

**FATTY AND AMINO ACIDS COMPOSITION OF SELECTED WILD
EDIBLE MUSHROOMS OF BUNYORO SUB-REGION, UGANDA****Nakalembe I^{1*} and JD Kabasa¹****Immaculate Nakalembe**

*Corresponding author email: immynakalembe@vetmed.mak.ac.ug

¹College of Veterinary Medicine, Animal Resources and Biosecurity, School of Biosecurity, Biotechnical and Laboratory Sciences, Department of Biomolecular Resources and Biolab Sciences, Makerere University, P.O. Box 7062, Kampala-Uganda.

ABSTRACT

For thousands of years, mushrooms have long been used for their health promoting properties. The aim of this study was to determine the fatty acids and amino acids contents in priority wild mushrooms: *Termitomyces microcarpus*, *Termitomyces* sp. (Bunyanaka), *Termitomyces globulus*, *Termitomyces eurrhizus* and *Polyporus tenuiculus* widely consumed in Bunyoro Sub-region, Uganda. The study revealed seventeen amino acids using pre-column derivatization with a phenylisothiocyanate followed by HPLC-UV-vis, and tryptophan using spectrophotometry. Fatty acids were determined by gas chromatography and detected by a flame ion detector. The essential amino acids ranged from 23.6% *P. tenuiculus* to 59.0% *T. microcarpus* of the total amino acids. *Termitomyces* sp. (Bunyanaka) and *T. globulus* were the most nutritious species based on essential amino acid indices. Generally, glutamic acid was high followed by phenylalanine, histidine, tryptophan and lysine concentrations, whereas threonine, valine, methionine, leucine, cysteine, arginine and tyrosine contents were either low or absent. *T. eurrhizus* and *T. globulus* were limited in leucine and valine, *T. microcarpus* in leucine and *Termitomyces* sp. (Bunyanaka) in isoleucine and valine. Unsaturated fatty acids predominated over saturated ones. *Termitomyces* sp. (Bunyanaka) and *T. globulus* had poor fatty acid profiles, rich in monounsaturated fatty acids (nervonic and erucic acids), had low PUFA:MUFA ratio and a PUFA+MUFA:SFA ratio greater than two. Docosahexaenoic (DHA) and a trans-fatty acid, elaidic were also identified in these mushroom species. This represents the first report on the occurrence of DHA in mushrooms. *T. microcarpus* had a fairly good profile, high in palmitoleic, λ -linolenic, α -linolenic, palmitic and oleic acids. Stearic DHA, linoleic and eicosapentaenic acids were detected in small quantities. The odd carbon number fatty acids (heptadecanoic, heneicosanoic and cis-10-heptadecanoic) were also identified. These results serve as a basis to encourage the local communities to exploit the nutritive potentials of these mushrooms in order to reduce the burden of nutritional deficiencies.

Key words: Amino acids, fatty acids, mushrooms

INTRODUCTION

Mushrooms have been recognized from time immemorial. Nowadays, edible mushrooms are eaten by people for their flavor, texture as well as for the health benefits. Some mushrooms are rich in minerals especially K, P, Ca, Mg, Mn and Se; vitamins most importantly D and B; and dietary fiber [1]. They are good sources of quality protein containing all the essential amino acids needed in the human diet [2, 3]. Mushrooms are extremely low in calories and cholesterol and contain low fat (high in unsaturated fatty acids) content and digestible carbohydrates [4], which are good characteristics of an ideal food for obese persons and for diabetic prevention. Some mushrooms are reportedly used as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia, atherosclerosis and/or cancer [5, 6].

Africa has a high diversity of wild mushroom biota that is poorly researched and documented [7]. The literature on East African mushrooms is limited, consisting of only a handful of published studies, mostly ethnomycological in scope [7]. Many Ugandan communities collect mushrooms during the rainy season for consumption. These wild mushrooms constitute a traditionally important nutritious food besides having broad ritual and medicinal acceptance [8, 9]. Mushrooms contribute to the welfare of some households when they are sold to earn additional income [9]. There is, therefore, a need to assess these natural resources as food basing on their chemical analysis, which has not been studied, explored and documented. In the absence of these scientific data, the contribution of Ugandan mushrooms to the overall nutritive value of the diet will remain speculative. In this study, there was determination of the amino acid profiles of five, and fatty acid profiles of three priority edible wild mushrooms species consumed in Bunyoro sub-region, Uganda. The results provide a preliminary guide on the relative nutritive potential of these natural resources.

