Proximate Chemical Composition, Free Amino Acid Contents, and Free Fatty Acid Contents of Some Wild Edible Mushrooms from Querétaro, México

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Some wild edible mushrooms, *Amanita rubescens* (Ar), *Boletus frostii* (Bf), *Lactarius indigo* (Li), and *Ramaria flava* (Rf), were analyzed to determine their proximate analysis and free amino acid and free fatty acid contents. The proximate composition was determined by AOAC and AACC methods. Total free amino acids were extracted (methanol/chloroform/water) at room temperature, purified on a cation resin, and derivatized with trifluoracetic anhydride. Free fatty acids were obtained by a Soxtec extraction with chloroform/methanol (2:1) and derivatized to their methyl ester form. The identification and quantitation of all compounds were performed by gas chromatography−mass spectrometry. The protein contents of the analyzed mushrooms (17.5, 15.8, 13.2, and 14.5%, respectively) were more significant than wheat (13.2%). The total free amino acid range of all analyzed edible mushrooms was 23.17−47.41 mg/g. *A. rubescens* had the largest amounts of glutamic acid, lysine, and alanine (17.53, 6.95, and 2.79 mg/g, respectively). The total free fatty acid composition (32.96−109.69 mg/g) were significantly different among all species, and on a quantitative basis, they were predominantly unsaturated in nature. *A. rubescens* presented the highest levels of C18:1 and C18:2, which were 69.3 and 21.7 mg/g, respectively, on a dry weight basis, followed by *B. frostii*.

Keywords: *Free amino acid; free fatty acid; wild edible mushroom; Amanita rubescens; Boletus frostii; Lactarius indigo; Ramaria flava; GC−MS*

INTRODUCTION

The socioeconomical and political crisis that México has lived during the past decade has caused a shortage of foods, mainly of dietary protein in small villages. Therefore, new dietary alternatives, such as mushrooms, seaweed, soybeans, among others, have been necessary.

In many parts of México, wild edible mushrooms have been part of the human diet for a long time. Mushrooms are very important nutritionally since most of them have a very high protein content (19−35%), in comparison to wheat (13.3%) and to milk (25.2%) (Martínez-Carrera and Larqué-Saavedra, 1990); besides, they are low-calorie foods and their fat fraction is mainly composed of unsaturated fatty acids, corresponding to ∼4.0% on dry weight basis. Furthermore, mushrooms contribute vitamins such as C and B (B_1, B_2, B_{12}, and niacin).

The quality, quantity, and *in vivo* availability of proteins (free and essential amino acids) play an important role in the assessment of the nutritional quality of mushrooms. Therefore, the determination of free amino acid profiles of mushrooms might be of great value from a nutritional, chemical, and biochemical point of view. It is also known that free amino acids such as glutamic and aspartic play an important role in the overall taste of food.

Relatively high concentration of unsaturated fatty acids, particularly linoleic acid, are relevant also from the nutritional standpoint (Bano and Rajarathnam, 1988). In addition, this fatty acid is a prostaglandin hormone precursor.

The wild edible mushrooms analyzed in this study belong to the *Basidiomycetes mycorrhizices*. At the present time, these mushrooms are only consumed in rural areas in Querétaro state in México.

The aims of this work were to determine the proximate composition, the type and amount of free amino acids and total free fatty acids in lyophilized wild edible mushrooms (*Amanita rubescens* (Ar), *Boletus frostii* (Bf), *Lactarius indigo* (Li), and *Ramaria flava* (Rf)) from Laguna de Servín (Amealco, Querétaro) by gas chromatography−mass spectrometry (GC−MS).

MATERIALS AND METHODS

Sample. The wild edible mushrooms were collected from Laguna de Servín in the state of Querétaro, Mexico. They were cut and frozen at −80 °C for 1 day, and after lyophilization (Virtis 5L) for 10 h, the milled samples were stored under vacuum at −20 °C. All reagents used for the extractions and derivatizations were analytical reagent grade. The helium used during the chromatographic runs was high-purity grade.

