

Research Article

Fatty Acid Profile of Wild and Cultivated Edible Mushrooms Collected from Ethiopia

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Abstract

Six wild (*A. campestris*, *L. sulphureus*, *T. clypeatus*, *T. microcarpus*, *T. letestui* and *Termitomyces* spps) and three cultivated (*P. ostreatus*, *L. edodes*, *A. bisporus*) edible mushrooms collected from Ethiopia were analyzed for their fatty acid profile. Fatty acids were extracted by one step lipid extraction and methylation procedure followed by gas chromatography with flame ionization detection. The dominant fatty acid in all mushroom species was linoleic acid (C18:2) ranging from 1044.5-2759.4 mg/100 g. The next three dominant fatty acid were oleic acid (C18:1n9c), palmitic acid (C16:0) and stearic acid (C18:0) ranging from 43.8-1558.8, 189.9-1081.5 and 13.5-374.1 mg/100 g respectively. Beside the four major fatty acids already described, more than 20 fatty acids were identified and quantified. The proportions of unsaturated fatty acids were of higher concentration than those of saturated fatty acids for all mushrooms. Moreover, the ratio of linoleic/oleic acid in all species are significantly different (P<0.05) and were greater than one.

Keywords: Mushroom; Wild; Cultivated; Fatty acids; GC-FID

Introduction

Fatty acid compositions have beneficial effects on blood lipid profiles. Substitution of saturated fatty acids (SFAs) with monounsaturated fatty acids (MUFAs) leads to increased high density lipoprotein (HDL) cholesterol and decreased low-density lipoprotein (LDL) cholesterol, triacylglycerol, lipid oxidation, and LDL susceptibility to oxidation [1]. In fact, the inclusion of edible mushrooms in a natural hypercholesterolemic and antisclerotic diet has been used in Oriental medicine [2].

The GC (or GLC) analysis of lipids has been much studied in the literature. Analysis of fatty acid composition by GC usually requires derivatization of fatty acids to increase their volatility. Fatty acid methyl esters (FAME) may be prepared by different Tran's methylation techniques and then separated on GC columns and detected by flame ionization detection (FID) [3]. Nevertheless all of the methods devised for the preparation of fatty acid methyl esters using either acid-or base-catalyzed them are time consuming and impractical for processing a high number of samples because lipids have to be extracted prior to FAMES preparation. Garces and Mancha [4] have developed a convenient general method using complex reagent mixture for the digestion of the tissue, lipid Tran's methylation and FAMES extraction in one step.

In Ethiopia, wild mushroom eating habit is variable among the various ethnic groups of the country. More important is the fact that cultivated mushrooms are too expensive for the ordinary Ethiopian and consequently mushroom buyers are predominantly foreigners [5]. The habit of wild mushroom eating in Ethiopia differs from region to region and among different ethnic groups. Interestingly the many tribes in southwest Ethiopia, such as the ethnic groups in Kaffa and Asosa Zone, have a strong tradition of consuming wild mushrooms and are mycophilic (mushroom-loving). The regions are also characterized by diver's vegetation with relatively higher precipitation and recognized as biodiversity hot spot. From field observations, it is evident that numerous species of wild growing mushrooms are widely consumed by many ethnic groups in these two regions.

There are a number of reports on the fatty acid profile of edible mushrooms on literature [6-11]. However, there is no information available on fatty acid of wild and cultivated edible mushrooms of Ethiopia, till now. Moreover, the analysis of fatty acids based on a one step lipid extraction and methylation was not exhaustive. Hence, the aim of the present study was to determine the fatty acid profile of wild and cultivated edible mushrooms of Ethiopia for the first time using a convenient one step lipid extraction and methylation, followed by gas chromatography with flame ionization (GC-FID) detection. Further to measure the proportion of saturated, monounsaturated and polyunsaturated fatty acids in order to determine the quality the lipid obtained from edible mushrooms.

