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Fatty Acid Composition of Six Mushroom Samples of Black Sea Region of Turkey

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The aim of this study is to determine the amount and composition of fatty acids in 6 edible mushroom species belonging to the different families grown in the middle Black sea region (Tokat, Amasya, Ordu provinces). The amount and composition of fatty acids in dried mushrooms samples were detected with a gas chromatography. The fatty acids were determined using the fatty acids methyl-esters standards. Fatty acids with 10-20 numbers were ocurred in mushrooms samples. Also, fatty acids with a single carbon and single double bound were found. Linoleic acid (18:2 ω6c) occurred more than 50 % in most of the samples studied. Fatty acit analysis of the mushroom in the present study showed that unsaturated fatty acids were higher than the unsaturated fatty acids.

Key Words: Fatty acid composition, Mushroom, Gas chromatography, Middle Black sea, Turkey.

INTRODUCTION

Mushrooms, low calorie food and rich in unsaturated fats, proteins, chitten, vitamins and minerals especially potassium and phosphorus, are healthy foods[1-4]. Mushrooms are the fungi that have been used as a food from ancient times. The benefits fatty acids in the diets of humans are well documented. Fatty acids play a major role in the functioning of the immune system and the maintenance of all hormonal systems in the body.

Edible mushrooms show a high proportion of unsaturated fatty acids[4]. Although mushrooms reveal highly variable fatty acid profiles, palmitic acid (16:0), oleic acid (9-*cis* 18:1) and linoleic acid (9-*cis*, 12-*cis* 18:2) are the main fatty acids found in members of the different species[5]. Nutritionally, linoleic acid and α-linolenic acid (9-*cis*, 12-*cis*, 15-*cis* 18:3) are essential for basal metabolism in humans, while long-chain polyunsaturated fatty acids have many beneficial effects on human health[6]. Many edible mushrooms have been used in the orient for medicinal purposes[7]. Some mushrooms have been reported as therapeutic foods, useful in preventing

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diseases such as hypertension, hypercholesterolemia, atherosclerosis and/or cancer. Considerable experimental evidence suggests that one of the most important food component that helps reduce serum cholesterols its poly unsaturated fatty acid content[8-11].

Interest in lipids, especially in fatty acid composition, is currently expanding. Such data are used for physiological, chemotaxonomic and intrageneric differentiation studies of many organisms such as bacteria, algae, fungi and vascular plants[12-15]. The fat fraction of mushrooms is mainly composed of unsaturated fatty acids, corresponding to 40 % on dry weight basis[3]. In this respect, knowledge of fatty acid content in edible mushrooms is of interest. Considering the interest for wild mushrooms for human consumption and the lack of data with regard to mushroom fatty acids, the objective of this study is to characterize the fatty acid profiles of mushrooms from middle Black sea belonging to the different families.

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 identification of these species in the different collection localities were recorded and photographed. Freshly, mushrooms samples collected from different forest and steps areas were completely cleaned before analysis. The samples were dried and stored in laboratuary. The habitat, edibility and the families of mushrooms used are listed in Table-1.

TABLE-1
HABITAT, EDIBILITY AND THE FAMILIES OF MUSHROOMS USED ARE LISTED IN

Class, family and species of mushrooms	Habitat	Edibility
Clitocybe infundibuliformis (Scop.)Fr	On acids soils in deciducus or coniferous woods	Edible
Collybia erythropus (Pers. Ex Fr) Kummer	Singly, scattered, or in groups on leaf litter under hardwoods, especially oak	Edible
Clavaria sp	In tufts, in leaf litter of deciduous woods	Edible
Lepista nuda (Bull. : Fr.) Cooke	In woodland, hedgerows and gardens	Edible
Marasmius oreades (Bolt. : Fr.)	Often forming rings in the short grass of pasture or lawns	Edible
Lactarius deliciosus (L. : Fr.) S.F. Gray	In woods, especially with conifers	Edible

