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Amino and Fatty Acids of Wild Edible Mushrooms of the Genus Boletus

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Abstract: A comparative study on the free amino acids of 15 wild edible mushroom species belonging to the genus *Boletus* (phylum Basidiomycota) was developed. The major amino acids in the fruit bodies were arginine, alanine, glutamine, and glutamic acid. The most abundant fatty acids were oleic (9-18:1), linoleic acid (9,12-18:2), and palmitic acid (16:0), but a great variation of the ester composition from one to another one was found. Chemical constituents were characterized by GC-MS, and other chemical methods.

Keywords: *Boletus*; mushrooms; amino acids; fatty acids; GC-MS

1. Introduction

The distinctive Kingdom of Fungi attracts the attention of chemists due to a great diversity of polyunsaturated [1-4], hydroxy [5-7], halogenated [8], and other unusual acids [9,10]. Arseno [11,12] and betaine containing compounds [13,14] have also been found in wild fungi. Many biological active enzymes [15], including peroxidases [16], haloperoxidases [17], and others [18] have been isolated from different fungi and used in the chemical science and industry [19]. Mushrooms are the fungi that have been used as a food from ancient times. Many species of mushrooms are traditionally used by many Asian, and some European countries, as well as in the former USSR Republics, Canada and USA, as food and medicine [20,21]. Some mushrooms have been reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia, atherosclerosis and/or cancer [22-25]. These functional characteristics are mainly due to their chemical composition [26].

The genus *Boletus* mushrooms has a worldwide distribution comprising of 24 species [27]. *Boletus* mushrooms are valuable health foods, low in calories, lipids, and essentials fatty acids, and high in vegetable proteins, minerals and vitamins [26,28-30]. The dry extract of *Boletus scaber* demonstrated antiulcer and antitumor activities and low acute toxicity [31]. The methanol extracts from the fruiting body of the mushroom *Boletus calopus* showed free radical-scavenging activity [32].

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Extract of *Boletius scaber* was active against *Escherichia coli* [33]. Four species of the genus *Boletus* produced pyridine-3-carboxylic acid: *Boletus versipellis*, 36 (mg % of air-dry matter), *B. bovinus*, 49; *B. scaber* 63; and *B. edulis*, 71-75 [34]. High fibrinolytic activity and low thrombolytic activity was shown by *Boletus* sp. [35].

Considering the interest for wild mushrooms for human consumption and the lack of data with regard to mushroom amino and fatty acids, the objective of this study was to characterize profiles of different mushroom species from East Mediterranean area belonging to the genus *Boletus*.

2. Materials and Methods

2.1. Mushroom Material

Mediterranean mushrooms belonging to the genus *Boletus* (Table 1) were harvested during December-February 2003-2007, in the Upper Galilee, Golan Heights, and Mount Carmel areas, and also in the forest around Jerusalem (mushroom names see in footnote in Table 1). Samples of the fruit bodies (3–8 mushrooms for each species) were homogenized, extracted and lyophilized.

2.2. General extraction procedures of fatty acids

Fresh mushrooms were extracted (Soxhlet) with ethanol-water-HCl (90:10:1, v/v/v) over 24 h. The ethanolic residue was further extracted with light petroleum, and then dichloromethane. All fractions were combined, evaporated to dryness at 5 ^{o}C under reduced pressure, and then dissolved in 2 mL of a cold mixture ethanol-dichloromethane (1:1, v/v). This solution was used for separation by HP-TLC and GC-MS, and followed chemical analysis [1,11,36].

2.2. General extraction procedures of amino acids

10g of each collected mushrooms were washed, drained, sliced into 5-10 mm thickness and homogenized in high speed blender in 80% aquatic ethanol during 10 min, at 75 ^{o}C. All homogenized parts were combined and fed into the soxhlet apparatus, add 100 mL 80% aquatic ethanol, and operated within 2 h, at 75 ^{o}C. Obtained extracts were lyophilized, and used for derivatization.

2.2. Derivatization of amino acids

Derivatization of amino acids (AA) with N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) causes the simultaneous silylation of the amino- and carboxyl groups in a single step with modifications procedure described by Buch et al. [37]. Each sample was spiked with 75 μL of the internal standard working solution (1,5 μg) and evaporated to complete dryness. Then, 10 μL of dimethylformamide and 60 μL of MTBSTFA were added and the vial was sealed with a polytetrafluoroethylene-lined cap. Finally, the sample was heated at 70 ◦C for 20 min to achieve the chemical derivatization of AA and the derivatives were analyzed by GC–MS.