Materials and Methods

Description of sampling areas and sites

The three mushroom sampling areas were Addis Ababa, Kaffa zone (site Bonga) and Benishangul gumuz region (site Asosa) of Ethiopia. Addis Ababa is the capital city of Ethiopia and located 9°01 N and 038°45 E. Kaffa zone is situated in the northwestern part of the southern nations, nationalities and people region state (SNNPR) and lies within 07°00'-7°25'N latitude and 35°55'-36°37'E Longitude. Benishangul gumuz region is located in western parts of Ethiopia located between 09.17°-12.06° North latitude and 34.10°-37.04° East longitude.

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Sample collection and identification

Identification of the wild edible mushrooms was made by making comparisons with authentic illustrations [12-14]. Moreover, confirmations of the wild mushrooms were made by mycological experts at the department of life sciences at Addis Ababa University.

Preparation of samples and storage

Cultivated mushrooms were dried at 105°C for 48 hours in oven in the same day they were collected. While the wild mushroom samples were pre-dried on the study areas before transporting to the laboratory using drying rack constructed as illustrated by Van Der Westhuizen and Eicker [12]. The mushroom samples were cleaned out of forest debris (without washing) with a plastic knife and sliced without separating the cap and the stipe of the mushrooms. Pre-dried samples in the field were further dried at 105°C for 24 hours in drying oven in the laboratory. The dried samples were milled to fine powder (20 meshes) using smashing machine (FW 100) and kept in plastic bottles until analysis.

Analysis of fatty acid profile

One step fat extraction and methylation: Fatty acids were methylated by acid methylation with 2,2-Dimethoxypropane (DMP) according to Garces and Mancha [4] and extracted in heptane. Briefly, 200 mg of mushroom powder was weighed into disposable extraction glass test tubes and 500 µl of internal standards (250 µl of C-13 and 250 µl of C-19) was added. Then 1.4 ml of aqueous reagent (methanol: DMP: sulfuric acid in 85: 11: 4 by volume) and 1.6 ml of organic reagent (Heptane + BHT: Toluene in 63:37 by volume) was added. The inside of the tube was sealed with rigid cap and out with Teflon tape, then vortexed for 1 minute. The tubes were then placed in shaking water bath at 80°C for 5 min and vortexed for 30 seconds. Tubes were then placed back into the 80°C water bath for additional 2 hours. Tubes were placed in the vortex until they come to room temperature (10 min). To separate the lipid phase 2 ml of saturated NaCl was added, vortexed and centrifuged for 10 min at 3500 rpm. The top layer was then transferred to new extraction tube and dried down under N₂ gas. The dried extract was then reconstituted with 2 ml of Heptane + BHT and transferred to GC vial for analysis with GLC. Heptane + BHT were prepared by dissolving 80 mg BHT in one liter of heptane.

Gas chromatography-Flame ionization detector (GC-FID): Fatty acid methyl esters were quantified by gas chromatography (GC; Agilent 6890A, Agilent Technologies, Palo Alto, CA) equipped with a fusedsilica capillary column (SP-2560; 100 m \times 0.25 mm (i.d.) with 0.2µm film thickness; Supelco, Bellefonte, PA), and a flame ionization detector (FID). The temperature program was 70°C for 4 min, 8°C/min to 110°C, 5°C/min to 170°C and held 10 min, and 4°C/min to 215°C and held for 23 min. Gas constant flows held hydrogen carrier at 1 ml/ min and detector hydrogen at 25 ml/min, airflow at 400 ml/min, and nitrogen plus carrier at 40 ml/min. Peaks were identified using pure methyl ester standards (GLC 780 & 68D; NuChek Prep Inc., Elysian, MN) and recoveries of individual FA determined using an equal weight reference standard (GLC 461; NuChek Prep Inc.). Total FAs were estimated using C13:0 or C19:0 as internal standards (I.S.; NuChek Prep Inc.).