Proximate Analysis. The proximate composition of all mushrooms was determined using the official AOAC methods (1984, 1990). Moisture was removed by oven dehydration at 98 °C for 5 h (section 14.004); ash was determined by weighing the incinerated residue obtained at 600 °C after 2 h (section

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S0021-8561(97)00640-7 CCC: $14.00

Table 1. Proximate Composition[a] (mg/g) of Wild Edible Mushrooms in Percentage

analysis	*A. rubescens*	*B. frostii*	*L. indigo*	*R. flava*
moisture (fresh)	92.90 ± 1.10	94.53 ± 0.83	95.15 ± 1.16	93.13 ± 1.9
ash	6.78 ± 0.26[b]	3.23 ± 0.02[d]	4.93 ± 0.06[c]	6.90 ± 0.33[b]
dietary fiber	19.97 ± 0.03[d]	30.24 ± 0.03[b]	18.73 ± 0.03[e]	21.61 ± 0.01[c]
fat	8.31 ± 0.19[b]	3.68 ± 0.09[d]	4.25 ± 0.10[c]	2.09 ± 0.12[e]
protein	17.40 ± 0.06[b]	15.81 ± 0.06[c]	13.42 ± 0.06[e]	14.47 ± 0.04[d]

[a] Mean values and standard error of three independent determinations. [b−e] Statistically significant ($p < 0.05$; Duncan).

Table 2. Free Amino Acid Composition[a] of Three Wild Edible Mushrooms Compared with *A. bisporus* and *U. maydis* (mg/g, Dry Basis)

amino acid	*A. rubescens*	*B. frostii*	*R. flava*	*A. bisporus*[b]	*U. maydis*[c]
N-Me-Gly (1)[d]	1.57	1.42	7.18	nd[h]	nd
Ala (2)	2.79	1.04	1.96	4.20	1.31
Gly (3)	2.39	3.03	7.14	0.35	2.63
Thr[e] (4)	1.36	0.60	0.66	0.96	0.65
β-amino isobutyric (5)	1.05	0.34	0.51	nd	nd
Ser (6)	0.88	0.51	1.34	1.18	0.98
Val[e]	nd	nd	nd	1.09	1.59
Leu[e] (7)	0.92	0.42	0.68	1.78	2.00
Ile[e] (8)	0.86	0.27	0.36	1.20	1.40
GABA (9)	1.03	0.82	2.91	3.33	0.75
Asp (10)	2.77	1.21	1.63	1.33	1.83
Pro (11)	1.19	0.57	1.02	3.45	0.66
Glu (12)	17.53	6.92	9.42	4.71	1.79
Met[e]	nd	nd	nd	nd	0.06
Phe[e] (13)	2.23	0.46	0.96	0.76	1.06
Orn (14)	2.38	3.10	5.70	nd	0.08
Lys[e] (15)	6.95	2.46	5.94	nd	2.97
Tyr	nd	nd	nd	nd	0.80
tricholomic acid	nd	nd	nd	nd	0.36
total	45.90 ± 1.73[f]	23.17 ± 0.73[g]	47.41 ± 1.40[f]	24.34	20.92
% essential	26.84 ± 0.08[f]	18.17 ± 1.01[g]	18.14 ± 0.73[g]	23.79	46.51

[a] Mean values and standard error of the sum of three independent determinations. [b] Dijkstra and Wikén (1976). [c] Lizárraga-Guerra and López (1996). [d] Peak number in chromatogram (Figure 1). [e] Essential amino acids. [f−g] Statistically significant ($p < 0.05$ Duncan). [h] nd, not detected.

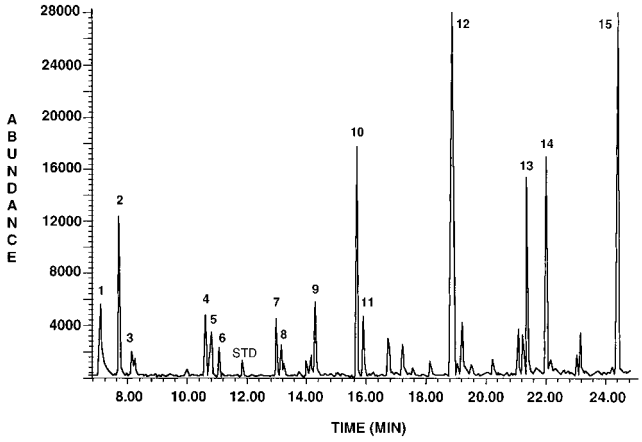

Figure 1. GC−MS profile of *N*-trifluoroacetyl-*N*-methyl esters of amino acids in *Amanita rubescens*.

