)	15.0 (0.58)	11:0 iso (0.30)	16:0 (10.95)	14:0 (0.70)	10:0 30H (0.54)	11.0 0.66	15.0 (3.26)	11:0 (0.15)
12:0 (0.37)	11:0 20H (0.29)	16:0 (9.55)	14:0 (0.74)	16:0 20H (1.19)	16:0 (12.81)	12:0 (0.44)	Unknown 0.43	16.0 (12.13)	Unknown (0.08)
12:0 iso (0.22)	11:0 antesio (0.68)	18:0 (5.49)	15:0 (1.88)	16:1 ω7c (1.00)	16:0 20H (0.88)	12.0 aldeh (1.18)	12:0 iso 0.21	16.0 20H (2.18)	11:0 Dimethyl acetat (0.09)
12:0 Antesio (0.21)	12.0 (1.76)	18:1 ω9c (47.82)	15: 0 iso (1.14)	18:0 (0.95)	18:0 (2.67)	12:0 (0.81) antesio	16:0 14.18	16.1 ω7c (1.47)	$C_{12:0}$ Primary alcohol (0.39)
C_{12} Primary alcohol (1.23)	12:0 Alde (0.33)	18:2 ω6c (26.60)	16.0 (45.06)	18:1 ω7c DMA (1.05)	18: ω19c (13.57)	12: ω6C (0.21)	16:0 20H 1.04	18:0 (1.38)	12:1 ω3c (0.37)
12:1 ω8C (0.17)	C_{12} Primary alcohol(1.71)	Unknown (1.42)	16:1 ω5c (4.42)	18:1 ω9c (17.46)	18:1 ω9t (4.35)	12:1 ω8C (0.13)	16:1 ω5c 0.52	18:1 ω9c (14.34)	14:0 (0.33)
14.0 (0.62)	12.1 ω7C (0.74)	19:0 (0.05)	16:1 ω7c (2.62)	18:2 ω6c (62.44)	18: ω6C (59.77)	14: 0 (0.36)	18:0 6.93	18:1 ω9t (1.83)	15.0 (3.21)
15:0 (0.37)	14:0 (0.83)	19.0 iso (2.14)	16:0 20H (3.53)	Unknown (1.34)	22:0 20H (1.84)	15:0 (1.42)	18:1 ω7C DMA 0.72	18:2 ω6c (55.83)	16:0 (10.50)
16:0 (18.94)	15:0 (1.09)	Unknown (0.79)	17.0 (0.45)	Unknown (1.22)	Unknown (2.10)	15:0 iso (0.12)	18:1 ω9c 2.24	22:0 (0.73)	16.0 20H (0.56)
16:0 20H (0.61)	16:0 (14.07)	18:20 H (0.77)	17:1 (2.46)			16.0 (10.56)	18:1 ω9t 1.96	22:0 20H (1.48)	16.1 ω7C (0.64)
16:1 ω 5C (0.41)	16:1 ω7c (0.92)	C_{20} N Alcohol (0.88)	18:0 20H (2.69)			16:0 iso (0.34)	18:2 ω6c 63.39	Unknown (4.00)	18:0 (0.82)
16:1 ω7C (2.68)	16:0 20H (0.71)	21:1 ω6C (0.91)	18:1 ω7c (3.98) DMA			16.0 20H (0.65)	Unknown 1.06		18:1 ω7c (1.09) DMA
18:0 1.21	17:0 (1.28)	24.0 20H (0.85)	18.1 ω9c (8.81)			C16 Alcohol (0.33)	C_{20} N alcohol 0.79		18:1 ω9c (16.61)
18:0 2OH (0.65)	17:1 ω8c (0.84)		18.1 ω9t (6.75)			16:1 ω5c (0.09)	24:0 0.72		18.2 ω6c (60.29)
18:1 ω7c DMA (0.63)	18:0 (4.37)		Unknown (4.10)			16.1 ω7c (1.16)	24:0 20H 2.89		Unknown (1.11)
18:1 ω9c (17.21)	18:1 ω9c (9.57)		C_{20} N alchol (2.15)			16:1 ω9c (0.35)	24:1 ω9c 0.57		22:6 ω3c (1.71)
18:1 ω7t (1.21)	18:2 ω6c (4.64)		22:0 2OH (0.72)			16:2 ω6c (0.67)			Unknown (1.07)
18.2 ω6c (49.55)	22:0 20H (1.98)		23:0 2OH (0.50)			18:0 (1.10)			
19:0 (0.74)	Unknown (2.08)		24:0 (0.73)			18.1 ω3c (0.31)			
Unknown (2.42)			24:0 20H (6.46)			18:1 ω6c (0.14)			
						18:1 ω9c (17.68)			
						18:1 ω9t (1.49)			
						18:2 ω6c (51.01)			
						18:3 ω6c (0.25)			
						18:1 ω7c DMA (0.89)			
						18:0 20H (0.63)			
						19:0 iso (1.46)			
						19:0 cyclo C_{11-12} (0.03)			
						19:0 cyclo C_{11-12} 20H (0.57)			
						19:1 ω8c (0.06)			
						19:1 ω8t (0.08)			
						19:1 ω9c (0.23)			
						Unknown (1.05)			
						C_{20} N Alchol (0.43)			
						C_{20} Alchol (1.11)			
						C 22:0 20H (0.27)			

composition and can be differentiated from one another on the basis of fatty acid content. Many of the mushrooms analyzed contained some fatty acids in different amounts.

Low-calorie and low-fat diets are recommended for people with high blood cholesterol. Therefore mushrooms are perfect, because of their low calories, low-fat composition and high essential fatty acid levels. Most of the studies on mushroom are limited to certain mushroom species and fatty acids. However, the present study contain a lot of economically important and edible mushroom samples that contain significant amounts of valuable fatty acids. The present study shows that unsaturated fatty acids are higher than the saturateds.

Different biolgical (anticarsinojenic, anticholesterol, immuno stimulating) effects of *Lentinus edodes* are studied[26]. The differences in the results can arise from physical and chemical factors of growth regions and genetic structures of species. Mushroom quality is influenced by variables such as habitats, the storage and pre/post harvest conditions. All these variables justify the variability in composition data published by different authors working with even the same species of mushrooms[27].

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