Statistical analysis

Completely randomized design (CRD) was used. All the experimental results were reported as mean \pm standard error (SE) of three parallel measurements. Data were evaluated by using one way

variance analysis (ANOVA) and means were separated by Duncan' multiple range test (p<0.05) by using SPSS version 15.0. For the construction pie graph Microsoft Excel was used.

Results and Discussion

The mushroom were selected purposefully and evaluated for their fatty acid composition by a one step fat extraction and methylation followed by gas chromatography-flame ionization detection (GC-FID) method of Garces and Mancha [4]. Fatty acids were identified using pure methyl ester standards (GLC 780 & 68D; NuChek Prep Inc., Elysian, MN) and quantified by the internal standard method by calculating the recoveries of individual FA determined using an equal weight reference standard (GLC 461; NuChek Prep Inc.).

Table 1 summarizes the fatty acid composition in mg/100 g of the analyzed Ethiopian edible mushrooms. The dominant fatty acid in all mushroom species was linoleic acid (C18:2) ranging from 1044.5-2759.4 mg/100 g. Similar observations have been made in lots of literatures [8,10,15]. The next three dominant fatty acid were oleic acid (C18:1n9c), palmitic acid (C16:0) and stearic acid (C18:0) ranging from 43.8-1558.8, 189.9-1081.5 and 13.5-374.1 mg/100 g respectively. Oleic acid is a bioactive compound and strongly inhibits the activity of human telomerase in a cell-free enzymatic assay, with an IC₅₀ value of 8.6 μ M. It was recently shown that oleic acid is an efficient inhibitor of glucosyltransferase [16].

Beside the four major fatty acids already described, more than 20 fatty acids were identified and quantified. The short-chain fatty acids (SCFs) from C4-C10 were not detected in any of the mushroom samples analyzed. This might be only due to the destruction and loss of these SCFs due to the heat treatment during lipid extraction and methylation.

SCFs are a liquid at room temperature but vaporize readily at high temperatures. The other fatty acid which is not detected in any of the mushroom samples analyzed was docosapentaenoic acid (C22:5) which commonly called clupanodonic acid. This implies mushrooms are not a good source of clupanodonic acid and we should obtain it from other diet. Another important finding was adrenic acid (C22:4) is only detected in *T. letestui*. It is also an interesting observation that odd carbon number fatty acids such as pentadecanoic (C15:0) and heptadecanoic acid (C17:0) were observed in all the nine mushrooms. This observation is similar to the reports of Stancher et al., [7] and Kavishree et al., [10].

However, the other important fatty acids α -linolenic acid (C18:3n3) and γ -linolenic acid (C18:3n6) amount was very low as compared to oleic and linoleic acid. Reports by Yilmaz et al., [8] and Kalač [17] suggested similar findings. It is known that linoleic acid is the precursor of 1-octen-3-ol, known as the alcohol of fungi, which is the principal aromatic compound in most fungi and might contribute to mushroom flavour [18]. The occurrence of Tran's fatty acids in mushrooms have not been reported and it is not expected Kalač [17]. Table 2 summarizes the proportion of saturated, monounsaturated and polyunsaturated fatty acids in the nine mushroom samples analyzed. It can be see that in all the mushrooms the unsaturated fatty acids were of higher concentration those saturated fats.

This is further verified by calculating the ratio of unsaturated: saturated fatty acids, which all are greater than one. This is consistent with the observation that, in mushrooms, unsaturated fatty acids Citation: Woldegiorgis AZ, Abate D, Haki GD, Ziegler GR, Harvatine KJ (2015) Fatty Acid Profile of Wild and Cultivated Edible Mushrooms Collected from Ethiopia. J Nutr Food Sci 5: 360. doi:10.4172/2155-9600.1000360