MATERIALS AND METHODS

Mushroom collection, identification and handling

Five priority mushroom species: *Termitomyces microcarpus*, *Termitomyces* sp. (Obunyanaka), *Termitomyces globulus*, *Termitomyces eurrhizus* and *Polyporus tenuiculus* collected from Bunyoro Sub-region, Uganda, between March and June, 2009. Major activities of identification were done by Dr. Ipulet Perpetua, Department of Botany, Makerere University. Herbarium specimen were prepared and deposited in the Makerere University Herbarium. The mushrooms including the cap and stalk were included. Fruit bodies were cleaned by gently wiping with cloth to remove soil debris. The wet weights of the mushrooms were recorded and then air-dried under the shade. Before analyses, the mushrooms were dried to constant weight in an oven (GallenKamp Sanyo OMT oven, UK) at 40°C for about 12 hours. All ground samples were transferred to airtight plastic bottles with well fitting caps, labeled and sealed in polythene bags to prevent further absorption of moisture and stored at 4°C till required for analysis. The cold samples were allowed to attain room temperature and mixed thoroughly with a spatula before analyses.

Amino acid determination

Procedure

Amino acid determination was carried out following an established high performance liquid chromatography protocol at Mubarak City for Scientific Research and Technology Applications, New Borg El-Arab, Egypt. Briefly, twenty grams (20.0g) of finely ground mushrooms were homogenized with 100ml of 70% (v/v) ethanol in water, centrifuged and the homogenate dried. From this, 1.0 g of the dried residues was hydrolyzed under nitrogen gas with 25 ml of 6 N HCl in an autoclave set at 110 °C for 24 h. The hydrolysates were thereafter neutralized to pH 7.00 by sodium citrate buffer. A separate portion of the samples was oxidized with performic acid and then hydrolyzed with 6 N HCl in order to obtain reliable cysteine and methionine values [10]. The hydrolysates were filtered through a 0.45µm cellulose acetate membrane filter before injection into the HPLC.

The samples were initially subjected to automatic pre-column derivatization with a phenylisothiocyanate (PITC) [11]. The amino acids derivatives were determined by the Agilent HPLC system (Agilent 1100 Series, American), with autosampler and a Hypersil, octadecylsilane (ODs) column (C18, 250 x 4.6 mm x 5µm). The amino acid detection was done under ultraviolet light (Hewlett-Packard, Agilent Technologies 1990-2002).

The mobile phase program consisted of 2 min run with 100% A (0.3N Na acetate and 0.5N tetrahydrofuran) followed by a linear gradient of 9 min of 70% A:30% B (0.1N Na acetate and acetonitrile) at a flow rate of 0.45 ml/min. A second linear gradient from 70% A: 30% B to 50% A: 50% B was then run over the next 4 min at a rate of 0.8ml/min, followed by a third linear gradient to 100% B for 5 min at a rate of 0.45ml/min, then 100% A for 5 min and finally 100% B was run over the next 8 min at the rate of 0.45 ml/min. At 33 min, the column was equilibrated for 5 min to the initial conditions for another sample. The chromatographic column temperature was set at 40°C and the UV detector set at 262 nm. The injection volume of the samples was 10µl and the elution time was set to run for 30 min. The concentrations of the amino acids were integrated on an "Autolab Computing Integrator" fixed with the HPLC.

Tryptophan was determined by spectrophotometric method [12]. Briefly, about 100mg sample from each of the finely grounded mushroom was put into a glass vial and 4 ml of papain solution added. The solutions were capped and vortexed to mix before placing them in an oven set at 65°C for 6 h. Then 1.0 ml of each hydrolysate was pipetted into a colorimetric tube containing 4.0 ml of the color development reagent (2.7 mg of FeCl₃.6H₂O, 10ml glacial acetic acid, 1 ml 30N H₂SO₄). The mixture was vortexed and left for 15 min at 65°C to allow color development. The solutions were cooled to room temperature and their absorbencies read at 545 nm in Bausch and Lomb spectronic-20. The tryptophan concentration was calculated from the standard curve (10-50µg/ml) of DL-tryptophan. The amino acids were scored using a FAO/WHO/UNU (1985) standard reference scoring pattern for pre-school child (2-5 yrs) [13].

Fatty acid determination

For fatty acid determination, only three mushroom species were available in appreciable quantities. Fatty acid determinations were determined according to Kavashree *et al.*'s procedure [14]. The fat from the mushrooms were extracted using the Soxhlet extraction procedure with petroleum ether at a boiling point of 40°C-60°C. Briefly, fatty acid methyl esters (FAMES) were prepared by direct trans esterification of the oil using 0.5N methanol/potassium hydroxide 2:1 (V/V) [15]. About 0.2 µl of the clear supernatant of FAMES was injected into the Varian Star model GC 3800 instrument equipped with a split/splitless injector, a flame ion detector and fused silica capillary column (30 m x 0.25 mm i.d. x 0.20 µm).