2.3. Gas Chromatography-Mass Spectrometry of amino acid derivatives

The analysis of AA derivatives, a Hewlett Packard 6890 (series II) gas chromatograph modified for glass-capillary work and a HP-GC mass selective detector (5973B MSD) were used. AA derivatives were separated on a 15 m DB5 capillary column (Agilent, phenyl arylene polymer, 0.25 mm ID 0.25 μm film thickness) operating with helium carrier gas (45 cm/sec) under the following temperature program: from 120 to 150 ◦C at 120 ◦C/min (5 min hold), to 240 ◦C at 7 ◦C/min and finally to 295 ◦C at 20 ◦C/min (16 min hold). The temperature of the injector, transfer line, ion source and quadrupole

filter was kept constant at 260, 300, 230 and 150 ◦C, respectively. The identification of AA derivatives was based on comparison of their MS spectra and retention times with those of authentic reference standards. Standard AA were obtained from Sigma-Aldrich.

2.5. Gas Chromatography-Mass Spectrometry of fatty acids

For analysis of fatty acid methyl esters (FAME), a Hewlett Packard 6890 (series II) gas chromatograph modified for glass-capillary work and a HP-GC mass selective detector (5973B MSD) were used. Fatty acid methyl esters were prepared and analyzed by GC fitted with serially coupled capillary columns as described [38]: the RTX 1 column (30 m, ID 0.32 mm, film thickness 0.25 µm; Restek, USA) was coupled with a second capillary column (RTX 1701, 30 m, 0.32 mm, 0.25 µm film; Restek, USA). The instrumental settings was as follows: initial temperature, 40°C; initial time, 2 min; rate, 2°C/min; final temperature, 300°C, final time, 20 min; injection port, 180°C; carrier gas, He: flow rate, 25.0 mL/min. The MS detector operated at 194°C; ionization energy, 70 eV. The scan range, 30 to 700 *m/z* at 0.9 scan per sec. Solvent delay, 9 min. FAME were identified using mass spectral libraries search (Wiley 7th, and NIST-98).

3. Results and Discussion

Separation and retention time of MTBSTFA AA derivatives are shown in Fig. 1. The major AA in the fruit bodies were arginine (133 µM/g dry wt), alanine (12.8), glutamine (7.7), and glutamic acid (4.0). Arginine was the predominant AA in all studied mushrooms representing 178.3 µM/g dry wt of the total AA content (Table 1).

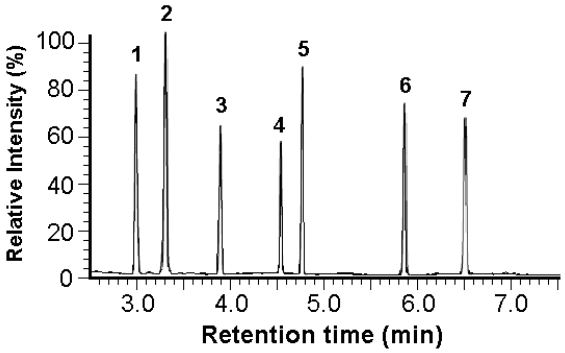

Figure 1. Chromatogram of GC–MS analysis of selected standard Amino acids.
Peaks and retention time (min): **1**, alanine (2.86); **2**, glycine (3.20); **3**, 2-aminobutyric acid (3.74); **4**, valine (4.51), **5**, norvaline (4.75); **6**, isoleucine (5.84); **7**, proline (6.49).
Amino acid derivatives not shown on GC chromatogram: **8**, methionine (10.06); **9**, serine (10.43); **10**, threonine (10.82); **11**, phenylalanine (11.83); **12**, aspartic acid (12.87); **13**, glutamic acid (14.47); **14**, asparagine (14.78); **15**, lysine (15.82); **16**, glutamine (16.31); **17**, arginine (17.11); **18**, histidine (18.06); **19**, tyrosine (18.62); and **20**, tryptophan (18.86)

Several publications of AA in Boletus mushrooms were published. Edible mushrooms of western Siberia: *Boletus edulis, B. sipellis, B. scaber*, and *B. variegatus*, contained 23 kinds of free AA, including dominated acids: lysine, arginine, threonine, valine, tryptophan, leuine, asparagine, and glutamine. *B. edulis* (8.8%) and *B. sipellis* (5.3%) were the richest in free AA, but the amino acid content and composition depended on environmental factors [39]. Krupa and Branstrom [40] detected of free and bound AA in the mushroom *Boletus variegatus* of aseptically grown *Pinus sylvestris* seedlings. Arginine was the major AA constituent in the mycelium of *B. variegatus* (18-22%) during the exponential phase of growth. 23 free AA (aspartic acid, glutamic acid, asparagine, glutamine,

serine, threonine, glycine, alanine, valine, proline, arginine, isoleucine, leucine, tryptophan, phenylalanine, cysteine, ornithine, lysine, histidine, and tyrosine) were detected in *Boletus edulis* by HPLC-UV [41].