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No.	Fatty acid	P. ostreatus	L. eddoes	A. bispours	A. campestris	L.sulphureus	T. clypeatus	T. microcarpus	T. letestui	T.spps
1	C12:0	1.65 ± 0.10e	2.40 ± 0.58e	1.99 ± 0.21e	14.3 ± 0.59b	3.18 ± 0.13e	7.08 ± 0.12d	20.8 ± 1.69a	11.8 ± 0.89c	2.47 ± 0.18e
2	C14:0	4.45 ± 0.07d,e	3.27 ± 0.12f	14.4 ± 1.23c	5.39 ± 0.02d,e	6.30 ± 0.14d	15.9 ± 0.26b,c	17.3 ± 0.6b	15.9 ± 0.53b,c	66.9 ± 2.11a
3	C14:1	1.77 ± 0.15e	ND f	ND f	ND f	ND f	2.70 ± 0.14d	4.08 ± 0.09c	8.03 ± 0.40b	9.31 ± 0.54a
4	C15:0	46.8 ± 0.15a	25.0 ± 0.46b	16.5 ± 1.33c	12.1 ± 0.07d	15.4 ± 0.12c	13.0 ± 0.21d	16.7 ± 0.51c	17.1 ± 0.16c	24.7 ± 0.77b
5	C16:0	310.1 ± 0.85f	223.7 ± 4.34g	472.8 ± 40.8e	439.1 ± 0.96e	189.9 ±1.36g	537.4 ± 4.85d	1081.5 ± 32.3a	852.6 ± 14.1b	649.4 ± 20.9c
6	C16:1	7.15 ± 0.85e,f	4.67 ± 0.04f	7.18 ± 0.59e,f	9.16 ± 0.18d,e	11.0 ± 0.29d	29.7 ± 0.55b	57.9 ± 2.43a	16.9 ± 0.47c	15.2 ± 0.45c
7	C17:0	4.66 ± 0.30e	2.40 ± 0.04f	14.3 ± 1.17c	18.2 ± 0.05b	17.2 ± 0.16b	11.0 ± 0.15d	21.2 ± 0.59a	14.9 ± 0.31c	20.4 ± 0.57a
8	C18:0	38.8 ± 0.11f	13.5 ± 0.22g	132.5 ± 10.9d	109.9 ± 0.19e	32.0 ± 0.24f	113.9 ± 0.72e	374.1 ± 11.2a	267.6 ± 4.18b	169.7 ± 5.55c
9	C18:1n9c	323.4 ± 1.00e	43.8 ± 0.68f	45.3 ± 3.79f	94.1 ± 0.19f	337.8 ± 1.94e	434.6 ± 3.21d	1558.8 ± 42.7a	783.9 ± 13.7b	670.5 ± 22.1c
10	C18:1n11c	2.88 ± 0.08g	9.32 ± 0.22f	9.07 ± 0.79f	18.5 ± 0.09e	7.32 ± 0.05f	74.2 ± 0.97a	59.6 ± 1.85c	41.5 ± 0.69d	66.5 ± 2.18b
11	C18:2	1663.2 ± 7.87e	1044.5 ± 17.8f	2759.4 ± 235.5a	2370.1 ± 4.69b	673.7 ±4.44g	1625.3 ±20.8e	1831.7 ± 49.6d,e	2222.8 ± 36.3b,c	2056.5 ± 64.3b,c
12	C18:3n6	ND g	5.59 ± 0.11a	1.75 ± 0.12c,d	1.87 ± 0.21c	1.02 ± 0.11e,f	1.41 ± 0.09d,e	0.89 ± 0.05f	3.64 ± 0.22b	1.77 ± 0.15c,d
13	C18:3n3	2.76 ± 0.02d	1.06 ± 0.07f	3.43 ± 0.17c	4.45 ± 0.09b	10.5 ± 0.26a	2.33 ± 0.11e	3.43 ± 0.03c	3.59 ± 0.13c	3.33 ± 0.16c
14	C20:0	1.89 ± 0.09f	0.95 ± 0.02f	60.8 ± 4.96a	31.2 ± 0.09b	1.40 ± 0.02f	5.32 ± 0.14f	13.7 ± 0.40c	12.7 ± 1.24c,d	7.75 ± 0.29d,e
15	C20:1	3.63 ± 0.23c	ND e	4.46 ± 0.11b	2.46 ± 0.02d	2.44 ± 0.29d	2.67 ± 0.04d	8.59 ± 0.53a	4.71 ± 0.09b	3.17 ± 0.13c,d
16	C20:2	5.27 ± 0.42b,c	1.37 ± 0.14e	4.80 ± 0.37c	5.67 ± 0.04b,c	2.91 ± 1.07d	5.29 ± 0.08b,c	10.9 ± 0.28a	6.37 ± 0.21b	2.51 ± 0.10d,e
18	C20:3	1.24 ± 0.43d	ND e	1.06 ± 0.08d	5.59 ± 0.08b	5.87 ± 0.39b	12.2 ± 0.07a	3.85 ± 0.20c	3.36 ± 0.12c	3.92 ± 0.32c
19	C20:4	ND c	ND c	0.84 ± 0.07b	1.08 ± 0.11b	ND c	ND c	ND c	2.31 ± 0.23a	ND c
20	C20:5n-3	4.13 ± 0.18b	3.06 ± 0.19b,c,d	3.52 ± 0.12b,c	2.59 ± 0.15c,d	4.17 ± 0.19b	6.77 ± 0.49a	7.30 ± 0.39a	6.17 ± 1.15a	1.74 ± 0.10d
21	C22:4	ND b	ND b	ND b	ND b	ND b	ND b	ND b	1.49 0.10a	ND b
22	C22:5	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	C22:6	ND c	ND c	ND c	ND c	ND c	1.48 ± 0.03b	3.38 ± 0.29a	ND c	ND c
24	C24:0	12.3 ± 0.39f	9.98 ± 0.19f	23.2 ± 1.72e	37.4 ± 0.11c	11.6 ± 0.09f	21.6 ± 0.17e	55.1 ± 1.89e	33.7 ± 0.43d	40.6 ± 1.44b
25	C24:1	17.4 ± 0.08a	ND e	ND e	3.31 ± 0.18c	ND e	ND e	4.19 ± 0.12b	ND e	1.90 ± 0.06d