The initial column temperature was set at 60°C and held for 2 min, then from 150°C to 260°C at a rate of 10°C/ min for 10 min. The total run time was 59.93 min. The carrier gas (helium) flow rate was 1 ml/min, measured at 50°C and the makeup gas was nitrogen (30ml/min). Splitless injection was carried out at 250°C. The same conditions applied for both the standard FAME and the test samples. Fatty acid identification in the samples was made by comparing the relative retention times of FAME peaks in the test sample with predetermined FAME standard peaks. The relative percentages of fatty acids in the test sample were done by peak area normalization and were finally scored accordingly [16].

Statistical analysis

Differences in means were analyzed using the t-test and one way ANOVA at the 95% confidence level in combination with the least significant difference. All analyses were computed using SPSS 12.0 Windows program [17].

RESULTS

Amino acid composition

The Table 1 shows the amino acid composition of the wild mushrooms under study. *Termitomyces microcarpus* had the highest nitrogen content (and, therefore, highest total protein) than the other mushrooms. Each one of the mushrooms lacked at least one of the amino acids. The total amounts of essential amino acids were less than the non essential amino acids except in *P. tenuiculus* and *T. eurrhizus*. The essential amino acids accounted for about 54% of the total amino acids in *Termitomyces* sp., 59% in *T. microcarpus* and 53.6% in *T. globulus*, 44.7% in *P.tenuiculus*, and 23.6% in *T. eurrhizus*. Glutamic acid, phenylalanine, histidine and lysine contents were high. Threonine, valine, methionine, leucine, cysteine, arginine and tyrosine contents were either low or absent.

Table 2 shows the amino acid scores of wild edible mushrooms as calculated basing on the FAO/WHO/UNU reference pattern (1985) for pre-school child (2-5 years). Histidine and tryptophan scores were high in all mushrooms, followed by lysine and phenylalanine (or + tyrosine). Mushrooms exhibited lowest scores in valine, threonine, leucine (except in *Termitomyces* sp.), and methionine (or cysteine). Valine was identified in *T. microcarpus* and leucine in *Termitomyces* sp. *Termitomyces* sp

had the best EAAI followed by *T. globulus*. *Termitomyces eurrhizus* had the lowest EAAI.

The limiting amino acids in the analyzed mushrooms are summarized in Table 3. The mushrooms had the poorest chemical score of zero, with the first limiting amino acids in *T. eurrhizus* and *T. globulus* being leucine and valine followed by threonine. *Termitomyces microcarpus* was limited in leucine, followed by threonine (10) and methionine (or cysteine, 18), respectively. *Termitomyces* sp. was limited in isoleucine and valine followed by traces of threonine and methionine (or cysteine, 8), respectively. Leucine, threonine, and valine were the first limiting amino acids in *P. tenuiculus*, followed by methionine (or cysteine, 5) and isoleucine (23), respectively.

Fatty acid composition

The results of fatty acid composition of three mushroom species are presented in Table 4. Unsaturated fatty acids dominated over saturated ones. Likewise, monounsaturated fatty acids dominated over polyunsaturated ones.

Termitomyces sp. and *T. globulus* had a poor fatty acid profile because of dominance of MUFAs over PUFAs. There are significant differences between the sum of different groups of fatty acids ($p < 0.01$). On average, *T. microcarpus* had 78.7%, *Termitomyces* sp. 91.7%, and *T. globulus* 92.5% of unsaturated fatty acids.

The most abundant fatty acids in *Termitomyces* sp. and *T. globulus* were erucic acid (20.36%, 20.7%), nervonic acid (33.18%, 25.7%) and DHA (19.48%, 33%), respectively, followed by elaidic acid, oleic acid and palmitic acid. *Termitomyces microcarpus* contained high palmitoleic acid (27.2%), γ -linolenic acid (19.7%) and palmitic acid (12.8%), with moderate quantities of α -linolenic acid (8.8%), oleic acid (7.6%), stearic acid (4%), DHA (3%), linoleic acid (2.8), myristic acid (2.65%) and vaccenic acid (2.5). Three odd carbon number fatty acids were also identified in very low levels in this mushroom species.

Unsaturated/saturated fatty acids ratios are shown in Figure 1. PUFA + MUFA:SFA and MUFA:SFA ratios were high in *Termitomyces* sp. (11, 8.7) and *T. globulus* (11.7, 7.83). *Termitomyces microcarpus* had a PUFA + MUFA:SFA ratio of 3.68 and MUFA:SFA ratio of 1.81. Basing on the ratios above, the best mushroom in terms of fatty acid composition was *T. microcarpus* and least in *Termitomyces* sp. and *T. globulus*.