14.006). The dietary fiber content was calculated by combining enzymatic and gravimetric methods (modified method, section 985.29). All Bran from Kellog's was used as a blank instead of the kit originally used. Crude fat was extracted by Soxtec extraction with methanol/chloroform (2:1 v/v) used in place of petroleum ether (modified method, section 14.019). Finally, crude protein was determined by a micro-Kjeldahl method (AACC (1983), section 46-13), and a conversion factor of 4.38 was used to quantify the nitrogen percentage of the crude protein (Bano and Rajarathnam, 1988).

Free Amino Acid Analysis. *Extraction Technique.* Sample analysis was conducted as follows: 0.25 g of mushroom (lyophilized and milled) plus norvaline (internal standard) was extracted with methanol/chloroform/water (12:5:3) at room temperature for 12 h and reextracted with 4 mL of water for 4 h (Bieleski and Turner, 1966). The extracts were combined and extracted with 10 mL of chloroform. The final extract pH was adjusted to 3 and passed through a Bio-Rad resin H^+ 200−400 mesh.

Derivatization. The amino acids in the eluent were derived first to their methyl ester forms with 600 μL of acetyl chloride in 2 mL of dry methanol; the esters were then trifluoroacetylated with 250 μL of trifluoracetic anhydride and 200 μL of dry dichloromethane for 1 h. The samples were evaporated under a stream of nitrogen and redissolved in 100 μL of CH_2Cl_2.

GC−MS. The derived free amino acids were separated in a HP5890 Serie II gas chromatograph from Hewlett-Packard, equipped with HP-MS detector 5972, and a 30 m × 0.2 mm × 0.25 μm 5% phenyl methyl silicone capillary column (HP-5MS). Operating conditions were as follows: Helium was the carrier gas, 0.44 mL/min; detector, 250 °C; injector, 300 °C; injected volume, 1 μL. The column was held for 3 min at 150 °C and programmed at 6 °C/min to a final temperature of 240 °C for 30 min.

Hydrolyzed Free Fatty Acid Analysis. *Extraction.* Sample (150 mg) plus heptadecanoic acid methyl ester (internal standard) was extracted with chloroform/methanol (2:1) on a Soxtec system HT (Tecator, Sweden) at 60 °C for 1 h. The final extracts were concentrated to 5 mL in the same equipment.

Derivatization. The fatty acids in the extract were simultaneously hydrolyzed and derived to their methyl ester forms with 1 mL of NaOH/methanol at 90 °C for 10 min and then a complete derivation was asured with 1 mL of BF_3 at 90 °C 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 stream of nitrogen and redissolved in 100 μL of isooctane.

GC−MS. The derived free fatty acids were separated in a HP5890 Serie II gas chromatograph equipped with a MS detector 5972, and a cross-linked (30 m × 0.2 mm × 0.25 μm) column with a stationary phase of 5% phenyl methyl silicone

Table 3. Free Fatty Acid Content[a] of Wild Edible Mushrooms Compared with *A. bisporus* and *U. maydis* (mg/g, Dry Basis)

fatty acid	*A. rubescens*	*B. frostii*	*L. indigo*	*R. flava*	*A. bisporus*[b]	*U. maydis*[c]
benzoic acid	0.074	nd[j]	nd	0.258	nd	nd
(C14)	0.067	0.052	nd	nd	0.142	0.145
(C15)	0.226	nd	nd	nd	nd	0.087
C14-9Me[d]	nd	0.106	0.134	nd	nd	nd
(C16:1)[e]	0.147	0.171	nd	nd	nd	0.874
(C16)	11.301	6.167	3.491	4.041	4.390	2.990
(C18:2)[e]	21.745	16.847	13.276	15.714	27.647	9.085
(C18:1)[e]	69.304	19.527	10.757	12.032	0.637	7.843
(C18)	6.254	1.754	32.057	0.910	1.274	0.552
(C20)	0.329	nd	0.213	nd	1.133	0.481
(C22)	0.245	0.331	nd	nd	nd	0.322
total	109.69 ± 0.57[f]	44.95 ± 0.74[h]	59.93 ± 0.96[g]	32.96 ± 0.21[i]	35.220	22.383
% essential	83.14 ± 0.02[f]	81.28 ± 0.30[g]	40.10 ± 0.08[h]	84.16 ± 0.06[f]	78.49	79.52