Means followed by different alphabets in the same column are significantly different (p<0.05)

Data are mean ± standard error of three parallel measurements (n=3)

Table 1: Fatty acid profile (mg/100 g) of wild and cultivated edible mushrooms of Ethiopia.

No.	P. ostreatus	L. eddoes	A. bispours	A. campestris	L. sulphureus	T. clypeatus	T. microcarpus	T. letestui	T. spps
Unknown FA	268.6 ± 10.8d	354.4 ± 24.8c	335.7 ± 6.30c	514.5 ± 32.9a	184.6 ± 5.89e	359.8 ± 2.64c	445.6 ± 18.3b	3495 15.5c	355.9 ± 16.6c
Total FA	2722.2 ± 19.8f	1749.1 ± 41.6g	3913.0 ± 309.1c,d	3701.0 ± 30.1d	1518.4 ± 13.3g	3283.7 ± 35.1e	5600.6 ± 163.2a	4680.7 ± 88.7b	4174.1 ± 134.3c
Total SFA	420.8 ± 1.60e	281.3 ± 5.53e	736.5 ± 62.3d	667.6 ± 0.81d	277.1 ± 1.77e	725.2 ± 6.53d	1600.4 ± 48.6a	1226.4 ± 20.9b	981.9 ± 31.8c
Total MUFA	356.3 ± 1.93e	57.8 ± 0.85g	66.0 ± 5.14g	127.5 ± 0.54f	358.6 ± 1.93e	543.9 ± 4.87d	1693.1 ± 47.4a	855.1 ± 15.3b	766.5 ± 25.3c
Total PUFA	1676.6 ± 7.75e	1055.6 ± 17.8f	2774.8 ± 236.2a	2391.4 ± 4.41b	698.2 ± 4.85g	1654.8 ± 21.1e	1861.5 ± 50.7d,e	2249.7 ± 38.1b,c	2069.8 ± 64.9c,d
UFA: SFA	4.83 ± 0.00a	3.96 ± 0.01b	3.86 ± 0.00c	3.77 ± 0.01e	3.81 ± 0.01d	3.03 ± 0.01f	2.22 ± 0.01i	2.53 ± 0.00h	2.89 ± 0.00g
Linoleic: Oleic	5.14 ± 0.01d	23.9 ± 0.16c	60.9 ± 0.19a	25.2 ± 0.06b	1.99 ± 0.00g	3.74 ± 0.02e	1.18 ± 0.00h	2.84 ± 0.01f	3.07 ± 0.01f