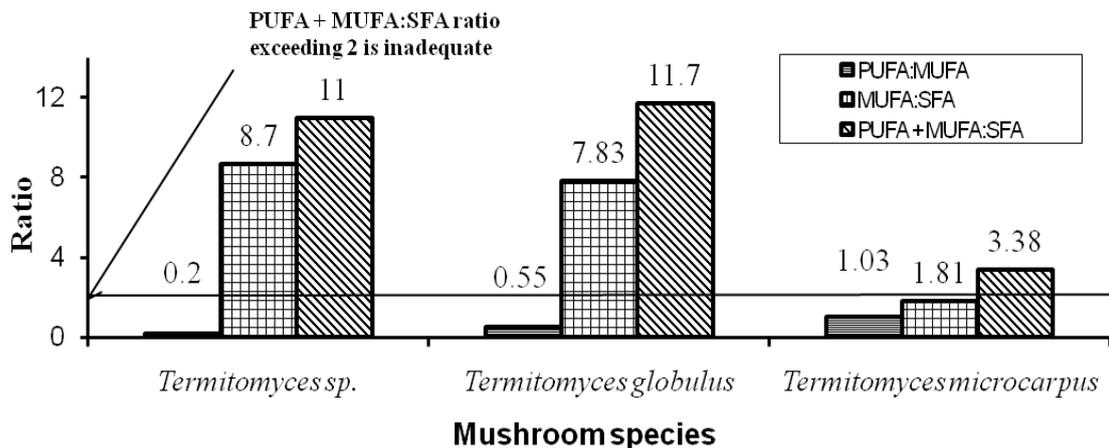


Figure 1: Unsaturated/saturated fatty acid ratios of wild mushrooms. High quality fat is considered to have: high PUFA:MUFA ratio, Low MUFA:SFA ratio and PUFA + MUFA : SFA ratio < 2.

DISCUSSION

Every mushroom lacked at least one of the amino acids. Furthermore, the amino acid levels are comparatively quite low compared to other studies [18, 19]. With regard to essential amino acids, the percentage composition in the analyzed mushrooms varied between 23.6 % *T. eurhizus* to 59% *T. microcarpus*. This was in agreement with other studies [12]. Glutamic acid, phenylalanine (or tyrosine), histidine, tryptophan and lysine were the highest amino acids, which was in line with other studies (18, 19). The observed amino acid pattern in this study may have been contributed by the differences in the growth substrate, size of the pilei and species or strain of mushroom among others [20].

The essential amino acids expressed as a percentage of FAO/WHO/UNU reference pattern (1985) for pre-school child (2-5 yrs) are presented in Table 2. It is observed that the histidine, lysine, phenylalanine (or tyrosine) and tryptophan dominated over other amino acids. The mushrooms were poor in valine, threonine, leucine and methionine (or cysteine). Many reports have shown different amino acids to be deficient in different mushroom species. Mushrooms are deficient in cysteine and methionine [18, 19], isoleucine, leucine, and lysine [21], tryptophan [18] and phenylalanine [22]. On the contrary, leucine was reported as the most abundant amino acid in wild mushrooms [18]. Limiting amino acids in these mushrooms are given in Table 3.

The presence of histidine, tryptophan, phenylalanine (or tyrosine), lysine and isoleucine in mushrooms indicates that they can substitute or supplement maize and beef [23, 24]. The presence of lysine in these mushrooms makes them very important in most poor families which depend on cereals as staple foods.

In this study, unsaturated fatty acid dominated over the saturated fatty acids, consistent to other studies [23, 25, 26]. In this study the monounsaturated fatty acids

(nervonic acid and erucic acid) were high in *T. globulus* and *Termitomyces* sp. than polyunsaturated fatty acids. This resulted in a high ratio of PUFA + MUFA:SFA (>2) and MUFA:SFA (1.81-8.7) and low PUFA:MUFA (0.2-1.03) in these mushroom species, making them less desirable from a nutritional point of view. High MUFA contents are reported to raise the total cholesterol and LDL cholesterol ("bad" cholesterol) while simultaneously lowering HDL cholesterol ("good" cholesterol) levels in the body [16].

Nevertheless, nervonic acid is an important factor in the biosynthesis of nerve cell myelin [27], and is recommended as a supplement to prevent disorders such as adrenoleukodystrophy and multiple sclerosis in elderly people [28]. Oleic acid is technically non-essential because the body can produce it in limited quantities provided the essential fatty acids are in good proportions in the body. Oleic acid is known to lower blood cholesterol levels, and insulin resistance; improve immune function and aid in some cancer prevention [29, 30, 31, 32]. It also has cytotoxic effects on cancer cells [33], as well as antimicrobial properties [34]. Therefore, consumption of these mushrooms by the elderly may be crucial in the prevention of the above mentioned conditions.