[a] Mean values and standard error of the sum of three independent determinations. [b] Beuchat et al. (1993). [c] Valverde and Paredes-López (1993). [d] 9-Methylmyristic acid methyl ester. [e] Essential fatty acid. [f–i] Statistically significant ($p < 0.05$ Duncan). [j] nd, not detected.

(HP-5 MS). Operating conditions were as follows: Helium was the carrier gas, 0.56 mL/min; detector, 250 °C; injector, 200 °C; injected volume, 2 μL. The column was held for 3 min at 130 °C and programmed at 3 °C/min to a final temperature of 270 °C for 35 min. The identification of all compounds (amino acids and fatty acids) was performed by means of their individual mass spectra and a comparison with the authentic standards or mass spectra reported by Leimer *et al.* (1977). The quantitative data were obtained by means of internal standards. However, the amino acid amounts were corrected using a correlation factor relative to the internal standard used (Lizárraga-Guerra and López, 1996).

RESULTS AND DISCUSSION

The proximate composition of the wild edible mushrooms analyzed is shown in Table 1. The moisture content in these mushrooms was 92.90−95.15%; this range falled into the 85−95% range, which is a normal percent for fresh mushrooms (Breene, 1990). The ash contents of Bf and Li were low compared to *Agaricus bisporus* (Ab) (9.1%) and *Ustilago maydis* (Um) (5.5%) reported by Beuchat *et al.* (1993) and Valverde and Paredes-López (1993), respectively. The dietary fiber content of the studied mushrooms was much higher in comparison to *A. bisporus* (6.6%) and *U. maydis* (16.0%), reported by Beuchat *et al.* (1992) and Valverde and Paredes-López (1993), respectively. The fat content of Bf (3.7%), Li (4.3%), and Ar (8.3%) was higher than the reported for Ab and Um, except for Rf (Table 1).

Finally, the protein contents of Ar, Bf, Rf, and Li were 17.4, 15.8, 14.5, and 13.4%, respectively, all of them higher than the reported for Um (11.5%) (Valverde and Paredes-López, 1992) but lower than the 35.0% reported for Ab (Beuchat *et al.*).

Figure 1 shows a characteristic GC−MS profile for *A. rubescens* of total free amino acids in their *N*-trifluoracetic-*N*-methyl ester form. The free amino acid compositions of all species are listed in Table 2. The total free amino acid values for Rf, Ar, and Bf were 47.41, 45.90, and 23.17 mg/g on a dry weight basis, respectively, these values are all higher than the values reported for Um (20.92 mg/g) and Ab (24.34 mg/g) by Lizárraga-Guerra and López (1996).

Glycine, aspartic acid, glutamic acid, ornithine, and lysine (eluting order) were among the most abundant amino acids present in all species. *A. rubescens* presented 17.53, 6.95, 2.79, 2.77, and 2.39 mg/g for glutamic acid, lysine, alanine, aspartic acid, and glycine, respectively. Besides lysine, *A. rubescens* contained other abundant essential amino acids such as threonine (1.36 mg/g) and phenylalanine (2.23 mg/g). *R. flava* contained