Methylation and GC analysis: All reagents used for the extractions and derivations were of analytical reagent grade. Sample extraction was performed using methods described by Folch *et al.*[16]. 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. 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 (2) 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. For analysis, the HP-Innowax chromatography column (30 m · 0.32 mm ID · 0.25 lm 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 of fatty acids in 6 different mushrooms were determined. The amount of saturated fatty acid (SFA), unsaturated fatty acid (UFA), poly unsaturated fatty acid (PUFA), oleic acid (18;1), linoleic acid (18:2), with single and double C fatty acids results for mushrooms species are shown in Table-2. Table-3 shows the amount of widespread fatty acids in the mushrooms studied. The fatty acids and percentages of mushrooms studied are shown at Table-4.

Carbon chain length of fatty acids was between 9-24 in the mushrooms studied. The most aboundant fatty acids were 15:0, 16:0, 16:1, 18:0, 18:1, 18:2. Essential fatty acids are important acids that are necessary substrates in animal metabolism and cannot be synthesized *in vivo*. It is reported that EFAs nourish skin, hair and nails. EFAs help eliminate eczema, psoriasis and dandruff and help in prevent hair loss[17]. The fatty acids 16:0, 18:0, 18:1, 18:2 were detected in all the species in present study. However, the fatty acid 18:2 ω6c, linoleic acid, was detected in all species and linolenic acid (18:3 ω3c) was not detected in any of the species. In all species, unsaturated fatty acids were higher than the saturated fatty acids. Generally, in all species, the amount of palmitic acid, oleic acid and linoleic acid was higher than others. In some mushrooms species were detected 22:0, 24:0 fatty acids. In *Lactarious deliciosus* was found eicosapentanoic acid which is very important for human diet (Table-2). 16:0, 18:0, 18:1, 18:2 Fatty acids were observed as 12.1, 25.3,

TABLE-2
SATURATED FATTY ACID (SFA), UNSATURATED FATTY ACID (UFA), MONOUNSATURATED FATTY ACID (MUFA), POLYUNSATURATED FATTY ACID (PUFA), PALMITIC ACID, WITH DIEN FATTY ACID, OLEIC ACID, EICOSAPENTANOIC ACID (EPA), DOCOSAHEXANOIC ACID (DHA), PUFA/SFA RATIO, WITH DOUBLE-C FATTY ACID, WITH SINGLE-C FATTY ACID PERCENTAGES IN THE FATTY ACIDS OF MUSHROOMS

	SFA	UFA	MUFA	PUFA	Palmitic acid	Dien	Oleic acid	Linoleic acid	EPA	DHA	UFA/SFA	With double-C fatty acid	With single-C fatty acid
Clitocybe infundibuliformis	18.38	78.13	5.40	73.73	11.72	73.74	2.06	73.74	–	–	4.30	96.58	0.94
Collybia erythropus	26.26	67.85	42.63	25.22	17.78	24.21	39.18	24.21	–	–	2.58	96.74	3.69
Clavaria sp	24.05	75.96	5.06	70.90	10.84	70.90	4.28	70.90	–	–	3.15	98.78	1.22
Lepista nuda	12.23	84.91	37.72	47.19	8.61	47.19	17.54	47.19	–	–	6.94	95.95	1.21
Marasmius oreades	20.41	76.06	3.33	72.73	14.533	72.73	3.33	72.73	–	–	3.72	96.47	–
Lactarious deliciosus	49.62	42.72	33.46	9.26	22.30	6.63	4.91	1.68	1.55	–	0.86	75.39	15.36

Dien: Double-unsaturated bound; Oleic acid: 18:1 ω9c ; UFA/SFA ratio; With Double-C fatty acids with single-C fatty acids.