Termitomyces globulus and *Termitomyces* sp. also contained an important fatty acid, docosahexaenoic acid (DHA). There has been no previous report of DHA in mushrooms and this may be the first report of this fatty acid in mushrooms. Docosahexaenoic acid is a major fatty acid in sperms and brainphospholipids [35], and especially in the retina [36]. It reduces the risk of heart disease by reducing the level of blood triglycerides in humans and its low levels cause reduction of brain serotonin levels [37]. Docosahexaenoic acid supplementation can effectively combat or prevent alzheimer's disease and depression, among other diseases [38]. Docosahexaenoic acid is naturally found primarily in fish, and animals can only produce very little on their own from alpha-linolenic acid found in plants, milk and animals [39]. The high content of DHA in *T. globulus* and *Termitomyces* sp. makes them suitable for consumption for newborns and pregnant mothers since the former depend critically on the presence of DHA for the development of their brains and nerves [40]. However, the high content erucic acid is an issue here because of its detrimental effects to the cardiac tissues [41]. In adults, erucic acid is broken down into short-chain fatty acids by the liver enzymes (long-chain acyl-coenzyme A (CoA) dehydrogenase).

T. microcarpus had relatively commendable profile due to their well-balanced fatty acid composition. The presence of the essential fatty acids γ -linolenic acid and α -linolenic acid in good proportions, in addition to relatively low levels of linoleic acid makes this mushroom species special. Several studies report high levels of linoleic acid in mushrooms [23, 25, 26]. Linoleic is known to be a precursor of 1-octen-3-ol (known as the alcohol of fungi) and a principal aromatic compound in most fungi contributing to mushroom flavor [42]. In addition to the nutritional value of linoleic acid, this acid is implicated to be a potent cytotoxic agent against HeLa cell, with IC50 = 6.5 mg/ml [33], and antibacterial activity [43].

CONCLUSION

The results of this study indicate the potential of these mushroom species as source of essential amino acids and fatty acids for infants and the elderly. The importance of erucic acid in these mushrooms may have little healthy impact to humans since the mushroom fat content is appreciably low. These results serve as a basis to encourage the local communities to exploit the nutritive potentials of these mushrooms in order to reduce the burden of nutritional deficiencies. Recommendation of wider raising awareness about and encouraging the consumption of these wild edible mushrooms.

ACKNOWLEDGEMENT

This study was sponsored by Carnegie Corporation, Science Initiative Group through the Regional Initiative in Science and Education -African Natural products Research and Training Network (RISE-AFNNET) Program, DAAD and staff Development fund of Makerere University for which we are very grateful. Our sincere thanks go to the Uganda Industrial Research Institute, Uganda and Mubarak City for Scientific Research and Technology Applications, Egypt for support in laboratory analyses. We are very grateful to the people of Bunyoro Sub-region for providing the relevant information about the edible mushrooms.

Table 1: Amino acid composition of five selected wild mushrooms

mg/g of protein dry weight ^a					
Amino acids	<i>T. globulus</i>	<i>P. tenuiculus</i>	<i>T. microcarpus</i>	<i>Termitomyces</i> sp.	<i>T. eurhizus</i>
%Nitrogen	3.16±0.06 ^b	2.64±.32 ^a	2.97±0.06 ^b	3.33±0.16 ^b	2.91±0.07 ^b
% Protein	13.84±0.26 ^b	11.56±0.09 ^a	13.00±0.71 ^b	14.59±0.26 ^b	12.76±1.4 ^a
Tryptophan*	28.8±0.001 ^a	26.7± 1.45 ^b	21.0±0.08 ^c	23.4±0.62 ^b	25.6±0.28 ^b
Threonine*	trace	ND	6.1±0.05	Trace	trace
Isoleucine*	10.5±0.19 ^a	6.3±0.66 ^b	46.7±0.47 ^c	ND	17.8±0.34 ^d
Leucine*	ND	ND	ND	68.9±0.06	ND
Lysine*	61.2±0.28 ^a	66.8±0.15 ^a	64.5±0.48 ^a	53.8±0.08 ^a	54.9±0.1 ^a
Methionine*	3.0±0.14 ^a	1.3±0.04 ^b	3.7±0.01 ^a	2.1±0.23 ^a	3.7±0.13 ^a
Phenylalanine*	138±0.47 ^a	15.5±0.46 ^b	63.9±0.25 ^c	142±0.15 ^a	33.5±0.49 ^e
Valine*	ND	ND	33.7±0.01	ND	ND
Histidine*	85.9±0.79 ^a	33.2±0.13 ^b	83.1±0.07 ^a	87.6±0.63 ^a	63.9±1.46 ^c
Cysteine	ND	ND	4.8±1.75	ND	ND
Tyrosine	ND	ND	14.1±0.11 ^a	2±0.01 ^b	ND
Arginine	7.2±1.15 ^a	4.3±0.68 ^b	ND	ND	7.1±0.05 ^a
Alanine	28.±0.19 ^a	2.1±0.37 ^b	14.2±0.00 ^c	1.3±0.05 ^b	trace
Aspartic acid	ND	3.94±1.1 ^a	30.9±0.001 ^a	32.9±0.04 ^a	131.8±2.01 ^b
Glutamic acid	171.1±0.3 ^a	12.52±0.7 ^b	45.2±1.24 ^c	130.6±0.05 ^b	494.5±0.01 ^d
Glycine	ND	4.3±0.57 ^a	12.2±0.32 ^b	ND	ND
Proline	63±0.62 ^a	7.7±0.28 ^b	66±0.08 ^a	29±0.2 ^c	6.7±0.91 ^b
Serine	14.6±1.1 ^a	2.1±0.37 ^b	69±0.77 ^c	118±0.51 ^d	6.8±0.23 ^e
Total amino acids	611.3 ^c	334.9 ^a	579.1 ^b	691.6 ^d	846.4 ^e
% Essential amino acids	53.6 ^a	44.9 ^b	59 ^c	54.9 ^a	23.6 ^d