Table 1. Detected free amino acids of the genus Boletus (μM/g dry wt)

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I	13.9	t	2.3	4.2	t	0.8	0.6	t	3.0	3.4	2.1	0.8	0.8	t	2.2	4.1	226.7	t	0.8	0.7
II	4.6	1.8	1.1	0.7	2.3	1.2	1.1	3.6	2.2	2.7	t	t	5.4	t	1.9	3.2	98.6	0.8	t	t
III	11.2	3.2	4.2	2.2	t	7.3	1.6	t	0.9	3.8	t	1.2	3.8	2.2	3.4	17.1	114.6	0.5	06	t
IV	9.2	t	0.9	5.8	t	2.3	0.8	t	t	5.1	0.9	0.9	2.2	1.1	5.2	10.4	58.4	1.9	1.3	t
V	20.2	t	0.8	2.3	3.1	3.1	0.6	0.9	0.6	1.4	t	2.1	1.7	0.9	1.1	5.6	69.4	2.4	1.7	0.9
VI	22.3	4.2	5.3	0.9	0.8	0.9	0.9	1.5	0.6	0.9	0.8	t	3.3	t	0.9	7.8	121.5	0.5	0.8	t
VII	13.2	1.6	6.2	3.2	0.7	1.1	0.7	t	t	0.7	t	0.9	8.1	0.7	6.2	2.9	137.8	t	t	0.8
VIII	9.9	t	1.4	2.0	1.2	1.4	1.2	t	t	1.3	t	0.6	5.4	0.8	0.8	12.6	144.1	2.1	1.4	0.6
IX	8.2	t	0.7	1.5	t	2.6	0.9	2.2	2.2	1.7	t	t	3.2	t	0.7	9.7	96.9	0.5	0.6	t
X	18.3	2.2	0.6	1.8	t	3.2	1.4	t	1.6	3.6	1.2	0.8	6.1	t	3.6	4.8	78.0	0.7	t	t
XI	11.9	1.6	3.8	3.5	t	5.2	2.3	t	1.1	2.4	t	0.9	4.8	1.4	5.1	8.3	88.9	t	0.7	t
XII	17.6	1.2	2.9	1.2	1.1	2.1	3.2	1.4	2.7	3.1	t	1.3	3.2	1.2	0.6	11.7	130.2	t	t	t
XIII	6.2	t	3.3	2.8	t	0.8	1.3	t	0.8	1.8	0.8	1.1	3.8	0.6	3.2	2.4	231.1	2.3	1.8	0.8
XIV	16.5	t	2.1	1.2	2.3	0.7	0.9	t	0.8	1.9	0.6	0.9	1.7	0.8	2.9	8.9	195.2	0.6	0.9	t
XV	9.3	2.3	0.8	2.2	0.9	1.7	1.2	t	t	4.2	t	0.7	6.2	t	4.4	6.7	204.3	t	t	t
Σ	**12.8**	**1.2**	**2.4**	**2.4**	**0.8**	**2.3**	**1.2**	**0.6**	**1.1**	**2.5**	**t**	**0.8**	**4.0**	**0.6**	**2.8**	**7.7**	**133.1**	**0.8**	**1.2**	**t**