Means followed by different alphabets in the same column are significantly different (p<0.05)

Data are mean ± standard error of three parallel measurements (n=3)

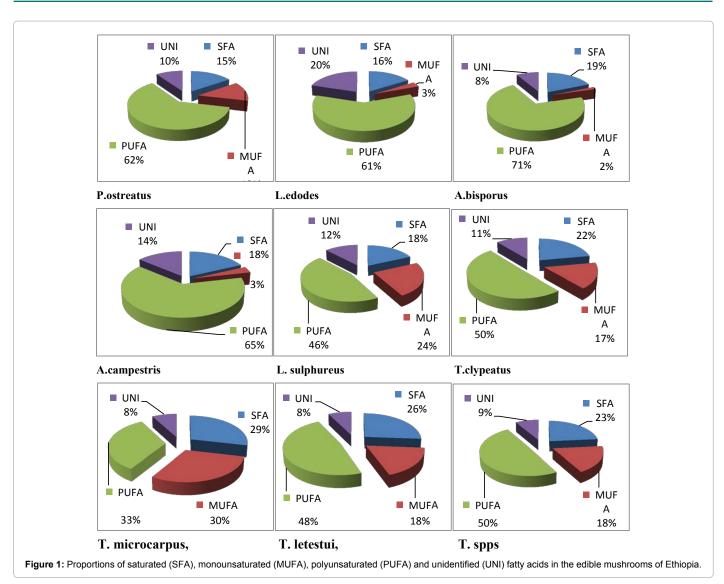
Table 2: Proportion of saturated, monounsaturated and polysaturated fatty acids (mg/100 g).

predominate over the saturated, in the total fatty acid content [19-21]. Considering total PUFA, *A. bisporus#2* had the highest value (2774.8 mg/100 g) or 71% of the total fat due to the high contribution of linoleic acid [22,23] (Figure 1).

Conclusions

Even though all the edible mushrooms evaluated in this study are generally low in lipids, their fat quality is good, mostly consisting Citation: Woldegiorgis AZ, Abate D, Haki GD, Ziegler GR, Harvatine KJ (2015) Fatty Acid Profile of Wild and Cultivated Edible Mushrooms Collected from Ethiopia. J Nutr Food Sci 5: 360. doi:10.4172/2155-9600.1000360

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of unsaturated fatty acids. Within the fatty acid composition the polyunsaturated linoleic acid (C18:2n6) was the most dominant. This fatty acid composition has beneficial effects on blood lipid profiles. Substitution of saturated fatty acids with monounsaturated leads to increased high density lipoprotein cholesterol and decrease low-density lipoproteins cholesterol. In fact, the inclusion of edible mushrooms in a natural hypercholesterolemic and antisclerotic diet has been used. Moreover, the simultaneous fat extraction and methylation procedure evaluated for the first time for mushroom samples to quantify their fatty acids with GC-FID was found to be simple, convenient and time saving with good recovery.

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Conflict of Interest

The author has no conflict of interest relevant to this study.

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