*Values along the row with different letters are significantly different at p<0.05.

^aCalculated using conversion factor of (N x 4.38); a correlation factor adopted for mushrooms in food composition tables (Crisan and Sands, 1978). Trace = quantities below 0.01mg/g, ND= Not detected

Table 2: Essential amino acid scores in wild mushrooms as percentage of FAO/WHO/UNU reference pattern (1985) for pre-school child (2-5 yrs)^b

Essential amino acid	Scoring pattern FAO/WHO/UNU pattern 1985 (mg/g protein)	Mushroom species				
		<i>T. globulus</i>	<i>T. eurrhizus</i>	<i>T. microcarpus</i>	<i>Termitomyces</i> sp.	<i>P. tenuiculus</i>
Histidine	14	614	239	593	630	456
Isoleucine	28	38	64	167	ND	23
Leucine	66	ND	ND	ND	104	ND
Lysine	58	106	95	111	93	115
Methionine (+ Cysteine)	25	12	15	34	8	5
Phenylalanine (+Tyrosine)	63	219	53	124	229	25
Threonine	34	T	T	18	T**	ND
Tryptophan	11	162	233	191	213	243
Valine	35	ND	ND	10	ND	ND
Total		1251	699	1248	1277	867
EAAI ^a		95	54.6	79.5	115	57.6

*Values above 20% indicate moderate amino acid content (rich), values between 20 and 10% indicate good level of the amino acid in question, < 10% indicate poor, trace= scores below of 0.02%. ^aEAAI are geometric means of chemical scores, T= Trace. ^bCalculated as amino acids in mushroom protein divided by amino acid in reference pattern and multiplied by 100

**Table 3: Chemical scores of wild mushrooms calculated by using
FAO/WHO/UNU reference pattern (1985) for pre-school child (2-5 yrs)**

Mushroom	Chemical score ^a	Limiting amino acids			
		First	Second	Third	
<i>T. globulus</i>	0	Leucine, Valine	Threonine (trace)	Methionine Cysteine) (12)	(+)
<i>T. eurrhizus</i>	0	Leucine, Valine	Threonine (trace)	Methionine Cysteine) (15)	(+)
<i>T. microcarpus</i>	0	Leucine	Valine (10)	Threonine (18)	
<i>Termitomyces</i> sp.	0	Isoleucine, Valine	Threonine (trace)	Methionine Cysteine) (8)	(+)
<i>P. tenuiculus</i>	0	Leucine, Threonine, Valine	Methionine (+ Cystine) (5)	Isoleucine (23)	

^aChemical score (0) indicates lack of such amino acids in the mushroom species. Values in parentheses are scores for the second and third limiting amino acid, respectively.

^aPercentage of the most limiting amino acid in protein as compared with the reference pattern