Species of the genus *Boletus*: I. *B. aestivalis* (=*B. reticulates* Jacob Schaeffer: Fries), **A**; II. *B. aereus* Fries, **A,D**; III. *B. appendiculatus* Schaeff., **A**; IV. *B. badius* (Fr.) Fr., **A**; V. *B. crocipodius* Letell., **B**; VI. *B. edulis* Fries, **C**; VII. *B. granulatus* L., **A**; VIII. *B. impolitus* Fr. (syn. *Xerocomus impolitus* (Fr.), **B**; IX. *B. luridus* (syn. *B. rubeolarius*), **A**; X. *B. luteus* (L. Fries Gray) (syn *Suillus luteus*), **A**; XI. *B. pinicola* Pilat & Dermak, **A**; XII. *Boletus* sp., **C, D**; XIII. *B. queletii* Schulzer, **C**; XIV. *B. scaber* Bull. Fr. (syn *Leccinum scabrum* (Bull. : Fr.) S.F. Gray), **A**; XV. *B. versipellis* Fr. & Hök (syn *Leccinum versipelle* (Fr. & Hök) Snell), **C.** Mushrooms were sampled: **A**, Upper Galilee area; **B**, Golan Heights area; **C**, Mount Carmel area; **D**, Jerusalem area

The major AA in the fruit bodies of *Boletus edulis* were glutamine (26.0%, of dry wt), alanine (24.9), glycine (6.6), serine (5.8%), and proline (4.0). Wild edible mushroom *Boletus frostii* from Queretaro (Mexico) contain as main free AA glutamine (6.9 mg/g dry wt), orthinine (3.1), glycine (3.0) and lysine (2.5) [42]. Among sixteen AA from Tanzanian wild mushroom species *Boletus pruinatus* (collected in Kwamngumi forest reserve in the East Usambara Mountains, and at Ununio on the outskirts of Dar es Salaam city), as major were detected glutamic acid (15.4% of dry wt), glutamine (11.9), alanine (11.5), leucine (8.4), serine (7.4), glycine (6.1), and valine (6.0) [43].

Table 2. Main fatty acids of mushrooms of the genus *Boletus*

Species	16:0	18:0	18:1(n-9)	18:1(n-11)	9,12-18:2	9,12,15-18:3	Other
I	8.3	3.5	19.1	3.4	57.6	1.0	7.1
II	11.1	3.0	21.7	1.6	51.7	1.2	9.7
III	9.7	2.2	28.4	1.3	50.9	2.1	5.4
IV	15.1	2.1	36.5	1.0	38.2	1.6	5.5
V	17.0	2.0	16.5	2.3	47.3	3.1	11.8
VI	8.9	3.0	29.4	1.4	51.7	1.2	4.4
VII	12.1	2.3	17.1	3.6	58.3	0.8	5.8
VIII	9.4	3.4	20.2	2.5	55.9	0.8	7.8
IX	10.2	2.4	24.1	3.1	53.4	0.9	5.9
X	7.6	4.1	37.8	1.0	44.1	0.7	4.7
XI	7.0	4.4	42.6	1.0	39.7	2.1	3.2
XII	11.4	2.1	35.2	1.7	39.4	0.8	9.4
XIII	13.4	1.9	17.1	2.9	52.1	2.5	10.1
XIV	9.7	3.4	31.7	1.0	45.8	3.1	5.3
XV	14.1	1.7	15.2	2.3	55.2	1.6	9.9

Mushroom species, see footnote in Table 1. Percentage of total fatty acids

FAME were identified from fifteen mushrooms by GC-MS as described by previously [8-10]. The proportion of saturated fatty acids in the examined species varied from 12 to 23% of total lipids (Table 1). The major saturated acid was 16:0. Six monoenoic acids were identified with major being oleic acid, 18:1(n-9) which varied from 15.2% in *B. versipelli* to 42.6% in *B. pinicola* (Table 2). This fatty acid is probably the most common acid among some plant oils, and the major source for human food are soybean, olive, rapeseed, lard and tallow.

Oleic acid is a major acid among monoenic acids in some mushrooms belonging to the genus *Boletus*: *B. edulis, B. erythropus, B. piperatus, B. subglabripes, B. subtomentosus* and *B. variipes* collected in the Province of Quebec (Canada) [44]. It is also a major fatty acid (38%) in *Boletus luteus* from Japan [45]. Oleic acid is a bioactive compound, and strongly inhibites the activity of human telomerase in a cell-free enzymic assay, with an IC_{50} value of 8.6 μM [46]. It was recently shown that oleic acid is an efficient inhibitor of glucosyltransferase [47]. Other monoenoic acids, 7-16:1, 9-17:1, 11-20:1 and 15-24:1 were also identified in some species. Hexadecadienoic acid (7,10-16:2), linoleic acid (9,12-18:2) and α-linolenic acid (ALA, 9,12,15-18:3) were identified among polyenoic acids. Significantly higher proportions of linoleic acid were observed in all examined species with maximum 58.3% in *B. granulatas*. The ALA content varied from 0.8 to 3.1%.

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