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
Clitocybe infundibuliformis	-	+	+	+	+	+	+	-	-	-	-
Collybia erythropus	+	+	+	-	+	+	+	-	+	+	+
Clavaria sp	+	-	+	+	+	+	+	-	-	+	+
Lepista nuda	+	+	+	+	+	+	+	-	-	+	-
Marasmius oreades	-	-	+	-	+	+	+	-	-	+	-
Lactarious deliciosus	+	+	+	+	+	+	+	-	-	-	-

41.3, 17.1 % in *Lactarious deliciosus*, 11.8, 2.4, 29.5, 51.5 % in *Lepista nuda*, respectively. Palmitic acid was detected in all the species, was lower than 10 % in *Lepista nuda*. Palmitic acid was very high amount in *Lactarious deliciosus* 22.30 %. Oleic acid level was 39.18 % in *Collybia erythropus* and it was from 3.33 to 39.18 % in the rest of the species studied.

Table-2 shows the amount of saturated fatty acids was greater than unsaturated fatty acid only in *Lactarious deliciosus*. However, unsaturated fatty acids were greater in the rest of the species. There are differences among mushrooms in the levels of major and minor fatty acids (Table-4).

Many monounsaturated fatty acids, 16.1 ω5,7,9c, 18:1 ω3,7c and 9t 16:1 ω9c, 19:1 ω8,9c and 9t, 17:1 ω8c, 24:1 ω9c, were found in studied species The abundant

TABLE-4
FATTY ACIDS AND PERCENTAGES OF MUSHROOMS STUDIED

Clitocybe infundibuliformis		*Clavaria* sp		*Collybia erythropus*		*Lepista nuda*		*Lactarious deliciosus*		*Marasmius oreades*	
Fatty acid	%	Fatty acid	%	Fatty acid	%	Fatty acid	%	Fatty acid	%	Fatty acid	%
C_9 Dicarboxylic acid	0.36	C_{12} Primary alcohol	0.37	C_9	1.04	C_{12}	0.32	C_{12} Primary alcohol	1.44	16: 0	14.53
14:0	0.76	12:0	0.33	12:0	0.63	14:0	0..36	12:0	1.89	16:0 2 0H	1.30
15:0	0.58	16:0	10.84	12:0 alde	0.55	16:0	8.61	12:1 ω7c	1.08	18:0	3.08
16:0	11.72	16:0 20H	0.85	14:0	0.82	15:0	0.68	12:1 ω9c	0.62	18:1 ω6c	3.33
16:0 20H	0.92	16:1 ω7c	0.78	15:0	1.10	16:0	8.61	12:0 2OH	0.39	18.2 ω6c	72.73
16.1 ω5c	0.51	18:0	3.48	16:0	17.78	16:0 2 0H	0.54	12:0 iso 3OH	0.33	22:0 20H	1.50
18:0	4.04	18:1 ω9c	4.28	16: 2 0H	1.31	16:1 ω5c	4.82	13:0	1.13	Unknown	3.43
18:1 ω7c DMA	0.68	18:2 ω6c	70.90	16:1 ω7c	2.52	16:1 ω7c	0.59	13:0 iso	1.33		
18:1 ω9C	2.06	C_{18} N-alcohol	1.01	18:0	1.99	17:0	0.30	13:0 anteiso	1.27		
18:1 ω9t	2.15	C_{20} N-alcohol	0.86	18:1 ω9c	39.18	17:1 ω8c	0.23	13:1 ω5c	0.36		
18:2 ω6c	73.74	20:0	0.69	18:2 ω6c	24.21	18:0	0.59	14:0	4.33		
Unknown	0.76	21:0 antesio	1.22	Unknown	1.61	18:1 ω7c	0.56	15:0	1.70		
Unknown	1.72	22:0	0.60	19:0 ωSO	1.55	18:1 ω9c	17.54	15:0 iso	2.20		
16:1 ω9c	0.35	22:20lt	1.76	20:3 ω6c	1.01	18:1 ω9t	13.98	15:0 3OH	2.53		
16:2 ω6c	0.67	24:0 20H	2.10	C_{22} Primary alcohol	3.29	18.2 ω6c	47.19	15:0 iso 3OH	3.21		