Table 4: Fatty acid content (percent) of selected wild mushrooms

Fatty acids	<i>Termitomyces</i> sp.	<i>T. globulus</i>	<i>T. microcarpus</i>
c6:0 (Caproic acid)	0.47	nd	0.08
c12:0 (Lauric acid)	nd	nd	0.43
c14:0 (Myristic acid)	nd	nd	2.65
c16:0, (Palmitic acid)	4.44	2.9	12.8
C17:0 (Heptadecanoic acid)	nd	nd	0.8
c20:0 (Arachidic acid)	nd	nd	0.24
C18:0 (Stearic acid)	nd	nd	4.0
c21 (Heneicosanoic acid)	nd	nd	0.12
c24:0 (Lignoceric acid)	3.38	4.7	nd
c16:1 (Palmitoleic acid)	nd	nd	27.2
c18:1w9cis (Oleic acid)	8.12	6.4	7.64
c18:1w9trans (Elaidic acid ^a (TFA)	10.6	6.7	nd
c18:1w7 (Vaccenic acid)	nd	nd	2.5
c22:1w9 (Erucic acid)	20.36	20.7	nd
c24:1 (Nervonic acid)	33.18	25.7	nd
C17:1(cis-10-Heptadecanoic acid)	nd	nd	0.70
c14:2 (Tetradecadienoic acid)	nd	nd	1.34
c18:2:cis (Linoleic acid)	nd	nd	2.81
c18:3:w6, γ -Linolenic acid	nd	nd	19.66
c18:3:w3, α -Linolenic acid	nd	nd	8.81
C22:5w3 (Docosapentaenoic)	nd	nd	1.83
C20:5w3 Eicosapentaenic acid (EPA)	nd	nd	1.3
c22:6w3 Docosahexaenoic acid (DHA)	19.48	33	3.0
Total saturated fatty acids (SFA)	8.29	7.6	21.4 ^a
Total monounsaturated fatty acids (MUFA)	72.17 ^a	59.5 ^b	38.8 ^c
Total polyunsaturated fatty acids (PUFA)	19.48 ^a	33 ^c	39.9 ^d

*Different letters along the rows are significantly different ($p < 0.01$) ^aTrans fatty acid, nd= not detected, C= Carbon

REFERENCES

1. **Kurtzman RH Jr** Nutrition from mushrooms, understanding and reconciling available data. *Mycoscience*. 1997; **38**: 247-253.
2. **Mattila P, Salo-Väänänen P, Könkö K, Aro H and T Jalava** Basic composition and amino acid contents of mushrooms cultivated in Finland. *J. Agric. Food Chem.* 2002; **50**: 6419-6422.
3. **Colak A, Faiz O and E Sesli** Nutritional Composition of some wild edible mushrooms. *Turk. J. Biochem.* 2009; **34**:25-31.
4. **Breene WM** Nutritional and medicinal value of specialty mushrooms. *J. Food Prod.* 1990; **53**: 883-894.
5. **Jakucs E** Therapeutic mushrooms, *Termesztet Vilaga*, 1996; **127**: 547.
6. **Tidke G and M Rai** Biotechnological potential of mushrooms: drugs and dye production. *Med. Mushr.* 2006; **8**:351-360.
7. **Chang ST and KE Mshigeni** Mushroom and their human health, their growing significance as potent dietary supplements. The University of Namibia, Windhoek, 2001.
8. **Opige M, Kateyo E, Kabasa JD and D Olila** Indigenous knowledge and usage of indigenous edible and medicinal mushrooms among the Teso people of Eastern Uganda. *J. Food Techn.* 2006; **4**: 325-330.
9. **Nakalembe I, Kabasa JD and D Olila** Indigenous knowledge and usage of wild mushrooms in Mid-Western, Uganda. *Afr. J. Anim Biomed Sci.* 2009; **4**: 63-73.
10. **Moore S** On the determination of cystine as cysteic acid. *J. Biol. Chem.* 1963; **238**: 235-237.
11. **Cohen SA and DJ Strydom** Amino acid analysis utilizing phenylisothiocyanate derivatives. *Anal. Biochem.* 1988; **174**:1-16.
12. **Nutri E, Tiessen A, Pixley K and N Paracios-Rojas** Reliable and inexpensive Colorimetric method for determining protein-bound tryptophan in maize kernels. *J. Agri. Food chem.* 2009; **57**:7233-7238.
13. **FAO.** Food and Agriculture Organization of the United Nations. FAOSTAT database. FAO, Rome, Italy, 2005.
14. **Kavishree S, Hemavathy J, Lokesh BR, Shashirekha MN and S Rajarathnam** Fat and fattyacids of Indian edible mushrooms. *Food chem.* 2008; **106**: 597-602.