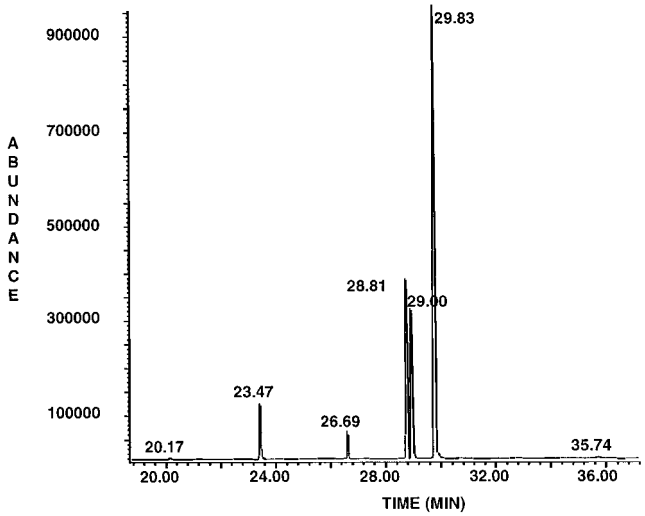

Figure 2. GC−MS profile of methyl esters of free fatty acids of *Lactarius indigo.* Myristic acid 9-methyl (20.17), palmitic acid (23.47), linoleic acid (28.81), oleic acid (29.00), stearic acid (29.83), and arachidonic acid (35.74).

9.42, 7.18, 7.14, 5.94, and 5.70 mg/g for glutamic acid, *N*-methylglycine, glycine, lysine, and ornithine, respectively. On the other hand, Bf showed 6.92, 3.10, 3.03, and 2.46 mg/g for glutamic acid, ornithine, glycine, and lysine, respectively.

The large amounts of glutamic acid in all mushrooms could be relevant since it is well-known that this amino acid is involved in the primary umami taste and also because its used commercially as a flavor potentiator (Yamaguchi *et al.*, 1971; Yojiro and Morely, 1987).

Four nonproteic amino acids were found in all the wild edible mushrooms: *N*-methylglycine, β-aminoisobutyric acid, γ-aminobutyric acid, and ornithine (Table 2). Other unusual amino acids were partially identified; however, they are not been reported here. The reason to believe this is the presence of characteristic amino acid fragment ions observed in their mass spectra. On the other hand, the absence of basic and sulfur-containing amino acids could be due to their instability or to the knowledge that these are not very common amino acids in mushrooms.

Figure 2 shows a typical GC−MS profile obtained for fatty acid methyl esters of *L. indigo.* The free fatty acid compositions of the studied wild edible mushrooms are shown in Table 3. The total free fatty acid values are

109.7 (Ar), 59.9 (Li), 44.9 (Bf), and 33.0 mg/g (Rf) on a dry weight basis; these values are higher than the amounts reported for Ab (35.2 mg/g) by Beuchat *et al.* (1993) and Um (22.4 mg/g) by Valverde and Paredes-López (1993), except for the Li species.

All the analyzed mushrooms in the present work contained larger quantities of essential fatty acids (91.19 (Ar), 36.54 (Bf), 27.74 (Rf), and 24.03 (Li) mg/g) than the published for Um (17.80 mg/g) by Valverde and Paredes-López (1993). Essential fatty acids common to all species included the C18:1 and C18:2. These two fatty acids compresed 83.1, 81.3, 40.1, and 84.2% for Ar, Bf, Li, and Rf, respectively, of the total fatty acids. On the other hand, *L. indigo* contained the highest saturated fatty acids such as the stearic acid with 32.06 mg/g, which corresponds to 53.5% of the total free fatty acids. Ar and Bf presented similar amounts of behenic acid (0.25 and 0.33 mg/g, respectively). And Li and Bf were the only two species that contained 9-methylmyristic acid (0.13 and 0.10 mg/g, respectively). Finally, Rf also presented a significant amount of benzoic acid (0.25 mg/g).

The high concentration of unsaturated fatty acids in these wild edible mushrooms is very significant from a nutritional standpoint (Bano and Rajarathnam, 1988).

Conclusions. The reported protein content for the studied wild edible mushrooms was lower than *A. bisporus* but higher than important food items such as wheat and milk powder. On the other hand, all the analyzed mushrooms presented large amounts of free lysine; therefore, they are a valuable supplement in the human diet. In general, the total unsaturated fatty acids for the studied mushrooms was larger than Ab and Um, except for Li. Finally, on the basis of the overall results, we believe that *A. rubescens* presents the best chemical and nutritional composition among the studied species. In the near future, all these mushrooms could be an important exotic food for export purposes.

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Received for review July 24, 1997. Accepted July 24, 1997.[⊗] This work was supported partly by the Consejo de Ciencia y Tecnología del Estado de Querétaro (CONCyTEQ) project Estudio Florístico y Faunístico de Algunas Regiones del Estado de Querétaro in México.

JF970640U

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.