18:0 1.10	24:1 20H 0.53	Unknown 0.68	16:0 22.30
18.1 ω3c 0.31	24:1 ω9c 0.57	22:0 20H 0.83	16:1 ω5c 1.14
18:1 ω6c 0.14		Unknown 1.77	16:1 ω7c 19.71
18:1 ω9c 17.68			16:2 ω6c 4.95
18:1 ω9t 1.49			16:1 ω7t alcohol 0.45
18:2 ω6c 51.01			17:0 iso 0.65
18:3 ω6c 0.25			17:0 anteiso 0.55
18:1 ω7c DMA 0.89			17:0 pri.alco. 070
18:0 20H 0.63			17:1 anteis. 0.69
19:0 iso 1.46			18:0 5.56
19:0 cyclo C_{11-12} 0.03			18:1 ω9c 4.95
19:0 cyclo C_{11-12} 20H 0.57			18:1 ω9t 3.94
19:1 ω8c 0.06			18:1 ω9t alco. 0.88
19:1 ω8t 0.08			18:2 ω6c 1.68
19:1 ω9c 0.23			20:4 ω6c 0.88
Unknown 1.05			20:5 ω3c 1.55
C_{20} N Alchol 0.43			Unknown 2.10
C_{20} Alchol 1.11			
C 22:0 20H 0.27			

mono unsaturated fatty acids were in *Collybia erythropus*. The fatty acids 16:1, 18:1 ω9c and 18:2 were the most dominant unsaturated fatty acids. However, The unsaturated fatty acid found in all species was linoleic acid. Linoleic acid was determined among 1.68- 73.74 % in the mushrooms species. This fatty acid is very important for human healty in studied species.

Linoleic acid was observed as 24.21 % in *Collybia erythropus*, 47.19 % in *Lepista nuda*, 70.90 % in *Clavaria* sp, 72.73 % *Marasmius oreades*. 73.74 % in *Clitocybe infundibuliformis* in our study. The percante of *Lactarious deliciosus* was very low (1.68 %). It is reported that linoleic acid level was as 17.1 % in *Lactarius deliciosus*, 51.5 % in *Lepista nuda*, respectively

Analyzed species are very important as linoleic acid takes important share for fatty acids in these species. As seen in Table-2, in unsaturated/saturated ratios, the amount of unsaturated fatty acid was more than (about 0.86-6.94 times) saturated fatty acids.

Díez and Alvarez[2] done compositional and nutrional studies on two wild edible mushrooms from northwest Spain. Pamale *et al.*[1] examined a comparative study for nutrients in edible mushrooms Ayer and Triponov[13] found that anofinic acid, choman-4-one, 3-hydroxyacetylindole and fatty acid mixture in *Lactarius delious*. Sun *et al.*[19] found therapeutic effect of some foods on hyperlipidemia in man. Russo[20] studied lipid content and fatty acid composition. Noel *et al.*[21] observed three different aromatic compounds in *Lepista nuda* and pointed out that the most aboundant fatty acids were converted to these aromatic compounds in mushroom. Dimou *et al.*[13] determined 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.*[22]. They found that this 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.*[22] observed as 34 % the decreasing of KC cholesterol level and the increasing of HDL-c level. They found level the unsaturation level of plasma fatty acid in either normolipidemic or hipercolesterolemic rats. Russo[20] studied lipid content and fatty acid composition in lemon wax.

A dependence of fatty acid composition on ambient temperature, during fructification, was reported. In cultivated oyster mushroom, growth temperatures below 17 ºC resulted in an increase of unsaturated fatty acid proportion as compared[23] with mushrooms produced at temperatures above 17 ºC.

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

Conclusion

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 fatty acids are limited to certain mushroom species. However, the present study contain a lot of economically important and edible mushroom samples that contain significant amounts of valuable fatty acids. Different biolgical (anticarcinogenic, anticholesterol, immuno stimulating) effects of *Lentinus edodes* are known[24]. The differences in the results can arise due to 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[25].

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