15. **ISO 5509** Preparation of methyl esters of fatty acids, 2000.
16. **Chang NW and PC Huang** Effects of ratio of polyunsaturated and monounsaturated fatty acid to saturated fatty acid on rat plasma and liver lipid concentrations. *Lipids* 1998; **33**: 481-487.
17. **SPSS inc.** SPSS 21.0 for Windows, Chicago, IL, USA, 2003.
18. **Agrahar-Murugkar D and G Subbulakshmi** Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chem.* 2005; **89**: 599-603.
19. **Mdachi SJM, Nkunya MHH, Nyigo VA and IT Urasa** Amino acid composition of some Tanzanian wild mushrooms. *Food Chem.* 2004; **86**: 179-182.
20. **Chang ST** Cultivated mushrooms. **In:** Marcel D. (Eds) Handbook of Applied Mycology., New York, 1991; **3**: 221-240.
21. **Stephanie I** The real value of fungi. Online at <http://www.mushroomcouncil.com> Accessed on July, 2010.
22. **Eisenhut R, Fritz D and P Tiefel** Investigations on nutritionally valuable constituents (mineral substances, amino acids, aromatic substances) of *Hericium erinaceus* (Bull.: Fr.) Pers. *Gartenbauwissenschaft.* 1995; **60**: 212-218.
23. **Die'z VA and A Alvarez** Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. *Food Chem.* 2002; **75**: 417-422.
24. **Sindayikengera S and W-S Xia** Nutritional evaluation of caseins and whey proteins and their hydrolysates from Protamex. *J. Zhejiang Univ Sci B.* 2006; **7**: 90-98.
25. **Lumír OH, Ilia S and MD Valery** Lipids and fatty acids of wild edible mushrooms of the genus *boletus*. *J. Food Lipids.* 2008; **15**: 370-383.
26. **Saiqa S, Haq NB and AH Muhammad** Studies on chemical composition and nutritive evaluation of wild edible mushrooms. *Iran. J. Chem. Chem. Eng.* 2008; **27**:151-154.
27. **Appelqvist A** Lipids in Cruciferae. **In:** Vaughan JG and AJ Macleod (Eds). The biology and the chemistry of *cruciferae*. Academic Press, London, UK, 1976: 221-277.
28. **Sargent JR, Coupland K and R Wilson** Nervonic acid and demyelinating disease. *Med. Hypoth.* 1994; **42**: 237-242.

29. **Chan JK, Bruce VM and BE McDonald** Dietary alpha-linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. *Am J Clin Nutr.* 1991; **153**: 1230-1234.
30. **Ryan M, McInerney D, Owens D, Collins P, Johnson A and GH Tomkin** Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. *QJM.* 2000; **93**: 85-91.
31. **Carrillo C, Cavia MM and S Alonso-Torre** Role of oleic acid in immune system; mechanism of action: a review. *Nutr. Hosp.* 2012; **4**:978-990.
32. **Win TD** Oleic acid- The anti-breast cancer component in olive oil. *AU.J.T.* 2005; **9**: 75-78.
33. **Mei W, Dai H and J Xu** Composition and cytotoxic activity of the organic acids from *Gracilaria lemaneiformis*. *Zhongguo. HaiyangYaowu.* 2006; **25**: 45-47.
34. **Seidel V and PW Taylor** *In vitro* activity of extracts and constituents of *Pelagonium* against rapidly growing mycobacteria. *Intl. J. Antimicrob. Agent.* 2004; **23**:613-619.
35. **O'Brien JS and L Sampson** Fatty acid and fatty aldehyde composition of the major brain lipids in normal human gray matter, white matter, and myelin. *J. Lipid Res.* 1965; **6**: 545-551.
36. **SanGiovanni JP and EY Chew** The role of omega-3 long chain polyunsaturated fatty acids in health and disease of the retina. *Progr. Retin. Eye Res.* 2005; **24**: 87-138.
37. **Levant B, Ozias MK, Davis PF, Winter M, Russell KL, Carlson SE, Reed GA and KE McCaaron** Decreased brain docosahexaenoic acid content produces neurobiological effects associated with depression: Interactions with reproductive status in female rats. *Psychoneuroendocrin.* 2008; **33**:1279-92.
38. **Quinn JF, Raman R, Thomas GR, Ernstrom K, Yurko-Mauro K, Nelson EB, Shinto L, Nair AK and P Aisen** A clinical trial of docosahexanoic acid (DHA) for the treatment of Alzheimer's disease. Therapeutic and therapeutic strategies: Clinical trial results. Alzheimer's Association, International Conference on Alzheimer's Disease. 2009.
39. **Burdge GC and PC Calder** Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.* 2005; **45**: 581-597.

40. **Birch EE, Garfield S, Hoffman DR, Uauy R and DG Birch** A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. *Dev. Med. Child Neurol.* 2000; **42**: 174-181.
41. **FSANZ** Erucic acid in food. A technical review and risk assessment. Technical report series No. 21. 2003: 4.
42. **Maga JA** Mushroom flavor. *J. Agric. Food Chem.* 1981; **29**: 1-4.
43. **Lee JY, Kim YS and DH Shin** Antimicrobial effects of linoleic acid and monoglyceride against *Bacillus cereus* and *Staphylococcus aureus*. *J. Agric. Food chem.* 2002; **50**: 2193-2199.